AN ABSTRACT OF THE THESIS OF

Michelle L. Moore for the degree of Master of Science in Nutrition and Food Management presented on May 21, 1999. Title: Effects of Selenium Supplementation on Plasma and Milk of Lactating Women of Habitually Low Selenium Status.

Abstract approved: _____

Rosemary C. Wander

Twenty-one women, lifelong residents of Xichang County, Sichuan Province, China, an area of very low soil selenium (Se), received tablets containing either 100 μg Se daily as Se-enriched yeast (+Se) or no additional Se (-Se), throughout the last trimester of pregnancy and the first three months of lactation. Diet was analyzed using diet recalls and proximate analysis of mixed diet samples. Milk and plasma samples were analyzed for Se content, glutathione peroxidase activity, and fatty acid profile and plasma alone was analyzed for vitamin E content and lipid peroxidation. At parturition and three months after delivery, milk and plasma Se levels and plasma GPx activities were significantly higher in the +Se women than the –Se women. Milk GPx activity did not change significantly with supplementation. Plasma vitamin E was not different between the treatment groups at either time. Plasma lipid peroxidation levels (TBARS) were significantly higher in the supplemented women at both time points. Fatty acid profiles at delivery and three months after delivery were

similar in both plasma and milk between the two groups. The data suggest that this level and length of supplementation, when given to pregnant women of very low Se status, are not adequate to influence the fatty acids in milk.

Effects of Selenium Supplementation on Plasma and Milk of Lactating Women of Habitually Low Selenium Status

by

Michelle L. Moore

A THESIS

submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Master of Science

Presented May 21, 1999
Commencement June 2000

Master of Science thesis of Michelle L. Moore presented on May 21, 1999

APRROVED:

Major Professor, representing Nutrition and Food Management

Chair of Department of Nutrition and Food Management

Dean of Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Michelle L. Moore, Author

ACKNOWLEDGEMENT

I would like to thank my advisor, Dr. Rosemary Wander, for the use of her lab, her time, and for her advice. I would like to thank Dr. Phil Whanger for providing the samples and laboratory time, space, and equipment for this study. I would especially like to thank Judy Butler for her help and advice in many areas of this research. I would also like to thank Shi-Hua Du and Ye-Sun Lee for their help and advice with laboratory procedures and statistics, respectively. Finally, I would like to thank my husband, Kelley Aders, for his love and support.

CONTRIBUTION OF AUTHORS

Dr. Rosemary Wander was involved in the design of the study and the analysis and interpretation of the data. Dr. Phil Whanger was also involved in the design of the study and the selenium, glutathione peroxidase, and diet analyses were conducted in his laboratory. These assays were performed by or with the help of Judy Butler. The other assays were conducted in the laboratory of Dr. Rosemary Wander with the help and guidance of Shi-Hua Du. Dr. Yiming Xia coordinated the subject selection and sample collection in China.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF APPENDICES (Continued)

LIST OF APPENDIX TABLES

Effects of Selenium Supplementation on Plasma and Milk of Lactating Women of Habitually Low Selenium Status

Introduction

Although the total lipid content of human milk remains fairly constant at about 50% of the caloric content, the amounts of specific fatty acids vary with diet and other factors (Jensen, 1989). The effects of macronutrients on the fatty acid profile of human milk are well documented while the effects of micronutrients are not. However, recent studies have hinted at a relationship between selenium and the fatty acid profile of human milk (Dodge et al., 1999a and 1999b). Also, studies have found a strong positive relationship between serum selenium and essential fatty acids and long chain n-6 polyunsaturated fatty acids in plasma phospholipids of humans (Cabre et al., 1992) and between selenium and polyunsaturated fatty acids in adipose tissue in rats (Viegas Crespo et al., 1995).

In a single blind study (Dodge et al., 1999b) conducted with women in New Zealand where dietary selenium intake is lower than in the United States, 12 of 22 women were supplemented with 50 μg daily of selenium as selenomethionine throughout pregnancy and lactation. The milk of these women had higher selenium concentrations but unchanged glutathione peroxidase activity (GPx) when compared with the women who received a placebo. The selenium supplement significantly increased the concentration of polyunsaturated fatty acids (PUFA), especially linoleic acid, in human milk.

The selenium content of plant foods is influenced by the soil selenium content. Although areas of low soil selenium exist in the United States, American citizens typically have adequate amounts of this element because they consume food grown in many different regions. In China, there are rural areas where soil selenium is extremely low. The residents of these areas consume only the foods grown locally and, therefore, consume less selenium than the residents of the United States and New Zealand (Dodge et al., 1999a). This population represents a unique area of the world where dietary selenium intake is extremely low (11 μg per day). This element is available in table salt although supplementation is not yet widespread.

Linoleic acid, an essential fatty acid, is important in the diets of infants for several reasons. It has been shown to be necessary to prevent developmental disturbances in infants (Fernstrom, 1999) and linoleic acid is the precursor to arachidonic acid (AA) (Levine 1988). From AA, the body synthesizes various eicosanoids such as prostaglandins, thromboxanes, and leukotrienes. Each of these groups of molecules has profound effects on the body. Some of their functions include lowering blood pressure and changing platelet aggregation. Eicosanoids also influence smooth muscle contraction, cell signaling, and membrane fluidity.

The purpose of this study was to determine the influence of selenium supplementation on the fatty acid profiles of plasma and human milk in Chinese women with habitually low intakes of dietary selenium. The habitual dietary selenium intake in the population studied is lower than that studied in New

Zealand by Dodge et al. (1999b) and the diet of the subjects is less varied than that of the subjects studied by Dodge et al. (1999a) in China.

Review of Literature

Lipids in Human Milk

Amount of Lipids in Human Milk

The total amount of lipids in human milk varies within a feeding, from breast to breast, diurnally, longitudinally, within individuals, and with diet (Jensen 1989). An average of 50% of the kilocalories of human milk are derived from lipids. The mean lipid content of human milk (Western diet) is about 3.9 wt% or 36 g/day (Jensen, 1989 and Jensen et al., 1992).

The majority of lipids in human milk (98%) are in the form of triglycerides (Jensen et al., 1992). Human milk contains fatty acids with as few as eight carbon atoms and as many as 22. The essential fatty acid, linoleic acid (18:2n-6), has been found in concentrations of 8-16 wt% in the human milk of European and African women but can be higher depending on dietary lipid intake (Koletzko et al., 1992). In China, milk 18:2n-6 has been shown to be as high as 23 wt% of fatty acids and as low as 10 wt% (Dodge et al., 1999a and Chulei et al., 1995). Linolenic acid, the other essential fatty acid, is found in much lower concentrations (Chulei et al., 1995). This fatty acid has been found in concentrations of 0.8 to 1.3 wt% in American, European, and African women (Jensen, 1989 and Koletzko et al., 1992) and 2-3 wt% in Chinese women (Chulei et al., 1995).

The shorter fatty acids such as 10:0, 12:0, and 14:0 are synthesized in the mammary gland while longer chain fatty acids come from the blood (Jensen et al., 1992). Lipids in the blood are derived from the diet, the liver, and the adipose tissue with about 30% coming from the diet.

Linoleic acid (18:2n-6) and linolenic acid (18:3n-3) stored in the adipose tissue can be desaturated and elongated in mammary tissue for secretion into human milk (Koletzko et al., 1992) via the actions of elongase and desaturases (Jumpsen and Clandinin, 1995). These enzymes function to add carbon atoms and double bonds to fatty acids, respectively, resulting in the production of LC-PUFA such as arachidonic acid (20:4n-6) and docosahexanoic acid (22:6n-3).

Functions of Lipids in Human Milk

Linoleic acid is necessary for the normal growth and development of infants (Hennig and Watkins, 1998) and decreased maternal linoleic acid levels have been associated with intra-uterine growth retardation (Al et al., 1995). A deficiency of n-6 fatty acids during development has been associated with altered central nervous system activities such as membrane-associated enzymes and receptors and cognitive behaviors (Jumpsen and Clandinin, 1995).

The brain has the highest concentration of lipid of any organ except adipose tissue with about 50% lipid on a dry-weight basis (Jumpsen and Clandinin, 1995). Over one-third of brain fatty acids are polyunsaturated fatty acids. LC-PUFA derivatives of linoleic acid such as arachidonic acid (AA) strongly correlate with fetal weight and head circumference (Hamosh, 1994). AA

is found in abundance in neural tissue and the retina of infants (Al et al., 1995) and is thought to promote growth during early life (Jumpsen and Clandinin, 1995).

The n-3 family of fatty acids has been hypothesized to play a role in various aspects of neurodevelopment and cognitive functions (Carlson and Neuringer, 1999 and Clandinin, 1999). The only definitive role is played by docosahexanoic acid (DHA) on visual function (Jumpsen and Clandinin, 1995). Other studies have tried to link α-linolenic acid deficiency with decreased cognitive ability, but no such studies have been completely successful (Carlson and Neuringer, 1999).

The amount of DHA needed by infants is currently unknown and widely debated (Carlson and Neuringer, 1999). DHA is present in human milk but not currently added to infant formula. The point at which an infant can begin to produce sufficient DHA from α-linolenic acid is unclear, so the question of whether formula-fed infants need an exogenous source of DHA is important (Clandinin, 1999). To make the situation even more complex, human milk DHA content has been shown to vary with maternal DHA intake making assessment of the amount needed by infants even more difficult (Cheruku et al., 1999).

Selenium in Human Milk

Amount of Selenium in Human Milk

The quantity of selenium in human milk is influenced by several factors including dietary selenium intake (Debski et al., 1987). Milk selenium concentration in the United States tends to vary from 13 ng/g in areas with low soil selenium such as Ohio to 28 ng/g in areas with high soil selenium such as South Dakota (Alaejos and Romero, 1995 and Mannan and Picciano, 1987). The average milk selenium concentration in the United States is about 16 ng/g (Mannan and Picciano, 1987, Debski et al., 1987, and Smith et al., 1981). In California, vegetarian and nonvegetarian women were found to have different milk selenium contents of 22.2 ng/g and 16.8 ng/g, respectively, despite similar dietary selenium intake (Debski et al., 1989).

In other areas of the world, milk selenium content can be very different than in the United States. In China, human milk selenium concentrations as low as 7.7 ng/g have been reported in an area with extremely low soil selenium content (Dodge et al., 1999a). In contrast, China also has areas of extremely high soil selenium where milk selenium levels reach 94 ng/g and areas of adequate soil selenium where milk selenium levels are comparable to those found in the United States (14 ng/g) (Dodge et al., 1999a).

Countries with chronically low milk selenium levels include New Zealand where milk selenium levels have been reported to be 9.4 ng/g (Dodge et al., 1999b). Residents of Finland and Belgium also typically have milk selenium levels of less than or equal to 10 ng/g (Mannan and Picciano, 1987).

Infant blood selenium levels are low at birth, drop during the first months after birth, and rise with age thereafter (Alaejos and Romero, 1995). Selenium requirements during the first six months of life are 10 μg/day (Alaejos and Romero, 1995) and selenium intake of human milk fed infants was calculated to be as low as 2.5 μg/day in areas of China with low soil selenium (Levander, 1987). Selenium deficiency can result in Keshan disease, an often fatal cardiomyopathy (Sunde, 1997). Keshan disease was most commonly seen in children under the age 15 years and women of childbearing age in regions of low soil selenium in China before the implementation of selenium supplementation. Therefore, adequate selenium intake is very important for infants.

Selenium supplementation has been shown to increase milk selenium levels. In New Zealand, women who consumed 50 μg/d of selenium (as selenomethionine) for nine months had milk selenium concentrations which were 37% higher than their counterparts who were given a placebo (12.9 ng/g compared to 9.4 ng/g) (Dodge et al., 1999b). In Illinois, lactating women who were given 200 μg/d of selenium (as selenomethionine) for four weeks increased their milk selenium concentration from 15.8 to 19.7 ng/g, a 25% increase (McGuire et al., 1993). On the other hand, supplementation with 200 μg/d selenium-enriched yeast consisting primarily of selenomethionine for four weeks did not increase milk selenium although it did prevent the decline in milk selenium seen in the lactating control subjects. Even 20 μg/d of selenium in the form of sodium selenate for three months has been shown to increase milk selenium concentrations 38% from 24 ng/g to 33 ng/g (Dylewski and Picciano,

1999). The study by Dylewski and Picciano was conducted in Pennsylvania where soil selenium is apparently high as evidenced by the high milk selenium content before supplementation. As mentioned previously, Ohio has low soil selenium content. This is particularly interesting considering that Ohio and Pennsylvania are neighboring states and shows the great variability in soil selenium content.

The differences seen in the abilities of various forms of selenium supplements to increase milk selenium concentrations probably has little to do with selenium absorption from the small intestine as selenium is well absorbed (Daniels, 1996). Selenomethionine and selenate are both absorbed at 95-98%. Selenium species in selenium-enriched yeast consist mainly of selenomethionine with other unidentified species (Myers et al., 1981) and 200 μg/d of selenium as selenium-enriched yeast for 16 weeks has been shown to increase plasma selenium concentrations in Finnish men (Alfthan et al., 1991). The inability of selenium-enriched yeast to increase milk selenium concentrations when selenomethionine increased milk selenium as seen by McGuire et al. (1993) may be a function of inadequate length of supplementation.

A strong, positive relationship has been demonstrated between whole blood selenium and milk selenium for residents of New Zealand (Williams, 1983). However, this same relationship was not seen in residents of Maryland (Levander et al., 1981). In Illinois, a positive correlation was seen between plasma selenium and milk selenium (Mannan and Picciano, 1987). Therefore, although it is not presently known how selenium is transported into human milk

(Burk and Levander, 1999), it is thought that a relationship between plasma selenium and milk selenium exists.

When Dodge et al. (1999a) supplemented women from New Zealand with 50 μg/d of selenium, plasma mean selenium concentration was about 75 ng/g while that of the control group was about 45 ng/g. In these same women, mean milk selenium concentration was 12.9 ng/g for the women taking the selenium supplement and 9.4 ng/g for those not taking the supplement. Plasma selenium was 67% higher in the supplemented women while milk selenium increased 37%. Another study conducted by Dodge et al. (1999b) in China reported plasma and milk selenium concentrations for women from an area with low soil selenium and women from an area of adequate soil selenium. The mean plasma selenium concentration was 380% higher (98 ng/g compared to 20.4 ng/g) for the women who lived in the area of adequate soil selenium while the mean milk selenium concentration was only 82% higher (14 ng/g compared to 7.7 ng/g) for these women. Therefore, although plasma selenium concentrations seem to influence milk selenium concentrations, milk is not as sensitive to increased selenium intake as is plasma.

Functions of Selenium in Human Milk

About 50-80% of selenium from food is absorbed (Daniels, 1996). Selenium is incorporated into selenoproteins as selenocysteine (Burk and Hill, 1993). Selenomethionine can be incorporated into proteins in place of methionine, and the amount of incorporation is influenced by the methionine

content of the diet (Butler et al., 1989). Sixty-one percent of selenium in the body is found in muscle, liver, blood, and kidneys (Sunde, 1997) with the largest pool residing in the muscle (Daniels, 1996). As many as 100 selenium-containing proteins may exist although little is known about most of them (Burk and Hill, 1993).

The most well known selenium-containing proteins are the glutathione peroxidases and there are four main forms of this enzyme (Sunde, 1997). Each glutathione peroxidase enzyme is thought to catalyze the same basic reaction:

$$2\ GSH + LOOH \rightarrow GSSG + LOH + H_2O$$

Each of the enzymes oxidizes glutathione although there are some differences in the specific molecules reduced by each isoenzyme.

Extracellular (or plasma) glutathione peroxidase (GPx) consists of four identical 23-kDa subunits with each subunit containing one atom of selenium as selenocysteine (Burk and Hill, 1993). Extracellular GPx is made mainly in the kidney (Sunde, 1997) and acts upon hydrogen peroxide and lipid peroxides (LOOH). It functions, with glutathione, to reduce these potentially dangerous oxidizing molecules (Sunde, 1997). It is thought that extracellular GPx may have other functions as well, due to the low concentration of glutathione in the blood.

About 15-30% of selenium in human milk is associated with glutathione peroxidase (L'Abbe and Friel, 1998) and 90% of the GPx activity in milk is due to the extracellular form of GPx (Avissar et al., 1991). It is unclear whether GPx in

milk functions only as a carrier of selenium or also protects milk from peroxidation (Sunde, 1997).

Cellular (or classical) GPx is found in almost all cells (Burk and Hill, 1993). It consists of four identical 23-kDa subunits containing one selenocysteine residue each (Sunde, 1997). This enzyme is thought to regulate intracellular hydroperoxide concentrations and consumes almost all hydroperoxides and hydrogen peroxide as its substrates. Gastrointestinal GPx, also a cellular enzyme, is produced in the human liver and the colon and has a 61% nucleotide homology with cellular GPx. Seventy-one percent of the GPx activity in the rat small bowel is due to this enzyme. The fourth GPx, phospholipid hydroperoxide GPx, is unique in that it is a 20-kDa monomer. This cellular enzyme reduces hydroperoxides and hydrogen peroxide as well as phospholipid hydroperoxides and cholesterol hydroperoxides which are not substrates for cellular GPx.

In addition to glutathione peroxidase enzymes, other selenium-containing proteins have been identified. Types I, II, and III iodothyronine 5'-deiodinases, another group of selenium-containing enzymes, are among several enzymes which function to convert thyroxine to triiodothyronine and are important for thyroid function (Sunde, 1997).

Selenoprotein P is a glycoprotein found in plasma and associated with endothelial cells (Burk and Levander, 1999). It is secreted by the kidney and liver and contains about 45% of the plasma selenium in a typical person with adequate dietary selenium (Sunde, 1997 and Burk and Levander, 1999). The

function of selenoprotein P is unknown although is has been associated with the antioxidant properties of selenium.

Found in muscle and other tissues, selenoprotein W is known to be lower in tissues from white muscle diseased sheep (Sunde, 1997 and Burk and Levander, 1999). Although its function is unknown at this time, the concentration of selenoprotein W is known to decrease in selenium deficiency (Yeh et al., 1997).

Lipid Peroxidation in Human Milk

Peroxidation

Reactive oxygen species (ROS) are oxygen-centered free radicals with one or more unpaired electrons (Halliwell and Gutteridge, 1989). Examples of ROS include hydrogen peroxide, superoxide, and hydroxyl radical. Peroxidation of lipids occurs when fatty acids (LH) react with ROS and a hydrogen radical is abstracted.

$$LH + OH^* \rightarrow L^* + H_2O$$

The reaction of a lipid with a ROS produces a carbon-centered radical which can react with oxygen to form a lipid peroxyl radical.

$$L^* + O_2 \rightarrow LOO^*$$

A lipid peroxyl radical can react with another lipid molecule producing a lipid hydroperoxide and generating another carbon-centered radical, perpetuating a free radical chain reaction (Halliwell and Gutteridge, 1989).

$$LOO^* + LH \rightarrow LOOH + L^*$$

The more unsaturated a fatty acid is, the more rapidly it oxidizes as rate of oxidation is associated with number of double bonds (Cosgrove et al., 1987). Consequently, larger amounts of unsaturated fatty acids require greater antioxidant protection. Free radicals are capable of abstracting hydrogen radicals from molecules and perpetuating radical reactions (Halliwell and Gutteridge, 1989). These reactions can result in the formation of lipid hydroperoxides which are cytotoxic and cause cell damage (Neuzil et al., 1995 and Halliwell and Gutteridge, 1989). Therefore, the body has an antioxidant defense system which helps to control oxidation.

Antioxidant Protection of Lipids

Antioxidants can protect lipids from peroxidation by scavenging free radicals before they react with lipids (Halliwell and Gutteridge, 1989). Antioxidants also break free radical chain reactions by reacting with lipid carbon-centered radicals and lipid peroxyl radicals and preventing them from reacting with other lipids.

Vitamin E (or α-tocopherol) is the major chain breaking antioxidant of extracellular fluids (Halliwell and Gutteridge, 1989). The α-tocopherol content of human milk has been reported to be about 1.3 - 4.5 mg/L and decreases with length of lactation (Packard, 1982 and Jensen et al., 1992). When α-tocopherol reacts with a ROS such as superoxide anion, the less reactive tocopheroxy radical (E*) is formed.

$$O_2^{-*} + EH \rightarrow H_2O_2 + E^*$$

α-tocopherol reacts with lipid peroxyl radicals to form lipid hydroperoxides which are reduced to alcohols.

$$LOO^* + EH \rightarrow LOOH + E^*$$

$$LOOH + EH \rightarrow LOH + H_2O + E^*$$

The most important aspect to these reactions is that α-tocopherol stops the free radical molecules from continuing to react with other molecules. The tocopheroxy radical formed by these reactions is relatively stable and α-tocopherol can be regenerated by reaction with another antioxidant such as ascorbic acid.

$$E^* + AH \rightarrow EH + A$$

Glutathione peroxidase, as mentioned earlier, is another antioxidant found in extracellular fluids (Sunde, 1997). A selenium-dependent enzyme, milk glutathione peroxidase activity has been shown to be associated with milk selenium concentration but does not decrease as much as might be expected in women with extremely low dietary selenium consumption (Mannan and Picciano, 1987; L'Abbe and Friel, 1998; Dodge et al., 1999b). For example, in a region of China with low soil selenium investigated by Dodge et al. (1999b), mean milk selenium was slightly more than half that of similar samples collected in an area of China where soil selenium is adequate. Mean plasma selenium, however, was one-fifth that seen in the area with adequate soil selenium suggesting that milk selenium is more conserved than plasma selenium. Mannan and Picciano (1987) found a strong, positive relationship between human milk selenium concentration and milk GPx activity. It is thought that protection of selenium in milk is through glutathione peroxidase activity and might, therefore, be important for conserving antioxidant function in milk.

Vitamin E and glutathione peroxidase function together to quench free radical reactions. Vitamin E can stop chain reactions and thereby forms hydrogen peroxide and lipid hydroperoxides. Hydrogen peroxide and lipid hydroperoxides while less harmful than ROS, are not completely benign. Glutathione peroxidase can convert these products into even less harmful molecules.

Antioxidants in human milk are important because ingestion of oxidized oils may negatively affect the immune system (Myrvik, 1994). The feeding of oxidized oil to mice has been shown to inhibit DNA synthesis of thymocytes and generation of low molecular weight molecules produced by oxidation damaged lymphocytes. Although more research is necessary, these findings highlight the importance of antioxidants in food and may be especially important for an infant whose only food source is human milk.

Materials and Methods

Overview

Plasma and human milk samples were obtained at two time points from twenty-one pregnant women living in Xichang County, China. Eleven of the subjects were supplemented with 100 μg/d of selenium-enriched yeast beginning in the third trimester of pregnancy while the remaining ten subjects received placebos. Plasma and human milk selenium concentrations and glutathione peroxidase activities were measured to assess selenium status. Fatty acid profiles were measured in plasma and human milk and α-tocopherol and lipid peroxidation were assessed in plasma alone. The samples were obtained with the help of Dr. Yiming Xia of the Chinese Academy of Preventive Medicine in Beijing.

Subject Recruitment

Twenty-one pregnant (second trimester) women between the ages of 20 and 30 y were recruited from Xichang County, Sichuan Province, a rural area in China with historically low selenium intake. Dietary selenium intake averages 11 μg/d in this area (Dodge et al., 1999a). Subject recruitment was conducted by village doctors in collaboration with our Chinese cohort, Dr. Yiming Xia.

The selected women were lifelong residents of several villages in Xichang County. The supplemented and unsupplemented women, however, lived in

different villages. The women had not taken selenium supplements in the year before becoming pregnant, had no known illness, and had been in good health for at least one year. The women had similar lifestyles and economic status. Each subject received $50 at the end of the study.

The Institutional Review Board of Oregon State University reviewed and approved the study protocol and oral consent was obtained from each participant. The study was also approved by a special institutional review board convened at the Chinese Academy of Preventive Medicine in Beijing, China.

Supplementation

Eleven of the women recruited for the study were supplemented daily with two selenium enriched yeast tablets containing a total of 100 μg of selenium (primarily in the form of selenomethionine) while the remaining subjects received placebo tablets containing yeast only (Nutrition 21, San Diego, CA). The subjects started taking their tablets at the beginning of the third trimester of pregnancy and continued until three months after the births of their babies. The study was double-blind. Each subject was given a packet of 60 pills at a time. To ensure compliance, subjects visited their doctor at the end of each month, turned in the used packet and received a full packet of pills for the next month. Compliance was determined by analyzing plasma and milk selenium concentration. All subjects completed the study. The data for one subject taking the selenium supplement was removed from the statistical analysis because

plasma and milk selenium concentrations were significantly lower than the other subjects in this group suggesting noncompliance.

Diet

Two 3-day diet recalls were collected from each woman and averaged to determine 1-day intakes. The first set of recalls was conducted at the time of delivery and the second was at the end of the study (three months after the birth of the baby). A protocol to accumulate the data for the dietary and nutritional status of the Chinese population was used in this study (Keyou, 1996). Also, mixed diets samples were collected at each of these times from each subject. The mixed diets contained one-eighth of all the food consumed at each meal by the women on the three days closest to the days when blood and milk samples were collected.

Sample Collection

Collection of blood samples occurred, on average, six days within the births of the babies (range = 0 – 8 days) and 3 months after the birth of the baby. Trained medical personnel collected the blood from the antecubital vein with a sterile needle into an evacuated glass tube containing 0.2 mL of 5% EDTA as an anticoagulant. The blood was centrifuged (5 min at 3000 rpm) and the plasma frozen immediately (-20°C) by the members of Dr. Xia's laboratory. At the end of the study period the samples were transported while frozen on dry ice via air to

OSU. The samples were kept frozen at all times (-20 °C) and transported on dry ice when not in a freezer. Once at Oregon State University, the plasma was briefly thawed and aliquoted into separate vials and refrozen for future measurement of selenium concentration, glutathione peroxidase activity, and α-tocopherol content. Samples were stored at -80°C at Oregon State University until they were analyzed. The lipid peroxidation and fatty acid assays were conducted immediately after thawing and aliquots for these assays were not refrozen.

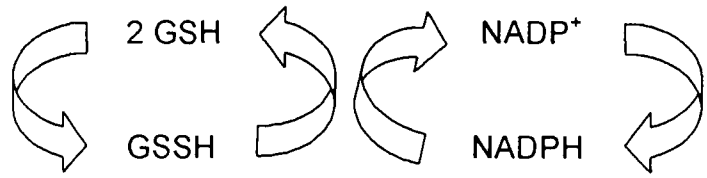
Human milk samples were collected on the same day that the plasma samples were collected. The village doctors instructed the subjects how to clean their breasts with 75% ethanol and express the milk by hand into a sterile, 15 mL container which was provided. They expressed approximately 10 mL of milk, from one breast, at the beginning of a feeding. The milk sample was aliquoted and frozen (-20°C) for the measurement of glutathione peroxidase activity, selenium concentration, and fatty acid content. Human milk samples were transported and stored in the same manner as the plasma samples.

Laboratory Procedures

Hematocrit and hemoglobin values were measured in China using a standard clinical procedure. Proximate composition of the diet samples, including percent moisture, percent ash, percent crude protein, and percent fat was analyzed using the methods of Harris (1970).

Selenium concentrations in plasma and human milk were determined using a semi-automated fluorimetric method (Brown and Watkinson, 1977). Samples were digested with nitric and perchloric acids which converted the selenium into the +6 oxidation state. The samples were heated briefly after adding hydrochloric acid to reduce the selenium to the +4 oxidation state. The pH was adjusted to between 2 and 3 and the selenium was complexed to 2,3-diaminonapthalene (DAN) at room temperature. The samples were extracted with cyclohexane and fluorescence was measured with a 325 nm filter for excitation and a 556 nm filter for emission using an Alpkem Autoanalyzer II, Perstorp Corporation (Beaverton, OR). Peak heights were measured in millimeters and the selenium concentration (ng/g) was calculated by referenced values obtained from known selenium concentrations.

