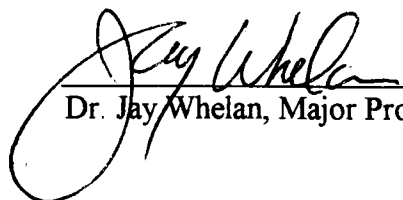
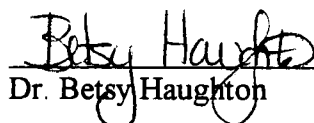


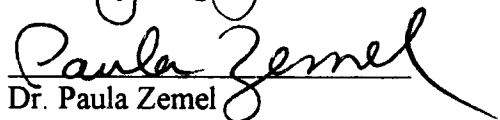
To the Graduate Council:

I am submitting herewith a thesis written by Laura Taber entitled "Assessment of the arachidonic acid content in a select group of foods commonly consumed in the American diet." I have examined the final 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.


Dr. Jay Whelan, Major Professor

We have read this thesis
and recommend its acceptance:


Dr. Betsy Haughton


Dr. Paula Zemel

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Associate Vice Chancellor and
Dean of The Graduate School

**Assessment of the Arachidonic Acid Content in a
Select Group of Foods Commonly Consumed
in the American Diet**

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

Laura Taber
December, 1998

DEDICATION

This thesis is dedicated to my parents

Ray Anderson Taber

and

Virginia Underwood Taber

who have loved, supported and encouraged me throughout my life, and without whom
this work would not have been possible.

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ABSTRACT

Arachidonic acid (AA) is a 20-carbon polyunsaturated fatty acid found in animal products, including red meat, poultry, fish and eggs. AA plays an important role in cell signaling and the function of all tissues are affected by AA and its metabolites. This study was designed to determine the levels of AA in foods known to be relatively rich in AA and commonly consumed in the American diet. Food items included were beef rib eye, chicken, pork loin, turkey and white tuna packed in water and were selected based on their frequency of consumption. Both raw and cooked samples were analyzed following procedures outlined in USDA Agriculture Handbook No. 8., the most comprehensive data bank on the nutrient composition of food. Experimental values were compared to those published by the USDA in an effort to validate published values. Experimental levels of AA were significantly higher than those reported by the USDA in all cooked foods except chicken thigh. Experimental levels of AA were significantly lower in tuna than those published by the USDA. In addition to the data presented in this study, uncertainty in the reliability of the USDA data for fatty acid analysis is based on the fact that some of their data is taken from empirical estimates. In addition, some of their data is over 30 years old, and hasn't been updated. Since then there have been changes in animal husbandry practices, as well as more reliable lipid analytical techniques. Because of the importance of USDA Agriculture Handbook No. 8 as a reliable source of nutrient content in foods and for determining average daily intakes of nutrients, a reevaluation of the fatty acid data may be warranted.

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LIST of ABBREVIATIONS

AA	Arachidonic acid
COX	Cyclooxygenase
DGLA	Di-homo gamma linolenic acid
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FA	Fatty acid
GLA	Gamma linolenic acid
HETE	Hydroxyeicosatetraenoic fatty acid
HpETE	Hydroperoxyeicosatetraenoic fatty acid
JNK	Jun N-terminal kinase
kDa	Kilodalton
LA	Linoleic acid
LOX	Lipoxygenase
LT	Leukotriene
MAP kinase; MAPK	Mitogen-activated protein kinase
MARCKS	Myristolated alanine-rich C-kinase substrate
mRNA	Messenger ribonucleic acid
MUFA	Monounsaturated fatty acid
NSAID	Nonsteroidal anti-inflammatory drug
PG	Prostaglandin
PGI ₂	Prostacyclin

PGI ₂ -M	Prostacyclin metabolite
PKC	Protein kinase C
PL	Phospholipid
PLA ₂	Phospholipase A ₂
PPAR	Peroxisome proliferator activated receptor
PUFA	Polyunsaturated fatty acid
SFA	Saturated fatty acid
TG	Triacylglycerol
TXA ₂	Thromboxane
TXA ₂ -M	Thromboxane metabolite
USDA	United States Department of Agriculture
w/w	Weight for weight

PART I

INTRODUCTION

There has been a great deal of concern about the biological and physiological effects of dietary fat and their relationship to chronic diseases. Over the past several decades the dangers of consuming too much saturated fat and its connection with increased levels of plasma cholesterol have been increasingly emphasized. More recently the role of polyunsaturated fat (PUFA) is being investigated.

Arachidonic acid is a 20-carbon PUFA with 4 double bonds. It is metabolized endogenously from dietary linoleic acid (18 carbons, 2 double bonds), which is found primarily in vegetable oils. However, its conversion from linoleic acid is limited. When AA is included in the diet in the form of products from animal sources, it by-passes regulatory mechanisms and tissues become enriched in AA.

AA has important roles in normal cellular function including modulation of cell signaling and gene expression which leads to the regulation of cell differentiation, cell proliferation and programmed cell death, or apoptosis. AA is converted intracellularly to the biologically active eicosanoids, which also play important roles in cellular function. Their expression has been linked to the regulation of thrombosis and platelet aggregation (e.g. thromboxane and prostacyclin), inflammation (e.g. prostaglandin E₂ and leukotriene B₄) and immune response (e.g. prostaglandin E₂).

Consuming dietary AA increases levels of tissue AA and eicosanoids *in vivo* and *in vitro* and it is believed that chronic production and elevated levels of eicosanoids are linked to cardiovascular disease, inflammatory disorders and some forms of cancer.

The amount of AA consumed has been estimated to be 170-200 mg/day. This estimate was based on food composition tables published by the United States Department

of Agriculture (USDA). The USDA publishes the most comprehensive data bank on the levels of AA in food, however, preliminary data from our laboratory suggest that data from the USDA may be inaccurate. In order to obtain accurate data for dietary intakes, the AA content in foods as published by the USDA must be validated.

Therefore, the purpose of this study is to determine the amount of AA in foods commonly consumed in the American diet and compare those results to data published by the USDA.

PART II

REVIEW OF THE LITERATURE

General Overview

Arachidonic acid (AA) is an important cellular fatty acid. It is found primarily in the plasma membrane and other membranes of cellular organelles, including the endoplasmic reticular and nuclear membranes. When released from membrane phospholipids by a variety of phospholipases, AA is oxidized to hundreds of compounds, i.e., eicosanoids, which are involved in the regulation of cell function, such as cell signaling, gene expression, cell differentiation, cell proliferation and apoptosis.

AA Metabolism

AA is derived from linoleic acid (18:2 n-6; LA) metabolically by a series of desaturases and elongases. The first step is the conversion of LA to gamma-linolenic acid (18:3 n-6; GLA) by delta-6 desaturase, the major rate-limiting step in this pathway (Figure 2.1). Two carbons then are added to GLA by an elongase, forming dihomo-gamma linolenic acid (20:3 n-6; DGLA), and desaturated to AA by delta-5 desaturase, a second rate-limiting step. AA can be elongated further to 22:4 n-6 and 24:4 n-6, and desaturated to 24:5 n-6, all of which occurs in the endoplasmic reticulum. The 24-carbon fatty acids are transferred then to peroxisomes, where they are shortened by 2 carbons (or retroconverted) to 22:5 n-6 (1). The equilibrium in these reactions lies towards AA.

The conversion of LA to AA is highly regulated. Intakes of LA over 3 energy percent do not result in changes in tissue AA levels (2, 3). Tissues can be enriched with AA by-passing the rate-limiting steps of delta-5 and delta-6 desaturase.

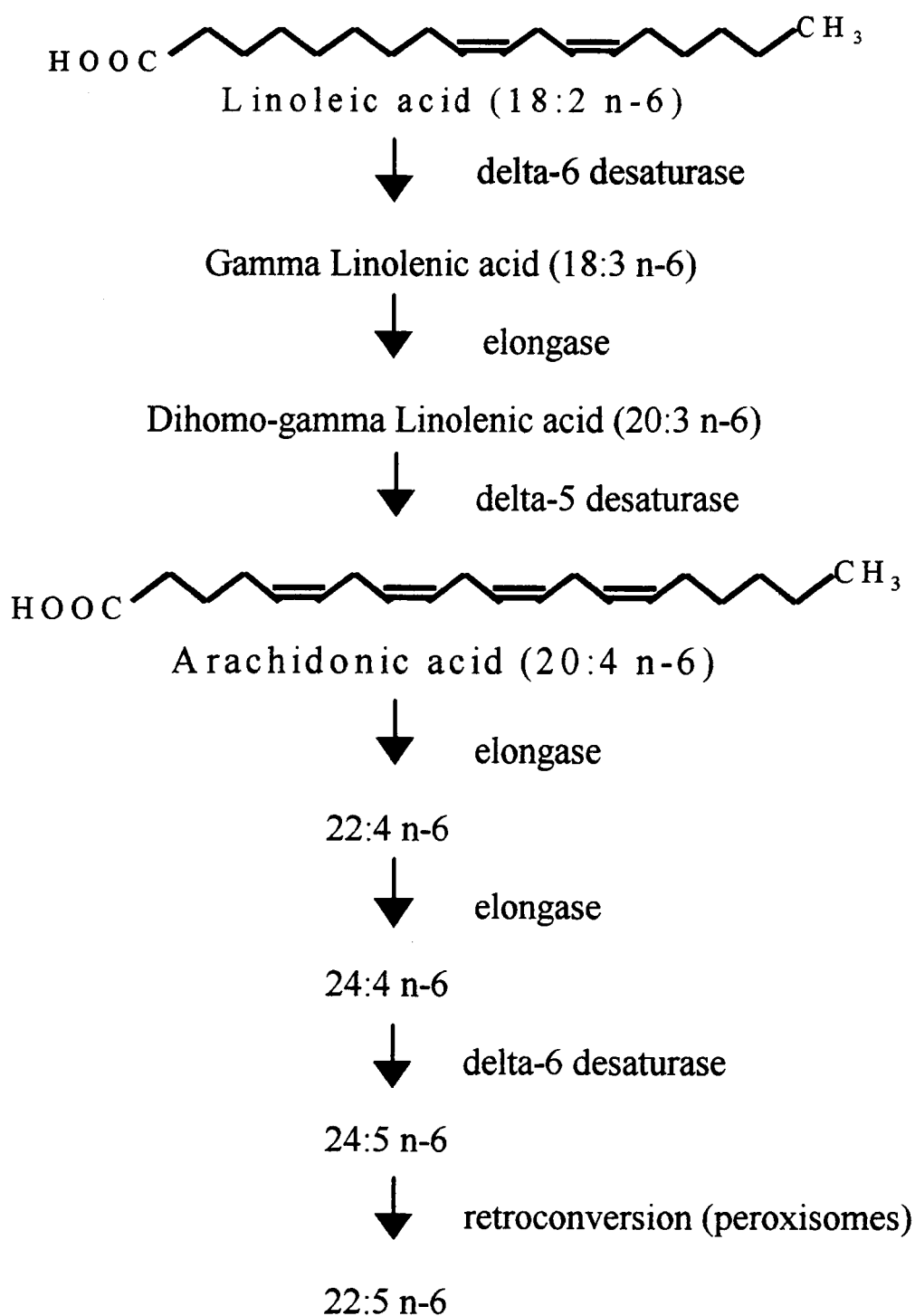


Figure 2.1 Metabolism of the n-6 PUFAs

AA release and conversion into eicosanoids

AA is released from triglycerides or membrane phospholipids by the activation of phospholipase A₂ (PLA₂), the rate-limiting step in the activation of the AA cascade in cells (4). AA is oxidized to a variety of 20-carbon biologically active fatty acids known as eicosanoids by two major pathways (Figure 2.2). One pathway involves cyclooxygenase (COX; also known as prostaglandin H synthase), which is found in all eukaryotic cell types. COX oxidizes AA into the unstable endoperoxide-containing prostaglandin, PGH₂. Iron, found within the structure of the enzyme, is the cofactor for the oxidation (4). PGH₂ is the substrate for further transformations to the major prostaglandins such as PGE₂, PGD₂, PGJ₂ and PGF_{2α}, and prostacyclin (PGI₂) and thromboxane (TXA₂) by specific synthases. Specific tissues contain different enzymes, thereby generating individualized prostaglandins.

Metabolic importance of AA derivatives

PGE₂ has many metabolic effects, such as regulating smooth muscle dilation and sodium excretion. It reduces hyperthermia, inhibits gastric secretion, catecholamine release, natural killer cell activity, and modulates renal homeostasis (5). As reviewed by Kinsella *et al.*, PGE₂ is important in regulating monocyte and macrophage functions and overall immune response. For example, the level of PGE₂ in a cell determines whether lymphocytes are differentiated into mature T-cells. In addition, chronic overproduction of PGE₂ can lead to suppression of the immune system (6).

Recent work with Swiss 3T3 fibroblasts has shown a direct connection between

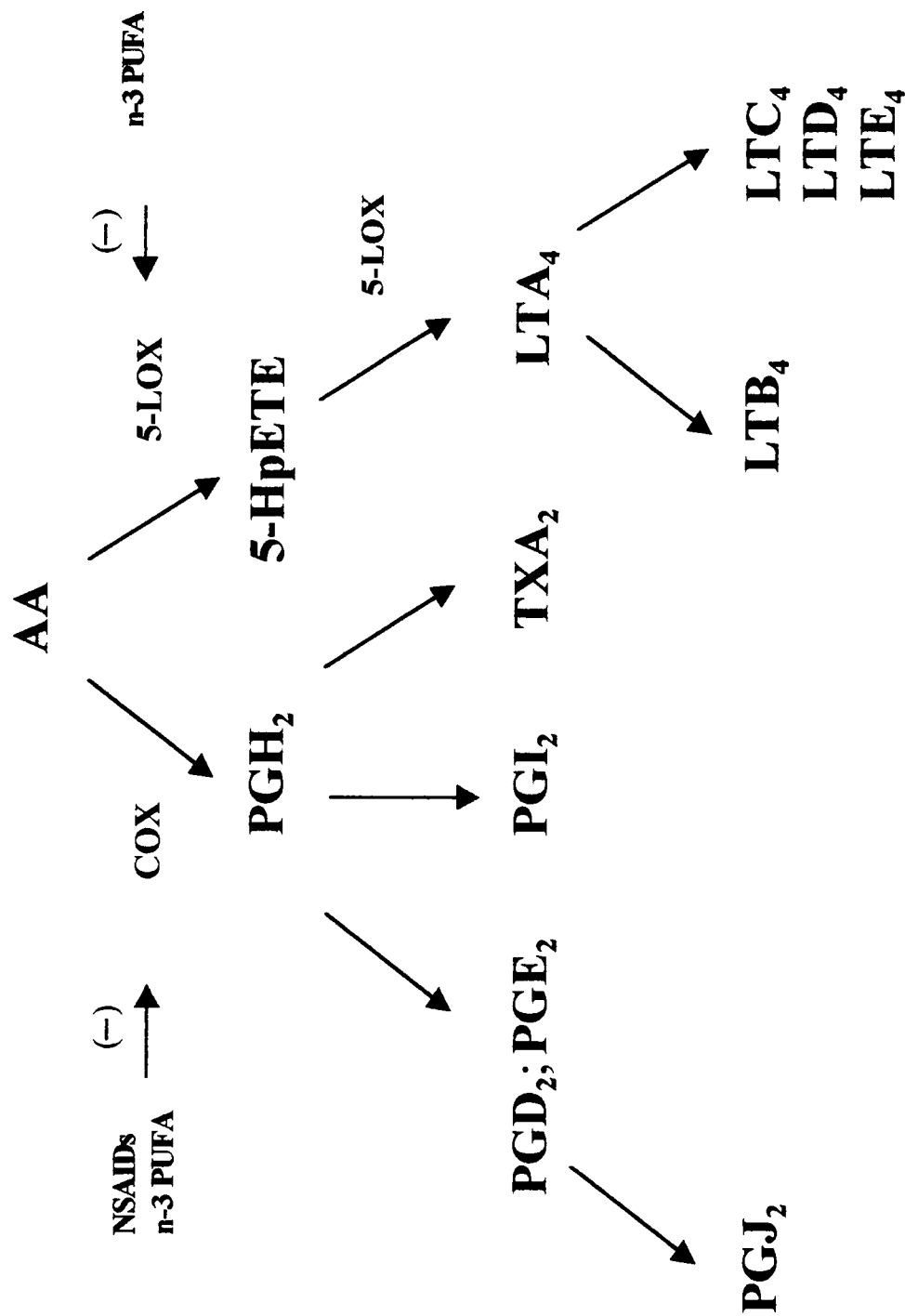


Figure 2.2 Cyclooxygenase (COX) and Lipoxygenase (5-LOX) pathways

PGE₂ levels and induced accumulation of mRNA of the oncogenes *c-fos* and *Erg-1* (7, 8). The addition of indomethacin, an inhibitor of COX, markedly reduced this effect, and the long chain n-3 fatty acids, eicosapentaenoic acid (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA), did not have the same effects as AA and PGE₂, suggesting specificity for AA (8). PGE₂ also induced *c-fos* mRNA expression in glomerular mesangial cells (5).

Prostacyclin (PGI₂) is produced by endothelial cells in vascular tissue where it is a potent vasodilator and inhibitor of platelet aggregation (9). However, vascular tissue can vary greatly in its ability to produce PGI₂ and is influenced by hormonal stimulants and the age of the individual (10, 11).

Thromboxane (TXA₂) is a potent vasoconstrictor and platelet aggregating agent. When it was first discovered in the 1970's, it was called "rabbit aorta contracting substance"(12). It is found in many tissues, including platelets, leukocytes, spleen, inflammatory granuloma, brain and kidney (12). In platelets, TXA₂ causes the formation of transient thrombi and facilitates platelet-vascular wall interaction and vasoconstriction (13).

There is increased excretion of the major urinary metabolite of TXA₂ in children with congenital heart disease. TXA₂ production decreases with the completion of successful cardiac surgery (14). Japanese researchers have shown that TXA₂ constricts the hepatic vein in isolated canine livers, which may contribute to TXA₂-induced liver injury (15).

There are three lipoxygenases (LOX) that convert AA to hydroperoxy

eicosatetraenoic fatty acids (HpETEs) which can be reduced subsequently to hydroxy eicosatetraenoic fatty acids (HETEs) via a variety of peroxidases. LOX also contain iron. The HpETEs have important physiological effects, but have very short half lives, and thus, are difficult to measure (4). One of these LOX, the 5-LOX, is the committed step in the biosynthesis of leukotrienes. 5-LOX oxidizes AA to 5-HpETE and leukotriene A₄ (LTA₄). LTA₄ is metabolized further to leukotriene B₄ (LTB₄) by LTA₄ hydrolase. LTB₄ is a potent chemotactic agent that initiates, coordinates, sustains and amplifies the inflammatory response (16).

LTA₄ can be converted also to the cysteinyl leukotriene, leukotriene C₄ (LTC₄), by the glutathione transferase enzyme, LTC₄ synthase (4). LTC₄ can be metabolized further to leukotrienes D₄ (LTD₄) and E₄ (LTE₄), key mediators of bronchial asthma, increasing bronchial constriction, mucus secretion and congestion of bronchial airways.

Cellular Effects of AA

AA and cell signaling

There are several different ways that AA functions in cell signaling. AA, through the binding of the eicosanoids to specific cell-surface receptors, modulates second messenger systems, such as phospholipase C, adenylyl cyclase and ion channels (17). AA activates protein kinase C (PKC) *in vitro* (7), and *in vivo* (18). As reviewed by Becker *et al.*, PKC has many roles in cellular functioning, including stimulation of cell growth, regulation of ion channels, changes in the cytoskeleton, modulation of cellular pH and secretion, and possibly the regulation of connections between microfilaments and the

plasma membrane (19). This is accomplished in part through the phosphorylation and activation of mitogen-activated protein kinases (MAP kinases or MAPKs), myristolated alanine-rich C-kinase substrate (MARCKS) and talin, proteins important in the regulation of cell growth (19). AA and its lipoxygenase metabolite, 12(S)-hydroperoxyeicosatetraenoic acid (12(S)-HpETE), activate jun N-terminal kinases (JNKs) in vascular smooth muscle cells, possibly mediated by PKC-dependent mechanisms (20). Rizzo and Carlo-Stella report stimulation of JNKs in mouse stromal cells by AA, independent of eicosanoids (21).

AA has been implicated as an important second messenger for insulin secretion in pancreatic beta cells by inducing the influx of intracellular calcium (22), and there appears to be a relationship between AA-stimulated calcium influx and the release of prolactin from rat pituitary cells (23).