Glutathione peroxidase activity in plasma and human milk was determined as described by Beilstein and Whanger (1983). This is a coupled enzyme procedure which uses 25 mM t-butyl hydroperoxide as a substrate. Reduced glutathione (GSH) concentration is kept constant by exogenous glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH) which reduce any oxidized glutathione (GSSG) produced back to GSH. The rate of GSSG formation is measured by following the decrease in absorbance of the reaction mixture because NADPH is oxidized to NADP at 340 nm (Paglia and Valentine, 1967).



To begin the assay, 25 μL of plasma diluted to 100 μL with water or 100 μL of milk was needed. Freshly prepared reaction mixture (0.8 mL) containing 0.125 M phosphate buffer (pH 7.0), 4.5 mM EDTA, 4.7 mM sodium azide, 2.8 nmol NADPH, 49.9 nmol reduced glutathione, and 0.67 units glutathione reductase were added to the sample. The addition of 0.1 mL of freshly prepared 25 mM t-butyl hydroperoxide solution initiated the reaction. Glutathione peroxidase activity was measured using a Beckman DU-64 Spectrophotometer (Fullerton, CA) at 340 nm. Activity is expressed as nmoles of NADPH oxidized per minute per milliliter of sample.

Lipids were extracted from the plasma samples by the method of Bligh and Dyer (1959) and from the diet and human milk samples with minor modifications to this method. Two milliliters of milk were homogenized with 14 mL of chloroform/methanol (1:1) (Dodge et al., 1999a). The chloroform (bottom) layer was used for fatty acid determination. The fatty acid profiles of the plasma, human milk, diet, and dietary fat samples were measured by gas chromatography as described by Song and Wander (1991). Fatty acid profile data are presented as the weight percent of total fatty acids measured for plasma and milk and as milligrams of fatty acid per gram of sample for the diet and fat samples. Since the total human milk fatty acid concentration can change with duration of nursing and between feedings, milk collection conditions affect fatty

acid concentrations. Despite this change in fatty acid concentrations, relative percentage of fatty acids remains the same (Koletzko et al., 1992). Therefore, it is appropriate to use weight percent to compare concentrations of fatty acids.

Lipid peroxidation was measured as thiobarbituric acid (TBA) reacting substances (TBARS) using a fluorescence spectrometer (LS-3B, Perkin-Elmer, San Jose, CA) (Wander et al., 1996a). Lipids were isolated by precipitation with phosphotungstic and sulfuric acids. TBA, which reacts with malondialdehyde, was added and the fluorimetric measurement was taken at 553 nm with excitation at 515 nm. TBARS values were calculated in nmol TBARS/mL using a six-point standard curve made from tetraethoxypropane solutions.

Alpha-tocopherol was measured in plasma using a fluorimetric HPLC method that has previously been used in our lab (Araund et al., 1991 and Wander et al., 1996b). In this method, ethanol was added to 200 μL of plasma in order to precipitate proteins and hexane was added to extract lipid-soluble components. The lipid-soluble fraction was dried and resuspended in methanol for injection into the HPLC. An external α-tocopherol standard was used to calculate α-tocopherol concentration in μg/mL of sample.

Statistical Analysis

The means and standard errors were calculated for all variables. Data were analyzed using 2-way analysis of variance (ANOVA) with the two factors being supplementation and time when the samples were obtained. The data were checked for interactions between the two variables and no data were

transformed. Significant changes over time indicated that a 2-way ANOVA was inappropriate. Therefore, the variable of time was not used and Student's t-tests were used to analyze differences between the supplemented and unsupplemented women at each time point (Ramsey and Schafer, 1997). Analysis was performed using SAS 6.11 (SAS Institute Inc., Cary, NC. 1985). Data were considered statistically significantly different if $P < 0.05$.

Results

Subjects

The ages of the subjects are given in Table 1. There were no statistically significant differences in mean hematocrit and hemoglobin values at the time of the births of the babies or three months after the births of the babies (data not shown) between the women who were supplemented with selenium and those who were not (Table 1).

Table 1: Age of Subjects and Selected Biological Values from Questionnaire and Blood Tests at the Time of Delivery[1]

	-Se group n = 10	+Se group n = 10
Age (years)	23 ± 1	25 ± 1
Hematocrit (%)	41 ± 2	39 ± 2
Hemoglobin (g/L)	130 ± 7	130 ± 7

[1]There were no statistically significant differences between the supplemented and unsupplemented groups for any of the parameters. Values are means ± SEM.

The dietary intakes from the recall data and the analysis of the mixed diets are given in Tables 2 and 3. There were interactions over time for both protein and fat intakes. Analysis of dietary intake over time shows an increase in caloric ($P = 0.01$) and fat ($P = 0.03$) intakes for the women who received the selenium supplement, a decrease in protein ($P = 0.002$) and α-tocopherol ($P = 0.008$)

Table 2: Diet Data Taken at the Time of Delivery and Three Months After Delivery

	0 months		3 months	
	-Se group	+Se group	-Se group	+Se group
Diet Recall:				
Energy (kJ)	6230 ± 710	5250 ± 710[1]	7070 ± 740	8600 ± 740[2]
Energy (kcal)	1490 ± 170	1250 ± 170[1]	1690 ± 180	2060 ± 180[2]
Protein (g)	110 ± 12[1]	83 ± 12	50 ± 6[a,2]	71 ± 6[b]
Fat (g)	73 ± 13	60 ± 13[1]	67 ± 13[a]	110 ± 13[b,2]
Carbohydrate (g)	98 ± 6[1]	95 ± 6[1]	220 ± 16[2]	200 ± 16[2]
Vitamin C (mg)	14 ± 5[1]	10 ± 5[1]	60 ± 10[2]	58 ± 10[2]
α-tocopherol (mg)	12 ± 2[1]	9 ± 2	3.8 ± 0.6[a,2]	6.3 ± 0.6[b]
Measured Diets:				
Protein (wt%)	7.8 ± 0.4[a,1]	6.6 ± 0.4[b,1]	3.2 ± 0.2[2]	3.7 ± 0.2[2]
Fat (wt%)	2.3 ± 0.5[a]	4.1 ± 0.5[b]	2.2 ± 0.6	3.3 ± 0.6
Carbohydrate (wt%)	11 ± 1[a,1]	15 ± 1[b,1]	19 ± 2[2]	20 ± 2[2]
Ash (wt%)	0.5 ± 0.1	0.6 ± 0.1[1]	0.6 ± 0.1	0.8 ± 0.1[2]
α-tocopherol (μg/g food)	1.8 ± 0.4[1]	1.3 ± 0.4	0.3 ± 0.11[2]	0.52 ± 0.11

[1]Values with different letters as superscripts are significantly different between level of selenium supplementation. Values with different numbers as superscripts are significantly different over time ($P < 0.05$). Values are mean ± SEM.

Table 3: Diet Recall Data Adjusted for Kilocalories[1]

	0 months		3 months	
	-Se group	+Se group	-Se group	+Se group
Protein (g/1000 kcal)	72 ± 5^{1}	71 ± 5^{1}	30 ± 2^{2}	35 ± 2^{2}
Fat (g/1000 kcal)	48 ± 4^{1}	43 ± 4	$36 \pm 3^{a,2}$	52 ± 3^{b}
Carbohydrate (g/1000 kcal)	69 ± 5^{1}	81 ± 5^{1}	140 ± 10^{2}	100 ± 10^{2}
Vitamin C (mg/1000 kcal)	10 ± 4^{1}	5.8 ± 4^{1}	39 ± 8^{2}	29 ± 8^{2}
α-tocopherol (mg/1000 kcal)	7.6 ± 0.4^{1}	7.0 ± 0.4^{1}	2.2 ± 0.3^{2}	3.1 ± 0.3^{2}

[1]Values with different letters as superscripts are significantly different between level of selenium supplementation. Values with different numbers as superscripts are significantly different over time ($P < 0.05$). Values are mean ± SEM.

intakes for the women who did not receive the supplement, and an increase in carbohydrate consumption for women in both groups ($P \leq 0.0003$). Also, vitamin C intake increased over time for both supplemented and unsupplemented women ($P \leq 0.007$).

Although caloric intake was not significantly different between the supplemented and unsupplemented women at the time of delivery or three months later, caloric intake did vary somewhat. In order to look for trends, the data were expressed per 1000 kilocalories (Table 3). This conversion caused some changes in significant differences between groups and over time. Protein intake was no significantly different between groups at three months after delivery but was lower over time for the supplemented women ($P = 0.0002$). Fat intake was no longer higher over time for the supplemented women but was lower over time for the women who did not receive the supplement ($P = 0.007$). α-tocopherol intake was lower over time for the supplemented women ($P = 0.0001$) but no longer significantly different between the supplemented and unsupplemented women three months after the births of the babies. Because of the differences seen over time in the dietary intake between the supplemented and unsupplemented women, the data were not compared between times.

At the time of the births of their babies, there were no differences in dietary intake in terms of α-tocopherol, kilocalories, or grams of protein, fat, or carbohydrate among the women as measured by dietary recall. Analysis of the mixed diets, however, revealed that the diets of the women who received the selenium supplement contained a smaller percentage of protein and larger

percentages of fat and carbohydrate than the women who did not take the supplement. The amount of α-tocopherol in the diets was the same for the supplemented and unsupplemented women.

Three months after the births of the babies, the dietary recalls indicate an increase in grams of protein and fat and milligrams of α-tocopherol in the diets of the women who received the selenium supplement (Table 2). The mixed diet sample analysis shows no statistically significant differences although the diets of the women who were supplemented with selenium tended to be higher in percentage of protein and fat (Table 2).

Fatty acid profiles were completed for the mixed diet samples taken three months after delivery (Table 4). The diets of the women who received the selenium supplement contained higher levels of the fatty acids 16:0 and 18:0 ($P = 0.02$) and 18:1n-9 ($P = 0.01$). These differences were reflected in the sum of saturated fatty acids ($P = 0.02$) and sum of monounsaturated fatty acids ($P = 0.001$) being significantly higher in the selenium supplemented group. The amounts of 18:2n-6, n-6, and n-3 fatty acids, and the n-6/n-3 ratio were not different between the groups. Despite the higher amount of 20:4n-6 ($P = 0.01$) in the selenium supplemented women, there was no difference in the sum of polyunsaturated fatty acids between the supplemented and unsupplemented women.

Table 4: Mixed Diet Fatty Acids[1]

	-Se group	+Se group
16:0	5.0 ± 1.2^a	9.2 ± 1.2^b
18:0	2.2 ± 0.7^a	4.9 ± 0.7^b
18:1n-9	7.1 ± 2^a	16 ± 2^b
18:2n-6	3.1 ± 0.6	4.2 ± 0.6
18:3n-3	0.42 ± 0.09	0.65 ± 0.09
20:1n-9	0.20 ± 0.09^a	0.52 ± 0.09^b
20:4n-6	0.07 ± 0.02^a	0.16 ± 0.02^b
22:1n-9	0.10 ± 0.04	0.07 ± 0.04
ΣSFA	7.5 ± 2^a	15 ± 2^b
ΣMUFA	8.5 ± 2.5^a	18 ± 3^b
ΣPUFA	4.1 ± 0.9	5.6 ± 0.9
Σn-6	3.6 ± 0.7	4.7 ± 0.7
Σn-3	0.51 ± 0.16	0.82 ± 0.16
Σn-6/Σn-3	9.6 ± 1.2^a	6.4 ± 1.2^b

[1]Values with different letters are significantly different ($P < 0.05$). Values are mean ± SEM.
Fatty acids are expressed in mg/g diet.

The fatty acid profiles of eight dietary fats that are used in different regions of China are given in Table 5. It is obvious that these profiles vary greatly. The concentration of linoleic acid, for example, varies 11-fold from lard from Xide County to soybean oil from Beijing.

Plasma Selenium Status

At the time of delivery, the mean plasma selenium concentration of the women who received the supplement was 100 ± 5.9 ng/g while that of the

Table 5: Fatty Acid Profiles of Chinese Fats[1]

	Rapeseed 1	Rapeseed 2	Rapeseed 3	Lard 1	Lard 2	Lard 3	Soybean	Mixed
16:0	32.06	27.51	33.10	214.7	116.4	205.1	98.55	57.88
18:0	9.41	9.78	11.00	108.6	78.17	111.6	40.56	22.26
18:1n-9	179.6	173.8	203.2	350.1	160.3	314.4	194.2	434.7
18:2n-6	130.0	106.3	130.2	73.85	40.30	75.45	450.5	231.6
18:3n-3	62.93	58.09	63.12	7.68	4.50	12.15	46.42	47.56
20:1n-9	55.12	39.64	36.42	11.52	4.50	8.06	2.65	9.70
20:4n-6	--	--	--	--	1.46	3.39	--	--
22:1n-9	119.2	186.8	169.3	2.07	1.30	3.00	--	3.56
ΣSFA	47.73	47.57	54.34	338.6	203.7	330.9	152.6	104.1
ΣMUFA	371.4	418.0	429.3	411.5	183.0	368.1	211.1	474.9
ΣPUFA	197.8	170.2	199.3	86.64	49.37	95.56	498.5	281.4
Σn-6	133.4	109.4	133.4	78.96	44.88	83.41	452.1	233.9
Σn-3	64.38	60.87	65.93	7.68	4.50	12.15	46.42	47.56
Σn-6/Σn-3	2.07	1.80	2.02	10.28	9.98	6.87	9.74	4.92

[1]Rapeseed oil 1 and Lard 1 are from Jianishi County. Soybean oil and Mixed oil are from Beijing. Rapeseed oil 2 and Lard 2 are from Xide County. Rapeseed oil 3 and Lard 3 are from Xichang County. Units are mg/g.

women who were not supplemented was 34.7 ± 5.9 ng/g (P = 0.0001, Figure 1). This is almost a 3-fold difference. The mean plasma GPx activity was 2.5-fold higher in the women who received the supplement, 430 ± 26 compared to 175 ± 26 nmoles NADPH oxidized / minute / mL plasma (P = 0.0001, Figure 2).

Three months after the births of their babies, the mean plasma selenium concentration of the supplemented women was 86.1 ± 4.2 ng Se / g while that of the women who were not supplemented was 28.0 ± 4.2 ng Se / g (P = 0.0001), again a 3-fold difference (Figure 1). Mean plasma GPx activity was almost 2-fold higher ($P < 0.05$) in women supplemented with selenium as compared to those not supplemented with selenium. The mean GPx value for the supplemented women was 293 ± 15 while that of the unsupplemented women was 175 ± 15 nmoles NADPH oxidized / minute / mL plasma (P = 0.0001, Figure 2).

Milk Selenium Status

At the time of delivery, mean human milk selenium concentration was 16.7 ± 0.9 ng/g milk for the women who were supplemented with selenium and 7.4 ± 0.9 ng/g milk for the women who were not supplemented (P = 0.0001, Figure 3), about a 2-fold difference. Even though there was this dramatic difference in the concentration of selenium in the human milk, mean milk GPx activity at the time of delivery was not significantly different for the women who were supplemented with selenium compared to the women who were not supplemented. Mean milk

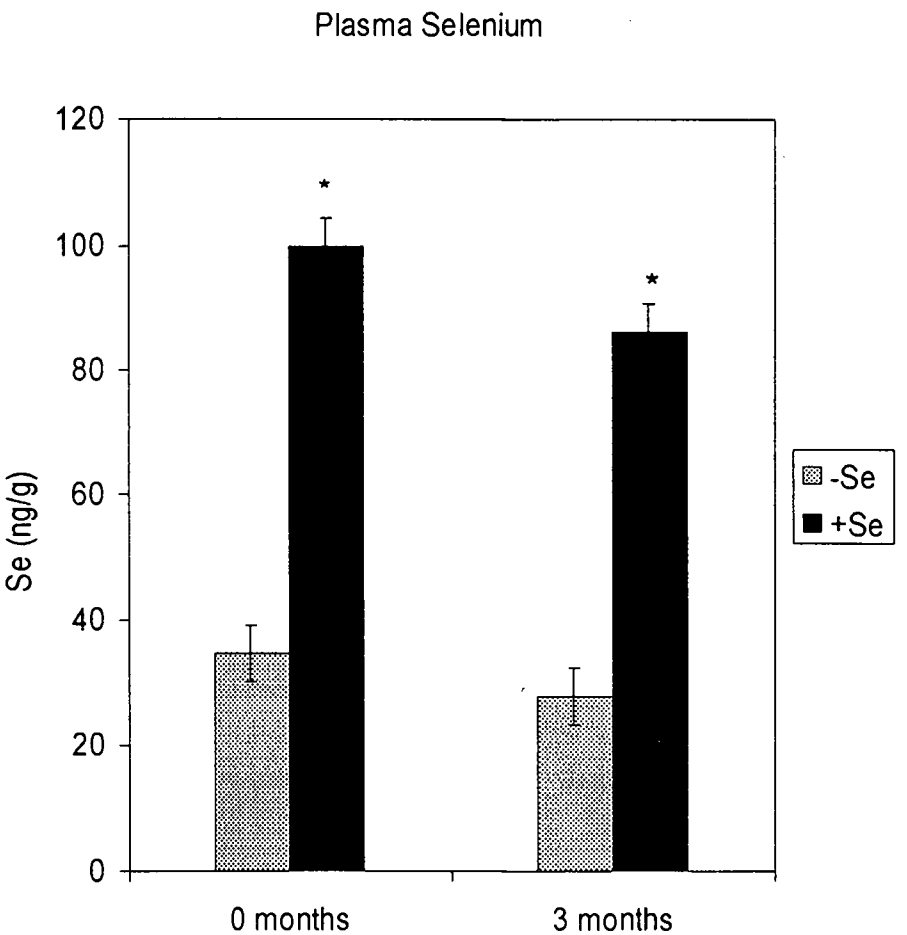


Figure 1. Plasma selenium concentrations in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. Asterisk above the bars indicate significant differences (*P<0.05).

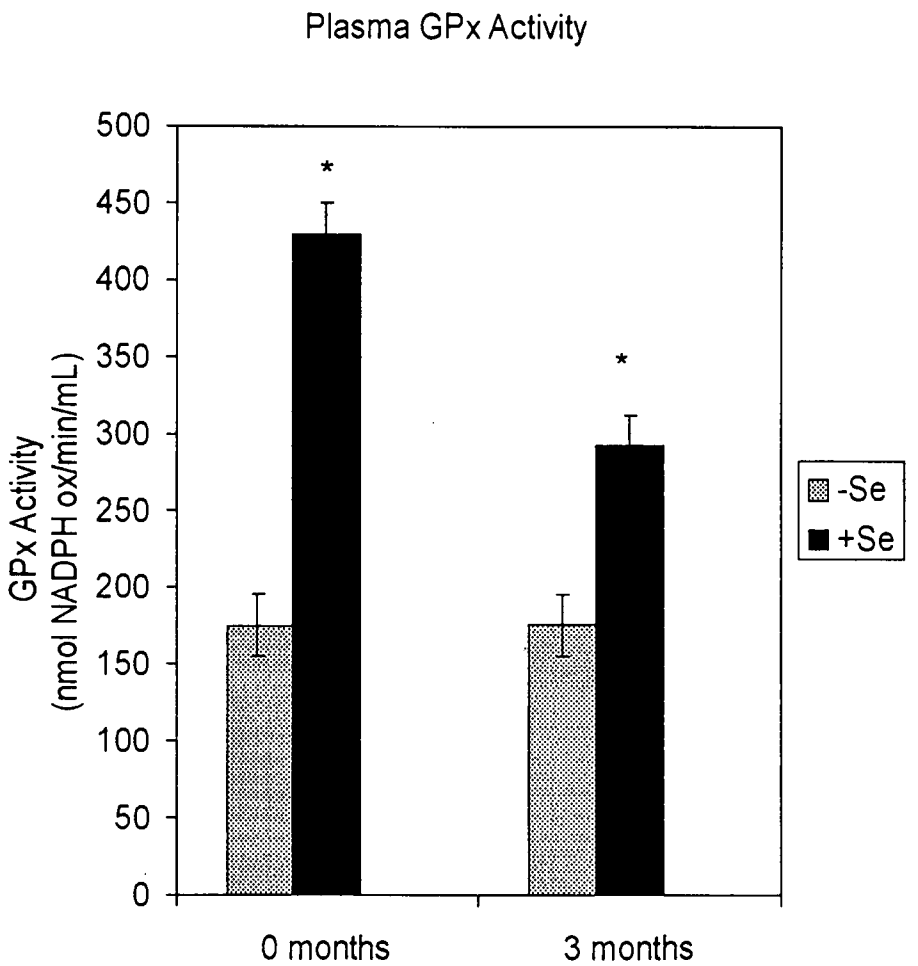


Figure 2. Plasma glutathione peroxidase activity in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. Asterisk above the bars indicate significant differences (*P<0.05).

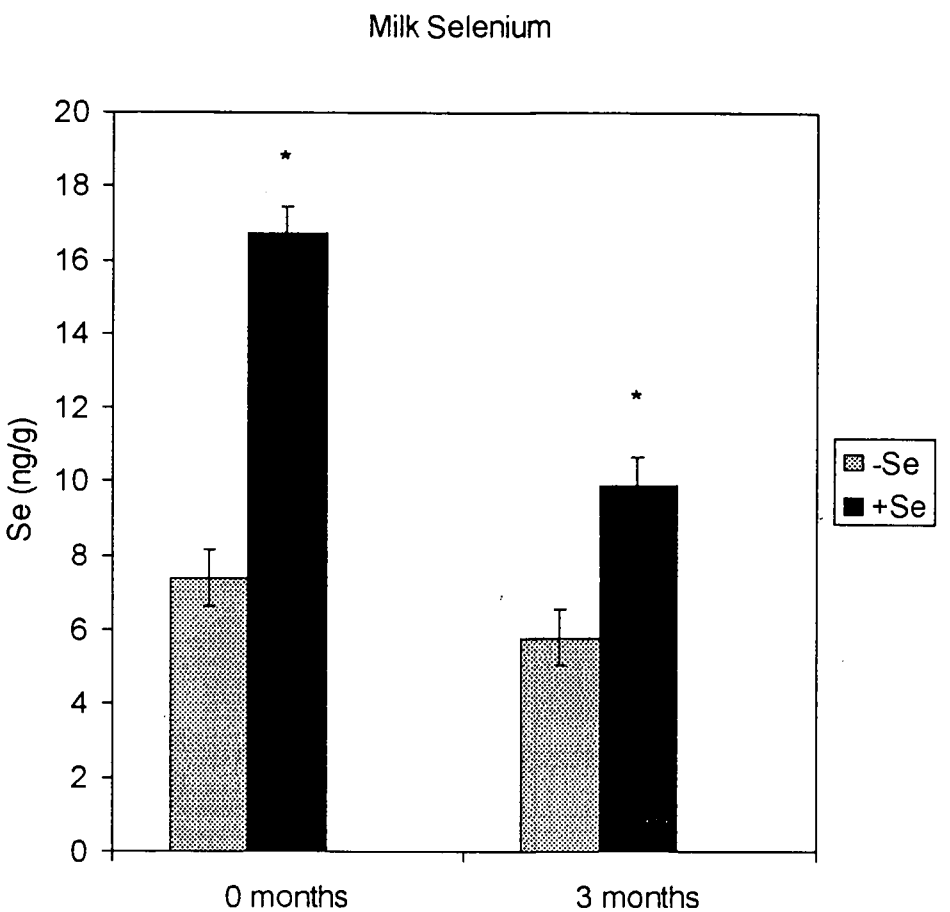


Figure 3. Milk selenium concentrations in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. Asterisk above the bars indicate significant differences (*P<0.05).

GPx activity for the women who were supplemented was 69 ± 6 nmoles NADPH oxidized / minute / mL milk while that of the women who did not receive the supplement was 56 ± 6 nmoles NADPH oxidized / minute / mL milk (P = 0.09, Figure 4).

The mean selenium concentration in the milk of the women that had been supplemented with selenium was 9.9 ± 0.6 ng Se / g milk and that of the women who had not been supplemented was 5.8 ± 0.6 ng Se / g milk (P = 0.0003) three months after the births of their babies (Figure 3). The milk selenium was about 2-fold higher in the samples taken from the supplemented women as compared to the women who were not supplemented. Milk GPx activity was not significantly different between the supplemented and unsupplemented women at three months after delivery (Figure 4). The mean milk GPx activity for the women who were supplemented with selenium was 44 ± 4 nmoles NADPH oxidized / minute / mL milk while that of the women who did not receive the supplement was at 37 ± 4 nmoles NADPH oxidized / minute / mL milk (P = 0.3).

Plasma α-tocopherol

At the time of delivery, there were no statistically significant differences in vitamin E content between the women supplemented with selenium and those not supplemented. The mean plasma vitamin E content for the supplemented women was 8.6 ± 0.8 μg/mL plasma while that of the women not supplemented was 10.3 ± 0.8 μg/mL plasma (P = 0.2, Figure 5).

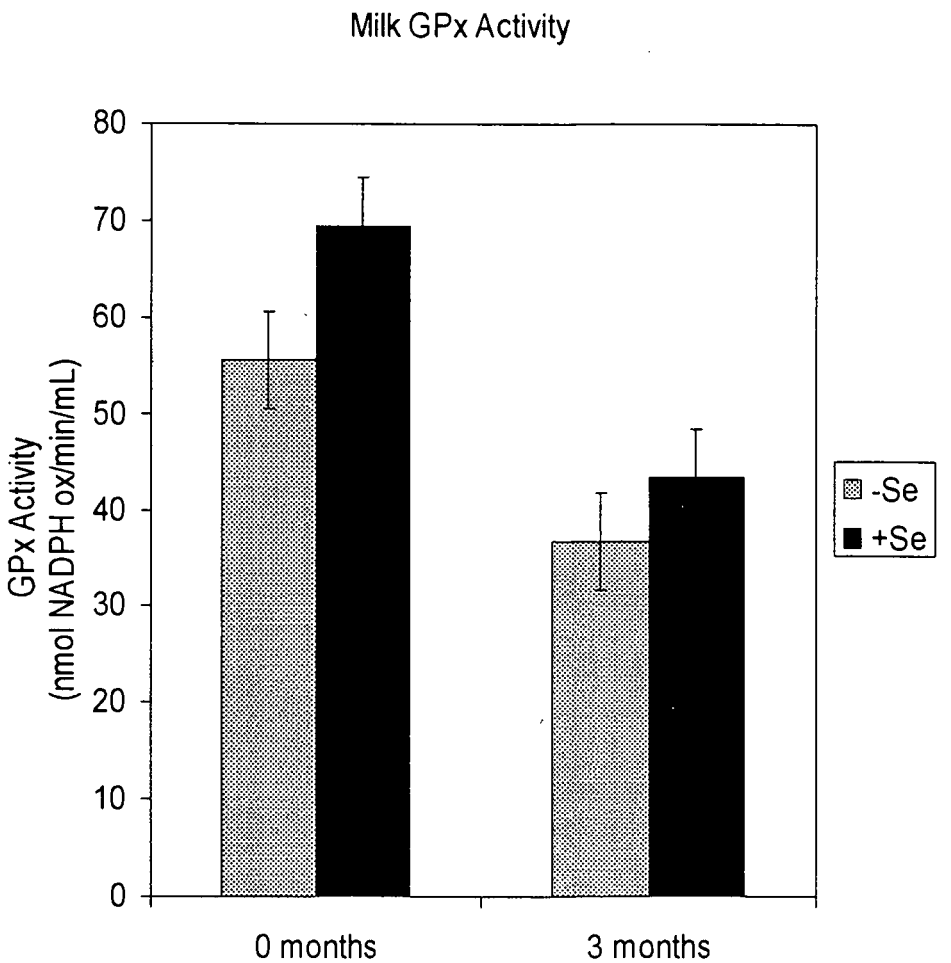


Figure 4. Milk glutathione peroxidase activity in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. There were no significant differences.

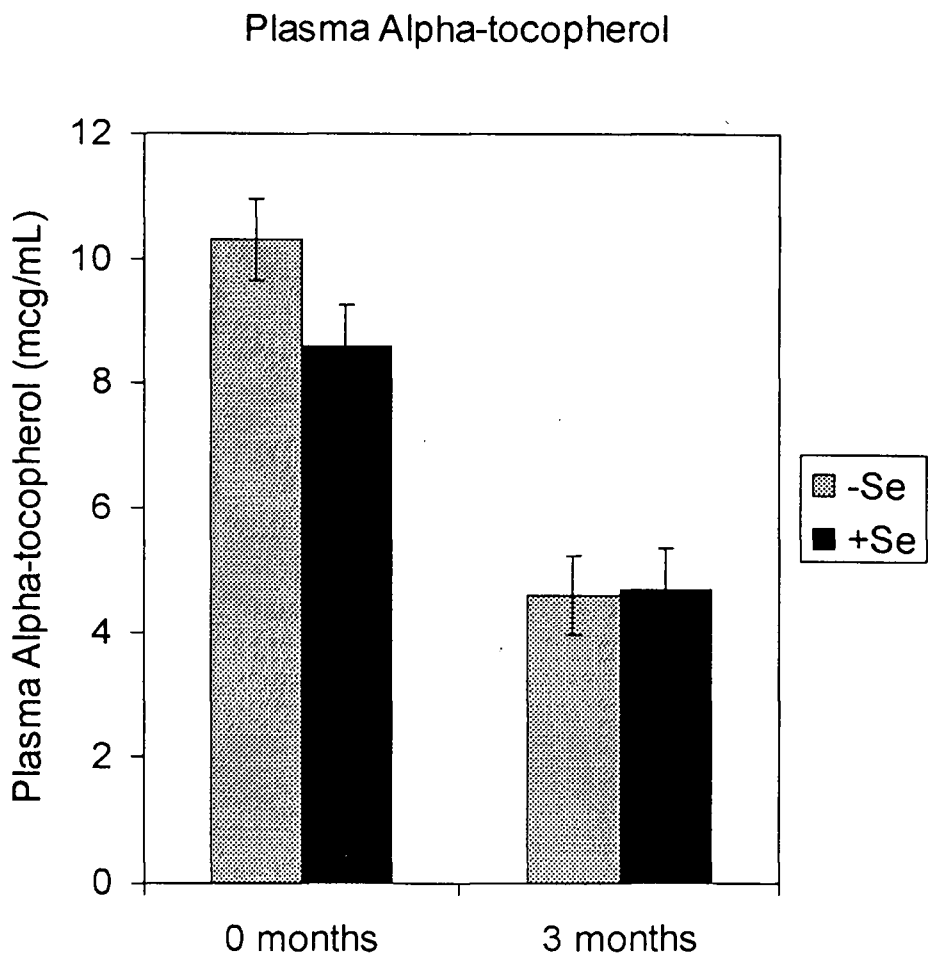


Figure 5. Plasma α-tocopherol concentrations in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. There were no significant differences.

Three months after the births of their babies, the mean plasma vitamin E levels were the same among all the women in the study. The mean plasma vitamin E content of the women who received the selenium supplement was 4.7 ± 0.5 while that of the women who did not receive the supplement was 4.6 ± 0.5 μg/mL plasma (P = 0.9).

Plasma Lipid Peroxidation

Mean plasma TBARS levels were about 40% higher at the time of delivery in the women who received the supplement than in the women who did not. Mean TBARS values were 1.7 ± 0.2 nmol TBARS/mL plasma in the supplemented women and 1.2 ± 0.1 nmol TBARS/mL plasma in the women who were not supplemented (P = 0.03, Figure 6).

At three months after the births of the babies, the mean plasma lipid peroxide level was 64% higher in the samples from the women who were supplemented with selenium than in the samples from the women who were not supplemented. Mean TBARS values were 1.2 ± 0.1 and 0.73 ± 0.1 nmol TBARS / mL plasma, in the supplemented and unsupplemented women, respectively (P = 0.001).

Fatty Acid Profiles in Plasma

There were very few statistically significant differences in plasma fatty acid profiles between the women who were supplemented with selenium and the

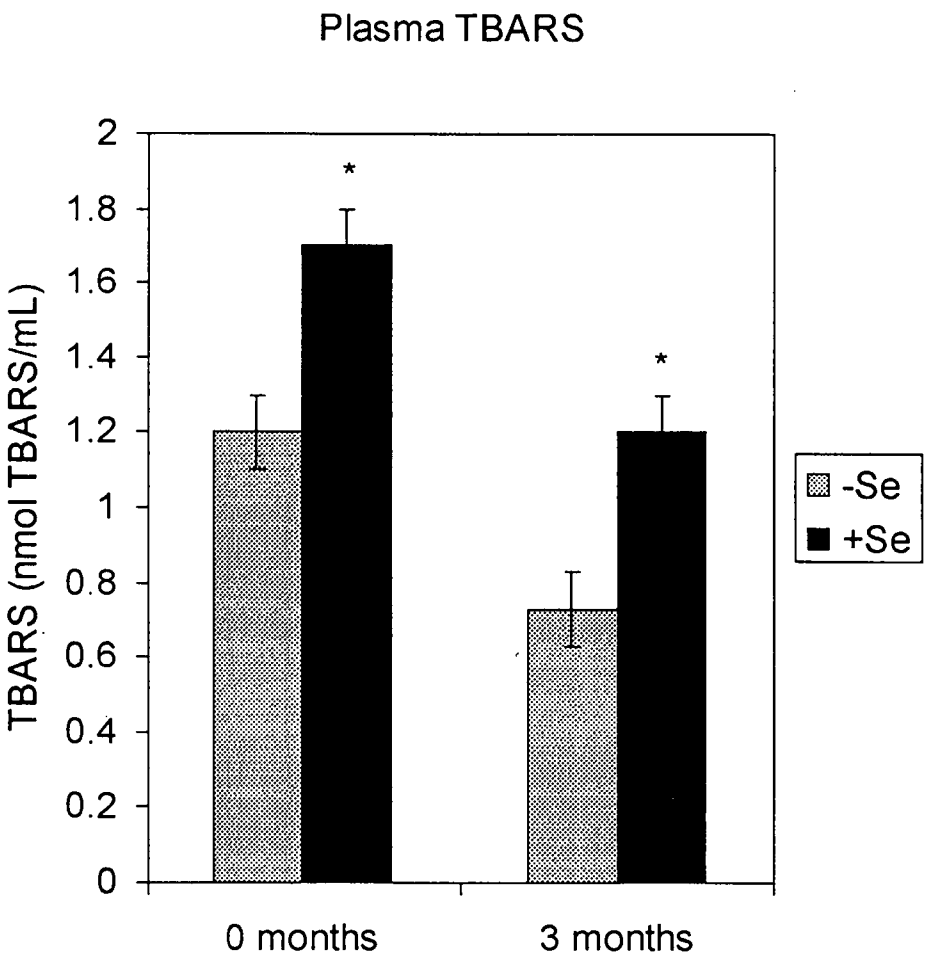


Figure 6. Plasma TBARS in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. Asterisk above the bars indicate significant differences (*P<0.05).

women who were not supplemented with selenium at the time of delivery. The mean plasma 18:1n-9t concentration at the time of delivery for the women taking the selenium supplement was 0.03 ± 0.02 wt% while that of the women not receiving the supplement was 0.1 ± 0.02 wt% (P = 0.03). The mean plasma 18:3n-3 concentration at the time of delivery was 0.7 ± 0.04 wt% for the women receiving the supplement and 0.5 ± 0.04 wt% for the women not receiving the supplement (P = 0.02). For 20:1n-9, the mean plasma concentration was 0.2 ± 0.05 wt% for the supplemented women and 0.4 ± 0.05 wt% for the unsupplemented women at the time of delivery (P = 0.01). The women taking the selenium supplement had a lower mean plasma 23:0 concentration at the time of delivery at 0.03 ± 0.01 wt% while that of the unsupplemented women was 0.07 ± 0.01 wt% (P = 0.046, Table 6, Figures 7 and 8).

There were no significant differences in individual fatty acids three months postpartum. There also were no statistically significant differences between the supplemented and unsupplemented women for the sums of saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids for plasma at either time point (Figure 8).

Fatty Acid Profiles In Milk

For milk there was one statistically significant difference for an individual fatty acid at the time of delivery. The mean milk 24:0 concentration for the women taking the selenium supplement was 0 wt% while that of the women not

Table 6: Plasma Fatty Acid Profiles (wt%)[1]

	0 months		3 months	
Fatty Acid	-Se group	+Se group	-Se group	+Se group
14:0	0.5 ± 0.05	0.43 ± 0.05	0.64 ± 0.08	0.59 ± 0.08
15:0	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02	0.02 ± 0.02
16:0	21.26 ± 0.50	21.33 ± 0.50	19.08 ± 0.76	18.92 ± 0.76
16:1n-7	2.12 ± 0.26	2.36 ± 0.26	1.64 ± 0.17	1.79 ± 0.17
16:2	0.26 ± 0.03	0.27 ± 0.03	0.39 ± 0.09	0.43 ± 0.09
18:0	7.09 ± 0.28	6.99 ± 0.28	8.70 ± 0.32	9.03 ± 0.32
18:1n-9t	0.10 ± 0.02[a]	0.03 ± 0.02[b]	0.44 ± 0.23	0.29 ± 0.23
18:1n-9c	19.55 ± 0.80	19.71 ± 0.80	20.06 ± 1.25	19.38 ± 1.25
18:1n-7	1.90 ± 0.09	2.18 ± 0.09	2.11 ± 0.14	2.12 ± 0.14
18:2n-6t	--	--	0.16 ± 0.10	0.03 ± 0.10
18:2n-6c	23.53 ± 0.95	24.57 ± 0.95	25.33 ± 1.40	24.03 ± 1.40
20:0	0.14 ± 0.02	0.11 ± 0.02	0.19 ± 0.04	0.11 ± 0.04
18:3n-3	0.51 ± 0.04[a]	0.66 ± 0.04[b]	0.83 ± 0.12	0.94 ± 0.12
20:1n-9	0.43 ± 0.05[a]	0.23 ± 0.05[b]	0.60 ± 0.06	0.43 ± 0.06
18:4n-3	--	--	--	--
20:2n-6	0.31 ± 0.04	0.26 ± 0.04	0.64 ± 0.20	0.31 ± 0.20
20:3n-9	0.31 ± 0.08	0.11 ± 0.08	0.19 ± 0.14	0.33 ± 0.14
20:3n-6	1.51 ± 0.09	1.64 ± 0.09	1.17 ± 0.11	1.36 ± 0.11
22:0	0.29 ± 0.02	0.30 ± 0.02	0.31 ± 0.04	0.30 ± 0.04
20:4n-6	9.88 ± 0.68	9.00 ± 0.68	5.85 ± 0.61	5.76 ± 0.61
22:1n-9	0.08 ± 0.02	0.08 ± 0.02	0.35 ± 0.13	0.28 ± 0.13
23:0	0.08 ± 0.01[a]	0.03 ± 0.01[b]	0.13 ± 0.04	0.02 ± 0.04

Table 6 (Continued)

	0 months		3 months	
Fatty Acid	-Se group	+Se group	-Se group	+Se group
20:5n-3c	0.44 ± 0.04	0.36 ± 0.04	0.46 ± 0.08	0.38 ± 0.08
24:0	0.18 ± 0.02	0.14 ± 0.02	0.21 ± 0.04	0.21 ± 0.04
22:4n-6	0.26 ± 0.02	0.22 ± 0.02	0.29 ± 0.2	0.46 ± 0.2
24:1	1.17 ± 0.09	1.11 ± 0.09	1.20 ± 0.08	1.24 ± 0.08
22:5n-3	--	0.09 ± 0.04	--	0.08 ± 0.04
22:6n-3	2.26 ± 0.11	2.21 ± 0.11	1.29 ± 0.12	1.22 ± 0.12
Others	3.47 ± 0.24	3.05 ± 0.24	5.04 ± 2.35	6.86 ± 2.35
ΣSFA	29.62 ± 0.47	29.41 ± 0.47	29.35 ± 0.87	29.21 ± 0.87
ΣMUFA	25.35 ± 0.92	25.70 ± 0.92	26.39 ± 1.35	25.53 ± 1.35
ΣPUFA	39.28 ± 0.99	39.40 ± 0.99	36.62 ± 1.60	35.35 ± 1.60
Σn-6	35.50 ± 1.02	35.70 ± 1.02	33.46 ± 1.63	31.95 ± 1.63
Σn-3	3.21 ± 0.10	3.33 ± 0.10	2.58 ± 0.20	2.63 ± 0.20
Σn-6/Σn-3	11.15 ± 0.53	10.89 ± 0.53	13.66 ± 0.96	12.31 ± 0.96
PI[2]	72.26 ± 2.19	70.48 ± 2.19	57.64 ± 2.99	56.70 ± 2.99

[1]Values within each time group with different superscripts are significantly different ($P < 0.05$).
Values are means ± SEM.
[2]PI = peroxidizability index.

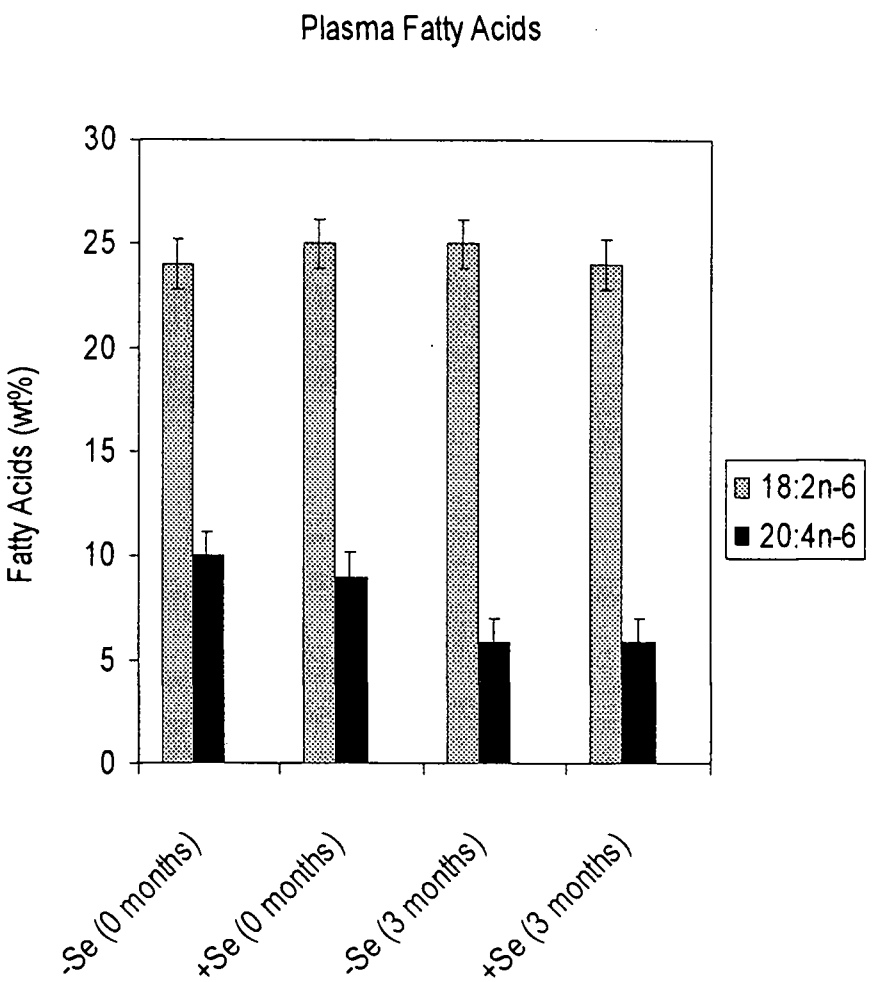


Figure 7. Selected n-6 plasma fatty acids in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. There were no significant differences.

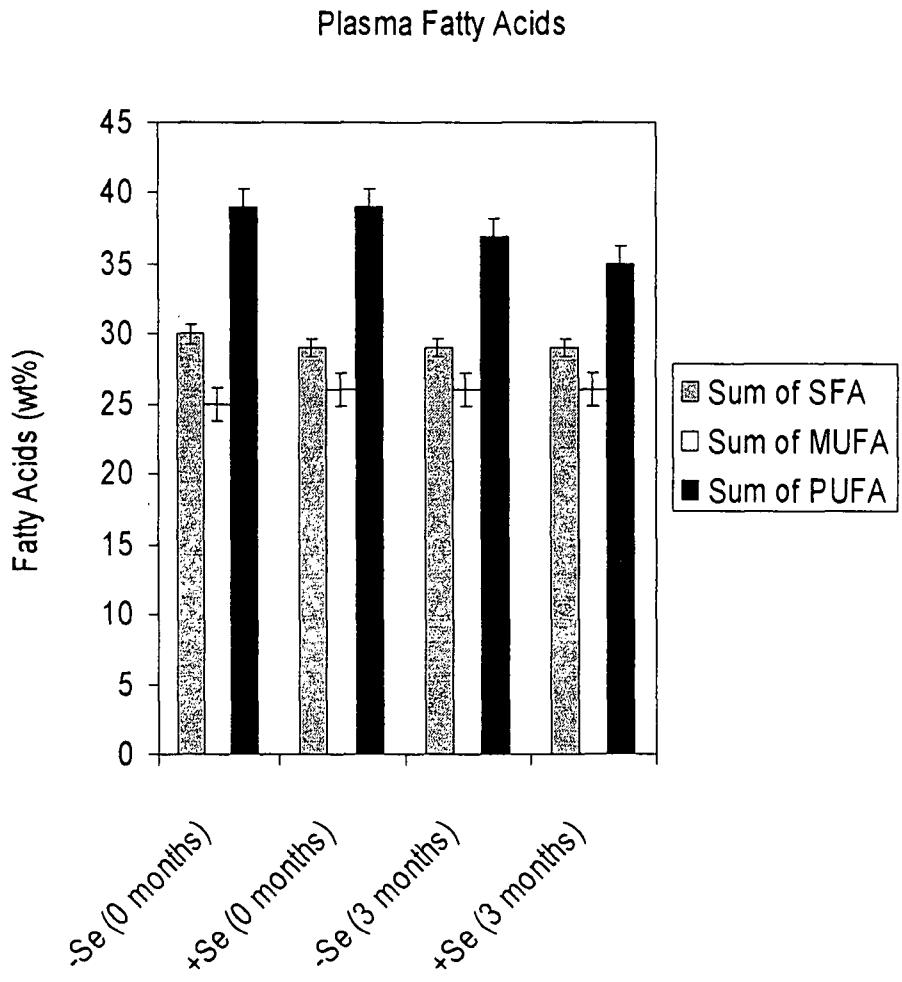


Figure 8. Sums of plasma fatty acid groups in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. There were no significant differences.

taking the supplement was 0.07 ± 0.01 wt% (P = 0.001, Table 7, Figures 9 and 10).

There were no statistically significant differences in individual milk fatty acids three months after delivery. There also were no statistically significant differences between the two groups for the sums of saturated fatty acids, monounsaturated fatty acids, or polyunsaturated fatty acids for milk at either time point (Figure 10).

Table 7: Human Milk Fatty Acid Profiles (wt%)[1]

	0 months		3 months	
Fatty Acid	-Se group	+Se group	-Se group	+Se group
10:0	0.86 ± 0.14	1.00 ± 0.14	1.08 ± 0.10	1.17 ± 0.10
12:0	4.83 ± 0.73	5.11 ± 0.73	4.46 ± 0.44	4.60 ± 0.44
14:0	5.49 ± 0.86	5.49 ± 0.86	4.01 ± 0.36	4.06 ± 0.36
14:1	0.01 ± 0.01	0.02 ± 0.01	--	--
15:0	0.10 ± 0.02	0.12 ± 0.02	0.05 ± 0.02	0.03 ± 0.02
16:0	22.67 ± 0.44	22.38 ± 0.44	19.85 ± 0.61	20.95 ± 0.61
16:1n-7	2.98 ± 0.16	2.99 ± 0.16	2.16 ± 0.12	2.32 ± 0.12
18:0	5.95 ± 0.17	6.01 ± 0.17	7.12 ± 0.41	7.35 ± 0.41
18:1n-9t	0.16 ± 0.02	0.14 ± 0.02	0.19 ± 0.03	0.25 ± 0.03
18:1n-9c	36.32 ± 1.11	35.49 ± 1.11	37.68 ± 0.81	36.73 ± 0.81
18:1n-7	2.75 ± 0.09	2.80 ± 0.09	2.56 ± 0.13	2.88 ± 0.13
18:2n-6c	10.71 ± 0.50	11.57 ± 0.50	12.15 ± 0.50	12.36 ± 0.50
20:0	0.18 ± 0.01	0.17 ± 0.01	0.23 ± 0.02	0.20 ± 0.02
18:3n-3	0.75 ± 0.08	0.98 ± 0.08	1.89 ± 0.20	1.87 ± 0.20
20:1n-9	0.88 ± 0.08	0.89 ± 0.08	1.48 ± 0.16	1.19 ± 0.16
20:2n-6	0.60 ± 0.07	0.63 ± 0.07	0.46 ± 0.01	0.47 ± 0.01
20:3n-6	0.65 ± 0.03	0.56 ± 0.03	0.28 ± 0.02	0.29 ± 0.02
20:4n-6	0.93 ± 0.05	0.86 ± 0.05	0.59 ± 0.03	0.60 ± 0.03
22:1n-9	0.29 ± 0.04	0.27 ± 0.04	1.61 ± 0.42	0.97 ± 0.42
20:5n-3c	0.13 ± 0.02	0.13 ± 0.02	0.10 ± 0.02	0.05 ± 0.02
24:0	-- [a]	0.07 ± 0.01[b]	--	--
22:4n-6	0.35 ± 0.05	0.27 ± 0.05	0.05 ± 0.02	0.03 ± 0.02

Table 7 (Continued)

	0 months		3 months	
Fatty Acid	-Se group	+Se group	-Se group	+Se group
24:1	0.29 ± 0.05	0.27 ± 0.05	0.28 ± 0.05	0.23 ± 0.05
22:6n-3	0.58 ± 0.05	0.49 ± 0.05	0.20 ± 0.02	0.18 ± 0.02
Others	1.54 ± 0.10 [a]	1.29 ± 0.10	1.50 ± 0.13	1.20 ± 0.13
ΣSFA	40.07 ± 1.70 [a]	40.35 ± 1.70	36.80 ± 0.98	38.37 ± 0.98
ΣMUFA	43.68 ± 1.20	42.87 ± 1.20	45.96 ± 0.78	44.57 ± 0.78
ΣPUFA	14.70 ± 0.60	15.48 ± 0.60	15.73 ± 0.59	15.85 ± 0.59
Σn-6	13.24 ± 0.56	13.89 ± 0.56	13.53 ± 0.51	13.75 ± 0.51
Σn-3	1.45 ± 0.07	1.60 ± 0.07	2.20 ± 0.22	2.10 ± 0.22
Σn-6/Σn-3	9.17 ± 0.40	8.82 ± 0.40	6.72 ± 0.56	6.95 ± 0.56
PI[2]	21.34 ± 0.81	21.62 ± 0.81	20.30 ± 0.79	20.15 ± 0.79

[1]Values within each time group with different superscripts are significantly different ($P < 0.05$).
Values are means ± SEM.
[2]PI = peroxidizability index.

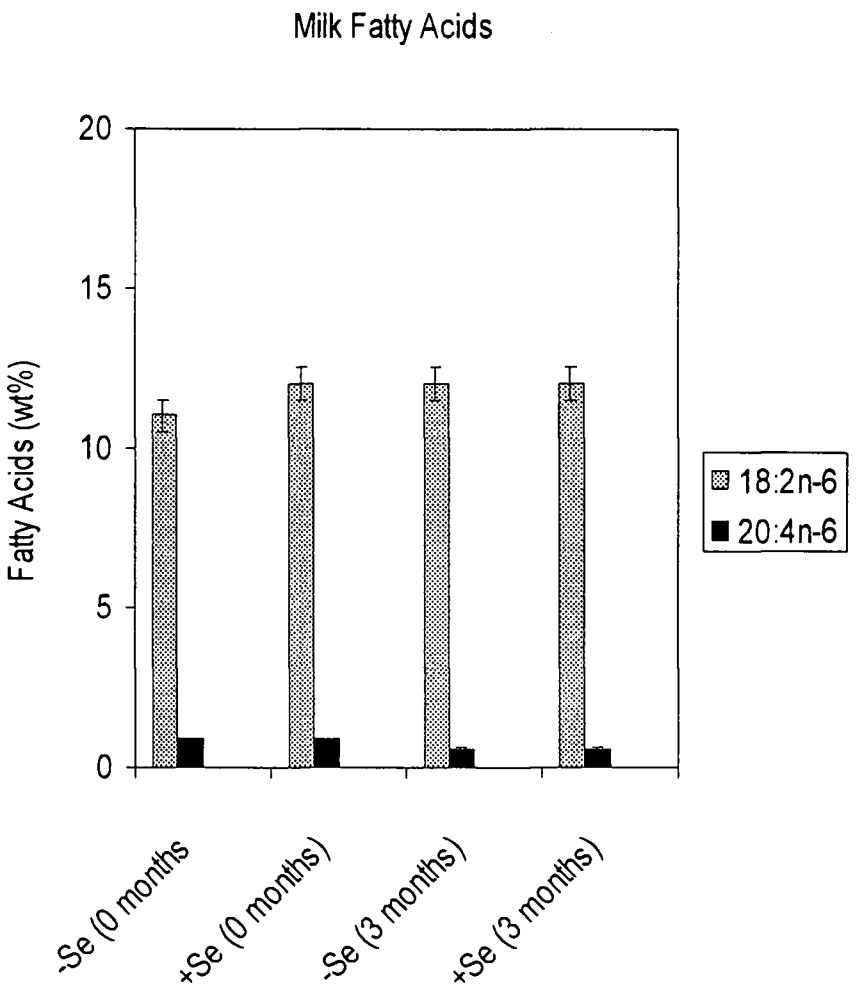


Figure 9. Selected n-6 milk fatty acids in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. There were no significant differences.