AA and gene expression

AA has been shown to down-regulate GLUT 4 and up-regulate GLUT 1 gene expression in 3T3-L1 adipocytes (24). Long and Pekala suggest that AA affects the GLUT 4 transport protein in 3T3-L1 cells by modulating the nuclear hormone receptors, peroxisome proliferator activated receptors (PPARs) (25).

AA suppresses the expression of a variety of lipogenic genes, including stearoyl-CoA desaturase-2 (26), and hepatic fatty acid synthase (27). AA is a potent modulator of human heat shock gene transcription in HeLa S3 cells (28), possibly blocking programmed cell death, (commonly referred to as apoptosis) (29). In some cell lines, gene transcription

is linked to the COX pathway (8), while in other cell lines it is linked to the LOX pathway (30-34).

AA and cell differentiation

Activation of PPAR γ by the AA-derived eicosanoid, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (PGJ₂), has been shown to be an important regulator of adipogenesis (35, 36), while PGF_{2 α} blocks adipogenesis in 3T3-L1 cells through the activation of MAP kinase and subsequent phosphorylation of PPAR γ . This balance between PGF_{2 α} and PGJ₂ signaling may be central to the development of obesity and diabetes (37). A number of investigators clearly have demonstrated that dietary AA up-regulates the prostaglandin pathway systemically and as such may be important in these signaling pathways (38-41).

AA and cell proliferation and apoptosis

Inhibition of an 85-kilo dalton (kDa) PLA₂ decreases proliferation of human coronary artery vascular smooth muscle cells, suggesting that the release of AA is central to the regulation of cell proliferation (42). This is supported by evidence that *exogenous* AA increases expression of the early response genes *c-fos* (30), *c-jun* (31) and MAP kinase (32) and that these effects are inhibited also by PLA₂ inhibitors (31) in rat vascular smooth muscle cells. The AA metabolites, 12 and 15 HpETEs, mediate the induction of *c-fos* and *c-jun* RNA expression in these hydrogen peroxide-stimulated rat vascular smooth muscle cells (33). The inhibition of PLA₂ in the human vascular smooth muscle cells did not result in apoptosis or phase-specific cell cycle arrest (42).

The release of AA has been linked also to cell proliferation in AR4-2J cells (rat pancreas carcinoma cells) (43). The addition of leukotrienes D₄ and C₄ had a proliferative effect on the growth of human gastric cancer cell line AGS, while prostaglandins D₂ and E₂ suppressed their growth (44).

Recently, Chan *et al.* reported that increasing intracellular levels of AA increase apoptosis in non-steroidal antiinflammatory drug (NSAID)-treated human colonic tumor cell lines HCT 116 and SW 480 (45). Increasing intracellular AA levels appear to stimulate sphingomyelinase and the conversion of sphingomyelin to ceramide, thereby increasing cellular apoptosis. Their data suggest that the antitumor effects of NSAIDs are not mediated by inhibition of PG synthesis, but rather by the accumulation of free arachidonate (45).

Other studies show conflicting results concerning the effects of AA on apoptosis (29). LOX inhibitors partially prevented apoptosis, while the addition of 12-HETE induced apoptosis in murine insulin-secreting cells. However, COX inhibitors did not inhibit apoptosis in the same cells (46). Rat carcinosarcoma cells undergo rapid apoptosis in response to inhibition of LOX activity. Subsequent addition of AA, LA or alpha-linolenic acid can reverse these effects (29).

In summary, AA is an important PUFA regulating cellular function. It modulates cell signaling through PKC and other proteins, regulates gene expression, and can modulate the activation of nuclear receptors. This results in the regulation of cell differentiation, cell proliferation and apoptosis.

Dietary Effects of AA

One of the most notable human studies evaluating dietary AA was conducted by Seyberth *et al.* in 1975, at a time when eicosanoids were being elucidated initially (47). This study was to assess the hypotensive effects of AA in a feeding trial designed to last 4 weeks. However, the study had to be terminated early due to hyperaggregation of platelets in 2 of the 4 patients, establishing the notoriety of dietary AA as a prothrombotic PUFA. This study also demonstrated that the feeding of AA (6 g/day) resulted in tissue enrichment of AA and resulted in an increase in the *in vivo* production of prostaglandins (i.e., PGE₂) (47). In contrast, subsequent studies in animal models and humans have failed to confirm the prothrombotic effects of dietary AA (39, 48, 52).

As a result of the Seyberth paper, there was a renewed interest in the biological effects of dietary AA. In 1982 O'Dea and Sinclair demonstrated that n-3 fatty acids of fish oil would reduce tissue AA levels in 12 Aborigine subjects maintained on a fish diet. To their surprise, tissue AA levels increased three-fold. Analysis of the diet revealed that AA was found in the fish fed to these subjects and was responsible for their results, revealing the potency of dietary AA in altering tissue AA content (49). Further studies by Sinclair *et al.* showed significant increases in AA levels in phospholipids and cholesterol esters in subjects fed diets containing tropical fish or kangaroo meat, both of which are good sources of AA (3). AA levels as low as 305 mg/day (in the form of kangaroo meat) showed a marked rise in plasma phospholipid levels of AA, as well as a significant increase in the urinary output of a PGI₂ metabolite (PGI₂-M), but not a significant increase in a TXA₂ urinary metabolite (TXA₂-M) (50). However, consuming 500 mg/day AA in the

form of white meat and eggs increased urinary levels of $\text{TXA}_2\text{-M}$ (50). A follow-up study providing approximately 140 mg AA/day in the form of meat or fish to 29 healthy, non-smoking adults for two weeks resulted in significant increases in the AA content of serum phospholipids but no significant changes in platelet AA levels, platelet aggregation, or *in vivo* or *ex vivo* eicosanoid production (48). Importantly, these studies demonstrated that consuming AA as part of a mixed diet could enrich tissues with AA and increase eicosanoid biosynthesis *in vivo* and *in vitro*, but these effects were dose dependent.

The most comprehensive human trial with dietary AA was performed by Nelson and colleagues of the USDA (41, 51-53). They supplemented diets with 1.5 g AA/day (as a triglyceride) to 10 healthy males for 50 days. They reported increases of AA in plasma phospholipids and cholesterol esters but no significant differences in adipose tissue fatty acid composition (51) or platelet aggregation (52). However, there was a 41% increase in urinary $\text{TXA}_2\text{-M}$ and a 27% increase in urinary $\text{PGI}_2\text{-M}$ (41), but minimal changes in immune function (53). As indicated by the authors, these results only apply to healthy individuals on short term supplementation. The investigators commented that the effects may not be the same with chronic intakes over long periods of time or with individuals at risk for health problems or individuals with pre-existing conditions (41, 53).

Notable differences in AA content in serum phospholipids have also been observed among individuals who were classified as vegetarians, semi-vegetarians and omnivores (54). AA levels in plasma lipids in vegetarians and semi-vegetarians were significantly lower than in omnivores. These biological effects are most likely associated with AA intake, since AA is found only in animal products.

Similar results have been reported in a number of animal studies. Dietary AA has been shown to enrich tissues in arachidonate and increase eicosanoid production *in vivo* and *in vitro* in Syrian hamsters (39), mice (38, 55, 56), and rats (40, 57). These effects are independent of dietary levels of LA, the metabolic precursor of AA (39), and attenuated by enrichment with n-3 fatty acids (38, 39, 56). One drawback of these animal studies is the difficulty of extrapolating AA levels provided in the diets of animals to those consumed by humans.

In summary, these results collectively indicate that: 1) consumption of AA by humans and animals results in its incorporation into tissue phospholipids and subsequent metabolism to biologically active eicosanoids, and 2) consumption of AA as part of a mixed diet at levels considered reasonable for humans can result in tissue enrichment of AA and increased eicosanoid formation.

AA and Cancer

AA has been linked to a number of chronic diseases, such as cardiovascular disease (13) and inflammatory disorders (6); however, more recently AA and its metabolism has been identified as an important mediator of cancer, especially intestinal cancer (58). Four key components link AA to tumor promotion.

First, dietary LA, the precursor of AA, fed to female rats resulted in a dose-dependent increase in the incidence and number of chemically-induced mammary tumors (59). Similarly, increasing dietary LA content from 0.3% to 5% increases the incidence and number of chemically-induced colon tumors in rats (60).

Second, dietary n-3 PUFAs, such as EPA and DHA which are found in fish oil, are known for their ability to antagonize AA metabolism, resulting in a reduction of AA-derived eicosanoids. EPA and DHA reduce tumor initiation and promotion in rats and mice (61-63). While the mechanism for this antithetic relationship of dietary n-6 and n-3 PUFA is not totally understood, it has been suggested that n-3 PUFAs may work through a down regulation of PKC, as reviewed by McCarty (64). This may inhibit the angiogenesis and metastasis of tumors (64). The reduction of colonic adenocarcinomas by EPA can be abrogated following the addition of LA to the diet (65).

Third, a unique feature of intestinal tumors is that they produce an excess of AA-derived eicosanoids, both in humans (66, 67) and mice (56) when compared to adjacent normal-appearing mucosa. This has been linked to the overexpression of COX-2 in rat (68) and human colonic tumors (69-71). Expression of COX-2 in human colorectal cancer cells has led to phenotypic changes that alter their metastatic potential (72).

Fourth, an inverse relationship has been shown to exist between NSAID use (which are known inhibitors of COX-1 and COX-2) and colorectal cancer. It has been demonstrated in a number of epidemiological studies that regular NSAID use reduces the relative risk of colorectal cancer by 40-50% (73). Sulindac, a commonly prescribed NSAID, exhibits antiproliferative activity against human colon cancer cells (74) and this has been linked to its ability to inhibit prostaglandin synthesis (75). Similarly, sulindac regresses tumors in patients who have familial adenomatous polyposis, a form of colon cancer related to the loss of the tumor suppressor gene *APC* (71). Other NSAIDs, such as indomethacin, suppress mammary cancer cell growth and metastasis in nude mice (76) and

these effects are attributed also to a suppression of AA-derived eicosanoids (77).

In summary, these data suggest that AA-derived prostaglandins are related to the promotion of tumors and that decreasing prostaglandin biosynthesis reduces the incidence and growth of tumors in animals and humans.