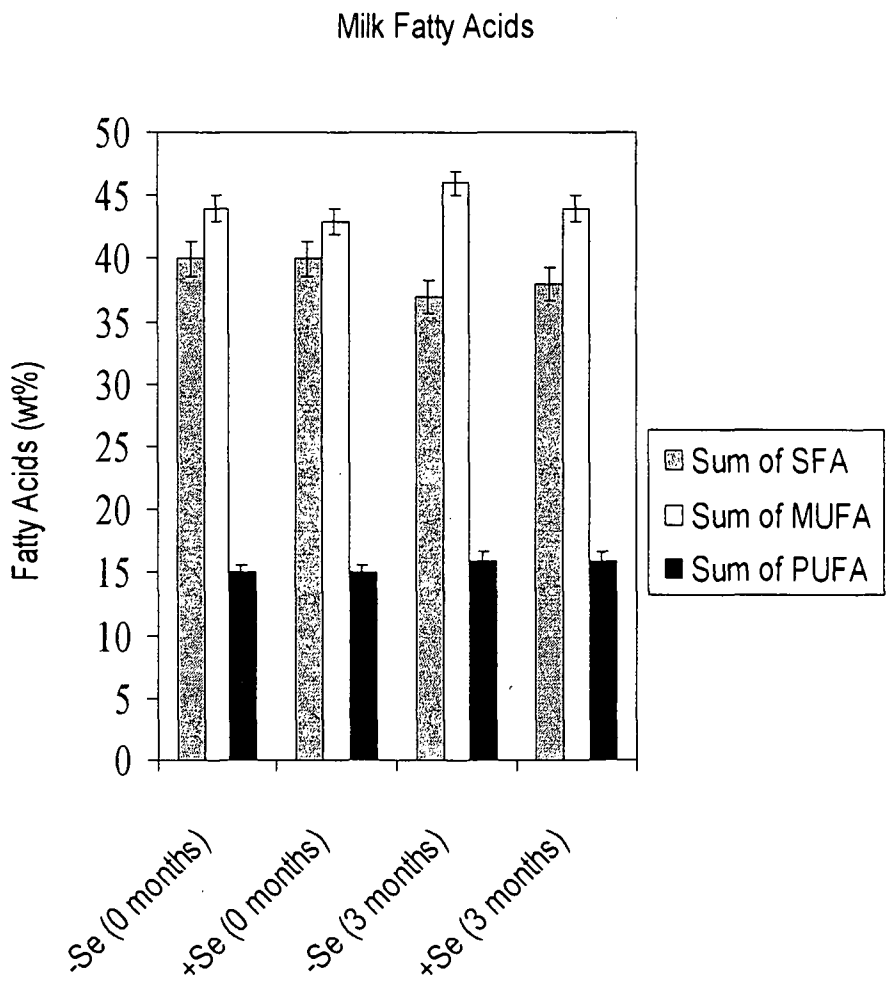


Figure 10. Sums of milk fatty acid groups in samples obtained from selenium-supplemented (n=10) and unsupplemented (n=10) lactating women from China. Values represent mean ± SEM. There were no significant differences.

Discussion

Because diet affects the selenium status and fatty acid content of human milk, it is important that dietary intake be the same for both the supplemented and unsupplemented women at all time points studied. To determine if this was the case, we evaluated diet two ways: by 3-day dietary recall and by analyzing the composition of the diet. Since the total weight of diet that the women consumed was not known, the results from the two methods cannot be compared directly. However, disagreement can be discerned if the same trends are not followed between the supplemented and unsupplemented women or between the two time points.

When evaluated in this manner, it was apparent that dietary intake varied greatly over time and, depending on the method used, varied somewhat between the supplemented and unsupplemented women as well. Protein, fat, carbohydrate, and α-tocopherol content were different between the two time points for one or both of the experimental groups depending on the type of analysis performed. Protein, fat, and α-tocopherol were significantly different between the supplemented and unsupplemented women three months after the births of their babies with the diet recalls. With the mixed diet analysis, protein, fat, and carbohydrate content were different between the two groups at the time of delivery. When the diet recalls were expressed per 1000 kilocalories, only fat intake was different between the groups three months postnatally.

When the mixed diets were collected, village doctors visited each woman and collected 1/8 of the amount of food that she ate at each meal. It is unclear why only 1/8 of the food was collected or how the 1/8 was measured. For this reason, we have decided that the diet recalls represent the most reliable measure of nutrient content. The database used to analyze the diet recalls, while unfamiliar to us, is used in China for national surveys. In addition, we prefer to use this data expressed per 1000 kilocalories because this evens out any slight differences in nutrient intake caused by a statistically insignificant difference in caloric intake between the groups.

Discussions with a Chinese colleague revealed that it is the custom of these villages to feed women high protein diets immediately after the births of their babies although total caloric intakes are relatively small. Consequently, we have elected to determine the effect of selenium supplementation at the two time points separately for all variables.

Fatty acid profiles are unavailable from diet recall data. However, as dietary fatty acids can critically influence human milk fatty acids, the data from the mixed diets were compared. The data are expressed in relative terms and, thus, not sensitive to errors made in measuring the total quantity of food. If, however, all foods were not represented appropriately when the samples were taken, these data are not reliable. Consequently, they are a 'best guess' at intake of fatty acids. The diets of the supplemented women contained a larger amount 16:0, 18:0, 18:1n-9, 20:1n-9, 20:4n-6, saturated fatty acids, and monounsaturated fatty acids. Lard is an especially good source of 16:0, 18:0,

18:1n-9, 20:4n-6, and saturated fatty acids while rapeseed oil is a good source of 20:1n-9 and monounsaturated fatty acids. It may be that the supplemented women consumed lard and rapeseed oil while the unsupplemented women consumed other types of fats more often. On the other hand, the supplemented women consumed more fat than the unsupplemented women three months after the births of their babies, making it hard to determine if only the amounts of fat consumed were different or if types of fat consumed were different too. In addition, the weight percent of fat in the diets varied form 0.09 to almost 9 wt%, a 100-fold difference. This huge range in dietary fat content would make it very hard to see a difference in milk fatty acid content between the supplemented and unsupplemented women.

It is also important, of course, that antioxidant intake be the same among all women in the study. Increased dietary α-tocopherol increases milk α-tocopherol content (Ortega et al., 1999). As α-tocopherol protects lipids from oxidation (Halliwell and Gutteridge, 1989), a difference in dietary α-tocopherol intake could have a substantial affect on milk fatty acids. Although α-tocopherol content of the diets was the same for the supplemented and unsupplemented women at each of the time points, it was higher at the time of the births of the babies. The lower plasma α-tocopherol seen in the samples obtained three months postnatal for both the supplemented and unsupplemented women was probably related to the lower intakes of α-tocopherol.

It was hypothesized that plasma lipid peroxidation would be decreased in the women who received the selenium supplement because, all other things

being equal, these women would have a greater antioxidant defense system through increased activity of glutathione peroxidase. However, this did not turn out to be the case and the women who took the supplements actually had higher TBARS levels compared to the women who did not receive the supplement at both time points. This finding may be due to the low level of specificity of the TBARS assay.

Despite the fact that it has previously been shown that selenium status influences the fatty acid profile of human milk (Dodge et al., 1999b), such is not the case with the data reported in this study. The mean milk selenium concentration of the women who received the selenium supplement reached almost 10 ng/g milk while that seen in New Zealand by Dodge et al. was about 13 ng/g. On the other hand, the mean milk GPx activity seen in this study was 44 nmoles NADPH oxidized / minute / mL while that seen in New Zealand was about 40 nmoles NADPH oxidized / minute / mL. These numbers are probably statistically equivalent. This may be unimportant, however, since Dodge et al. attributed the change in fatty acid profile they saw in New Zealand to something other than GPx activity. Dodge et al. reported a 50% increase in milk linoleic acid and a 41% increase in milk PUFA for women taking a selenium supplement while no increases were seen in this study.

In another study conducted by Dodge et al. (1999a), women from different areas of China with high, adequate, and low habitual selenium intakes were studied. The women who consumed an adequate amount of selenium had

higher milk selenium content, higher milk GPx activity, and about 150% more linoleic acid when compared to the women with habitually low selenium intake.

The reasons for the differences between this study and the ones conducted by Dodge et al. (1999a, b) are not readily apparent. In the area of New Zealand where Dodge et al. (1999b) conducted their study, average daily intake of selenium is 45 μg while that in Xichang County, China is only 11 μg. It is possible that the women in this study were not repleted enough for the supplement to affect milk fatty acids. Also, the lifestyles of New Zealand women and rural Chinese women are very different. It is difficult to determine if a comparison between the women of these two regions is appropriate.

Diet was very hard to control and diet samples were not analyzed in the study Dodge et al. (1999a) conducted in China. The differences in dietary intake seen in the present study where the subjects lived in the same area demonstrates the possibility that differences in dietary intake between Chinese women living in the different areas Dodge et al. (1999a) studied could be great. These differences in dietary intake could account for the differences Dodge et al. saw in human milk linoleic acid across regions of China. The fatty acid profiles of the oils are especially enlightening in this regard considering that soybean oil from Beijing has 3.5 times the concentration of linoleic acid as rapeseed oil from Xichang County.

Thompson and Robinson (1980) demonstrated in New Zealand women that plasma Se and GPx are highly correlated at plasma selenium levels lower than 100 ng/g. Mannan and Picciano (1987) found such a correlation in lactating

U.S. women that was absent in control (nonlactating) women. They concluded that this relationship was only present when the amount of selenium needed for milk production is not met by the diet. Since the mean plasma selenium concentration in the women supplemented with selenium in this study was about 86 ng/g three months after delivery, we can conclude that the subjects did not receive an amount of selenium adequate for milk production.

In a study published in 1993, McGuire et al. found that plasma GPx activity decreased over time in lactating U.S. women not supplemented with selenium and that this decline was prevented by 200 μg selenium daily. This suggests that adequate selenium intake prevents a decline in plasma GPx activity that might likely occur with continued lactation. Although we are unable to draw definitive conclusions at differences observed at the two time points due to dietary differences, a decline in plasma GPx activity was seen with the women who received the selenium supplement in this study and further supports the idea that the supplemented women did not receive an adequate amount of selenium to cause the differences in fatty acids observed by Dodge et al. (1999a, b).

In summary, this study suggests that the women in this study were not given enough selenium to overcome their habitually low selenium intake and protect human milk PUFA from oxidation. Future studies in China should include a longer supplementation time or a larger amount of selenium supplementation in order to replete the subjects with selenium. Thus the effect of selenium on milk fatty acids can be better studied and understood.

Conclusions

Although plasma and human milk selenium concentrations and plasma GPx activity were increased with selenium supplementation in these lactating women from Xichang County, China, the expected changes in milk fatty acids did not occur. Milk GPx activity was similar to that seen in New Zealand lactating women, but milk selenium levels were not quite as high. Further studies are needed to determine if selenium influences the polyunsaturated fatty acids in human milk. Such studies in China should start supplementation earlier than the third trimester (supplementation began during the second month of pregnancy in New Zealand) or should include a larger daily dose of selenium to overcome chronically low dietary intake. Future studies should also control diet including dietary fat and antioxidant intake in order to rule out their influences on human milk fatty acids.

When compared to the literature on the effects of selenium on milk fatty acids, this study is valuable not because it confirms what has been seen previously, but because it adds another piece to the puzzle. We know a little bit more about the selenium nutriture of Chinese women and how supplementation affects them. We also know what needs to be done in the future to study this population more effectively with possible applications to American subjects.

Literature Cited

Al M, Van Houwelingen A, Kester A, Hasaart T, De Jong A, and Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. Brit J Nutr. 1995;74:55-68.

Alaejos MS and Romero CD. Selenium in human lactation. Nutrition Reviews. 1995;53:159-166.

Alfthan G, Aro A, Arvilommi H, and Huttunen JK. Selenium metabolism and platelet glutathione peroxidase activity in healthy Finnish men: effects of selenium yeast, selenite, and selenate. Am J Clin Nutr. 1991;53:120-125.

Araund J, Blachier S, Kia D, and Favier A. Simultaneous determination of retinol, α-tocopherol and β-carotene in serum by isocratic high-performance liquid chromatography. Journal of Chromatography. 1991;572:103-16.

Avissar N, Slemmon JR, Palmer IS, and Cohen HJ. Partial sequence of human plasma glutathione peroxidase and immunologic identification of milk glutathione peroxidase as the plasma enzyme. J Nutr. 1991;121:1243-1249.

Beilstein M and Whanger PD. Distribution of selenium and glutathione peroxidase in blood fractions from human, rhesus and squirrel monkeys, rats, and sheep. J Nutr. 1983;113:2138-2146.

Bjerve KS, Brubakk AN, Fougner KJ, Johnsen H, Midthjell K, and Vik T. Omega-3 Fatty Acids: Essential fatty acids with important biological effects, and serum phospholipid fatty acids as markers of dietary omega-3-fatty acid intake. Am J Clin Nutr. 1993;57(supp):801S-6S.

Bligh EG and Dyer WJ. A rapid method of total lipid extraction and purification. Canadian J. Biochem. 1959;37:911-917.

Bray TM and Levy MA. The Role of Antioxidants in Free Radical-Mediated Diseases in Premature Infants. In: Huang YS and Sinclair AJ, eds. Lipids in Infant Nutrition. Champaign, Illinois: AOCS Press, 1998, pp. 111-121.

Brown M and Watkinson J. An automated fluorimetric method for the determination of nanogram quantities of selenium. Ana Chimica Acta. 1977;89:29-35.

Burk RF and Hill KE. Regulation of selenoproteins. Ann Rev Nutr. 1993;13:65-81.

Burk RF and Levander OA. Selenium. In: Shils ME, Olson JA, Shike M, and Ross AC, Eds. Modern Nutrition in Health and Disease, 9th Ed. Baltimore: Williams and Wilkins. 1999.

Butler JA, Beilstein MA, and Whanger PD. Influence of dietary methionine on the metabolism of selenomethionine in rats. J Nutr. 1989;119:1001-1009.

Cabre E, Periago JL, Mingorance MD, Fernandez-Banares F, Abad A, Esteve M, Gil A, Lachica M, Gonzalez-Huix F, and Gassull MA. Factors related to the plasma fatty acid profile in healthy subjects, with special reference to antioxidant micronutrient status: a multivariate analysis. Am J Clin Nutr. 1992;55:831-7.

Carlson SE and Neuringer M. Polyunsaturated fatty acid status and neurodevelopment: a summary and critical analysis of the literature. Lipids. 1999;34:171-178.

Cheruku S, Ohajunwa U, Wijendran V, Bendel R, and Lammi-Keefe CJ. Docosahexanoic acid (DHA) status of normal term breastfed infants: relationship with maternal diet and breastmilk composition. The FASEB Journal. 1999;14:A697.

Chulei R, Xiaofang L, Hongsheng M, Xiulan M, Guizheng L, Gianhong D, DeFrancesco C, and Connor W. Milk composition in women from five different regions of China: the great diversity of milk fatty acids. J Nutr. 1995;125:2993-2998.

Clandinin MT. Brain development and assessing the supply of polyunsaturated fatty acid. Lipids. 1999;34:131-137.

Cosgrove JP, Church DF, and Pryor WA. The kinetics of the autoxidation of polyunsaturated fatty acids. Lipids. 1987;22(5):299-304.

Daniels LA. Selenium metabolism and bioavailability. Biological Trace Element Research. 1996;54:185-199.

Debski B, Finley DA, Picciano MF, Lonnerdal B, and Milner J. Selenium content and glutathione peroxidase activity of milk from vegetarian and nonvegetarian women. J Nutr. 1989;119:215-220.

Debski B, Picciano MF, and Milner JA. Selenium content and distribution of human, cow and goat milk. J Nutr. 1987;117:1091-1097.

Dodge ML, Wander RC, Xia Y, Butler JA, and Whanger PD. Glutathione peroxidase activity modulates fatty acid profiles of plasma and breast milk in

Chinese women. Journal of Trace Elements in Medicine and Biology (in press). 1999a.

Dodge ML, Wander RC, Butler JA, Du SH, Thomson CD, and Whanger PD. Selenium supplementation increases the polyunsaturated fatty acid content of human breast milk. Journal of Trace Elements in Experimental Medicine. 1999b;12:37-44.

Dolamore BA, Brown J, Darlow BA, George PM, Sluis KB, and Winterbourn CC. Selenium status of Christchurch infants and the effect of diet. New Zealand Medical Journal. 1992;106:139-42.

Dylewski ML and Picciano MF. Modest selenium supplementation enhances milk selenium content in extended lactation. The FASEB Journal. 1999;13:A255.

Fernstrom JD. Effects of dietary polyunsaturated fatty acids on neuronal function. Lipids. 1999;34:161-169.

Folch J, Lees M, and Sloane-Stanley GH. A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem. 1957;226:497-509.

Halliwell B and Gutteridge JMC. Free Radicals in Biology and Medicine, 2nd Ed. Oxford: Clarendon Press. 1989

Hamosh M. Editorial: long chain polyunsaturated fatty acids in neonatal nutrition. J Am Coll Nutr. 1994;13:546-548.

Harris LE. Nutrition Research Techniques for Domestic and Wild Animals. Logan, Utah: Lorin E. Harris, 1970.

Hennig B and Watkins BA. Dietary lipid guidelines for infants and children: considerations for growth and disease risk. In: Huand YS and Sinclair AJ, eds. Lipids in Infant Nutrition. Champaign, Illinois: AOCS Press, 1998, pp. 235-251.

Insull W and Ahrens EH. The fatty acids of human milk from mothers on diets taken ab libitum. Biochem J. 1959;72:27-33.

Jensen R. The Lipids of Human Milk. Boca Raton, FL: CRC Press. 1989.

Jensen R, Ferris A, Lammi-Keefe C. Lipids in human milk and infant formulas. Ann Rev Nutr. 1992;12:417-441.

Jumpsen J and Clandinin T. Brain Development: Relationship to Dietary Lipid and Lipid Metabolism. Champaign, Illinois: AOCS Press, 1995.

Keyou G, ed. The Dietary and Nutritional Status of the Chinese Population, Vol. 1. 1992 National Survey. Beijing, China: People's Medical Publishing House. 1996.

Koletzko B, Thiel I, and Abiodun PO. The fatty acid composition of human milk in Europe and Africa. The Journal of Pediatrics. 1992;120:S62-69.

L'Abbe MR and Friel JK. Enzymes in human milk. In: Huang YS and Sinclair AJ, eds. Lipids in Infant Nutrition. Champaign, Illinois: AOCS Press, 1998, pp. 133-147.

Levander OA. A global view of human selenium nutrition. Ann Rev Nutr. 1987;7:227-50.

Levander OA, Morris VC, Moser PB. Dietary selenium (Se) intake and Se content of breast milk and plasma of lactating and non-lactating women. Fed Proc. 1981;40:890.

Levine L, ed. Progress in Allergy: Arachidonate Metabolism in Immunologic Systems. Vol. 44. New York: Karger. 1988. p. 7.

Mannan S, and Picciano M. Influence of maternal selenium status on human milk selenium concentration and glutathione peroxidase activity. Am J Clin Nutr. 1987;46:95-100.

McGuire MK, Burgert SL, Milner JA, Glass L, Kummer R, Deering R, Boucek R, and Picciano MF. Selenium status of lactating women is affected by the form of selenium consumed. Am J Clin Nutr. 1993;58:649-52.

Myers G, Wang C-S, Novelli R, Buffington B, and Spallholz JE. Selenium Yeast Studies. In: Spallholz JE, Martin JL, and Ganther HE, Eds. Selenium in Biology and Medicine. Westport, Conn.: AVI Publishing Company, Inc., 1981, pp. 531-534.

Myrvik QN. Immunology and Nutrition. In: Shils ME, Olson JA, Shike M, Eds. Modern Nutrition in Health and Disease, 8th Ed., vol. 1. Baltimore: Lea and Ferbiger, 1994, p. 654.

Neuzil J, Darlow BA, Inder TE, Sluis KB, Winterbourn CC, and Stocker R. Oxidation of parenteral lipid emulsion by ambient and phototherapy lights: potential toxicity of routine parenteral feeding. J Pediatr. 1995;126:785-790.

Nommsen LA, Lovelady CA, Heinig MJ, Lonnerdal B, and Dewey KG. Determinants of energy, protein, lipid, and lactose concentrations in human milk during the first 12 mo of lactation: the DARLING Study. Am J Clin Nutr. 1991;53:457-465.

Ortega RM, Lopex-Sobaler AM, Andres P, Martinez RM, Quintas ME, and Requejo AM. Maternal vitamin E status during the third trimester of pregnancy in Spanish women: influence on breast milk vitamin E concentration. Nutr Res. 1999;19:25-36.

Packard VS. Human Milk and Infant Formula. New York: Academic Press, 1982.

Paglia DE and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967;70:15-169.

Ramsey FL and Schafer DW. The Statistical Sleuth: A course in methods of data analysis. New York: Duxbury Press, 1997.

Sattler W, Mohr D, and Stocker R. Rapid isolation of lipoproteins and assessment of their peroxidation by high-performance liquid chromatography postcolumn chemiluminescence. Methods in Enzymology. 1994;233:469-89.

Silber GH, Hachey DL, Schanler RJ, and Garza C. Manipulation of maternal diet to alter fatty acid composition of human milk intended for premature infants. Am J Clin Nutr. 1988;47:810-814.

Smith AM, Picciano MF, Milner JA. Selenium intakes and status of human milk and formula fed infants. Am J Clin Nutr. 1981;35:521-6.

Song J and Wander RC. Effects of dietary selenium and fish oil (MaxEPA) on arachidonic acid metabolism and hemostatic function in rats. J. Nutr. 1991;121:284-292.

Sunde RA. Selenium. In: O'Dell BL and Sunde RA, eds. Handbook of Nutritionally Essential Mineral Elements. New York: Marcel Dekker, Inc., 1997.

Thompson CD and Robinson MF. Selenium in human health and disease with emphasis on those aspects peculiar to New Zealand. Am J Clin Nutr. 1980;33:303-323.

Viegas Crespo AM, Reis MA and Lanca MJ. Effect of selenium supplementation on polyunsaturated fatty acids in rats. Biological Trace Element Research. 1995;47:335-341.

Wander RC, Du SH, Ketchum SO, and Rowe KE. α-Tocopherol influences in vivo indices of lipid peroxidation in postmenopausal women given fish oil. J Nutr. 1996a;126:643-652.

Wander RC, Du SH, Ketchum SO, and Rowe KE. Effects of interaction of RRR-α-tocopheryl acetate and fish oil on low-density-lipoprotein oxidation in postmenopausal women with and without hormone-replacement therapy. Am J Clin Nutr. 1996b;63:184-93.

Whanger PD et al. Selenium metabolism studies in humans living in the United States, New Zealand, and China. In: Proc. Fourth Intern. Symp. on Uses of Se and Te, Baniff, Canada. Darein, CT: Se-Te Development Assoc. Press. 1989. p. 361.

Williams MMF. Selenium and glutathione peroxidase in mature human milk. Proc Univ Otago Med Sch. 1983;61:20-1.

Xia, Y. Academy of Preventive Medicine, Department of Food Hygiene, Beijing, China. Personal Communication. 1998, 1999.

Yamamoto Y, Brodsky MH, Baker JC, and Ames BN. Detection and characterization of lipid hydroperoxides at picomole levels by high-performance liquid chromatography. Analytical Biochemistry. 1987;160:7-13.

Yeh J-H, Gu Q-P, Beilstein MA, Forsberg NE, and Whanger PD. Selenium influences tissue levels of selenoprotein W in sheep. J Nutr. 1997;127:394-399.

Appendices

Appendix A

Laboratory Methodology

Semi-Automated Selenium Analysis for Blood, Plasma, and Animal Tissues

1. Tare a 50 mL acid washed Erlenmeyer flask for each sample. Label flasks on two sides with a 'Vis-à-vis' or other suitable water-soluble marker.
2. Add to the flask a known quantity of standard, unknown, or blank containing between 0 and 400 ng selenium. Be sure that standards will bracket the unknowns. If pipeting, calibrate the pipettes. Best accuracy is obtained by weighing samples and basing calculations on gram weight.
3. Digest the samples: use extreme caution and wear appropriate safety equipment including a lab coat, gloves, and safety glasses.

    a. Low fat samples (blood, tissues)

        1. Add 10 mL concentrated HNO_3 and 3 mL concentrated $HClO_4$ to the flask and predigest overnight (or a minimum of 4 hours) at room temperature.

        2. Digest on hot plate to "white fume" digestion and digest for 15 minutes. Remove from heat and let cool. Heat setting for hot plate is usually #4. Hood fan should be turned on as soon as the hot plate is turned on.

        3. Add 1 mL concentrated HCl and continue "white fume" digestion for 15 minutes.

        4. Remove from heat and cool.

    b. Medium fat samples (fish, diets)

        1. Add 10 mL concentrated HNO_3 to the flask and predigest

overnight (or a minimum of 4 hours) at room temperature.

2. Digest on hot plate to light brown fume. Watch carefully for foaming or spitting. Remove, cool, and examine for floating black globules. If present, they indicate a high fat sample and the sample will need to be digested using O_2 combustion methodology, therefore, stop at this point.

3. If globules are absent, add 3 mL concentrated $HClO_4$ and continue digestion to "white fumes." Digest for 15 minutes past "white fumes", add 1 mL concentrated HCl and continue digestion for additional 15 minutes.

4. Remove from heat and cool.

c. Vegetative samples are the same as low fat samples (a) except:

1. Digest for 7 minutes following "white fumes."

2. Continue "white fumes" after addition of HCl for 7 minutes.

5. Add 15 mL of 0.009 M EDTA and 2 drops of the combination indicator (cresol red and brom creasol green.)

6. Titrate to yellow with 5 N NH_4OH. Peach color has a pH of 1.2-1.5, yellow color has a pH of 2-3, and blue-green or blue has a pH > 4.5. If you overshoot during the titration, you can back-titrate with concentrated HCl. If you catch the transition from blue-green to yellow with HCl, the pH will probably be fine. However, the yellow color may not be indicative of proper pH if you overshoot in the reverse direction. Desired pH range is 2-3.

7. Weigh the flask after titration (final weight).

8. Subtract tare weights from final weights (Δ final weight).

9. Calculate standard concentrations:

$$ng/mL = (ml\ of\ std\ sol'n \times 312\ ng/mL) / \Delta\ final\ weight$$

10. Run samples through automated system.

11. Measure peak height of each standard, blank, and unknown sample from the chart.

12. Plot peak height versus standard concentrations (ng/mL) and determine the equation for the line using the least squares fitting routine on the HP calculator. Record r^2, m (or a), and b parameters.

13. Determine the solution concentration (ng/mL) for the unknowns using the above equation.

14. Calculation of selenium concentration in unknown:

$$\frac{\text{solution concentration (step 13)} \times \Delta\ \text{final weight}}{\text{weight of sample (step 3)}} = ng\ Se / g\ sample$$

15. Keep a dated and signed copy of all calculations for Dept. records.

Reference:

Brown, MW & Watkinson, JH. An Automated Fluorimetric Method for the Determination of Nanogram Quantities of Selenium. Analytica Chimica Acta 89:(1977) pp. 29-35.

Standard Glutathione Peroxidase Procedure

Preparation of Hemolysate:

A. Erythrocytes (RBC's) from fresh, whole blood

1. Add 0.25 mL whole blood to 1.5 mL cold 0.9% NaCl in graduated conical tip centrifuge tubes.
2. Centrifuge approximately 1 minute in clinical centrifuge (1250 x g) and discard supernatant.
3. Dilute pellet (packed RBC's) to 1.5 mL with doubly distilled water (DDH_2O). Mix well.
4. Discard all but 0.5 mL.
5. Add 0.5 mL Drabkins solution.
6. Dilute to 7 mL with DDH_2O. This is the hemolysate.

B. RBC lysate from previously frozen RBC's.

1. Pipette 0.25 mL of thawed, well mixed RBC's into conical centrifuge tubes.
2. Dilute to 3 mL with DDH_2O. Mix well.
3. Follow steps 4-6 above.

Note: If sample is small, use 0.125 mL RBC and dilute to 1.5 mL with DDH_2O.

C. Whole blood lysate from fresh or frozen (thawed) whole blood

1. Pipette 0.25 mL whole blood into conical centrifuge tubes.
2. Dilute to 1.5 mL with DDH_2O. Mix well.

3. Follow steps 4-6 above.

Note: Under ideal conditions, lysates should be assayed within 15-30 minutes after preparation. Frozen samples should be prepared immediately after thawing. This prevents possible unwanted and uncontrolled activation.

Assay of hemoglobin (RBC and Whole Blood samples)

Read absorbance of prepared lysate at 540 nm against DDH_2O blank. Lysate with Drabkins solution added should be stable for 4-6 hours at least if kept cool.

Assay Sequence:

1. Adjust wavelength of recording spectrophotometer to 340 nm. Adjust recorder span from 0 to 1.0 absorbance units.
2. Add 0.1 mL hemolysate, blank, or other sample to 1 mL quartz cuvette.
3. Add 0.8 mL reaction mixture.
4. Add 0.1 H_2O_2, t-butyl, or Cumene solution.
5. cover cuvettes with parafilm, mix by inversion three times.
6. Place cuvettes in spectrophotometer. Read at least 3 minutes for H_2O_2 and at least 5 minutes for t-butyl and Cumene (there is usually a lag phase for the organic hydroperoxides).
7. IMPORTANT: if the change in absorbance at 340 nm is greater than 0.120 per minute, the hemolysate should be diluted.

Calculation for nmoles NADPH oxidized per minute per mL hemolysate:

$$\frac{\Delta A_{340}\text{ (sample)} - \Delta A_{340}\text{ (blank)}}{\text{aliquot of sample (mL) x 1607.7}} = \text{nmol NADPH oxidized / min / mL sample}$$

Procedure for Human Plasma Fatty Acid Profile

Extraction:

1. Get PC di(17:0) standard out of freezer and briefly warm in hands until not cloudy.
2. Vortex standard for 5 seconds.
3. Add 5 μL of standard to a test tube with a screw top.
4. Dry standard on evaporator under N_2 (this should only take a few minutes).
5. Vortex plasma sample for 5 seconds.
6. Add 300 μL of sample to tube with dried standard.
7. Add 700 μL of distilled water to the tube.
8. Add 3.75 (3.8 is close enough) mL of 2:1 methanol:chloroform mixture (with 10-100 mg BHT).
9. Vortex for 5 seconds.
10. Cap tubes and shake for 1 hour on rocker
11. Centrifuge for 10 minutes at 3000 rpm and 4°C with brake on "high".
12. Transfer the supernatant into a larger tube (still with screw top).
13. Extract the residue left in tube 1 with 1 mL distilled water and 3.75 mL methanol:chloroform mixture.
14. Centrifuge again for 10 minutes.
15. Transfer the resulting supernatant into tube 2 with the first portion of supernatant.
16. Add 2.5 mL distilled water and 2.5 mL chloroform to the supernatant in

second tube. Vortex.

17. Centrifuge for 10 minutes.
18. There will be three layers. Aspirate off the first layer.
19. Quickly insert pipette through middle layer and remove bottom portion into another tube. This is a good place to stop and store samples.

Methylation (for milk also):

20. Add 5 μL of methyl 21:0 standard and dry sample under N_2.
21. Add 1 mL boron trichloride (or trifluoride) and 0.2 mL of benzene to each test

    tube. Vortex for 5 seconds.
22. Put the cap on and cover with Teflon tape.
23. Heat at 95°C in heat block for 90 minutes checking initially that gas is not escaping from the tubes.
24. Cool tubes to room temperature.
25. Add 3 mL hexane and 3 mL distilled water.
26. Vortex for 5 seconds.
27. Centrifuge for 10 minutes.
28. Transfer the upper hexane layer to another test tube using a transfer pipette.
29. Add 2 mL hexane to the first tube
30. Vortex for 5 seconds.
31. Centrifuge for 10 minutes.
32. Transfer upper hexane layer to tube 2 with other hexane layer.

33. Dry sample under N_2 (this will take about 15 minutes).
34. Resuspend in 100 μL of iso-octane. Roll liquid around in tube to dissolve all fatty acids. This is a good place to stop and store samples.
35. Inject 2 μL of sample into the GC and save the rest of the sample in case you need to run another GC.

Fatty Acid Extraction of Human Milk

1. Put 200 μL of 17:0 standard into test tubes large enough to hold over 20 mL of liquid.
2. Dry standard using evaporator.
3. Thaw frozen human milk samples in a warm water bath.
4. Vortex samples and withdraw 2 mL and put into test tubes with dried standard.
5. Add 14 mL of 1:1 methanol –chloroform with BHT added.
6. Vortex.
7. Homogenize using a Polytron set on setting #7 for 30 seconds.
8. Add 4 ml distilled water.
9. Homogenize 30 seconds.
10. Clean Polytron with 20 mL of chloroform for 10 seconds.
11. Wipe Polytron dry with Kim Wipe.
12. Centrifuge tubes for 15 minutes (with big centrifuge in Rm. 6 set on setting 27).
13. Carefully aspirate off top layer.
14. Punch through milky layer and withdraw bottom, chloroform layer. Put chloroform layer into small test tube.
15. To methylate, add 100 μL methyl 21:0 standard.
16. Dry samples on evaporator and proceed with methylation as for plasma samples.

Notes:

Concentration of 17:0 standard is 9.604 mg/mL.

Concentration of 21:0 standard is 13.86 mg/mL.

Concentration of BHT in chloroform-methanol is 71 mg/mL.

Getting some of the milky layer into the sample doesn't seem to make a difference in the chromatogram.

Using the GC for Fatty Acid Profiles

The GC uses helium as the mobile phase. Never turn the He off, just turn it down at the end of the day. The air and hydrogen tanks can be turned completely off at the end of the day.

Turning on the GC:

1. Turn on the He to about 40 psi.
2. Turn on the auxiliary gas detector in the lower right corner of the panel on the top left of the GC.
3. Turn on the air and the H_2.
4. Turn on the computer.
5. ⇒Specify method. "?" lists all methods. For plasma, use method 3.M which goes down to 13:0.
6. Select item.
7. Load method.
8. ⇒Edit datafile.
9. ⇒Change "raw data" to new sample name.
10. ⇒Change "integration" to same name as sample name above.
11. ⇒Quit.
12. ⇒Run method.
13. Hit "FID ignitor".

Injecting the sample:

1. Vortex sample.
2. Draw up 2 μL into the syringe with no bubbles. Wipe the outside of the syringe before injecting it into the GC.
3. Inject into the first column and hit "start" immediately.
4. Wash the syringe with iso-octane about 5 times in between samples and 20 times at the end of the day.

Turning off the GC:

1. Press "oven temperature".
2. Then "100", and "enter". This will lower the oven temperature to 100 ° and must be done before turning off the gases.
3. Turn off the air and H_2.
4. Turn off the computer.
5. Lower the pressure of the He to about 18 psi. Turn the lever about 2 turns and wait to see where the pressure is. It takes awhile for the pressure to decrease.
6. Turn auxiliary gas detector knob to off.

General Notes:

Push "FF" on the printer to advance the paper. Pushing it once will put the paper in the right position to continue printing the next day. Pushing it twice

allows you to tear off the pages you have printed. For consecutive runs, there is no need to push "FF".

"⇒" means that you have to repeat this step on the computer for each separate run.

Plasma TBARS

1. Put 100 μL of plasma into a tube with a cap.
2. Add 4 mL N/12 sulfuric acid in the tube and vortex.
3. Add 0.5 mL 10% phosphotungstic acid ($H_3[P(W_3O_{10})_4]\cdot xH_2O$) and vortex.
4. Let stand at room temperature for 5 minutes.
5. Centrifuge at 3000 rpm for 10 minutes.
6. Discard the supernatant. Add 2 mL N/12 sulfuric acid and 0.3 mL of 10% phosphotungstic acid. Vortex.
7. Centrifuge at 3000 rpm for 10 minutes.
8. Discard the supernatant. The sediment is suspended in 4 mL distilled water and 1 mL TBA reagent. Vortex.
9. Heat the mixture for 60 minutes at 95°C in a water bath.
10. After cooling with tap water, add 4 mL butanol. Vortex for 15 seconds.
11. Centrifuge for 15 minutes at 3000 rpm.
12. Transfer the upper butanol layer into a 4-sided plastic cuvette and measure on fluorimetric spectrometer at 553 nm with 515 nm excitation.
13. Standard curve: use 1×10^{-5} M TEP (tetraethoxypropane) solution (in N/12 sulfuric acid) as working standard solution. Make a 6 point curve using the following volumes of TEP: 0, 10, 15, 20, 25, 30 μL. Be sure to wipe the tip before adding TEP. For each standard tube, add 4 mL distilled water and 1 mL TBA reagent. Then follow steps 9-12.

Notes:

The elimination of TBA-reacting substances other than lipid peroxides is necessary for the measurement of lipid peroxides in plasma. One of the best procedures isolates lipids by precipitating them along with plasma protein with the phosphotungstic acid-sulfuric acid system. This procedure removes water-soluble substances which may react with TBA. Perform all steps after removal from the water bath quickly as product is time sensitive.

Calculation: nmol TBARS/mL plasma = A/10, where A is the data collected from the standard curve.

Reference:

Kunio Yagi: In Methods of Enzymology, Vol. 105, pp. 328-331.

Using the Fluorimetric Spectrometer:

1. Log use in the logbook.
2. Turn on switch at back of machine. The machine does not need to be warmed up.
3. Set excitation and emission numbers. For example, press "ex", 515, and "goto" to set excitation to 515. Set emission to 553.
4. Quickly put each cuvette into the spectrometer and record the reading when it has stabilized a little.
5. Run a standard curve each day. The reading for the standard solution containing no TEP is the blank and each reading should be corrected using this reading.

When computing the standard curve, only use data if $r \geq .99$

HPLC Vitamin E Assay for Plasma

1. Place 200 μL plasma into a 12x75 mm Borosilicate Disposable Culture tube.
2. Add 200 μL ethanol.
3. Vortex 30 seconds. (Ethanol precipitates the protein.)
4. Add 500 μL hexane and vortex 3 minutes on the multi-vortexer or 1 minute each, three times, using a single vortexer.
5. Centrifuge at 3000 rpm at 4°C for 15 minutes.
6. Take 250 μL from the upper phase using a displacement pipette and put it into another tube.
7. Dry contents of second tube under N_2 at 55°C.
8. Add 500 μL methanol to each tube.
9. Vortex 30 seconds and transfer into injection vial.

Note: All procedures must be done avoiding bright light. A yellow light such as a 100W Sylvania Bugs Poiler may be used in a relatively dark room. Use external standards. For human plasma, about 6 μg/mL is a good concentration for α-tocopherol and about 3 μg/mL is good for γ-tocopherol.

HPLC Conditions:

Detector Control:

File 6,	detector A (RF)
EX wav	292 nm
EM wav	330 nm
Range	1
Resp.	2
Gain	1
Sens	2
Specty	2
Exstrl	300 nm
Exstop	400 nm
EMstrt	350
EMstop	450
Sc. Spd	2
PLT spd	1

Other conditions:

Run time	10 min.
Oven temp.	35°C
Mobile phase	methanol
Flow rate	1.5 mL/min
Inject volume	20 μL
Detector	Fluorimetric
Pump pressure	117-124

Inject control parameters:

Rack select	small
Syringe volume	500 μL
Needle stroke	39 mm
Rinse volume	500 μL
Rinsing syringe speed	150 μL/s
Sampling syringe speed	10 μL
Excess volume	10 μL
Kvol	1

Analysis parameters of integrator:

Width	10	Slope	500
Drift	50	Min. Area	200
T. DBL	99	Stop.TM	9
Atten	2	Speed	5
Method$	2021	Format$	0001
Spl. Wt	100	IS.Wt	1
Window	5		

References:

1. J. Araund et al. Journal of Chromatography, 572 (1991), 103-116.

2. Sharon Ketchum's procedure.

Using the HPLC for Vitamin E Assay

1. Turn controller on
2. Turn on both pumps
3. Turn oven on
4. To purge system, take syringe off of (yellow) tube and put tube into waste bottle
5. Open drain valve
6. Push "purge"
7. Purge for about 1 minute
8. To stop purging, push "purge" again and close drain valve
9. Press "activate" (act) to get mobile phase moving – pressure should increase (pressure over 140 @ 35°C is bad and filter may be plugged)
10. Turn on detector
11. Push "monitor" to turn on display of integrator
12. Push "LLIST", "PARA", and return arrow which will print out parameters
13. Check to see that parameters are same as those already listed (print parameters out again at the end of using the HPLC – if power goes off or integrator is turned off, parameters are lost)
14. Press "A.SAVE" and the number of samples you plan to run, ex. "1,30", return arrow – will save data from assay 1 through 30 for the day
15. Press "zero" on integrator and on detector
16. To run the HPLC, press "menu"

17. Auto injector #5
18. Put in #'s that your samples are in, ex. If samples are in slots 10 through 15, push "10 enter, 15 enter"; NOTE: running duplicates is suggested over injecting twice from one sample.
19. 1 injection
20. 20 μL volume
21. Run time 10 minutes
22. Pret. File # = 0
23. Fract. File # = "-"
24. Zero detector
25. Press "run"
26. Note: in an emergency, hit "run" again to stop the run
27. To shut down the HPLC, press "act" to lower pressure
28. At about pressure 1, turn controller off
29. Then turn off pumps, oven, detector
30. "Esc" on integrator turns to monitor off

Using the UV/Spec

1. Turn on two switches
2. Push "auto-zero" which will blank
3. Push "step" and go to #2
4. Press "start" to record data
5. Push "step" until it goes to #1
6. Press "return"
7. Y
8. Change parameter 1
9. Change parameter 5 (wavelength)
10. Auto zero
11. When done, press "return"
12. Then, "mode"
13. Turn off 2 switches

Calculations for Determination of Concentration Using UV/Spec

1. Absorbance = A x PL x C where Absorbance = number given by UV/Spec
   A = absorptivity and is different depending on sample
   PL = path length and is 1 cm for quartz cuvettes
   C = concentration and is the variable to solve for
2. Abs ÷ A = C

Ex. 0.2 ÷ 75.8 = 0.002638 = 26.38 μg/mL

According to NIST:

1. absorptivity for α-tocopherol = 75.8 dL/g•cm, lambda max. = 292
2. absorptivity for δ-tocopherol = 91.2 dL/g•cm, lambda max. = 297 (don't often use)
3. absorptivity for γ-tocopherol = 91.4 dL/g•cm, lambda max. = 298

Useful information:

1. for people, an α-tocopherol standard concentration of about 6 μg/mL is good – at step (D) in protocol, concentration is about 12.5 μg/mL – measure at this step and then carefully dilute further because UV/Spec is not sensitive enough to detect 6 μg/mL very well - UV detects to about 0.1 absorbance well

for people, a γ-tocopherol standard concentration of about 3 μg/mL is good – at step (D) in protocol, concentration is about 13 μg/mL – measure at this step

Calculations for Vitamin E (Plasma) HPLC Method

1. Know the concentration of the standards as figured out by UV/Spec
2. (Sample area x standard concentration) ÷ standard area = sample concentration (usually in μg/mL)

Ex. (2534 x 11.54 μg/mL) ÷ 25964 = sample concentration

(Areas are determined by HPLC integrator.)

3. Then multiply by dilution factors; in original plasma procedure, 500 μg of hexane is added and only half is used for the assay, therefore, multiply by 2; also, 500 μL of methanol is added as the last step and only 200 μL of plasma was used initially, therefore, multiply by 500/200 or 5/2 or 2.5.

Ex. (2534 x 11.54 μg/mL) ÷ 25964 x 2 x 2.5 = 5.63 μg/mL

TLC Procedure for Human Plasma Phospholipid Fatty Acid Profile

Extraction:

1. Get PC di(17:0) standard out of freezer and briefly warm in hands until not cloudy.
2. Vortex standard for 5 seconds.
3. Add 15 μL of standard to a test tube with a screw top.
4. Dry standard on evaporator under N_2 (this should only take a few minutes).
5. Vortex plasma sample for 5 seconds.
6. Add 300 μL of sample to tube with dried standard.
7. Add 700 μL of distilled water to the tube.
8. Add 3.75 (3.8 is close enough) Ml of 2:1 methanol:chloroform mixture (with 10-100 mg BHT).
9. Vortex for 5 seconds.
10. Cap tubes and shake for 1 hour on rocker
11. Centrifuge for 10 minutes at 3000 rpm and 4°C with brake on "high".
12. Transfer the supernatant into a larger tube (still with screw top).
13. Extract the residue left in tube 1 with 1 Ml distilled water and 3.75 Ml methanol:chloroform mixture.
14. Centrifuge again for 10 minutes.
15. Transfer the resulting supernatant into tube 2 with the first portion of supernatant.
16. Add 2.5 mL distilled water and 2.5 mL chloroform to the supernatant in tube 2.

17. Centrifuge for 10 minutes.
18. There will be three layers. Aspirate off the first layer.
19. Quickly insert pipette through middle layer and remove bottom portion into another tube.
20. Dry on evaporator (this will take about 15 minutes).
21. Resuspend in 0.3 Ml of chloroform. Roll the liquid around in the tube to redissolve the fatty acids.
22. Transfer solution into a 2 mL microcentrifuge tube. This is a good place to stop and store samples.
23. Dry sample and resuspend in 50 μl of chloroform.

TLC:

24. Wash the plate in 1:1 methanol:chloroform (55 mL of each) for 2 hours. Washing solution should be made fresh each week.
25. Activate the plate in a 110°C oven for 1 hour right before using.
26. Lightly score the plate at the origin and at the solvent front. Mark the areas where the samples are to be applied leaving at least 1.5 scale between each sample.
27. Apply 10 μL of PC standard to each side of the plate in a spot, one drop at a time, letting the first drop dry before adding the second.
28. Apply 50 μL of sample to the plate in a row of dots along the origin, rinsing the syringe in between each standard and sample about 5 times with chloroform. Rinse the syringe about 10 times at end of use. The plate will hold 3 samples and 2 standards.

29. Develop the plate in 15% diethylether and 85% hexane (18 mL diethylether and 102 mL hexane) for 35 minutes or until solvent reaches solvent front line. Developing solution must be made fresh each day.
30. Blow plate dry with N_2 to prevent lipid oxidation.
31. Put plate in the I_2 jar for 2-3 minutes until spots are clearly visible.
32. Using clean aluminum foil as a "drop cloth", scrape individual samples off the plate and onto a piece of weighing paper using a clean razor blade.
33. Carefully pour into a test tube.
34. Add 5 μL of methyl 21:0 standard to each tube.

Methylation:

35. Add 1 mL boron trichloride (or trifluoride) and 0.2 mL of benzene to each test tube. Vortex for 5 seconds.
36. Put the cap on and cover with Teflon tape.
37. Heat at 95°C in heat block for 90 minutes checking initially that gas is not escaping from the tubes.
38. Cool tubes to room temperature.
39. Add 3 mL hexane and 3 mL distilled water.
40. Vortex for 5 seconds.
41. Centrifuge for 10 minutes.
42. Transfer the upper hexane layer to another test tube using a Pasteur pipette.
43. Add 2 mL hexane to the first tube
44. Vortex for 5 seconds.

45. Centrifuge for 10 minutes.
46. Transfer upper hexane layer to tube 2 with other hexane layer.
47. Dry sample under N_2 (this will take about 15 minutes).
48. Resuspend in 100 μL of iso-octane. Roll liquid around in tube to dissolve all fatty acids. This is a good place to stop and store samples.

    Inject 2 μL of sample into the GC and save the rest of the sample in case you need to run another GC.

Appendix B

Raw Data

Table 1: The Effect of Supplementation on Variables in Plasma at the Time of Delivery and Three Months after Delivery[1]

	0 Months		3 Months	
Variable	-Se group	+Se group	- Se group	+Se group
Se (ng/g plasma)	34.7 ± 5.9^a	100 ± 5.9^b	28 ± 4.2^a	86.1 ± 4.2^b
GPX Activity[2]	175 ± 26^a	430 ± 26^b	175 ± 15^a	293 ± 15^b
TBARS[3]	1.2 ± 0.1^a	1.7 ± 0.2^b	0.73 ± 0.1^a	1.2 ± 0.1^b
Vitamin E[4]	10.3 ± 0.8	8.6 ± 0.8	4.6 ± 0.5	4.7 ± 0.5

[1]Values within each time group with different superscripts are significantly different ($P < 0.05$). Values are means ± SEM calculated using Student's t-tests.
[2]GPX activity is measured in units of nmoles NADPH oxidized per minute per mL of sample.
[3]TBARS is measured in units of nmol TBARS/mL plasma.
[4]Vitamin E is measured in units of μg/mL plasma.

Table 2: The Effect of Supplementation on Variables in Human Milk at the Time of Delivery and Three Months after Delivery[1]

	0 Months		3 Months	
Variable	-Se group	+Se group	-Se group	+Se group
Se (ng/ g milk)	7.4 ± 0.9^a	16.7 ± 0.9^b	5.8 ± 0.6^a	9.9 ± 0.6^b
GPX Activity[2]	56 ± 6	69 ± 6	37 ± 4	44 ± 4

[1]Values within each time group with different superscripts are significantly different ($P < 0.05$). Values are means ± SEM calculated using Student's t-tests.
[2]GPX activity is measured in units of nmoles NADPH oxidized per minute per mL of sample.

Definitions

I = initial time point = birth of babies

F = final time point = three months postpartum

0 before subject number = subject received selenium supplement

1 after subject number = initial time point = birth of babies

4 after subject number = final time point = three months postpartum

Ages, Delivery Dates, and Sample Collection Dates

Supplemented Group

No.	Age	Tablets start	Delivery	Sampling (Milk and Blood)					
				wk 0	wk 1	wk 2	wk 3	wk 4	wk 12
1	23	9/26/97	11/10/97	11/17/97	11/24/97	12/1/97	12/8/97	12/15/97	2/5/98
2	20	9/26/97	11/12/97	11/18/97	11/25/97	12/2/97	12/9/97	12/16/97	2/16/98
3	27	9/26/97	11/25/97	12/2/97	12/9/97	12/16/97	12/23/97	12/30/97	3/28/98
4	20	10/5/97	12/3/97	12/9/97	12/16/97	12/23/97	12/30/97	1/6/98	3/6/98
5	22	9/26/97	12/24/97	12/31/97	1/7/98	1/14/98	1/21/98	1/28/98	3/28/98
6	26	9/26/97	1/1/98	1/8/98	1/15/98	1/22/98	1/29/98	2/5/98	4/5/98
7	31	9/27/97	1/12/98	1/19/98	1/25/98	2/2/98	2/9/98	2/16/98	4/16/98
8	26	10/8/97	1/19/98	1/26/98	2/2/98	2/9/98	2/16/98	2/23/98	4/23/98
9	21	10/8/97	2/3/98	2/10/98	2/17/98	2/25/98	3/3/98	3/10/98	5/8/98
10	23	10/19/97	2/4/98	2/11/98	2/18/98	2/25/98	3/2/98	3/19/98	5/8/98
11	28	10/19/97	3/11/98	3/17/98	3/24/98	3/31/98	4/7/98	4/14/98	6/9/98

Control Group

No.	Age	Tablets start	Delivery	Sampling (Milk and Blood)					
				wk 0	wk 1	wk 2	wk 3	wk 4	wk 12
1	22	9/26/97	11/11/97	11/17/97	11/24/97	12/1/97	12/8/97	12/15/97	2/5/98
2	21	9/26/97	12/2/97	12/8/97	12/15/97	12/22/97	12/29/97	1/5/98	3/5/98
3	23	9/26/97	12/7/97	12/15/97	12/22/97	12/29/97	1/5/98	1/12/98	3/12/98
4	25	9/26/97	12/8/97	12/15/97	12/22/97	12/29/97	1/5/98	1/12/98	3/12/98
5	20	9/26/97	12/17/97	12/22/97	12/29/97	1/5/98	1/12/98	1/19/98	3/19/98
6	23	10/5/97	12/28/97	1/3/98	1/9/98	1/16/98	1/23/98	1/30/98	3/30/98
7	23	10/5/97	1/5/98	1/9/98	1/16/98	1/23/98	1/30/98	2/6/98	4/6/98
8	22	10/5/97	1/7/98	1/12/98	1/19/98	1/26/98	2/2/98	2/9/98	4/9/98
9	28	10/19/97	3/9/98	3/9/98	3/16/98	3/23/98	3/30/98	4/5/98	6/5/98
10	23	10/19/97	3/20/98	3/23/98	3/30/98	4/6/98	4/13/98	4/20/98	6/9/98

Mixed Diet Data as % of Dry Matter

subject	% dry matter I	% dry matter F	% moisture I	% moisture F	% crude pro I	% crude pro F	% ash I	% ash F	% fat I	% fat F	% CHO I
0-1	28.05	28.80	71.95	71.20	25.60	11.90	1.89	3.89	31.20	2.35	41.31
0-2	26.10	30.87	73.90	69.13	22.60	9.40	2.84	3.80	11.01	10.54	63.55
0-3	25.67	26.29	74.33	73.71	27.70	12.40	2.29	2.82	15.14	4.30	54.87
0-5	27.03	26.87	72.97	73.13	25.40	12.10	2.42	3.16	10.77	12.59	61.41
0-6	27.03	27.90	72.97	72.10	23.40	11.20	1.21	2.42	13.57	7.83	61.82
0-7	25.59	24.85	74.41	75.15	27.30	21.90	4.20	4.88	21.54	14.79	46.96
0-8	28.17	22.42	71.84	77.58	19.30	16.10	0.97	2.36	5.45	21.47	74.28
0-9	30.96	25.87	69.04	74.13	25.50	15.60	2.77	3.27	20.07	26.18	51.66
0-10	23.97	28.93	76.03	71.07	26.60	14.70	4.44	3.05	11.97	20.72	56.99
0-11	23.98	33.69	76.02	66.31	23.90	11.50	1.51	1.04	10.61	4.32	63.98
ave	26.66	27.65	73.35	72.35	24.73	13.68	2.45	3.07	15.13	12.51	57.68
STD	2.10	3.17	2.10	3.17	2.52	3.57	1.17	1.03	7.35	8.19	9.48
1	26.23	25.52	73.77	74.48	33.50	14.30	2.29	2.36	13.01	22.24	51.20
2	17.64	21.62	82.36	78.38	43.40	9.80	1.55	4.40	11.36	1.17	43.69
3	17.56	30.14	82.44	69.86	35.50	12.10	2.35	3.30	11.95	8.94	50.20
4	16.48	26.54	83.52	73.46	31.60	11.50	3.16	1.14	11.44	9.21	53.80
5	20.98	17.64	79.02	82.36	40.90	16.10	3.02	4.51	14.39	29.97	41.69
6	23.08	30.00	76.92	70.00	34.60	12.80	1.71	1.33	8.62	7.06	55.07
7	26.22	20.03	73.78	79.97	38.80	16.30	1.46	2.05	10.53	5.44	49.21
8	26.04	24.54	73.96	75.46	32.90	11.20	2.43	1.46	8.11	6.36	56.56
9	19.55	26.95	80.45	73.05	37.50	13.80	2.77	1.97	12.15	1.36	47.58
10	22.25	30.75	77.75	69.25	35.20	11.30	1.67	2.99	7.52	0.31	55.61
ave	21.60	25.37	78.40	74.63	36.39	12.92	2.24	2.55	10.91	9.21	50.46
STD	3.77	4.47	3.77	4.47	3.73	2.17	0.62	1.22	2.22	9.62	5.05

% CHO F	g. pro/g food I	g. pro/g food F	g fat/g food I	g fat/g food F	g CHO/g food I	g CHO/g food F
81.86	0.07	0.03	0.09	0.01	0.12	0.24
76.26	0.06	0.03	0.03	0.03	0.17	0.24
80.48	0.07	0.03	0.04	0.01	0.14	0.21
72.15	0.07	0.03	0.03	0.03	0.17	0.19
78.55	0.06	0.03	0.04	0.02	0.17	0.22
58.43	0.07	0.05	0.06	0.04	0.12	0.15
60.07	0.05	0.04	0.02	0.05	0.21	0.13
54.95	0.08	0.04	0.06	0.07	0.16	0.14
61.53	0.06	0.04	0.03	0.06	0.14	0.18
83.14	0.06	0.04	0.03	0.01	0.15	0.28
70.74	0.07	0.04	0.04	0.03	0.15	0.20
10.88	0.01	0.01	0.02	0.02	0.03	0.05
61.10	0.09	0.04	0.03	0.06	0.13	0.16
84.63	0.08	0.02	0.02	0.00	0.08	0.18
75.66	0.06	0.04	0.02	0.03	0.09	0.23
78.15	0.05	0.03	0.02	0.02	0.09	0.21
49.42	0.09	0.03	0.03	0.05	0.09	0.09
78.81	0.08	0.04	0.02	0.02	0.13	0.24
76.21	0.10	0.03	0.03	0.01	0.13	0.15
80.98	0.09	0.03	0.02	0.02	0.15	0.20
82.87	0.07	0.04	0.02	0.00	0.09	0.22
85.40	0.08	0.03	0.02	0.00	0.12	0.26
75.32	0.08	0.03	0.02	0.02	0.11	0.19
11.41	0.01	0.01	0.01	0.02	0.03	0.05