USDA Food Composition Tables

The United States Department of Agriculture (USDA) has compiled and evaluated the nutritive value of foods since the 1890's when the first data were published by W.O. Atwater and C.D. Woods (78). Since then, tables have been expanded to include vitamins, minerals and other dietary essentials in foods (78). At the present time the data in USDA's Nutrient Data Bank System are available in printed form through a 21 volume series of books called Agriculture Handbook No. 8, Composition of Foods: Raw, Processed, Prepared. It also is available in computerized form, the USDA Nutrient Data Base for Standard Reference (79). Four supplements have been issued for Agriculture Handbook No. 8, and new releases are issued periodically for the computerized data base, the last of which was Release #12, March 1998. There are several sources of food composition data, including USDA contracts with universities and commercial laboratories, and information from the food industry, academia, trade groups and other government agencies (79).

USDA conversion factors for fatty acids

Lipid conversion factors were developed by the USDA as a means of calculating

the fatty acid content of foods (80). They were developed as a way to convert methyl ester fatty acid data, expressed as weight percent, to the amount of individual fatty acid per 100 grams of food. The conversion factors are a way of relating fatty acid content to total lipid content (80).

The conversion factor is based on the average molecular weight of the fatty acids and the amount of triglyceride (TG) and phospholipid (PL) in the fat. For example, the average amount of TG and PL in the separable lean part of beef was determined to be 87% and 12% respectively. The fatty acid content of PLs and TGs is estimated to be 95.6% and 70.7% respectively (81). A typical computation for deriving the conversion factor for separate lean of beef is:

$$\text{conversion factor} = (.87 \times .956) + (.12 \times .707) = .831 + .085 = .916 \text{ (81)}$$

This number, 0.916, is now referred to as the "lipid factor" by USDA personnel. The current lipid factors quoted for raw beef rib eye and broiled beef rib eye are 0.9 and 0.91, respectively (personal communication, Dr. Juliet Howe, USDA, May 1998).

To determine the amount of fatty acid per 100 grams of food, the weight percent of the fatty acid methyl ester is multiplied by the conversion factor for that food, and then multiplied by the amount of lipid that has been determined gravimetrically for that food (81). For example, if the weight percent of the fatty acid methyl ester for AA in raw beef rib eye is 26.77%, the conversion factor is 0.9, and the amount of lipid is 8.3 g/100g; the equation for determining the amount of AA/100 g sample would be:

$$\text{amount of AA/100 g} = .2677 \times 0.9 \times .083 = 0.0199 \text{ mg AA/100 g sample}$$

Background of USDA database

It is important to know when USDA samples were analyzed in order to evaluate the reliability of the data. Fatty acid analyses of poultry samples, as reported by the USDA, were determined between 1963 and 1976, and these data have not been updated. Best estimates indicate that beef samples were analyzed in 1987 or 1988, egg samples in 1988 and 1989, pork samples in 1989, saltine crackers in 1989 or 1990 and tuna in 1991 (personal communication, Dr. Jacob Exler, USDA). Therefore, data in general are seven to 35 years old.

When the USDA lists a number in the "N" column of their data, it means the data were analyzed empirically. In most cases, data for cooked samples are computed from data for raw samples, using a slightly different conversion factor. However, the origin of some data is unclear. For example, there is a printed "N" for hard cooked eggs, but the data, as explained by the USDA, appear to be extrapolated from other sources. No "N" exists for the fatty acid data for white tuna. These data may have originated from unpublished experiments on albacore (personal communication, Dr. Jacob Exler, USDA). In addition, data for raw beef rib eye were taken from that of another undetermined cut of beef. This reflects the USDA policy of only analyzing some cuts from each species, and extrapolating that data to other cuts in the same primal area (81).

The USDA gets lipid values by contracting analyses out to different laboratories, sometimes generating conflicting data (personal communication, Margaret Hoke, USDA). Sometimes data is "normalized", i.e., the amount of fatty acids quantified from multiplying the fatty acid profile by the conversion factors is adjusted to equal the amount of lipid that

has been gravimetrically determined for any particular sample (personal communication, Dr. Juliet Howe, USDA, May 1998).

Compilation of fatty acid data is not trivial. The USDA uses a combination of sources in compiling fatty acid data published in Agriculture Handbook No. 8, and its computerized counterpart; and these data are derived from direct analytical and empirical methodology and indirect estimates. Some of the data (i.e., poultry) are over 30 years old. Since that time there have been significant changes, for example, in animal husbandry practices for beef cattle, where these animals were primarily range-fed but are now primarily grain-fed (82). There also have been advances in lipid analytical techniques (83). However, modifications of the USDA data base only reflect some of these changes.

Scope and Rationale of the Study

AA has very important roles in cellular signaling and gene expression which leads to modification and regulation of cell differentiation, cell proliferation and apoptosis, many of which are mediated by eicosanoids. Consumption of dietary AA by animals and humans results in increased AA levels in tissue phospholipids and subsequent metabolism to biologically active eicosanoids. Decreasing eicosanoid biosynthesis can have profound effects on cardiovascular disease, inflammatory diseases and cancer. However, there is controversy as to how much AA is consumed on a daily basis. This uncertainty is likely related to errors associated with food composition data.

The USDA is responsible for providing accurate nutrient composition data for foods in the American diet (79). Fatty acid data provided by the USDA is outdated and

incomplete, many times based on extrapolated values, rather than from empirical data. Therefore, the current study is designed to determine the levels of AA in some foods commonly consumed in the American diet and considered to be relatively rich in AA, and to compare these results to those published by the USDA in order to validate that data.

Criteria for selection

Foods analyzed included: beef, chicken, pork, turkey, eggs and tuna fish, which are animal products commonly consumed in the American diet (84, 85). Saltine crackers were analyzed also because another American database reported them to be high in AA. Cooked and raw samples of each food group were analyzed, as applicable. All items were selected from local stores in a supermarket chain, The Kroger Store™, in Knoxville, Tennessee. The best selling cuts of meat (e.g. beef rib eye steak, pork loin chop) and brands of tuna (Starkist) and crackers (Nabisco) were selected based on the advice of the store manager. Cuts of meat most closely similar to those presented in Agriculture Handbook No. 8 were selected based on the advice of an experienced butcher in the meat department.

Objectives

1. To determine the levels of AA in foods, raw and cooked, commonly consumed in the American diet, considered to be relatively rich in AA.
2. To compare the AA levels of a select sample of foods, raw and cooked, to the AA levels in Agriculture Handbook No. 8.

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PART III

ASSESSMENT OF THE ARACHIDONIC ACID CONTENT IN A SELECT GROUP OF FOODS COMMONLY CONSUMED IN THE AMERICAN DIET

This manuscript is being prepared for publication with co-author Whelan, J.
in *Lipids*

Abstract

Arachidonic acid (AA) is an extremely important fatty acid involved in cell regulation. When provided in the diet, it is cogently incorporated in membrane phospholipids and enhances eicosanoid biosynthesis *in vivo* and *in vitro*; however, there is controversy as to the levels of AA in food and in the diet. This study determined the amount of AA in cooked and raw portions of beef (rib eye), chicken (breast and thigh), eggs, pork (loin), turkey (breast), tuna and saltine crackers and compared these results to values published by the USDA in Agriculture Handbook No. 8. The cooked portions were prepared as described in Handbook No. 8. With the exception of chicken thigh and tuna, the levels of AA (w/w) in the selected foods analyzed were significantly higher, in general, than those values published in Handbook No. 8. The greatest differences were observed in beef (raw and cooked), turkey breast (raw and cooked) and pork (cooked) where AA levels were twice that of the USDA values. In contrast, the AA and n-3 fatty acid content in tuna were almost half the USDA values. The present data indicate that the USDA tends to under report the amounts of AA in a number of foods commonly consumed in the American diet and new initiatives should be considered to validate and update the current database for fatty acid composition of foods.

Introduction

Arachidonic acid (5,8,11,14 eicosatetraenoic acid, 20:4 n-6) is a polyunsaturated fatty acid (PUFA) located in membrane phospholipids where it is released by a variety of phospholipases and subsequently oxidized. Recent advances in lipid metabolism have

demonstrated a variety of roles for AA and its metabolic derivatives, the eicosanoids, in modulating cell signaling and gene expression. AA is derived metabolically from linoleic acid (9,12 octadecadienoic acid, 18:2 n-6; LA), the major PUFA in the diet. Although dietary LA is the major source of tissue AA, this metabolic pathway is regulated such that variations in the LA content of the Western diet do not significantly alter AA content of tissue phospholipids (1). Both animal (2-4) and human (5, 6) studies have demonstrated that consuming AA increases plasma and tissue levels of AA and significantly augments eicosanoid biosynthesis *in vivo* and *in vitro*. However, there is conflicting information as to how much AA is actually consumed and how much is in the foods we eat (3, 7-9). A series of comprehensive studies on the Australian diet suggests the amount of AA in their foods and in their diet may be lower than anticipated, but that these numbers are influenced by the lean and visible fat portions of the food samples (8, 9).

The United States Department of Agriculture (USDA) is responsible for publishing Agriculture Handbook No. 8, a comprehensive compilation of the nutrient composition of virtually all foods consumed in the American diet (10). Most, if not all, major software programs assessing human nutrient intakes rely on this database. Understandably, it is difficult for this database to remain current by keeping pace with technological advances in analytical procedures and changes associated with animal feeding and breeding practices. Therefore, despite volumes of nutrient composition tables, there is a lack of direct empirical data on the fatty acid composition, in particular AA, of many foods. Preliminary data from our laboratory suggested that the USDA was under reporting the amount of AA in US food products (11). Therefore, the purpose of this study was to

determine the amount of AA in foods known to be relatively high in AA and commonly consumed in the American diet and compare these results to those published by the USDA in Agriculture Handbook No. 8.

Materials and Methods

Samples

The selection criteria for samples was based on foods known to be relatively rich in AA that are commonly consumed in the American diet. These included beef, chicken, eggs, pork and turkey. White tuna canned in water was included as a food known to be rich in omega-3 fatty acids and saltine crackers were included as a negative control.

With the exception of the tuna and crackers, all samples were obtained fresh from local outlets of a large multi-state supermarket chain. They were refrigerated at 4°C after purchase, and processed on the same day, usually within one hour of purchase. Beef rib eye (BRE, n=4), chicken breast (CB, n=5), chicken thigh (CT, n=5), pork loin (PL, n=4) and turkey breast (TB, n=4) samples were skinned, trimmed of excess fat, cut in half and weighed. One half of each BRE sample was broiled and one half of each sample of CB, CT, PL and TB was roasted according to protocol established by the USDA (12-14). All meat samples were cooled for one hour after cooking prior to further processing and analysis. Approximately 77 g samples of commercially canned and processed (in water) tuna were analyzed. The fatty acid composition of single raw eggs (WE) weighing approximately 50 g was determined. In addition, four eggs were hard cooked in boiling water for 10 minutes, cooled for 20 minutes, shelled, weighed and analyzed.

All samples were finely chopped in a Cuisinart™ food processor (Model DLC-10E, Greenwich, CT) for 2 minutes in 0.9% cold NaCl at a concentration of 380 g sample/L buffer. The average weight of the samples was approximately 70 g (after cooking). Fifty milligrams of the internal standard nonadecanoic acid methyl ester (19:0) were added to each sample prior to processing. The chopped samples then were finely pureed in a Waring™ blender (New Hartford, CT) for 3 minutes at a final concentration of 210 g sample/L buffer. The homogenates were filtered through cheesecloth to remove connective tissue and under constant stirring three aliquots were removed for fatty acid analysis. Four to five individual food samples were prepared and analyzed as indicated above.

Lipid extraction and fatty acid analysis

Lipids were extracted by a modified method of Bligh and Dyer (15). Briefly, one part sample was suspended in 3 parts methanol/chloroform (2:1 v/v) and lipids were extracted following the addition of chloroform and 200 μ l saturated NaCl solution. The chloroform extraction was repeated (x 2). Following complete evaporation of the pooled chloroform fractions under a stream of nitrogen, the lipids were solubilized with toluene and saponified with KOH (0.5 N) in methanol for 8 minutes at 86°C. The samples were cooled and acidified with HCl (0.7 N) in methanol, extracted with two volumes of hexane (x 2), evaporated under an atmosphere of nitrogen and methylated with ethereal diazomethane. The fatty acid methyl esters were resuspended in hexane and separated by gas chromatography (Hewlett Packard 5890 Series II gas chromatograph (Palo Alto, CA)

equipped with a flame ionization detector) using a DB-23 capillary column (J & W Chromatography, Folsom, CA) with hydrogen as the carrier gas. The fatty acid methyl esters were identified by comparison of retention times with those of known standards (Nu-Chek Prep, Elysian, MN) and quantified based on the internal standard. It had been established previously that each sample did not contain an appreciable amount of 19:0.

Statistical analysis

The Statistical Analysis System (SAS Institute, Inc., Cary, N.C.) was used to analyze the data. Data were expressed as means $\pm$ SEM of 4-5 samples, each of which was expressed as the means of three aliquots. Differences between experimental values and those of the USDA were analyzed using the one-sample *t*-test. Data were considered significant at $p < 0.05$.

Results

Polyunsaturated fatty acid composition of raw samples.

Levels of AA varied in raw samples from a low of 46 mg/100 g in raw BRE to a high of 156 mg/100 g in raw WE, and varied significantly from those values reported by the USDA (Table 3.1). AA content was significantly higher in BRE (46 mg/100 g vs. 20 mg/100 g), CB (64 mg/100 g vs. 40 mg/100 g) and TB (59 mg/100 g vs. 30 mg/100 g) compared to the USDA values. Overall, AA content was significantly higher in half of the raw foods analyzed (Figure 3.1). LA content was significantly lower in PL (262 mg/100g vs. 440 mg/100 g) and higher in WE (1272 mg/100 g vs. 1148 mg/100 g).

TABLE 3.1 Polyunsaturated fatty acid composition of raw samples (mg/100g sample)¹

Raw samples	n-6 series						n-3 series				Total	
	18:2	20:3	20:4	22:4	22:5	Total	18:3	20:5	22:5	22:6	Total	n-3
Beef rib eye	178 (±20)	16 (±2)	46 ^a (±3)	7.3 (±0.4)	1.5 (±0.4)	248 (±25)	9.5 (±1.5)	5.1 (±0.4)	11.9 (±0.6)	1.7 (±0.5)	28 (±2)	28
Beef /USDA ²	240	---	20	---	---	260	10	---	---	---	10	10
Chicken breast	222 (±31)	13 (±1)	64 ^a (±5)	16 (±1)	5.5 (±0.6)	320 (±32)	7.5 (±1.6)	2.6 (0.3)	6.4 ^a (±0.8)	6.0 ^a (±0.7)	22 (±2)	22
ChB ³ /USDA	170	---	40	---	---	210	10	---	10	20	40	40
Chicken thigh	698 (±58)	18 (±1)	106 (±7)	27 (±2)	8.4 (±0.8)	858 (±65)	28 (±3)	3.2 ^a (±0.7)	9.0 ^a (±0.4)	7.5 ^a (±0.7)	48 (±3)	48
ChT ³ /USDA	750	---	90	---	---	840	30	10	20	40	100	100
Egg (whole)	1272 ^a (±38)	18 (±1)	156 (±7)	14 (±2)	40 (±3)	1501 (±47)	31 (±2)	0.4 ^a (±0.2)	6.3 (±1.2)	44 (±2)	82 (±5)	82
Egg /USDA	1148	---	142	---	---	1290	33	4	---	37	74	74
Pork loin	262 ^a (±46)	8.7 (±1.0)	53 (±5)	12 (±3)	1.3 (±0.4)	338 (±53)	12 (±5)	2.8 (±1.5)	7.2 (±1.8)	2.1 (±0.8)	24 (±8)	24
Pork /USDA	440	---	60	---	---	500	20	---	---	---	20	20
Turkey breast	399 (±130)	4.9 (±1.2)	59 ^a (±2)	13 (±1)	2.5 (±0.4)	478 (±132)	25 (±11)	5.1 (±1.0)	10.7 (±0.4)	16 ^a (±1)	57 (±10)	57
TurB ³ /USDA	110	---	30	---	---	140	---	---	10	10	20	20

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²USDA NDB numbers: Beef: #13097; Chicken breast: #05062; Chicken thigh: #05096; Whole egg: #01123; Pork loin: #10040; Turkey breast: #05219.

³Abbreviations: ChB., Chicken breast; ChT., Chicken thigh; TurB., Turkey breast.

^ap < 0.05 significant difference compared with USDA.

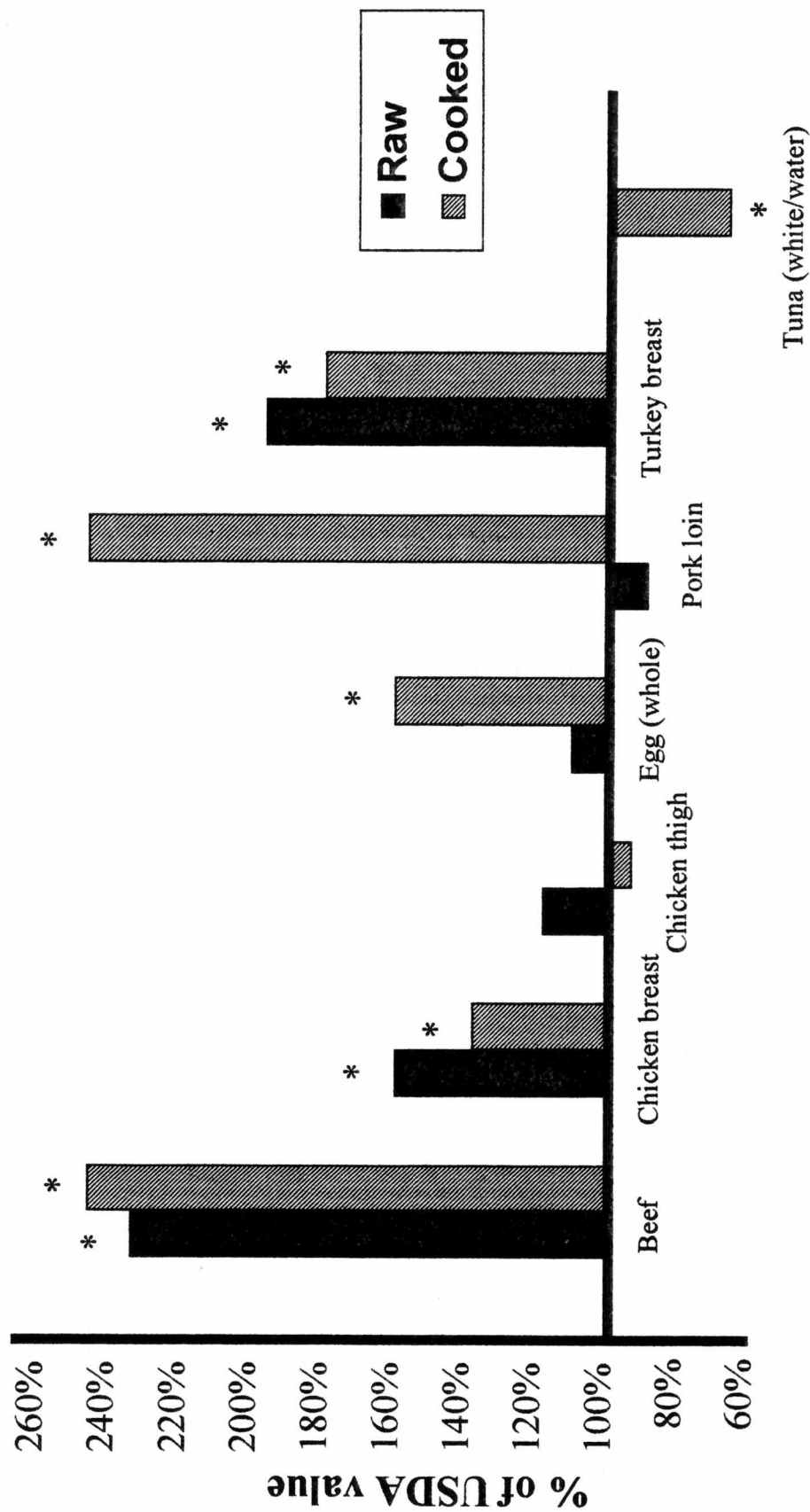


Figure 3.1 Relative amounts of AA in selected foods as compared to values published by the USDA.

Asterisks denote values that were significantly different from the USDA at $p < 0.05$.

compared to USDA (Table 3.1). Similar inconsistencies among the experimental and USDA values were observed with the n-3 PUFA. The USDA reports significantly higher levels of 22:6 n-3 in CB and CT, and significantly lower levels in TB. They failed to report the presence of a number of n-3 and n-6 PUFAs that we observed, including 20:3 n-6, 22:4 n-6, 22:5 n-6, 20:5 n-3, 22:5 n-3 and 22:6 n-3 in many of the food items.