Fatty acid profile of Chinese diets (mg/g)

	12:0	14:0	15:0	16:0	16:1n7	16:4n1	18:0	18:1n9t	18:1n9c	18:1n7
1	0.00	0.64	0.00	12.90	1.00	0.00	6.79	0.29	19.43	1.22
2	0.00	0.03	0.00	0.61	0.00	0.00	0.05	0.00	0.36	0.03
3	0.00	0.33	0.00	6.13	0.45	0.00	2.20	0.34	7.04	0.58
4	0.00	0.31	0.00	5.62	0.60	0.00	1.96	0.00	8.57	0.77
5	0.04	0.64	0.04	11.54	1.17	0.00	7.07	0.63	18.07	1.04
6	0.00	0.24	0.00	4.69	0.43	0.00	1.70	0.05	6.81	0.52
7	0.00	0.05	0.00	1.77	0.19	0.00	0.52	0.00	2.76	0.13
8	0.00	0.22	0.00	4.00	0.38	0.00	1.39	0.00	5.86	0.47
9	0.00	0.04	0.00	1.36	0.07	0.00	0.28	0.00	1.28	0.08
10	0.00	0.05	0.00	1.27	0.02	0.00	0.15	0.00	0.91	0.05
0-1	0.00	0.19	0.00	3.88	0.31	0.03	1.71	0.05	5.80	0.42
0-2	0.03	0.62	0.02	10.89	0.83	0.11	6.38	0.13	15.83	1.02
0-3	0.00	0.37	0.00	6.80	0.46	0.06	3.70	0.07	9.21	0.63
0-4	0.03	0.44	0.02	7.85	0.89	0.14	3.50	0.12	14.06	1.18
0-5	0.04	0.62	0.02	11.08	0.92	0.12	5.98	0.13	16.10	1.11
0-6	0.03	0.44	0.02	8.21	0.66	0.10	4.22	0.10	12.32	0.80
0-7	0.03	0.43	0.04	7.23	1.21	0.17	2.84	0.14	13.78	1.48
0-8	0.04	0.70	0.02	12.32	1.61	0.18	5.63	0.18	21.14	1.94
0-9	0.04	0.68	0.02	13.23	1.05	0.15	7.74	0.21	27.24	1.44
0-10	0.04	0.68	0.04	12.59	1.45	0.16	7.69	0.61	23.44	1.41
0-11	0.00	0.28	0.00	5.97	0.44	0.07	2.92	0.07	10.27	0.62

18:2n6t	18:2n6c	20:0	18:3n3c	20:1n9	18:4n3	21:0	20:2n6	20:3n9	20:3n6	22:0
0.07	6.42	0.13	1.01	0.56	0.00	0.07	0.34	0.00	0.00	0.00
0.00	0.62	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.02	0.00
0.11	3.97	0.05	0.38	0.18	0.00	0.05	1.91	0.14	0.00	0.00
0.00	4.00	0.00	0.43	0.21	0.00	0.00	0.15	0.00	0.00	0.00
0.10	5.82	0.14	0.95	0.69	0.20	0.12	0.51	0.00	0.11	0.00
0.00	3.85	0.04	0.59	0.15	0.00	0.00	0.13	0.00	0.00	0.00
0.00	0.87	0.06	0.04	0.06	0.00	0.00	0.00	0.00	0.00	0.05
0.00	2.56	0.00	0.34	0.14	0.00	0.00	0.10	0.00	0.00	0.00
0.00	1.57	0.00	0.12	0.02	0.00	0.00	0.02	0.00	0.00	0.00
0.00	1.66	0.01	0.18	0.01	0.00	0.00	0.02	0.00	0.01	0.00
0.00	2.42	0.04	0.36	0.14	0.00	0.02	0.08	0.00	0.00	0.00
0.04	4.08	0.12	0.56	0.45	0.00	0.05	0.25	0.00	0.04	0.00
0.00	3.78	0.08	0.51	0.24	0.00	0.03	0.13	0.00	0.00	0.00
0.00	3.57	0.08	0.61	0.39	0.00	0.05	0.18	0.00	0.03	0.00
0.05	5.32	0.12	0.82	0.40	0.00	0.05	0.26	0.00	0.04	0.02
0.03	4.36	0.09	0.67	0.32	0.00	0.04	0.19	0.00	0.03	0.02
0.02	1.74	0.08	0.61	0.42	0.00	0.04	0.13	0.00	0.03	0.00
0.03	2.19	0.15	0.77	0.62	0.00	0.05	0.20	0.00	0.00	0.00
0.05	6.94	0.19	0.92	1.10	0.00	0.12	0.72	0.02	0.06	0.03
0.06	7.33	0.18	1.00	1.11	0.26	0.20	0.69	0.00	0.13	0.04
0.00	3.56	0.08	0.32	0.37	0.00	0.06	0.19	0.00	0.00	0.00

20:4n6	22:1n9	20:5n3c	24:0	22:4n6	24:1	22:5n3	22:6n3	Others	Sum	SFA
0.21	0.43	0.00	0.00	0.00	0.00	0.00	0.00	0.44	51.95	20.53
0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.86	0.68
0.08	0.00	0.05	0.00	0.00	0.00	0.00	0.00	1.12	25.12	8.77
0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	22.76	7.88
0.21	0.39	0.06	0.07	0.00	0.00	0.06	0.51	2.82	52.94	19.66
0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	19.29	6.67
0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.05	6.70	2.44
0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	15.50	5.61
0.05	0.02	0.00	0.00	0.00	0.00	0.00	0.02	0.04	4.97	1.68
0.01	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.02	4.41	1.49
0.07	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	15.57	5.84
0.16	0.03	0.00	0.00	0.02	0.00	0.04	0.02	0.35	42.03	18.11
0.08	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.09	26.27	10.98
0.17	0.03	0.00	0.00	0.00	0.00	0.05	0.00	0.28	33.64	11.97
0.18	0.04	0.00	0.00	0.02	0.00	0.05	0.03	0.36	43.84	17.93
0.15	0.03	0.00	0.02	0.02	0.02	0.04	0.04	0.26	33.19	13.08
0.21	0.03	0.06	0.00	0.02	0.04	0.08	0.05	0.42	31.30	10.68
0.18	0.02	0.02	0.00	0.00	0.03	0.07	0.00	0.59	48.63	18.91
0.18	0.20	0.03	0.00	0.05	0.02	0.07	0.05	1.17	63.71	22.05
0.24	0.24	0.06	0.00	0.03	0.00	0.09	0.62	4.05	64.40	21.45
0.11	0.09	0.00	0.00	0.00	0.00	0.03	0.00	0.22	25.66	9.31

MUFA	PUFA	n6	n3	n6/n3	PI	UI	17:0	Sample
22.93	8.05	7.03	1.01	6.94	9.47	17.52	1035	0.507
0.41	0.77	0.65	0.12	5.18	0.92	1.69	3630	0.554
8.59	6.64	6.07	0.43	14.14	7.46	14.10	1384	0.545
10.15	4.64	4.21	0.43	9.82	5.18	9.82	1048	0.514
21.99	8.51	6.73	1.78	3.78	12.79	21.30	1159	0.686
7.96	4.64	4.05	0.59	6.86	5.36	10.00	1402	0.548
3.30	0.91	0.87	0.04	19.88	0.95	1.86	1033	0.543
6.85	3.04	2.70	0.34	8.02	3.47	6.51	1048	0.537
1.47	1.78	1.64	0.14	12.11	2.07	3.85	3655	0.568
1.01	1.89	1.70	0.18	9.30	2.11	3.99	4437	0.609
6.71	2.97	2.57	0.36	7.17	3.53	6.50	2193	0.609
18.29	5.33	4.59	0.62	7.38	6.72	12.05	2467	0.595
10.64	4.56	3.99	0.51	7.84	5.36	9.92	2027	0.537
16.68	4.74	3.94	0.66	5.96	6.13	10.87	2097	0.578
18.70	6.89	5.88	0.89	6.62	8.64	15.52	2226	0.570
14.24	5.63	4.79	0.75	6.41	7.13	12.76	2954	0.597
17.10	3.12	2.16	0.79	2.74	5.16	8.28	2233	0.576
25.54	3.63	2.59	0.86	3.01	5.39	9.03	2152	0.605
31.26	9.25	8.00	1.08	7.41	11.55	20.81	2211	0.562
28.25	10.68	8.49	2.03	4.18	16.12	26.80	1377	0.626
11.85	4.28	3.86	0.35	11.02	5.06	9.35	2219	0.557

Fatty acid profile of Chinese Fats (mg/g)

	14:0	16:0	16:1n7	18:0	18:1n9t	18:1n9c	18:1n7	18:2n6c
Rapeseed oil, Jianshi County	0.00	32.06	1.96	9.41	0.00	179.55	12.67	129.99
Lard, Jianshi County	13.56	214.69	19.45	108.63	2.54	350.15	25.80	73.85
Soybean oil, Beijing	0.00	98.55	1.35	40.56	0.00	194.24	12.86	450.47
Mixed oil, Beijing	0.00	57.88	1.89	22.26	0.00	434.71	23.50	231.64
Rapeseed oil, Xide County	0.00	27.51	1.79	9.78	0.00	173.80	11.83	106.33
Rapeseed oil, Xichang County	0.00	33.10	2.06	11.00	0.00	203.24	13.89	130.20
Lard, Xide County	8.00	116.40	6.45	78.17	1.27	160.26	9.24	40.30
Lard, Xichang County	12.51	205.07	18.72	111.61	3.57	314.38	20.38	75.45

20:0	18:3n3c	20:1n9	20:2n6	22:0	20:4n6	22:1n9	20:5n3c	24:0	24:1	Others
4.17	62.93	55.12	3.41	2.08	0.00	119.19	1.45	0.00	2.94	7.33
1.69	7.68	11.52	5.11	0.00	0.00	2.07	0.00	0.00	0.00	8.59
11.88	46.42	2.65	1.61	0.00	0.00	0.00	0.00	1.66	0.00	14.63
17.40	47.56	9.70	2.22	4.15	0.00	3.56	0.00	2.45	1.50	13.09
5.42	58.09	39.64	3.04	3.55	0.00	186.75	2.78	1.31	4.19	12.21
5.30	63.12	36.42	3.18	3.56	0.00	169.34	2.81	1.39	4.33	12.23
1.13	4.50	4.50	3.12	0.00	1.46	1.30	0.00	0.00	0.00	1.82
1.73	12.15	8.06	4.57	0.00	3.39	3.00	0.00	0.00	0.00	7.18

Sum	SFA	MUFA	PUFA	n6	n3	n6/n3	PI	UI	17:0	Sample
624.26	47.73	371.43	197.78	133.40	64.38	2.07	265.06	462.84	1440	0.215
845.33	338.57	411.53	86.64	78.96	7.68	10.28	94.32	180.96	1500	0.236
876.86	152.64	211.10	498.49	452.07	46.42	9.74	544.92	1043.41	861	0.219
873.51	104.13	474.87	281.42	233.86	47.56	4.92	328.98	610.40	1400	0.214
648.02	47.57	418.00	170.24	109.37	60.87	1.80	236.67	406.91	1387	0.264
695.15	54.34	429.28	199.31	133.38	65.93	2.02	270.85	470.16	1369	0.267
437.90	203.70	183.01	49.37	44.88	4.50	9.98	56.79	106.17	1338	0.265
801.76	330.92	368.11	95.56	83.41	12.15	6.87	114.49	210.04	1318	0.245

PLASMA VITAMIN E

sample	area (alpha	conc. (ug/mL)	ave.		area (gamma)	conc. (ug/mL)
0-1-1	3302	8.368	8.298			
0-1-1	3246	8.227				
0-2-1	3390	8.591	9.150			
0-2-1	3831	9.709				
0-3-1	5467	7.588	7.378			
0-3-1	5164	7.168				
0-4-1	6469	8.979	8.866			
0-4-1	6307	8.754				
0-5-1	5282	7.574	7.873			
0-5-1	5698	8.171				
0-6-1	8056	11.719	11.412			
0-6-1	7635	11.106				
0-7-1	7676	11.166	12.362			
0-7-1	9321	13.559				
0-8-1	3540	5.149	5.181			
0-8-1	3583	5.212				
0-9-1	6397	10.279	10.125			
0-9-1	6205	9.971				
0-10-1	1637	2.630	2.438			
0-10-1	1398	2.246				
0-11-1	6846	11.655	12.186			
0-11-1	7470	12.717				
ave			8.661			
sd			2.850			
0-1-4	1422	3.604	3.601			
0-1-4	1420	3.599				
0-2-4	1891	4.792	4.786		0	
0-2-4	1886	4.780			706	0.9338
0-3-4	4047	5.617	5.410			
0-3-4	3749	5.204				
0-4-4	3185	4.567	4.678			
0-4-4	3339	4.788				
0-5-4	2314	3.318	3.619			
0-5-4	2733	3.919				
0-6-4	3416	4.969	4.733			
0-6-4	3091	4.496				
0-7-4	4972	7.233	7.151			
0-7-4	4860	7.070				
0-8-4	1746	2.540	2.754			
0-8-4	2041	2.969				
0-9-4	1444	2.320	2.352			
0-9-4	1483	2.383				
0-10-4	4321	7.356	7.685			
0-10-4	4707	8.013				
0-11-4	3030	5.158	5.269			
0-11-4	3160	5.380				
ave			4.731			
sd			1.579			

sample	area (alpha	conc. (ug/mL)	ave.		area (gamma)	conc. (ug/mL)
1-1	4761	12.066	11.866			
1-1	4603	11.666				
2-1	4241	10.748	10.447			
2-1	4003	10.145				
3-1	5852	8.123	7.486			
3-1	4935	6.850				
4-1	9417	13.071	14.442			
4-1	11393	15.813				
5-1	7161	10.269	10.533			
5-1	7529	10.797				
6-1	7353	10.696	9.873			
6-1	6221	9.049				
7-1	6481	9.428	9.795			
7-1	6986	10.162				
8-1	5901	8.584	9.114			
8-1	6630	9.644				
9-1	6655	10.694	10.506			
9-1	6422	10.319				
10-1	5574	8.957	8.879			
10-1	5477	8.801				
ave			10.294			
sd			1.775			
1-4	1497	3.794	4.249			
1-4	1856	4.704				
2-4	2400	6.082	5.506			
2-4	1945	4.929				
3-4	2299	3.191	3.490			
3-4	2730	3.789				
4-4	5521	7.917	8.076		1634	1.4173
4-4	5742	8.234			1839	1.5951
5-4	2600	3.728	3.542			
5-4	2340	3.356				
6-4	1954	2.842	2.751			
6-4	1829	2.661				
7-4	4114	5.984	5.443			
7-4	3369	4.901				
8-4	3618	5.263	4.472			
8-4	2530	3.680				
9-4	2758	4.432	4.479		850	0.7412
9-4	2817	4.527			0	
10-4	2656	4.522	4.425			
10-4	2542	4.328				
ave			4.643			
sd			1.398023			

Milk and Plasma Selenium

Milk Se	Ave (ng/g)
0-1-1	17.773
0-2-1	20.088
0-3-1	24.774
0-4-1	6.81
0-5-1	17.853
0-6-1	14.426
0-7-1	15.49
0-8-1	12.681
0-9-1	13.419
0-10-1	16.591
0-11-1	14.177
Ave	15.82564
SD	4.353485
0-1-6	8.381
0-2-6	12.083
0-3-6	14.381
0-4-6	4.652
0-5-6	9.916
0-6-6	7.527
0-7-6	7.835
0-8-6	6.791
0-9-6	11.643
0-10-6	9.707
0-11-6	10.872
Ave	9.435273
SD	2.626778

Plasma Se	ave (ng/g)
0-1-1	96.235
0-2-1	113.16
0-3-1	104.121
0-4-1	29.172
0-5-1	97.601
0-6-1	85.094
0-7-1	103.253
0-8-1	63.639
0-9-1	156.431
0-10-1	77.711
0-11-1	105.659
Ave	93.825
SD	30.26467
0-1-4	99.583
0-2-4	88.849
0-3-4	108.028
0-4-4	27.817
0-5-4	67.471
0-6-4	81.295
0-7-4	88.017
0-8-4	48.918
0-9-4	96.283
0-10-4	81.254
0-11-4	101.017
Ave	80.77564
SD	23.08335

Milk Se	Ave (ng/g)
1-1	7.834
2-1	5.395
3-1	6.342
4-1	6.919
5-1	6.522
6-1	8.004
7-1	8.305
8-1	8.367
9-1	6.475
10-1	10.314
Ave	7.4477
SD	1.333161
1-6	9.591
2-6	4.612
3-6	3.642
4-6	4.212
5-6	7.28
6-6	5.66
7-6	5.961
8-6	5.287
9-6	5.858
10-6	6.015
Ave	5.8118
SD	1.597154

Plasma Se	ave (ng/g)
1-1	44.222
2-1	14.159
3-1	35.353
4-1	46.106
5-1	39.517
6-1	39.832
7-1	33.483
8-1	23.083
9-1	35.066
10-1	35.865
Ave	34.6686
SD	9.113629
1-4	36.784
2-4	18.488
3-4	16.369
4-4	28.553
5-4	29.421
6-4	29.588
7-4	34.238
8-4	25.206
9-4	29.934
10-4	31.261
Ave	27.9842
SD	6.073342

Milk and Plasma Glutathione Peroxidase Activities

Milk

SAMPLE	Gpx
0-1-1	-145.406
0-2-1	-120.606
0-3-1	-131.156
0-4-1	-82.606
0-5-1	-106.556
0-6-1	-171.656
0-7-1	-122.456
0-8-1	-168.306
0-9-1	-145.406
0-10-1	-167.006
0-11-1	-138.906
ave	-136.37

0-1-6	-80.206
0-2-6	-136.906
0-3-6	-100.656
0-4-6	-56.506
0-5-6	-92.106
0-6-6	-94.156
0-7-6	-98.956
0-8-6	-93.656
0-9-6	-66.706
0-10-6	-99.156
0-11-6	-101.106
ave	-92.7378

SAMPLE	Gpx
1-1	-114.156
2-1	-71.356
3-1	-87.956
4-1	-131.206
5-1	-85.956
6-1	-92.506
7-1	-91.156
8-1	-172.456
9-1	-66.556
10-1	-95.106
ave	-100.841

1-6	-124.606
2-6	-52.756
3-6	-56.956
4-6	-47.106
5-6	-58.556
6-6	-68.906
7-6	-72.756
8-6	-83.006
9-6	-66.706
10-6	-78.006
ave	-70.936

Plasma

SAMPLE	Gpx
0-1-1	-484.676
0-2-1	-488.876
0-3-1	-418.876
0-4-1	-144.696
0-5-1	-404.676
0-6-1	-477.876
0-7-1	-536.276
0-8-1	-318.808
0-9-1	-575.276
0-10-1	-308.876
0-11-1	-613.196
ave	-433.828

0-1-4	-322.076
0-2-4	-309.276
0-3-4	-307.208
0-4-4	-166.476
0-5-4	-280.076
0-6-4	-377.076
0-7-4	-376.276
0-8-4	-267.476
0-9-4	-303.744
0-10-4	-285.876
0-11-4	-488.144
ave	-316.7

SAMPLE	Gpx
1-1	-295.476
2-1	-79.756
3-1	-185.344
4-1	-331.476
5-1	-213.676
6-1	-205.476
7-1	-214.676
8-1	-128.636
9-1	-245.476
10-1	-223.076
ave	-212.307

1-4	-272.276
2-4	-131.676
3-4	-131.376
4-4	-255.476
5-4	-217.076
6-4	-223.876
7-4	-248.876
8-4	-200.876
9-4	-196.076
10-4	-246.944
ave	-212.453

Milk 2nd run

SAMPLE	Gpx
0-1-1	-56.48
0-2-1	-61.93
0-3-1	-72.13
0-4-1	-42.26
0-5-1	-50.73
0-6-1	-98.73
0-7-1	-63.23
0-8-1	-77.28
0-9-1	-63.73
0-10-1	-82.78
0-11-1	-67.78
ave	-67.0055

0-1-6	-26.49
0-2-6	-69.73
0-3-6	-53.93
0-4-6	-29.73
0-5-6	-43.33
0-6-6	-44.29
0-7-6	-40.61
0-8-6	-51.73
0-9-6	-30.56
0-10-6	-41.53
0-11-6	-32.8
ave	-42.25

SAMPLE	Gpx
1-1	-51.53
2-1	-41.25
3-1	-44.83
4-1	-65.43
5-1	-40.52
6-1	-61.18
7-1	-59.13
8-1	-103.68
9-1	-29.19
10-1	-38.33
ave	-53.507

1-6	-64.28
2-6	-22.35
3-6	-33.15
4-6	-28.04
5-6	-30.04
6-6	-44.07
7-6	-44.18
8-6	-54.28
9-6	-23.9
10-6	-23.79
ave	-36.81

Plasma 2nd run

SAMPLE	Gpx
0-1-1	-444.34
0-2-1	-480.26
0-3-1	-406.14
0-4-1	-146.06
0-5-1	-345.16
0-6-1	-490.16
0-7-1	-478.46
0-8-1	-283.86
0-9-1	-530.46
0-10-1	-271.36
0-11-1	-568.03
ave	-404.03

0-1-4	-288.56
0-2-4	-280.46
0-3-4	-281.86
0-4-4	-158.86
0-5-4	-251.56
0-6-4	-346.96
0-7-4	-353.9
0-8-4	-218.56
0-9-4	-257.54
0-10-4	-237.53
0-11-4	-409.36
ave	-280.47

SAMPLE	Gpx
1-1	-273.86
2-1	-72.26
3-1	-168.76
4-1	-259.26
5-1	-165.16
6-1	-175.16
7-1	-150.56
8-1	-104.55
9-1	-212.36
10-1	-164.46
ave	-174.64

1-4	-241.16
2-4	-131.56
3-4	-122.69
4-4	-197.56
5-4	-190.06
6-4	-176.66
7-4	-199.06
8-4	-158.16
9-4	-167.26
10-4	-169.7
ave	-175.39

Plasma TBARS

Subject	T-BARS (ave)
P0-1-1	2.312
P0-2-1	2.153
P0-3-1	
P0-4-1	1.624
P0-5-1	1.433
P0-6-1	1.663
P0-7-1	1.915
P0-8-1	1.518
P0-9-1	2.078
P0-10-1	1.032
P0-11-1	0.909
ave	1.664

P0-1-4	1.65
P0-2-4	1.314
P0-3-4	1.279
P0-4-4	0.9
P0-5-4	0.946
P0-6-4	0.975
P0-7-4	1.316
P0-8-4	1.234
P0-9-4	
P0-10-4	
P0-11-4	1.08
ave	1.188

Subject	T-BARS (ave)
P-1-1	1.174
P-2-1	1.681
P-3-1	1.647
P-4-1	1.472
P-5-1	1.218
P-6-1	0.925
P-7-1	1.001
P-8-1	
P-9-1	0.696
P-10-1	0.851
ave	1.185

P-1-4	0.91
P-2-4	0.982
P-3-4	1.069
P-4-4	0.787
P-5-4	0.635
P-6-4	0.567
P-7-4	0.602
P-8-4	0.596
P-9-4	0.38
P-10-4	0.613
ave	0.714

PLASMA FATTY ACID PROFILE AREA % INITIAL

	13:0		14:0		14:1		15:0		16:0		16:1n7	
Subject ID	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
PO-1-1	0	0	154	0.46	0	0.00	35	0.10	6468	19.20	688	2.04
PO-2-1		0	94	0.63		0.00	31	0.21	3448	22.93	542	3.61
PO-3-1		0	154	0.76		0.00	43	0.21	4650	22.91	851	4.19
PO-4-1		0	231	0.56		0.00	42	0.10	8872	21.65	1022	2.49
PO-5-1		0	61	0.43		0.00		0.00	2890	20.17	281	1.96
PO-6-1		0	35	0.26		0.00		0.00	2621	19.67	208	1.56
PO-7-1		0	82	0.34		0.00		0.00	5584	23.38	386	1.62
PO-8-1		0	30	0.36		0.00		0.00	1674	20.27	170	2.06
PO-9-1		0	137	0.40		0.00	38	0.11	7361	21.68	941	2.77
PO-10-1		0	151	0.38		0.00	42	0.11	9466	23.78	806	2.02
PO-11-1		0	85	0.24		0.00	26	0.07	6845	19.30	629	1.77
ave		0		0.44		0.00		0.08		21.36		2.37
sd		0		0.15		0.00		0.07		1.63		0.80
P-1-1		0	356	0.70		0.00	94	0.19	11280	22.33	1822	3.61
P-2-1		0	52	0.28		0.00		0.00	3749	20.53	320	1.75
P-3-1		0	153	0.60		0.00	37	0.15	5560	21.89	741	2.92
P-4-1		0	293	0.64		0.00	47	0.10	9679	21.16	1127	2.46
P-5-1		0	115	0.32		0.00		0.00	8103	22.43	498	1.38
P-6-1		0	196	0.34		0.00	42	0.07	11179	19.17	875	1.50
P-7-1		0	472	0.60		0.00	76	0.10	16768	21.29	1649	2.09
P-8-1		0	337	0.52		0.00	62	0.10	14014	21.61	1039	1.60
P-9-1		0	203	0.37		0.00	56	0.10	10627	19.19	841	1.52
P-10-1		0	141	0.58		0.00		0.00	5562	22.99	585	2.42
ave		0		0.50		0.00		0.08		21.26		2.13
sd		0		0.15		0.00		0.06		1.24		0.69

16:2n4		X		18:0		18:1n9t		18:1n9c		18:1n7	
Area	Area %	Area	Area%	Area	Area %	Area	Area %	Area	Area %	Area	Area %
170	0.50	1011	3.00	2371	7.04	37	0.11	6460	19.18	722	2.14
	0.00	199	1.32	909	6.05	0	0.00	3785	25.18	415	2.76
77	0.38	125	0.62	1726	8.50		0.00	3698	18.22	365	1.80
99	0.24	637	1.55	2671	6.52		0.00	8567	20.90	867	2.12
39	0.27	705	4.92	1058	7.38		0.00	2565	17.90	280	1.95
38	0.29	454	3.41	1008	7.57		0.00	2471	18.55	277	2.08
56	0.23	425	1.78	1460	6.11		0.00	5049	21.14	501	2.10
28	0.34	215	2.60	631	7.64		0.00	1571	19.02	185	2.24
78	0.23	979	2.88	2080	6.13	25	0.07	6570	19.35	919	2.71
92	0.23	749	1.88	2576	6.47	37	0.09	8862	22.26	939	2.36
74	0.21	669	1.89	2490	7.02		0.00	5798	16.35	609	1.72
	0.27		2.35		6.95		0.03		19.82		2.18
	0.12		1.13		0.74		0.04		2.31		0.31
198	0.39	1118	2.21	4002	7.92	127	0.25	9823	19.45	809	1.60
41	0.22	561	3.07	1212	6.64		0.00	3400	18.62	411	2.25
59	0.23	438	1.72	1905	7.50	26	0.10	4809	18.94	501	1.97
103	0.23	601	1.31	3482	7.61	68	0.15	9377	20.50	843	1.84
80	0.22	731	2.02	2077	5.75	42	0.12	7705	21.33	676	1.87
157	0.27	2330	3.99	4847	8.31	121	0.21	8739	14.98	974	1.67
221	0.28	1700	2.16	5522	7.01	61	0.08	17432	22.13	1368	1.74
177	0.27	1297	2.00	4142	6.39	57	0.09	14036	21.65	1373	2.12
187	0.34	1527	2.76	4547	8.21		0.00	8679	15.67	938	1.69
40	0.17	356	1.47	1342	5.55		0.00	5376	22.22	555	2.29
	0.26		2.27		7.09		0.10		19.55		1.91
	0.06		0.77		0.94		0.08		2.44		0.23