Polyunsaturated fatty acid composition of cooked samples.

Values of AA in cooked samples varied from 33 mg/100 g in white tuna packed in water to 239 mg/100 g in WE hard boiled (in the shell) (Table 3.2). In general, AA content (per 100 g cooked sample) was found to be significantly higher in the experimental samples compared to the reported USDA values, i.e., BRE (77 mg vs. 30 mg), CB (83 mg vs. 60 mg), WE (239 mg vs. 149 mg), PL (74 mg vs. 30 mg) and TB (72 mg vs. 40 mg); and significantly lower in white tuna packed in water (33 mg vs. 51 mg). There were no detectable levels of AA in saltine crackers. Overall, AA content was significantly different in 6 out of the 7 foods analyzed as compared to the USDA (Figure 3.1).

Significant differences also were observed in the 18:2 n-6 content of 7 of the 8 experimental foods as compared to the USDA values. For example, the USDA reports more than twice the levels of 18:2 n-6 in CT, but one-third the levels in TB. In addition, the USDA reports that 18:2 n-6 content increases 3 fold in CT following cooking (Tables 3.1 and 3.2). Similar discrepancies were observed with the n-3 PUFA. For example, in tuna, the experimental value (per 100 g sample) for 20:5 n-3 was significantly lower

TABLE 3.2 Polyunsaturated fatty acid composition of cooked samples (mg/100g sample)¹

Cooked samples	n-6 series					Total	n-3 series				Total
	18:2	20:3	20:4	22:4	22:5		n-6	18:3	20:5	22:5	
Beef rib eye	320 (±51)	27 (±5)	77 ^a (±14)	13 (±2)	1.9 (±0.9)	439 (±69)	17 (±3)	6.9 (±0.7)	20 (±2)	2.4 (±0.6)	46 (±5)
Beef /USDA ²	280	---	30	---	---	310	20	---	---	---	20
Chicken breast	272 ^a (±17)	16 (±1)	83 ^a (±8)	20 (±2)	7.2 (±0.9)	398 (±21)	8.8 ^a (±0.9)	3.1 ^a (±0.3)	8.0 (±0.8)	8.0 ^a (±1.0)	28 (±1)
ChB ³ /USDA	590	---	60	---	---	650	30	10	10	20	70
Chicken thigh	838 ^a (±27)	22 (±2)	121 (±6)	30 (±2)	9.5 (±0.7)	1020 (±29)	34 ^a (±1)	4.1 ^a (±0.9)	10 ^a (±1)	9.0 ^a (±0.6)	57 (±3)
ChT ³ /USDA	2100	---	130	---	---	2230	100	10	30	50	190
Egg (whole)	1884 ^a (±183)	30 (±3)	239 ^a (±21)	14 (±8)	92 (±11)	2264 (±215)	41 ^a (±1)	---	3.6 (±1.6)	68 ^a (±9)	113 (±12)
Egg /USDA	1188	---	149	---	---	1337	35	5	---	38	78
Pork loin	341 ^a (±33)	12 (±1)	74 ^a (±4)	15 (±3)	2.0 (±0.4)	444 (±36)	16 (±6)	3.3 (±1.7)	10 (±3)	3.4 (±1.0)	33 (±11)
Pork /USDA	630	---	30	---	---	660	20	---	---	---	20
Turkey breast	415 ^a (±71)	7.0 (±0.7)	72 ^a (±4)	16 (±1)	4.2 (±0.6)	514 (±76)	23 (±5)	4.9 (±1.1)	13 (±1)	19 ^a (±2)	60 (±8)
TurB ³ /USDA	130	---	40	---	---	170	---	---	10	10	20
Tuna (white in water)	25 ^a (±2)	1.5 (±0.3)	33 ^a (±4)	3.4 (±0.4)	18 (±2)	81 (±8)	5.8 ^a (±0.9)	94 ^a (±20)	20 (±3)	417 (±67)	537 (±91)
Tuna/USDA	55	---	51	---	---	106	71	233	18	629	951
Saltine crackers	932 ^a (±41)	---	---	---	---	932 (±41)	53 (±6)	---	---	---	53 (±6)
S.Cr ² /USDA	1804	---	---	---	---	1804	45	---	---	---	45

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²USDA NDB numbers: Beef: #13098; Chicken breast: #05064; Chicken thigh #05098; Whole egg #01129; Pork loin #10043; Turkey breast #05220; Tuna #15126; Saltine crackers #18228.

³Abbreviations: ChB., Chicken breast; ChT., Chicken thigh; TurB., Turkey breast; S.Cr., Saltine crackers.

^ap < 0.05 significant difference compared with USDA.

compared to the USDA value (94 mg vs. 233 mg). In addition, the USDA failed to report the presence of a number of n-3 and n-6 PUFAs, including 20:3 n-6, 22:4 n-6, 22:5 n-6, 20:5 n-3, 22:5 n-3 and 22:6 n-3 in many of the food items. Some of the data were represented as having no fatty acids, or no data were provided.

Saturated fatty acid composition of raw samples.

Of the major saturated fatty acids, 16:0 was significantly lower in BRE and WE, and 16:0 and 18:0 were significantly lower (per 100 g of sample) in PL compared to USDA values (Table 3.3). In many of the food items, the USDA failed to report the presence of a number of saturated fatty acids, i.e., 10:0, 12:0, 15:0 and 17:0.

Saturated fatty acid composition of cooked samples.

The 12:0, 14:0, 16:0 and 18:0 content of CB, CT and PL, and 14:0 and 16:0 of white tuna were significantly lower in the experimental foods compared to the USDA values, while the 16:0 and 18:0 content of TB were significantly higher (Table 3.4).

Monounsaturated fatty acid composition of raw samples.

Monounsaturated fatty acid values for 16:1 and 18:1 n-9 were significantly lower in BRE, WE and PL, and 16:1 was higher in CT compared to the USDA (Table 3.5).

Monounsaturated fatty acid composition of cooked samples.

Monounsaturated fatty acid values for 16:1 and 18:1 n-9 were significantly lower

TABLE 3.3 Saturated fatty acid composition of raw samples (mg/100g sample)¹

Raw samples	10:0	12:0	14:0	15:0	16:0	17:0	18:0	Other ²
Beef rib eye	21 (±4)	3.7 (±0.3)	122 ^a (±17)	35 (±12)	1122 ^a (±191)	63 (±13)	697 (±158)	---
Beef /USDA ³	---	---	240	---	1930	---	1060	---
Chicken breast	3.5 (±1.2)	0.9 (±0.2)	7.1 (±1.2)	1.2 (±0.3)	283 (±45)	1.7 (±0.5)	109 (±10)	---
ChB ⁴ /USDA	---	---	10	---	210	---	100	---
Chicken thigh	11 (±4)	2.3 ^a (±0.2)	22 (±2)	3.8 (±0.2)	779 (±66)	5.2 (±0.4)	267 (±17)	---
ChT ⁴ /USDA	---	20	20	---	670	---	260	---
Egg (whole)	5.1 (±2.0)	0.6 ^a (±0.1)	28 ^a (±1)	4.3 (±0.4)	2092 ^a (±35)	13 (±1)	778 (±16)	---
Egg /USDA	3	3	34	4	2226	17	784	28
Pork loin	16 (±4)	2.4 ^a (±0.4)	27 ^a (±7)	1.4 (±0.3)	552 ^a (±132)	6.0 (±1.2)	308 ^a (±66)	---
Pork /USDA	---	10	60	---	1110	---	550	---
Turkey breast	8.0 (±1.8)	3.6 (±0.9)	22 (±8)	3.9 (±1.3)	413 (±163)	6.6 (±2.7)	215 (±57)	---
TurB ⁴ /USDA	---	---	---	---	90	---	60	---

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²Other fatty acids include: 8:0; 20:0, 22:0 and 24:0.

³USDA NDB numbers: Beef: #13097; Chicken breast: #05062; Chicken thigh: #05096; Whole egg: #01123; Pork loin: #10040; Turkey breast: #05219.

⁴Abbreviations: ChB., Chicken breast; ChT., Chicken thigh; TurB., Turkey breast.

^a $p < 0.05$ significant difference compared with USDA.

TABLE 3.4 Saturated fatty acid composition of cooked samples (mg/100g sample)¹

Cooked samples	10:0	12:0	14:0	15:0	16:0	17:0	18:0	Other ²
Beef rib eye	29 (±6)	7 (±1)	254 (±48)	56 (±6)	2193 (±401)	122 (±25)	1365 (±297)	---
Beef/USDA ³	10	10	370	---	2800	---	1540	---
Chicken breast	43 (±2.0)	1.1 ^a (±0.1)	7.9 ^a (±0.8)	1.7 (±0.1)	334 ^a (±33)	2.3 (±0.2)	139 ^a (±6)	---
ChB ⁴ /USDA	---	10	30	---	690	---	250	---
Chicken thigh	8.9 (±3.3)	2.5 ^a (±0.3)	27 ^a (±1)	4.2 (±0.2)	940 ^a (±16)	6.0 (±0.2)	313 ^a (±5)	---
ChT ⁴ /USDA	---	30	80	---	2120	---	690	---
Egg (whole)	8.6 ^a (±0.6)	0.5 ^a (±0.2)	43 (±4)	6.3 (±0.2)	2927 ^a (±138)	20 (±1)	1168 (±107)	---
Egg/USDA	3	3	35	---	2349	---	828	3
Pork loin	21 ^a (±3)	3.4 ^a (±0.5)	48 ^a (±6)	1.7 (±0.3)	930 ^a (±121)	8.2 (±1.2)	505 ^a (±75)	---
Pork/USDA	10	10	120	---	2060	---	1080	---
Turkey breast	11 (±3)	3.3 (±0.6)	21 (±5)	3.6 (±0.8)	420 ^a (±93)	7.9 (±1)	237 ^a (±33)	---
TurB ⁴ /USDA	---	---	---	---	110	---	70	---
Tuna (white in water)	3.4 (±1)	1.3 (±0.2)	26 ^a (±6)	7.8 (±1.3)	273 ^a (±40)	13 (±2)	97 (±13)	---
Tuna/USDA	---	---	82	---	592	---	118	---
Saltine crackers	13 (±1)	1.7 (±0.1)	8.1 (±0.5)	2.5 (±0.2)	1123 (±63)	12 (±1)	788 (±39)	84
S.Cr ⁴ /USDA	---	---	---	---	1204	---	837	---

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²Other fatty acids include: 8:0; 20:0; 22:0 and 24:0.

³USDA NDB numbers: Beef: #13098; Chicken breast: #05064; Chicken thigh #05098; Whole egg #01129; Pork loin #10043; Turkey breast #05220; Tuna #15126; Saltine crackers #18228.

⁴Abbreviations: ChB., Chicken breast; ChT., Chicken thigh; TurB., Turkey breast; S.Cr., Saltine crackers.

^a $p < 0.5$ significant difference compared with USDA.

TABLE 3.5 Monounsaturated fatty acid composition of raw samples (mg/100g sample)¹

Raw samples	14:1	16:1	18:1n-9	18:1n-7	20:1	22:1
Beef rib eye	21 (±3)	108 ^a (±13)	1565 ^a (±327)	71 (±11)	6.5 ^a (±1.1)	---
Beef /USDA ²	---	290	3260	---	10	---
Chicken breast	2.3 (±0.5)	72 (±16)	404 (±77)	41 (±6)	4.5 (±0.8)	---
ChB ³ /USDA	---	30	250	---	---	---
Chicken thigh	8.6 (±0.8)	254 ^a (±25)	1257 (±112)	93 (±10)	12.4 ^a (±0.6)	---
ChT ³ /USDA	---	180	1010	---	10	---
Egg (whole)	6.4 (±0.6)	229 ^a (±13)	3192 ^a (±66)	165 (±9)	20 ^a (±1)	---
Egg /USDA	8	298	3473	---	28	3
Pork loin	0.4 (±0.2)	62 ^a (±18)	883 ^a (±237)	97 (±26)	16 ^a (±4)	---
Pork /USDA	---	160	2070	---	40	---
Turkey breast	3.0 (±1.8)	51 (±30)	567 (±249)	48 (±15)	6.6 (±2.0)	---
TurB ³ /USDA	---	10	90	---	---	---

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²USDA NDB numbers: Beef: #13097; Chicken breast: #05062; Chicken thigh: #05096; Whole egg: #01123; Pork loin: #10040; Turkey breast: #05219.

³Abbreviations: ChB., Chicken breast; ChT., Chicken thigh; TurB., Turkey breast.

^a*p* < 0.05 significant difference compared with USDA value.

in CB, CT, PL and tuna compared to the USDA values. The 18:1 n-9 levels in TB were significantly higher than the USDA values (Table 3.6).

Total fatty acid composition of raw and cooked samples.

For the most part, total fatty acid, SFA, MUFA and PUFA content in the raw samples reported by USDA were similar in the experimental foods with the exception of BRE and PL (Table 3.7).

Total fatty acid, SFA, MUFA and PUFA content were significantly lower in the cooked samples of CB, CT, PL and tuna compared to USDA (Table 3.8). In contrast, overall fatty acid content was higher in WE (hard boiled) and TB compared to USDA. Saltine crackers, the only non-animal product included in the analyses, contained significantly lower amounts of PUFA, in the form of 18:2 n-6, compared to the value reported by USDA.

Discussion

The diverse biological functions of AA are unraveling with increased scrutiny of this fatty acid (16). AA can modulate cell differentiation (17-19), cell proliferation (20-22), gene expression (23-27) and signal transduction (28-32). The best described functions of AA are mediated through its oxidized derivatives, the eicosanoids. Eicosanoids have been shown to regulate immune function, vascular reactivity, renal function and blood pressure. Chronically elevated levels have been associated with inflammatory disorders, cardiovascular disease, and cancer. Over the last two decades, a

TABLE 3.6 Monounsaturated fatty acid composition of cooked samples (mg/100g sample)¹

Cooked samples	14:1	16:1	18:1 n-7	20:1	Other ²
Beef rib eye	47 (±12)	222 ^a (±41)	160 (±32)	13 (±3)	---
Beef /USDA ³	---	400	4540	---	---
Chicken breast	2.7 (±0.4)	80 ^a (±13)	471 ^a (±52)	5.5 ^a (±0.5)	---
ChB ⁴ /USDA	---	150	1040	30	---
Chicken thigh	10.1 (±0.3)	311 ^a (±11)	1499 ^a (±40)	14.2 ^a (±0.4)	---
ChT ⁴ /USDA	---	560	3460	70	---
Egg (whole)	8.6 (±0.6)	303 (±27)	4447 ^a (±120)	30 (±1)	---
Egg /USDA	---	310	3725	30	3
Pork loin	1.2 (±0.1)	118 ^a (±15)	1582 ^a (±173)	29 ^a (±3)	---
Pork /USDA	---	300	3570	80	---
Turkey breast	3.3 (±1.1)	47 (±18)	551 ^a (±126)	6.4 (±1.0)	---
TurB ⁴ /USDA	---	20	110	---	---
Tuna (white in water)	0.6 (±0.2)	36 ^a (±7)	211 ^a (±37)	33 ^a (±7)	---
Tuna /USDA	---	144	518	78	44
Saltine crackers	---	6.0 ^a (±0.4)	7092 (±418) ⁵	18 (±1)	7
S.Cr ⁴ /USDA	---	12	6465	---	---

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²Other fatty acids include 22:1 and 24:1.

³USDA NDB numbers: Beef: #13098; Chicken breast: #05064; Chicken thigh #05098; Whole egg #01129; Pork loin #10043; Turkey breast #05220; Tuna #15126; Saltine crackers #18228.

⁴Abbreviations: ChB., Chicken breast; ChT., Chicken thigh; TurB., Turkey breast; S.Cr., Saltine crackers.

⁵Includes all isomers of 18:1.

^a*p* < 0.05 significant difference compared with USDA value.

TABLE 3.7 A summary of the fatty acid composition of raw samples
(mg/100g sample)¹

Raw samples	SFA	MUFA	PUFA	Total FA	20:4n-6	%AA/total FA
Beef rib eye	2064 ± 369	1772 ± 351 ^a	282 ± 25	4118 ± 742 ^a	47 ± 4 ^a	1.2 ± 0.2 ^a
Beef rib eye /USDA ^{2,3}	3230	3560	270	7060	20	0.3
Chicken breast	406 ± 57	523 ± 99	352 ± 34	1281 ± 185	64 ± 5 ^a	5.5 ± 0.9
Chicken breast /USDA	320	280	250	850	40	4.7
Chicken thigh	1091 ± 88	1625 ± 147 ^a	922 ± 69	3637 ± 293	106 ± 7	3.0 ± 0.2
Chicken thigh /USDA	970	1200	940	3110	90	2.9
Egg (whole)	2922 ± 33 ^a	3614 ± 78	1603 ± 53 ^a	8138 ± 87	156 ± 7	1.9 ± 0.1
Egg (whole) /USDA	3096	3810	1364	8270	142	1.7
Pork loin	913 ± 208 ^a	1059 ± 285 ^a	373 ± 59	2345 ± 546 ^a	53 ± 5	2.7 ± 0.6
Pork loin /USDA	1730	2270	520	4520	60	1.3
Turkey breast	672 ± 233	675 ± 298	541 ± 144	1889 ± 675	59 ± 2 ^a	4.0 ± 0.8
Turkey breast /USDA	150	100	160	410	30	7.3

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²USDA NDB numbers: Beef: #13097; Chicken breast: #05062; Chicken thigh: #05096; Whole egg: #01123; Pork loin: #10040; Turkey breast: #05219.

³USDA values for SFA, MUFA, PUFA and Total FA are equal to the total of the individual fatty acids reported in Tables 3.1, 3.3 and 3.5.

^ap < 0.05 significant difference compared with USDA value.

TABLE 3.8 A summary of the fatty acid composition of cooked samples
(mg/100g sample)¹

Cooked samples	SFA	MUFA	PUFA	Total FA	20:4n-6	% AA/total FA
Beef rib eye	4025 ± 752	3537 ± 692	494 ± 73	8056 ± 1500	74 ± 12 ^a	1.0 ± 0.2 ^a
Beef rib eye /USDA ^{2,3}	4730	4940	330	10,000	30	0.3
Chicken breast	490 ± 38 ^a	607 ± 69 ^a	437 ± 22 ^a	1534 ± 119 ^a	83 ± 8 ^a	5.6 ± 0.7 ^a
Chicken breast /USDA	980	1220	720	2920	60	2.1
Chicken thigh	1301 ± 19 ^a	1949 ± 51 ^a	1096 ± 29 ^a	4345 ± 72 ^a	121 ± 6	2.8 ± 0.1 ^a
Chicken thigh /USDA	2920	4090	2420	9430	130	1.4
Egg (whole)	4173 ± 236 ^a	5006 ± 154 ^a	2409 ± 233 ^a	11,587 ± 570 ^a	239 ± 21 ^a	2.1 ± 0.1 ^a
Egg (whole) /USDA	3218	4068	1415	8701	149	1.7
Pork loin	1518 ± 206 ^a	1897 ± 208 ^a	492 ± 46 ^a	3907 ± 444 ^a	74 ± 4 ^a	1.9 ± 0.2 ^a
Pork loin /USDA	3280	3950	680	7910	30	0.4
Turkey breast	704 ± 131 ^a	659 ± 153 ^a	582 ± 84 ^a	1945 ± 367 ^a	72 ± 4 ^a	4.0 ± 0.6 ^a
Turkey breast /USDA	180	130	190	500	40	8.0
Tuna (white/H ₂ O)	420 ± 61 ^a	314 ± 58 ^a	623 ± 100 ^a	1357 ± 219 ^a	33 ± 4 ^a	2.4 ± 0.2 ^a
Tuna (white/H ₂ O) /USDA	792	784	1057	2633	51	1.9
Saltine crackers	2032 ± 107	7123 ± 418	984 ± 47 ^a	10,139 ± 571	---	---
Saltine crackers /USDA	2041	6477	1849	10,367	---	---

¹Values are means of 4-5 independent samples, each analyzed in triplicate. SEMs are in parentheses.

²USDA NDB numbers: Beef: #13098; Chicken breast: #05064; Chicken thigh #05098; Whole egg #01129; Pork loin #10043; Turkey breast #05220; Tuna #15126; Saltine crackers #18228.