16:4n1		19:0		18:2n6t		18:2n6c		18:3n6		20:0	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00		0.00		0.00	8736	25.94		0.00	60	0.18
	0.00		0.00	0	0.00	2969	19.75		0.00		0.00
	0.00		0.00		0.00	4360	21.48		0.00	33	0.16
	0.00		0.00		0.00	10225	24.95		0.00	60	0.15
	0.00		0.00		0.00	3884	27.11		0.00		0.00
	0.00		0.00		0.00	3376	25.34		0.00	27	0.20
	0.00		0.00		0.00	5592	23.41		0.00	29	0.12
	0.00		0.00		0.00	2149	26.02		0.00		0.00
	0.00		0.00		0.00	7099	20.91		0.00	47	0.14
	0.00		0.00		0.00	10115	25.41		0.00	47	0.12
	0.00		0.00		0.00	10765	30.36		0.00	78	0.22
	0.00		0.00		0.00		24.61		0.00		0.12
	0.00		0.00		0.00		2.91		0.00		0.08
	0.00		0.00		0.00	9433	18.68		0.00	68	0.13
	0.00		0.00		0.00	4888	26.76		0.00	27	0.15
	0.00		0.00		0.00	4926	19.40		0.00	39	0.15
	0.00		0.00		0.00	10964	23.96		0.00	61	0.13
	0.00		0.00		0.00	9024	24.98		0.00	51	0.14
	0.00		0.00		0.00	14942	25.62		0.00	86	0.15
	0.00		0.00		0.00	17786	22.58		0.00	122	0.15
	0.00		0.00		0.00	17274	26.64		0.00	95	0.15
	0.00		0.00		0.00	12440	22.47		0.00	86	0.16
	0.00		0.00		0.00	5855	24.20		0.00	34	0.14
	0.00		0.00		0.00		23.53		0.00		0.15
	0.00		0.00		0.00		2.64		0.00		0.01

18:3n3c		20:1n9		18:4n3		21:0		20:2n6		20:3n9	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
216	0.64	104	0.31		0.00		0.00	93	0.28	36	0.11
122	0.81	48	0.32		0.00		0.00	44	0.29	42	0.28
135	0.67	36	0.18		0.00		0.00	51	0.25	35	0.17
293	0.71	95	0.23		0.00		0.00	104	0.25	80	0.20
94	0.66	37	0.26		0.00		0.00	34	0.24		0.00
112	0.84	51	0.38		0.00		0.00	35	0.26		0.00
160	0.67	72	0.30		0.00		0.00	52	0.22	38	0.16
41	0.50		0.00		0.00		0.00	25	0.30		0.00
174	0.51	100	0.29		0.00		0.00	82	0.24	53	0.16
304	0.76		0.00		0.00		0.00	104	0.26	39	0.10
210	0.59	97	0.27		0.00		0.00	77	0.22	48	0.14
	0.67		0.23		0.00		0.00		0.26		0.12
	0.11		0.12		0.00		0.00		0.03		0.09
165	0.33	117	0.23		0.00		0.00	119	0.24	120	0.24
110	0.60	117	0.64		0.00		0.00	51	0.28	41	0.22
99	0.39	77	0.30		0.00		0.00	49	0.19	77	0.30
336	0.73	172	0.38		0.00		0.00	103	0.23	83	0.18
253	0.70	221	0.61		0.00		0.00	274	0.76	442	1.22
170	0.29	106	0.18		0.00		0.00	154	0.26	95	0.16
488	0.62	401	0.51	36	0.05		0.00	200	0.25	185	0.23
347	0.54	405	0.62		0.00		0.00	265	0.41	102	0.16
183	0.33	249	0.45		0.00		0.00	100	0.18	108	0.20
130	0.54	82	0.34		0.00		0.00	73	0.30	46	0.19
	0.51		0.43		0.00		0.00		0.31		0.31
	0.15		0.16		0.01		0.00		0.16		0.31

20:3n6		22:0		20:3n3		20:4n6		22:1n11		22:1n9	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
549	1.63	102	0.30		0.00	2989	8.87		0.00	33	0.10
267	1.78	29	0.19		0.00	1092	7.26		0.00		0.00
478	2.36	71	0.35		0.00	1957	9.64		0.00		0.00
604	1.47	120	0.29		0.00	3217	7.85		0.00		0.00
236	1.65	42	0.29		0.00	1143	7.98		0.00		0.00
180	1.35	52	0.39		0.00	1274	9.56		0.00	34	0.26
294	1.23	67	0.28		0.00	2232	9.34		0.00	28	0.12
158	1.91	28	0.34		0.00	890	10.78		0.00		0.00
445	1.31	99	0.29		0.00	3645	10.73		0.00	43	0.13
680	1.71	88	0.22		0.00	2187	5.49		0.00	29	0.07
540	1.52	110	0.31		0.00	3672	10.36		0.00	30	0.08
	1.63		0.30		0.00		8.90		0.00		0.07
	0.30		0.05		0.00		1.55		0.00		0.08
880	1.74	129	0.26		0.00	5084	10.07		0.00		0.00
333	1.82	62	0.34		0.00	1666	9.12		0.00		0.00
428	1.69	84	0.33		0.00	3188	12.55		0.00	27	0.11
632	1.38	115	0.25		0.00	4355	9.52		0.00	27	0.06
398	1.10	91	0.25		0.00	2767	7.66		0.00	57	0.16
886	1.52	176	0.30		0.00	6932	11.89		0.00	28	0.05
993	1.26	199	0.25		0.00	6617	8.40		0.00	110	0.14
1125	1.73	199	0.31		0.00	4229	6.52		0.00	61	0.09
785	1.42	200	0.36		0.00	8224	14.85		0.00	113	0.20
345	1.43	60	0.25		0.00	2005	8.29		0.00		0.00
	1.51		0.29		0.00		9.89		0.00		0.08
	0.22		0.04		0.00		2.40		0.00		0.07

20:4n3		23:0		20:5n3c		22:2n6		22:3n9		22:3n6	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00	29	0.09	112	0.33		0.00		0.00		0.00
	0.00		0.00	78	0.52		0.00		0.00		0.00
	0.00		0.00	66	0.33		0.00		0.00		0.00
	0.00	36	0.09	167	0.41		0.00		0.00		0.00
	0.00		0.00	37	0.26		0.00		0.00		0.00
	0.00		0.00	66	0.50		0.00		0.00		0.00
	0.00		0.00	66	0.28		0.00		0.00		0.00
	0.00		0.00	27	0.33		0.00		0.00		0.00
	0.00	34	0.10	119	0.35		0.00		0.00		0.00
	0.00	25	0.06	140	0.35		0.00		0.00		0.00
	0.00	34	0.10	115	0.32		0.00		0.00		0.00
	0.00		0.04		0.36		0.00		0.00		0.00
	0.00		0.04		0.08		0.00		0.00		0.00
	0.00	40	0.08	244	0.48		0.00		0.00		0.00
	0.00		0.00	88	0.48		0.00		0.00		0.00
	0.00	28	0.11	191	0.75		0.00		0.00		0.00
	0.00	44	0.10	237	0.52		0.00		0.00		0.00
	0.00	26	0.07	68	0.19		0.00		0.00		0.00
	0.00	65	0.11	272	0.47		0.00		0.00		0.00
	0.00	62	0.08	275	0.35		0.00		0.00		0.00
	0.00	62	0.10	202	0.31		0.00		0.00		0.00
	0.00	77	0.14	222	0.40		0.00		0.00		0.00
	0.00		0.00	100	0.41		0.00		0.00		0.00
	0.00		0.08		0.44		0.00		0.00		0.00
	0.00		0.04		0.14		0.00		0.00		0.00

24:0		22:4n6		24:1		22:5n6		22:5n3		22:6n3	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
67	0.20	72	0.21	441	1.31		0.00		0.00	804	2.39
	0.00	41	0.27	127	0.84		0.00	82	0.55	259	1.72
37	0.18	73	0.36	188	0.93		0.00	82	0.40	379	1.87
66	0.16	103	0.25	398	0.97		0.00	145	0.35	804	1.96
	0.00	32	0.22	111	0.77		0.00		0.00	313	2.18
36	0.27	30	0.23	209	1.57		0.00		0.00	331	2.48
37	0.15	55	0.23	285	1.19		0.00		0.00	583	2.44
	0.00		0.00	95	1.15		0.00		0.00	169	2.05
69	0.20	99	0.29	364	1.07		0.00		0.00	1054	3.10
47	0.12	74	0.19	342	0.86		0.00		0.00	715	1.80
79	0.22	76	0.21	497	1.40		0.00		0.00	748	2.11
	0.14		0.22		1.10		0.00		0.12		2.19
	0.09		0.08		0.24		0.00		0.20		0.38
104	0.21	188	0.37	469	0.93		0.00		0.00	1189	2.35
51	0.28	40	0.22	217	1.19		0.00		0.00	380	2.08
52	0.20	83	0.33	284	1.12		0.00		0.00	587	2.31
72	0.16	100	0.22	415	0.91		0.00		0.00	1095	2.39
45	0.12	87	0.24	469	1.30		0.00		0.00	766	2.12
119	0.20	139	0.24	587	1.01		0.00		0.00	1366	2.34
101	0.13	194	0.25	947	1.20		0.00		0.00	1604	2.04
66	0.10	133	0.21	830	1.28		0.00		0.00	1169	1.80
140	0.25	152	0.27	1028	1.86		0.00		0.00	1451	2.62
45	0.19	70	0.29	217	0.90		0.00		0.00	612	2.53
	0.18		0.26		1.17		0.00		0.00		2.26
	0.05		0.05		0.27		0.00		0.00		0.23

Others		Sum		SFA	MUFA	PUFA	n6	n3	n6/n3	PI	UI
Area	Area %	Area	Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %
1125	3.34	33684	100	27.57	25.19	40.90	36.93	3.36	10.99	72.00	112.90
411	2.73	15034	100	30.01	32.71	33.23	29.35	3.60	8.16	61.25	94.49
626	3.08	20296	100	33.08	25.32	37.90	34.09	3.26	10.45	70.76	108.66
1460	3.56	40985	100	29.52	26.71	38.65	34.78	3.44	10.12	67.37	106.02
486	3.39	14328	100	28.27	22.85	40.56	37.19	3.10	12.00	68.78	109.35
398	2.99	13323	100	28.36	24.39	40.85	36.74	3.82	9.62	74.04	114.88
753	3.15	23886	100	30.39	26.46	38.21	34.43	3.39	10.17	70.02	108.23
173	2.09	8259	100	28.61	24.47	42.22	39.01	2.87	13.59	75.35	117.57
1302	3.83	33956	100	29.05	26.39	37.84	33.48	3.97	8.44	75.34	113.17
1157	2.91	39813	100	31.25	27.67	36.29	33.05	2.91	11.35	58.46	94.76
1057	2.98	35458	100	27.49	21.60	46.04	42.67	3.03	14.10	78.84	124.88
	3.10		100	29.42	25.80	39.34	35.61	3.34	10.82	70.20	109.54
	0.44		1E-06	1.61	2.75	3.19	3.32	0.34	1.79	5.83	8.60
2529	5.01	50507	100	31.82	26.07	34.89	31.09	3.16	9.83	68.93	103.82
447	2.45	18264	100	28.21	24.45	41.82	38.21	3.16	12.07	72.92	114.74
947	3.73	25395	100	30.94	25.46	38.15	34.16	3.45	9.89	77.79	115.93
1320	2.89	45751	100	30.15	26.29	39.36	35.31	3.65	9.68	72.26	111.62
1061	2.94	36127	100	29.09	26.76	39.19	34.74	3.01	11.55	67.06	106.26
2740	4.70	58323	100	28.65	19.60	43.06	39.53	3.10	12.75	80.05	123.11
3184	4.04	78773	100	29.61	27.89	36.31	32.74	3.05	10.73	65.00	101.30
1748	2.70	64846	100	29.26	27.45	38.59	35.51	2.65	13.40	62.61	101.20
2211	3.99	55374	100	28.78	21.40	43.07	39.19	3.35	11.69	86.95	130.03
559	2.31	24190	100	29.70	28.17	38.35	34.51	3.48	9.91	69.02	107.36
	3.47		100	29.62	25.35	39.28	35.50	3.21	11.15	72.26	111.54
	0.90		1E-06	1.05	2.68	2.57	2.60	0.27	1.27	7.08	9.07

PLASMA FATTY ACID PROFILE AREA % FINAL

	13:0		14:0		14:1		15:0		16:0	
Subject ID	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
PO-1-4		0.00	44	0.48		0.00		0.00	1703	18.64
PO-2-4		0.00	35	0.26		0.00		0.00	2464	18.29
PO-3-4		0.00	163	0.70		0.00	28	0.12	4386	18.81
PO-4-4		0.00		0.00		0.00		0.00	2005	18.10
PO-5-4		0.00	68	0.90		0.00		0.00	1595	21.06
PO-6-4		0.00	64	0.28		0.00		0.00	4188	18.30
PO-7-4		0.00	169	0.64		0.00		0.00	5130	19.31
PO-8-4		0.00	64	0.81		0.00		0.00	1818	23.07
PO-9-4		0.00	54	0.60		0.00		0.00	1034	11.41
PO-10-4		0.00	156	0.56		0.00		0.00	5864	21.08
PO-11-4		0.00	250	0.72		0.00	39	0.11	6651	19.26
ave		0.00		0.54		0.00		0.02		18.85
sd		0.00		0.26		0.00		0.04		2.77
P-1-4		0.00	248	0.64		0.00	41	0.11	8130	20.96
P-2-4		0.00	51	0.42		0.00		0.00	2135	17.67
P-3-4		0.00	220	0.84		0.00	34	0.13	5290	20.22
P-4-4		0.00	235	0.45		0.00	33	0.06	8353	15.99
P-5-4		0.00	40	0.34		0.00		0.00	2370	20.01
P-6-4		0.00	397	0.96		0.00	34	0.08	8200	19.80
P-7-4		0.00	154	0.41		0.00	29	0.08	7145	19.16
P-8-4		0.00	153	0.56		0.00		0.00	5481	20.21
P-9-4		0.00	314	0.61		0.00	49	0.10	9042	17.70
P-10-4		0.00	663	1.19		0.00	113	0.20	10634	19.11
ave		0.00		0.64		0.00		0.08		19.08
sd		0.00		0.26		0.00		0.06		1.45

16:1n7		16:2n4		x		18:0		18:1n9t		18:1n9c	
Area	Area %	Area	Area %	Area	Area%	Area	Area %	Area	Area %	Area	Area %
198	2.17	33	0.36	346	3.79	781	8.55		0.00	1964	21.50
159	1.18	76	0.56	50	0.37	991	7.35	28	0.21	2021	15.00
352	1.51	71	0.30	563	2.41	2170	9.31		0.00	4708	20.19
77	0.69	87	0.79	0	0.00	857	7.73		0.00	739	6.67
108	1.43	40	0.53	354	4.67	864	11.41		0.00	1697	22.41
333	1.46	69	0.30	1040	4.54	1912	8.36		0.00	4264	18.63
397	1.49	79	0.30	906	3.41	2137	8.04	42	0.16	5140	19.34
76	0.96	78	0.99	0	0.00	781	9.91	171	2.17	650	8.25
283	3.12	47	0.52	685	7.56	949	10.47		0.00	2136	23.58
644	2.32	58	0.21	906	3.26	2151	7.73	61	0.22	6620	23.80
785	2.27	94	0.27	156	0.45	3179	9.21	52	0.15	7270	21.05
	1.69		0.47		2.77		8.92		0.26		18.22
	0.67		0.23		2.30		1.22		0.61		5.60
854	2.20	72	0.19	0	0.00	3512	9.05	135	0.35	8861	22.84
206	1.70	38	0.31	479	3.96	1061	8.78		0.00	2611	21.60
522	1.99	76	0.29	820	3.13	2366	9.04	40	0.15	5149	19.68
520	1.00	430	0.82	192	0.37	3822	7.32	264	0.51	8147	15.60
133	1.12		0.00	297	2.51	950	8.02	33	0.28	2532	21.37
754	1.82	117	0.28	1361	3.29	3978	9.60	175	0.42	10078	24.33
492	1.32	107	0.29	1340	3.59	3352	8.99		0.00	5831	15.63
398	1.47	72	0.27	1029	3.79	2499	9.22		0.00	6087	22.45
822	1.61	181	0.35	1758	3.44	4386	8.59	46	0.09	8990	17.60
1209	2.17	622	1.12	1055	1.90	4689	8.43	1449	2.60	10855	19.51
	1.64		0.39		2.60		8.70		0.44		20.06
	0.40		0.31		1.34		0.62		0.74		2.86

18:1n7		16:4n1		19:0		18:2n6t		18:2n6c	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
232	2.54		0.00		0.00		0.00	2621	28.69
251	1.86		0.00		0.00	45	0.33	3556	26.39
451	1.93		0.00		0.00		0.00	6225	26.70
92	0.83		0.00		0.00		0.00	1217	10.98
159	2.10		0.00		0.00		0.00	1899	25.08
460	2.01		0.00		0.00		0.00	5933	25.93
551	2.07		0.00		0.00		0.00	6592	24.81
75	0.95		0.00		0.00		0.00	718	9.11
242	2.67		0.00		0.00		0.00	1973	21.78
795	2.86		0.00		0.00		0.00	6791	24.42
775	2.24		0.00		0.00		0.00	9477	27.45
	2.01		0.00		0.00		0.03		22.85
	0.61		0.00		0.00		0.10		6.28
869	2.24		0.00		0.00	74	0.19	9286	23.94
297	2.46		0.00		0.00		0.00	3380	27.97
551	2.11		0.00		0.00		0.00	6331	24.20
806	1.54		0.00		0.00	711	1.36	11285	21.60
245	2.07		0.00		0.00		0.00	3546	29.93
969	2.34		0.00		0.00		0.00	9373	22.63
683	1.83		0.00		0.00		0.00	10651	28.56
648	2.39		0.00		0.00		0.00	7330	27.03
889	1.74		0.00		0.00		0.00	12538	24.54
1306	2.35		0.00		0.00	33	0.06	12775	22.96
	2.11		0.00		0.00		0.16		25.34
	0.29		0.00		0.00		0.40		2.69

18:3n6		20:0		18:3n3c		20:1n9		18:4n3	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00		0.00	105	1.15	33	0.36		0.00
	0.00	28	0.21	113	0.84	54	0.40		0.00
	0.00	49	0.21	453	1.94	214	0.92		0.00
	0.00		0.00	76	0.69	35	0.32		0.00
	0.00		0.00	90	1.19	31	0.41		0.00
	0.00	46	0.20	124	0.54	107	0.47		0.00
	0.00	42	0.16	148	0.56	98	0.37		0.00
	0.00		0.00	68	0.86	27	0.34		0.00
	0.00		0.00	59	0.65	35	0.39		0.00
	0.00	41	0.15	263	0.95	118	0.42		0.00
	0.00	63	0.18	242	0.70	86	0.25		0.00
	0.00		0.10		0.91		0.42		0.00
	0.00		0.09		0.39		0.17		0.00
	0.00	58	0.15	228	0.59	117	0.30		0.00
	0.00		0.00	208	1.72	92	0.76		0.00
	0.00	114	0.44	142	0.54	167	0.64		0.00
	0.00	152	0.29	446	0.85	327	0.63	45	0.09
	0.00		0.00	137	1.16	90	0.76		0.00
	0.00	70	0.17	341	0.82	159	0.38		0.00
	0.00	89	0.24	232	0.62	157	0.42		0.00
	0.00	43	0.16	146	0.54	235	0.87		0.00
	0.00	98	0.19	416	0.81	325	0.64		0.00
	0.00	157	0.28	350	0.63	313	0.56		0.00
	0.00		0.19		0.83		0.60		0.01
	0.00		0.13		0.35		0.17		0.03

21:0		20:2n6		20:3n9		20:3n6		22:0	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00	33	0.36		0.00	115	1.26	35	0.38
	0.00	45	0.33	51	0.38	144	1.07	49	0.36
	0.00	108	0.46		0.00	427	1.83	74	0.32
	0.00	1117	10.08	220	1.99	124	1.12	51	0.46
	0.00	28	0.37		0.00	93	1.23		0.00
	0.00	65	0.28	34	0.15	282	1.23	79	0.35
	0.00	72	0.27	48	0.18	288	1.08	87	0.33
	0.00		0.00	162	2.06	94	1.19	29	0.37
	0.00	30	0.33	30	0.33	146	1.61	29	0.32
	0.00	85	0.31	26	0.09	383	1.38	66	0.24
	0.00	136	0.39	50	0.14	587	1.70	111	0.32
	0.00		1.20		0.48		1.34		0.31
	0.00		2.81		0.73		0.25		0.11
	0.00	148	0.38	77	0.20	652	1.68	116	0.30
	0.00	50	0.41		0.00	189	1.56	31	0.26
	0.00	91	0.35	57	0.22	451	1.72	83	0.32
	0.00	1667	3.19	348	0.67	673	1.29	250	0.48
	0.00	35	0.30		0.00	99	0.84	39	0.33
	0.00	169	0.41	50	0.12	418	1.01		0.00
	0.00	92	0.25	47	0.13	424	1.14	141	0.38
	0.00	136	0.50	43	0.16	114	0.42	76	0.28
	0.00	144	0.28	112	0.22	646	1.26	175	0.34
	0.00	208	0.37	119	0.21	432	0.78	242	0.43
	0.00		0.64		0.19		1.17		0.31
	0.00		0.85		0.18		0.40		0.12

20:3n3		20:4n6		22:1n11		22:1n9		20:4n3		23:0	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00	461	5.05		0.00		0.00		0.00		0.00
	0.00	989	7.34		0.00	33	0.24		0.00		0.00
	0.00	1083	4.64		0.00	383	1.64		0.00		0.00
	0.00	280	2.53		0.00		0.00		0.00		0.00
	0.00	282	3.72		0.00		0.00		0.00		0.00
	0.00	1908	8.34		0.00	85	0.37		0.00		0.00
	0.00	2326	8.75		0.00	59	0.22		0.00	26	0.10
	0.00	146	1.85		0.00		0.00		0.00		0.00
	0.00	661	7.30		0.00		0.00		0.00		0.00
	0.00	1194	4.29		0.00	29	0.10		0.00		0.00
	0.00	2166	6.27		0.00	61	0.18		0.00	40	0.12
	0.00		5.46		0.00		0.25		0.00		0.02
	0.00		2.21		0.00		0.46		0.00		0.04
	0.00	2533	6.53		0.00	42	0.11		0.00	44	0.11
	0.00	651	5.39		0.00	57	0.47		0.00		0.00
	0.00	1838	7.02		0.00	37	0.14		0.00	30	0.11
	0.00	1979	3.79		0.00	481	0.92		0.00	47	0.09
	0.00	624	5.27		0.00	71	0.60		0.00		0.00
	0.00	1742	4.21		0.00	26	0.06		0.00	37	0.09
	0.00	3160	8.47		0.00	122	0.33		0.00	45	0.12
	0.00	1248	4.60		0.00	38	0.14		0.00		0.00
	0.00	4054	7.94		0.00	325	0.64		0.00	61	0.12
	0.00	2952	5.30		0.00	49	0.09		0.00	373	0.67
	0.00		5.85		0.00		0.35		0.00		0.13
	0.00		1.50		0.00		0.28		0.00		0.19

20:5n3c		22:2n6		22:3n9		22:3n6		24:0		22:4n6	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
31	0.34		0.00		0.00		0.00	27	0.30		0.00
69	0.51		0.00		0.00		0.00	72	0.53	171	1.27
107	0.46		0.00		0.00		0.00	29	0.12	42	0.18
	0.00		0.00		0.00		0.00	97	0.88	252	2.27
	0.00		0.00		0.00		0.00		0.00		0.00
121	0.53		0.00		0.00		0.00	37	0.16	45	0.20
168	0.63		0.00		0.00		0.00	49	0.18	58	0.22
	0.00		0.00		0.00		0.00	33	0.42	171	2.17
71	0.78		0.00		0.00		0.00		0.00	27	0.30
79	0.28		0.00		0.00		0.00	38	0.14		0.00
108	0.31		0.00		0.00		0.00	70	0.20	92	0.27
	0.35		0.00		0.00		0.00		0.27		0.62
	0.25		0.00		0.00		0.00		0.25		0.82
130	0.34		0.00		0.00		0.00	79	0.20	150	0.39
48	0.40		0.00		0.00		0.00	38	0.31		0.00
200	0.76		0.00		0.00		0.00	49	0.19	53	0.20
140	0.27		0.00		0.00		0.00	178	0.34	711	1.36
55	0.46		0.00		0.00		0.00		0.00		0.00
168	0.41		0.00		0.00		0.00	77	0.19	81	0.20
238	0.64		0.00		0.00		0.00	89	0.24	72	0.19
	0.00		0.00		0.00		0.00	44	0.16	61	0.22
348	0.68		0.00		0.00		0.00	129	0.25	110	0.22
344	0.62		0.00		0.00		0.00	121	0.22	86	0.15
	0.46		0.00		0.00		0.00		0.21		0.29
	0.22		0.00		0.00		0.00		0.09		0.37

24:1		22:5n6		22:5n3		22:6n3		Others		Sum	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
114	1.25		0.00	38	0.42	112	1.23	109	1.19	9135	100
219	1.63		0.00	52	0.39	220	1.63	1489	11.05	13474	100
251	1.08		0.00		0.00	240	1.03	739	3.17	23316	100
139	1.25		0.00		0.00	81	0.73	3534	31.90	11080	100
67	0.88		0.00		0.00	68	0.90	130	1.72	7573	100
334	1.46		0.00		0.00	421	1.84	933	4.08	22884	100
359	1.35		0.00		0.00	392	1.48	1208	4.55	26571	100
94	1.19		0.00		0.00	48	0.61	2578	32.71	7881	100
119	1.31		0.00		0.00	121	1.34	329	3.63	9060	100
274	0.99		0.00		0.00	265	0.95	907	3.26	27814	100
425	1.23		0.00		0.00	429	1.24	1136	3.29	34530	100
	1.24		0.00		0.07		1.18		9.14		100
	0.20		0.00		0.15		0.36		11.19		1E-06
479	1.23		0.00		0.00	540	1.39	1319	3.40	38794	100
140	1.16		0.00		0.00	146	1.21	178	1.47	12086	100
333	1.27		0.00		0.00	351	1.34	771	2.95	26166	100
556	1.06		0.00		0.00	579	1.11	8862	16.96	52239	100
180	1.52		0.00		0.00	131	1.11	239	2.02	11846	100
327	0.79		0.00		0.00	539	1.30	1780	4.30	41420	100
650	1.74		0.00		0.00	776	2.08	1181	3.17	37299	100
272	1.00		0.00		0.00	232	0.86	732	2.70	27117	100
702	1.37		0.00		0.00	870	1.70	3554	6.96	51084	100
449	0.81		0.00		0.00	432	0.78	3616	6.50	55646	100
	1.20		0.00		0.00		1.29		5.04		100
	0.29		0.00		0.00		0.37		4.32		0.00