³USDA values for SFA, MUFA, PUFA and Total FA are equal to the total of the individual fatty acids reported in Tables 3.2, 3.4 and 3.6.
^ap < 0.05 significant difference compared with USDA value.

number of studies have demonstrated that dietary AA enriches tissue arachidonate levels and elevates eicosanoid formation *in vivo* and *in vitro* (2-4, 6). However, there has been a problem estimating overall intake of AA because of the lack of complete data on AA content in foods. Among the most referenced sources of lipid and fatty acid composition in foods is Agriculture Handbook No. 8, published by the United States Department of Agriculture (USDA) (10). The importance of this database is underscored by the fact that most, if not all, commercially available diet analysis programs are based on data from the USDA database. Of concern is the apparent lack of empirical fatty acid data, among them AA. This study was designed to evaluate the AA content in some commonly consumed U.S. foods known to be high in AA and to compare these values to those published by the USDA.

Fatty acid analysis of eggs and a variety of meats revealed that the values reported by the USDA for AA content were significantly different ($p < 0.05$), in general, than those observed in our experimental foods (Figure 3.1). AA content in terrestrial-based meats tended to be under reported by the USDA, while AA content in the marine source meat (tuna) was significantly lower than the USDA value. In a number of instances, the USDA fatty acid values for raw and cooked portions of the same food were inconsistent. For example, the USDA reports that the total PUFA content (weight/weight: w/w) of pork loin increased 31% following cooking, but the AA content decreased by 50%; and in chicken breast, the total fatty acid content increased 244% following cooking, but the AA content increased only 50%. We did not observe a decrease in AA content in any of the samples tested following cooking. In general, we and others (33, 34) observed consistent

increases in fatty acid and AA content (w/w) in cooked portions compared to raw samples. Consistent with the USDA values, we did not observe reductions in the PUFA content following cooking. Sinclair *et al.* (7) reported that cooking lean beef resulted in significant reductions in PUFA content, in particular the 20-carbon PUFA. It is possible that the incongruity of these data may be related to different methods of cooking (i.e., roasting versus grilling and frying) and how the cooked data were reported (i.e., mg/100 g cooked sample weight versus mg/100 g original raw sample weight). Loss of moisture due to cooking would result in a higher fatty acid content on a w/w basis, and as such, the relative abundance of AA to the total fatty acid content would remain similar (35). Cooking can result in 30% moisture loss and enrich the amount (w/w) of extractable lipid in the muscle component (36, 37). However, Chang and Watts reported that they did not observe consistently an enrichment of fatty acids in meats following cooking, including AA. They suggest that these results could be attributed to the non-uniform distribution of triglycerides rather than the destruction of unsaturated fatty acids (38). More recently, though, Li *et al.* reported that the amount of AA (w/w) in the visible fat of animal meats (beef, lamb, turkey, pork, chicken and duck) is equal to or greater than that of comparable lean portions even though the relative amounts of AA (wt% of total fatty acids) were 10-100 times higher in the lean portions (9). These data indicate that the triglyceride portion of meat can have a significant impact on AA content and care must be taken when selecting and comparing meat portions for fatty acid analysis.

There are a number of possible explanations for the discrepancies observed between the USDA values and the experimental values of this study. First of all, studies

conducted for the USDA report that the relative contributions of AA to the total fatty acid profile of the triglyceride portion (separable fat) of meats (i.e., beef) were considered quantitatively insignificant (34), while more recent data indicate this is not the case (9).

Many of the USDA values are estimates. When analytical data were not available, it was sometimes necessary to calculate or impute nutrient values (10). The USDA routinely generated fatty acid data by using mathematical formulas based on the weight % of the total lipid in a food, multiplied by a set of conversion factors (34, 39-41). The conversion factors were based primarily on the relative abundance of triglycerides and phospholipids and their fatty acid content. For example, the lipids in lean beef were estimated to be 87% triglycerides and 12% phospholipids and the fatty acid portions of triglycerides and phospholipids account for an estimated 95.6% and 70.7% of their total weights, respectively (34). The conversion factors vary among different foods, raw and cooked samples (39). For a given sample, the fatty acid composition (w/w) would be determined by first determining the amount of total lipid gravimetrically and multiplying this value by 1) its conversion factor and 2) the weight percent (sometimes assumed) of the individual fatty acid methyl esters. Based on preliminary experiments, fatty acid profiles for related anatomical portions of meat from a beef carcass, for example, are assumed to be similar (39). When necessary, the fatty acid data was adjusted so the amount of fatty acid was consistent with the total amount of extracted lipid (personal communication, Dr. Juliet Howe, USDA).

Fatty acid composition data can be influenced by scientific advances in breeding, feeding practices and analytical techniques. Over the last 30 years feeding practices for

beef cattle has increasingly shifted from forage-fed to grain-fed animals resulting in significantly higher levels of AA in the muscle of grain-fed animals (42). It also is assumed that cuts of meat presented in Agriculture Handbook No. 8 reflect retail cuts of meat. Retail beef is leaner than the values published by the USDA, possibly reflecting the health-conscience trend of the public. Furthermore, a retail cut may be associated with more than one muscle group (43). Fatty acid composition also can be affected by breed and age of an animal and an interaction of these two variables (44). PUFA content of muscle phospholipids increase and SFA and MUFA decrease with age in Jersey and Limousin cows and the level of AA in muscle phospholipids in mature Limousin cows is 28% higher compared to Jersey cows. In addition, the AA content in muscle phospholipids in heifers is almost 2.5 times higher than that of steers (44).

Most of the food analyses for Agriculture Handbook No. 8 were performed in the 1970's and 1980's (10). For example, the Agricultural Handbook No. 8 Series for poultry, pork and beef products were issued in 1979, 1992 and 1990, respectively, but some of the data, such as poultry, originated from the mid 1960's and 1970's and have not been updated (personal communication, Dr. Jacob Exler, USDA). The food analysis data published by the USDA is derived from multiple sources including contracts with universities and commercial laboratories, contacts with the food industry, trade groups, academia and other government agencies (10). Earlier, fatty acid data was generated using packed column gas chromatography, gas liquid chromatography (35-37) and spectrophotometric methods (38) and this may explain many of the missing values for the polyunsaturated fatty acids (see Tables 3.1-3.8). Recent advances in analytical techniques

using internal standards and capillary gas chromatography has improved the separation, identification and measurement of fatty acids, in particular longer chain unsaturated fatty acids.

Finally, the discordance between our results and those of the USDA's could be related to the sources of the samples analyzed. We selected samples, as would the public, from a regional supermarket chain and analyzed only small sample sizes. The data published in Agriculture Handbook No. 8 is based on a compilation of data from a variety of sources.

In summary, our results suggest there are wide differences in the fatty acid content of individual foods when compared to the USDA published values. The content of AA in foods analyzed in this study were, for the most part, significantly higher than that reported in Agriculture Handbook No. 8. Because of the importance of Agriculture Handbook No. 8 as a reliable source of nutrient content in foods and for determining average daily intakes of nutrients, a reevaluation of the fatty acid data may be warranted.

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APPENDIX

FATTY ACID ANALYSIS

Preparation of samples

1. Weigh sample (approximately 70 g), and place in Cuisinart food processor.
2. Add 50 mg internal standard (19:0 in 250 μ l iso-octane).
3. Add 0.9% cold NaCl to achieve concentration of 380 g sample/L buffer.
4. Chop mixture in Cuisinart food processor for 2 minutes.
5. Transfer mixture to Waring blender, adding 0.9% cold NaCl to achieve concentration of 210 g sample/L buffer.
6. Blend mixture for 3 minutes.
7. Filter homogenates through cheesecloth to remove connective tissue and under constant stirring, remove 3 one ml aliquots for fatty acid analysis.

Lipid Extraction

1. Add 3 ml MeOH:CHCl₃ (2:1)
vortex 30 sec.
let sit in ice for 30 min.
2. Add 1 ml CHCl₃; and 200 μ l saturated NaCl
vortex 30 sec.
let sit in ice for 15 min.
centrifuge for 3 min.
extract CHCl₃ layer (bottom layer) into 12 x 75 mm test tube.
3. Add 1 ml CHCl₃
vortex 30 sec.
let sit in ice for 15 min.
centrifuge for 3 min.
extract CHCl₃ layer into 12 x 75 mm test tube.
4. Add 1 ml CHCl₃
vortex 30 sec.
let sit in ice for 15 min.
centrifuge for 3 min.
extract CHCl₃ layer into 12 x 75 mm test tube.

5. Evaporate CHCl_3 to dryness with N_2 gas under hood.
6. Add 200 μl CHCl_3
vortex 5 sec.
transfer to screw-top test tube with 500 μl Hamilton syringe.
7. Repeat step #5 one time to insure that all the lipid is transferred.
8. Evaporate CHCl_3 to dryness with N_2 gas under hood.
9. Saponify by adding 250 μl toluene; and 500 μl KOH:MeOH (0.5 N)
10. Place caps on TIGHTLY and place tubes in 86°C water bath for 8 min.
11. Cool tubes to room temperature.
12. Acidify with 1 ml HCl:MeOH (0.7 N)
13. Extract with hexane
add 2 ml hexane
vortex 30 sec.
centrifuge for 3 min.
extract hexane (top layer) to 12 x 75 mm test tube.
14. Repeat step #12 one time to insure that all lipid is extracted.
15. Evaporate to dryness with N_2 gas under hood.
16. Add 250 μl hexane
vortex 5 sec.
transfer to GC vial with Hamilton syringe or pipette.
17. Repeat step #15 one time to insure that all lipid is transferred.
18. Evaporate hexane to dryness with N_2 gas under hood.
19. To methylate fatty acids, add 400 μl ethereal diazomethane under hood
put caps on lightly immediately after addition of diazomethane and let sit under hood with fan on for 10 min.
20. Evaporate diazomethane to dryness with N_2 gas under hood with fan on.
21. Add 1 ml hexane.

22. Cap TIGHTLY
vortex 10 sec.
23. Sample is now ready to be analyzed via gas chromatography.

VITA

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She received a Bachelor of Science degree in Secondary Education from the University of Kansas in June, 1968. After graduating from college, she taught school in California and Oregon, moving to Knoxville, Tennessee in 1979. She returned to the University of Tennessee, where she participated in the Professional Dietetics Program and became a Registered Dietitian in June, 1995. In December, 1998 she will receive her Master of Science degree in Nutrition Science from the University of Tennessee.

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