SFA	MUFA	PUFA	n6	n3	n6/n3	PI	UI
Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %
28.35	27.82	38.85	35.36	3.13	11.29	58.52	97.37
27.01	20.52	41.05	36.74	3.37	10.90	69.78	110.83
29.59	27.27	37.55	33.82	3.43	9.86	56.47	94.03
27.17	9.77	31.17	26.99	1.42	19.04	47.49	78.66
33.37	27.23	33.01	30.40	2.09	14.57	46.47	79.48
27.64	24.40	39.34	35.98	2.91	12.36	67.27	106.61
28.75	25.01	38.28	35.14	2.66	13.19	65.84	104.12
34.58	13.87	18.84	14.33	1.47	9.73	33.43	52.28
22.80	31.07	34.93	31.31	2.77	11.30	60.41	95.34
29.90	30.71	32.88	30.39	2.18	13.93	48.54	81.42
30.13	27.38	38.75	36.08	2.26	15.99	60.28	99.04
29.03	24.09	34.97	31.50	2.52	12.92	55.87	90.83
3.02	6.47	5.92	6.18	0.66	2.70	10.38	16.01
31.52	29.28	35.80	33.11	2.31	14.30	58.68	94.48
27.44	28.16	38.97	35.33	3.33	10.62	59.05	98.02
31.28	25.98	36.65	33.49	2.65	12.65	61.25	97.90
25.02	21.25	36.40	32.59	2.32	14.07	54.91	91.31
28.69	27.72	39.06	36.33	2.73	13.33	57.40	96.46
30.89	30.15	31.38	28.45	2.53	11.24	48.56	79.94
29.61	21.27	42.36	38.60	3.34	11.56	71.81	114.17
30.59	28.31	34.60	32.78	1.39	23.52	48.79	83.39
27.90	23.68	38.01	34.24	3.20	10.71	65.47	103.48
30.54	28.09	32.98	29.63	2.02	14.64	50.48	83.46
29.35	26.39	36.62	33.46	2.58	13.66	57.64	94.26
1.97	3.07	3.03	2.83	0.58	3.58	7.05	9.81

MILK FATTY ACID PROFILE AREA % INITIAL

	10:0		12:0		13:0		14:0		14:1	
Subject ID	Area	Area%	Area	Area %	Area	Area %	Area	Area %	Area	Area %
0-1-1	549	1.29	2660	6.27		0.00	2617	6.17		0.00
0-2-1	367	1.79	2102	10.25		0.00	2406	11.73		0.00
0-3-1	292	0.90	1943	5.96		0.00	2686	8.24	26	0.08
0-4-1	272	0.90	1549	5.14		0.00	1874	6.21	28	0.09
0-5-1	323	1.04	1726	5.54		0.00	1994	6.40		0.00
0-6-1	249	0.91	1039	3.78		0.00	946	3.44		0.00
0-7-1	97	0.65	483	3.23		0.00	457	3.05		0.00
0-8-1	894	1.22	4185	5.69		0.00	3713	5.05	58	0.08
0-9-1	443	1.24	1733	4.84		0.00	1444	4.03	32	0.09
0-10-1	65	0.22	464	1.55		0.00	864	2.89		0.00
0-11-1	260	0.75	1369	3.96		0.00	1360	3.93		0.00
AVE		0.99		5.11		0.00		5.56		0.03
SD		0.39		2.10		0.00		2.52		0.04
1-1	177	1.04	1419	8.38		0.00	1913	11.29		0.00
2-1	303	1.33	1596	7.00		0.00	1574	6.91		0.00
3-1	352	1.18	1532	5.12		0.00	1476	4.93	29	0.10
4-1	406	1.30	2296	7.34		0.00	2300	7.35		0.00
5-1	240	0.68	1140	3.22		0.00	1061	3.00		0.00
6-1	421	1.09	2416	6.23		0.00	2563	6.61		0.00
7-1	88	0.33	756	2.82		0.00	1338	4.99		0.00
8-1	265	0.64	1355	3.26		0.00	1472	3.54		0.00
9-1	205	0.97	770	3.65		0.00	534	2.53		0.00
10-1	0	0.00	230	1.27		0.00	674	3.72		0.00
AVE		0.86		4.83		0.00		5.49		0.01
SD		0.41		2.21		0.00		2.51		0.03

15:0		16:0		16:1n7		16:2n4		X	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area%
43	0.10	9016	21.24	1093	2.57		0.00		0.00
42	0.20	4602	22.44	553	2.70		0.00		0.00
58	0.18	8069	24.76	989	3.03		0.00		0.00
37	0.12	7167	23.77	1146	3.80		0.00		0.00
47	0.15	7274	23.33	895	2.87		0.00		0.00
27	0.10	5591	20.34	882	3.21		0.00		0.00
	0.00	3576	23.89	360	2.41		0.00		0.00
81	0.11	15478	21.05	2406	3.27		0.00		0.00
46	0.13	7513	20.98	1347	3.76		0.00		0.00
34	0.11	7286	24.38	820	2.74		0.00		0.00
35	0.10	7392	21.37	1159	3.35		0.00		0.00
	0.12		22.51		3.07		0.00		0.00
	0.05		1.50		0.44		0.00		0.00
29	0.17	4029	23.78	514	3.03		0.00		0.00
26	0.11	4900	21.51	659	2.89		0.00		0.00
44	0.15	7108	23.76	1344	4.49		0.00		0.00
34	0.11	6937	22.16	986	3.15		0.00		0.00
36	0.10	8201	23.17	1081	3.05		0.00		0.00
41	0.11	8522	21.98	1031	2.66		0.00		0.00
30	0.11	6353	23.71	761	2.84		0.00		0.00
50	0.12	8664	20.86	999	2.41		0.00		0.00
	0.00	4605	21.82	651	3.08		0.00		0.00
	0.00	4333	23.91	402	2.22		0.00		0.00
	0.10		22.67		2.98		0.00		0.00
	0.05		1.07		0.58		0.00		0.00

18:0		18:1n9t		18:1n9c		18:1n7		16:4n1	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
2800	6.60	72	0.17	14695	34.62	1051	2.48		0.00
1074	5.24	35	0.17	5786	28.22	597	2.91		0.00
1966	6.03	45	0.14	9897	30.37	897	2.75		0.00
1696	5.62	46	0.15	10469	34.72	921	3.05		0.00
1791	5.74	48	0.15	10526	33.76	945	3.03		0.00
1532	5.57	47	0.17	10647	38.73	815	2.97		0.00
853	5.70		0.00	5760	38.49	420	2.81		0.00
4374	5.95	96	0.13	28186	38.34	1632	2.22		0.00
2104	5.88	65	0.18	13215	36.91	970	2.71		0.00
2066	6.91	44	0.15	11331	37.92	1020	3.41		0.00
2230	6.45	53	0.15	12984	37.54	940	2.72		0.00
	5.97		0.14		35.42		2.82		0.00
	0.47		0.05		3.35		0.30		0.00
961	5.67		0.00	5110	30.16	443	2.61		0.00
1361	5.97	50	0.22	8052	35.34	671	2.94		0.00
1613	5.39	46	0.15	10872	36.35	812	2.71		0.00
1906	6.09	56	0.18	10254	32.76	761	2.43		0.00
2005	5.66	58	0.16	13869	39.18	889	2.51		0.00
2658	6.85	72	0.19	13546	34.93	1002	2.58		0.00
1631	6.09	47	0.18	9857	36.79	745	2.78		0.00
2048	4.93	66	0.16	16507	39.75	1138	2.74		0.00
1434	6.79	40	0.19	8695	41.20	578	2.74		0.00
1094	6.04	34	0.19	6661	36.76	620	3.42		0.00
	5.95		0.16		36.32		2.75		0.00
	0.56		0.06		3.13		0.26		0.00

19:0		18:2n6t		18:2n6c		18:3n6		20:0	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00		0.00	5014	11.81		0.00	94	0.22
	0.00		0.00	1691	8.25		0.00	31	0.15
	0.00		0.00	3476	10.66		0.00	60	0.18
	0.00		0.00	3031	10.05		0.00	48	0.16
	0.00		0.00	3449	11.06		0.00	59	0.19
	0.00		0.00	3447	12.54		0.00	46	0.17
	0.00		0.00	2010	13.43		0.00		0.00
	0.00		0.00	7979	10.85		0.00	140	0.19
	0.00		0.00	4458	12.45		0.00	68	0.19
	0.00		0.00	3362	11.25		0.00	66	0.22
	0.00		0.00	4639	13.41		0.00	59	0.17
	0.00		0.00		11.43		0.00		0.17
	0.00		0.00		1.46		0.00		0.06
	0.00		0.00	1375	8.12		0.00	32	0.19
	0.00		0.00	2211	9.70		0.00	43	0.19
	0.00		0.00	2785	9.31		0.00	50	0.17
	0.00		0.00	3371	10.77		0.00	56	0.18
	0.00		0.00	4376	12.36		0.00	58	0.16
	0.00		0.00	3827	9.87		0.00	77	0.20
	0.00		0.00	2877	10.74		0.00	48	0.18
	0.00		0.00	5754	13.86		0.00	69	0.17
	0.00		0.00	2233	10.58		0.00	40	0.19
	0.00		0.00	2140	11.81		0.00	43	0.24
	0.00		0.00		10.71		0.00		0.19
	0.00		0.00		1.56		0.00		0.02

18:3n3c		20:1n9		18:4n3		21:0		20:2n6	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
359	0.85	332	0.78		0.00		0.00	252	0.59
136	0.66	153	0.75		0.00		0.00	109	0.53
258	0.79	244	0.75		0.00		0.00	217	0.67
315	1.04	204	0.68		0.00		0.00	155	0.51
249	0.80	288	0.92		0.00		0.00	259	0.83
343	1.25	314	1.14		0.00		0.00	171	0.62
127	0.85	133	0.89		0.00		0.00	89	0.59
683	0.93	461	0.63		0.00		0.00	294	0.40
598	1.67	241	0.67		0.00		0.00	160	0.45
265	0.89	475	1.59		0.00		0.00	305	1.02
389	1.12	264	0.76		0.00		0.00	197	0.57
	0.99		0.87		0.00		0.00		0.62
	0.27		0.27		0.00		0.00		0.17
87	0.51	117	0.69		0.00		0.00	79	0.47
177	0.78	188	0.83		0.00		0.00	127	0.56
202	0.68	177	0.59		0.00		0.00	103	0.34
350	1.12	188	0.60		0.00		0.00	147	0.47
322	0.91	365	1.03		0.00		0.00	207	0.58
182	0.47	285	0.73		0.00		0.00	200	0.52
214	0.80	327	1.22		0.00		0.00	216	0.81
334	0.80	475	1.14		0.00		0.00	329	0.79
166	0.79	170	0.81		0.00		0.00	64	0.30
122	0.67	215	1.19		0.00		0.00	218	1.20
	0.75		0.88		0.00		0.00		0.60
	0.18		0.23		0.00		0.00		0.25

20:3n9		20:3n6		22:0		20:3n3		20:4n6	
Area	Area %		Area %	Area	Area %	Area	Area %	Area	Area %
	0.00	202	0.48	31	0.07		0.00	378	0.89
	0.00	125	0.61		0.00		0.00	172	0.84
	0.00	202	0.62		0.00		0.00	297	0.91
	0.00	151	0.50		0.00		0.00	243	0.81
	0.00	227	0.73		0.00		0.00	250	0.80
	0.00	175	0.64		0.00		0.00	295	1.07
	0.00	79	0.53		0.00		0.00	155	1.04
	0.00	373	0.51		0.00		0.00	533	0.72
	0.00	198	0.55		0.00		0.00	304	0.85
	0.00	154	0.52		0.00		0.00	215	0.72
	0.00	143	0.41		0.00		0.00	266	0.77
	0.00		0.55		0.01		0.00		0.86
	0.00		0.08		0.02		0.00		0.11
	0.00	93	0.55		0.00		0.00	153	0.90
	0.00	116	0.51		0.00		0.00	181	0.79
	0.00	187	0.63		0.00		0.00	326	1.09
	0.00	193	0.62		0.00		0.00	245	0.78
	0.00	176	0.50		0.00		0.00	289	0.82
	0.00	263	0.68		0.00		0.00	394	1.02
	0.00	221	0.82		0.00		0.00	263	0.98
	0.00	306	0.74		0.00		0.00	351	0.85
	0.00	126	0.60		0.00		0.00	169	0.80
	0.00	152	0.84		0.00		0.00	238	1.31
	0.00		0.65		0.00		0.00		0.93
	0.00		0.12		0.00		0.00		0.16

22:1n11		22:1n9		20:4n3		23:0		20:5n3c	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00	84	0.20		0.00		0.00	61	0.14
	0.00	42	0.20		0.00		0.00	32	0.16
	0.00	66	0.20		0.00		0.00	51	0.16
	0.00	46	0.15		0.00		0.00	39	0.13
	0.00	71	0.23		0.00		0.00	58	0.19
	0.00	107	0.39		0.00		0.00	43	0.16
	0.00	44	0.29		0.00		0.00		0.00
	0.00	99	0.13		0.00		0.00	70	0.10
	0.00	46	0.13		0.00		0.00	37	0.10
	0.00	196	0.66		0.00		0.00	47	0.16
	0.00	82	0.24		0.00		0.00	40	0.12
	0.00		0.26		0.00		0.00		0.13
	0.00		0.14		0.00		0.00		0.05
	0.00	31	0.18		0.00		0.00		0.00
	0.00	49	0.22		0.00		0.00	38	0.17
	0.00	53	0.18		0.00		0.00	42	0.14
	0.00	48	0.15		0.00		0.00	39	0.12
	0.00	125	0.35		0.00		0.00	39	0.11
	0.00	62	0.16		0.00		0.00	56	0.14
	0.00	127	0.47		0.00		0.00	43	0.16
	0.00	143	0.34		0.00		0.00	71	0.17
	0.00	104	0.49		0.00		0.00		0.00
	0.00	66	0.36		0.00		0.00	44	0.24
	0.00		0.29		0.00		0.00		0.13
	0.00		0.12		0.00		0.00		0.07

22:2n6		22:3n9		22:3n6		24:0		22:4n6	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00		0.00		0.00	37	0.09	103	0.24
	0.00		0.00		0.00	26	0.13	55	0.27
	0.00		0.00		0.00	33	0.10	119	0.37
	0.00		0.00		0.00	41	0.14	77	0.26
	0.00		0.00		0.00	39	0.13	113	0.36
	0.00		0.00		0.00		0.00	84	0.31
	0.00		0.00		0.00		0.00	41	0.27
	0.00		0.00		0.00	25	0.03	134	0.18
	0.00		0.00		0.00		0.00	71	0.20
	0.00		0.00		0.00	41	0.14	83	0.28
	0.00		0.00		0.00	25	0.07	73	0.21
	0.00		0.00		0.00		0.07		0.27
	0.00		0.00		0.00		0.05		0.06
	0.00		0.00		0.00		0.00	53	0.31
	0.00		0.00		0.00		0.00	54	0.24
	0.00		0.00		0.00		0.00	88	0.29
	0.00		0.00		0.00		0.00	57	0.18
	0.00		0.00		0.00		0.00	84	0.24
	0.00		0.00		0.00		0.00	119	0.31
	0.00		0.00		0.00		0.00	113	0.42
	0.00		0.00		0.00		0.00	110	0.26
	0.00		0.00		0.00		0.00	49	0.23
	0.00		0.00		0.00		0.00	179	0.99
	0.00		0.00		0.00		0.00		0.35
	0.00		0.00		0.00		0.00		0.22

24:1		22:5n6		22:5n3		22:6n3		Others	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
80	0.19		0.00		0.00	288	0.68	536	1.26
57	0.28		0.00		0.00	102	0.50	210	1.02
86	0.26		0.00		0.00	182	0.56	434	1.33
57	0.19		0.00		0.00	144	0.48	399	1.32
101	0.32		0.00		0.00	175	0.56	269	0.86
97	0.35		0.00		0.00	164	0.60	426	1.55
23	0.15		0.00		0.00	96	0.64	163	1.09
82	0.11		0.00		0.00	253	0.34	1292	1.76
30	0.08		0.00		0.00	96	0.27	588	1.64
214	0.72		0.00		0.00	80	0.27	385	1.29
82	0.24		0.00		0.00	161	0.47	387	1.12
	0.26		0.00		0.00		0.49		1.30
	0.16		0.00		0.00		0.13		0.26
35	0.21		0.00		0.00	108	0.64	185	1.09
50	0.22		0.00		0.00	115	0.50	244	1.07
45	0.15		0.00		0.00	159	0.53	465	1.55
53	0.17		0.00		0.00	161	0.51	457	1.46
112	0.32		0.00		0.00	165	0.47	497	1.40
64	0.17		0.00		0.00	289	0.75	687	1.77
129	0.48		0.00		0.00	138	0.52	470	1.75
154	0.37		0.00		0.00	141	0.34	728	1.75
41	0.19		0.00		0.00	135	0.64	297	1.41
107	0.59		0.00		0.00	157	0.87	393	2.17
	0.29		0.00		0.00		0.58		1.54
	0.14		0.00		0.00		0.14		0.32

Sum		SFA	MUFA	PUFA	n6	n3	n6/n3	PI	UI
Area	Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %
42447	100.00	42.05	41.01	15.68	14.02	1.67	8.40	22.42	38.10
20505	100.00	51.94	35.23	11.81	10.50	1.32	7.97	17.76	29.57
32593	100.00	46.35	37.58	14.73	13.23	1.51	8.78	21.40	36.13
30155	100.00	42.06	42.84	13.78	12.13	1.65	7.34	19.74	33.52
31176	100.00	42.51	41.29	15.33	13.79	1.55	8.92	21.99	37.32
27487	100.00	34.31	46.96	17.18	15.18	2.00	7.59	24.68	41.86
14966	100.00	36.52	45.04	17.35	15.86	1.49	10.65	23.91	41.27
73521	100.00	39.29	44.91	14.04	12.67	1.37	9.26	18.95	32.98
35807	100.00	37.29	44.53	16.54	14.50	2.04	7.10	22.24	38.78
29882	100.00	36.43	47.19	15.10	13.78	1.31	10.51	20.04	35.13
34589	100.00	36.80	45.00	17.08	15.37	1.71	9.01	22.79	39.87
	100.00	40.50	42.87	15.33	13.73	1.60	8.68	21.45	36.78
	0.00	4.96	3.62	1.62	1.49	0.24	1.12	2.02	3.59
16943	100.00	50.52	36.89	11.50	10.35	1.15	8.99	17.54	29.04
22785	100.00	43.02	42.66	13.25	11.80	1.45	8.15	19.12	32.37
29910	100.00	40.71	44.73	13.01	11.66	1.35	8.66	19.63	32.64
31301	100.00	44.52	39.44	14.58	12.82	1.76	7.30	20.67	35.25
35395	100.00	36.00	46.61	15.99	14.50	1.49	9.76	21.70	37.68
38777	100.00	43.06	41.42	13.75	12.39	1.36	9.11	20.95	34.70
26792	100.00	38.24	44.76	15.25	13.77	1.47	9.34	22.22	37.47
41529	100.00	33.53	46.91	17.81	16.49	1.31	12.55	23.44	41.25
21106	100.00	35.95	48.70	13.94	12.51	1.43	8.77	19.95	33.89
18122	100.00	35.17	44.72	17.93	16.15	1.78	9.06	28.24	46.18
	100.00	40.07	43.69	14.70	13.25	1.455	9.169	21.346	36.046
	0.00	5.01	3.43	1.97	1.88	0.18	1.30	2.79	4.64

MILK FATTY ACID PROFILE AREA % FINAL

	10:0		12:0		13:0		14:0		14:1	
Subject ID	Area	Area%	Area	Area %	Area	Area %	Area	Area %	Area	Area %
0-1-6	248	0.93	873	3.26		0.00	859	3.21		0.00
0-2-6	516	1.59	1876	5.76		0.00	1454	4.47		0.00
0-3-6	238	1.52	950	6.05		0.00	858	5.46		0.00
0-4-6	321	1.48	1444	6.65		0.00	1342	6.18		0.00
0-5-6	283	0.95	1026	3.44		0.00	1107	3.71		0.00
0-6-6	130	0.60	449	2.07		0.00	423	1.95		0.00
0-7-6	167	0.98	599	3.51		0.00	544	3.19		0.00
0-8-6	391	1.06	1468	3.99		0.00	1348	3.66		0.00
0-9-6	281	1.46	1276	6.64		0.00	1072	5.57		0.00
0-10-6	614	1.52	2308	5.71		0.00	1834	4.54		0.00
0-11-6	161	1.12	795	5.51		0.00	700	4.85		0.00
Ave		1.20		4.78		0.00		4.25		0.00
SD		0.31		1.49		0.00		1.19		0.00
1-6	601	0.97	1850	2.97		0.00	1691	2.72	26	0.04
2-6	502	1.62	2060	6.64		0.00	1749	5.63		0.00
3-6	345	0.90	1542	4.03		0.00	1722	4.50		0.00
4-6	467	0.62	2263	3.00		0.00	2278	3.02		0.00
5-6	253	0.83	1151	3.79		0.00	876	2.89		0.00
6-6	710	1.02	3383	4.85		0.00	3694	5.29		0.00
7-6	315	1.31	1350	5.62		0.00	1015	4.22		0.00
8-6	461	1.34	1918	5.56		0.00	1671	4.85		0.00
9-6	435	1.04	1349	3.23		0.00	1033	2.47		0.00
10-6	245	1.13	1068	4.92		0.00	973	4.48		0.00
Ave		1.08		4.46		0.00		4.01		0.00
SD		0.27		1.19		0.00		1.09		0.01

15:0		16:0		16:1n7		16:2n4		X	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area%
	0.00	5692	21.28	729	2.73		0.00		0.00
32	0.10	6130	18.84	786	2.42		0.00		0.00
	0.00	2852	18.16	239	1.52		0.00		0.00
	0.00	4541	20.90	451	2.08		0.00		0.00
	0.00	7279	24.41	559	1.87		0.00		0.00
	0.00	4138	19.10	485	2.24		0.00		0.00
	0.00	3980	23.34	374	2.19		0.00		0.00
50	0.14	8042	21.85	847	2.30		0.00		0.00
	0.00	4009	20.85	487	2.53		0.00		0.00
35	0.09	8334	20.63	1144	2.83		0.00		0.00
	0.00	3039	21.07	378	2.62		0.00		0.00
	0.03		20.95		2.30		0.00		0.00
	0.05		1.77		0.37		0.00		0.00
45	0.07	13885	22.32	1635	2.63		0.00		0.00
30	0.10	5144	16.57	547	1.76		0.00		0.00
42	0.11	8340	21.81	932	2.44		0.00		0.00
41	0.05	14193	18.81	1478	1.96		0.00		0.00
	0.00	5450	17.95	487	1.60		0.00		0.00
42	0.06	13960	20.00	1437	2.06		0.00		0.00
	0.00	4442	18.48	596	2.48		0.00		0.00
	0.00	7104	20.60	654	1.90		0.00		0.00
34	0.08	8278	19.81	1023	2.45		0.00		0.00
	0.00	4812	22.17	502	2.31		0.00		0.00
	0.05		19.85		2.16		0.00		0.00
	0.04		1.82		0.33		0.00		0.00

18:0		18:1n9t		18:1n9c		18:1n7		16:4n1	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
2101	7.86	46	0.17	10553	39.45	894	3.34		0.00
1794	5.51	68	0.21	11582	35.59	901	2.77		0.00
1224	7.79	59	0.38	5081	32.35	361	2.30		0.00
1490	6.86	47	0.22	7287	33.54	567	2.61		0.00
3349	11.23	55	0.18	10177	34.13	686	2.30		0.00
1674	7.73	55	0.25	9017	41.63	635	2.93		0.00
1226	7.19	79	0.46	6558	38.46	521	3.06		0.00
2871	7.80	84	0.23	13306	36.15	942	2.56		0.00
1105	5.75	40	0.21	6956	36.17	669	3.48		0.00
2259	5.59	76	0.19	14981	37.08	1299	3.21		0.00
1023	7.09	32	0.22	5230	36.26	417	2.89		0.00
	7.31		0.25		36.44		2.86		0.00
	1.51		0.09		2.56		0.38		0.00
5009	8.05	121	0.19	25686	41.29	1589	2.55		0.00
2001	6.45	110	0.35	10110	32.57	729	2.35		0.00
2681	7.01	119	0.31	14498	37.92	1008	2.64		0.00
5794	7.68		0.00	29194	38.70	1689	2.24		0.00
1874	6.17	77	0.25	11520	37.94	678	2.23		0.00
5442	7.79		0.00	28709	41.12	1627	2.33		0.00
1453	6.04	56	0.23	9018	37.51	623	2.59		0.00
2779	8.06	25	0.07	12403	35.97	1182	3.43		0.00
2690	6.44	93	0.22	15301	36.61	925	2.21		0.00
1634	7.53	52	0.24	8063	37.15	657	3.03		0.00
	7.12		0.19		37.68	1070.70	2.56		0.00
	0.75		0.12		2.38	405.03	0.37		0.00

19:0		18:2n6t		18:2n6c		18:3n6		20:0	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00		0.00	3100	11.59		0.00	53	0.20
	0.00		0.00	4873	14.97		0.00	70	0.22
	0.00		0.00	1764	11.23		0.00	41	0.26
	0.00		0.00	3090	14.22		0.00	41	0.19
	0.00		0.00	3456	11.59		0.00	62	0.21
	0.00		0.00	2833	13.08		0.00	45	0.21
	0.00		0.00	1846	10.83		0.00	37	0.22
	0.00		0.00	5155	14.01		0.00	83	0.23
	0.00		0.00	2239	11.64		0.00	32	0.17
	0.00		0.00	4827	11.95		0.00	67	0.17
	0.00		0.00	1834	12.71		0.00	28	0.19
	0.00		0.00		12.53		0.00		0.20
	0.00		0.00		1.31		0.00		0.03
	0.00		0.00	6367	10.23		0.00	118	0.19
	0.00		0.00	3595	11.58		0.00	102	0.33
	0.00		0.00	4422	11.56		0.00	72	0.19
	0.00		0.00	9605	12.73		0.00	260	0.34
	0.00		0.00	4865	16.02		0.00	71	0.23
	0.00		0.00	6610	9.47		0.00	134	0.19
	0.00		0.00	3301	13.73		0.00	43	0.18
	0.00		0.00	4311	12.50		0.00	71	0.21
	0.00		0.00	4847	11.60		0.00	120	0.29
	0.00		0.00	2614	12.04		0.00	42	0.19
	0.00		0.00		12.15		0.00		0.23
	0.00		0.00		1.73		0.00		0.06

18:3n3c		20:1n9		18:4n3		21:0		20:2n6	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
591	2.21	275	1.03		0.00		0.00	132	0.49
633	1.94	387	1.19		0.00		0.00	157	0.48
504	3.21	321	2.04		0.00		0.00	86	0.55
323	1.49	150	0.69		0.00		0.00	97	0.45
597	2.00	321	1.08		0.00		0.00	119	0.40
382	1.76	324	1.50		0.00		0.00	108	0.50
279	1.64	203	1.19		0.00		0.00	69	0.40
679	1.84	321	0.87		0.00		0.00	201	0.55
255	1.33	194	1.01		0.00		0.00	88	0.46
569	1.41	413	1.02		0.00		0.00	182	0.45
191	1.32	140	0.97		0.00		0.00	61	0.42
	1.83		1.14		0.00		0.00		0.47
	0.52		0.34		0.00		0.00		0.05
623	1.00	651	1.05		0.00		0.00	285	0.46
1051	3.39	698	2.25		0.00		0.00	157	0.51
531	1.39	358	0.94		0.00		0.00	169	0.44
1527	2.02	1475	1.96		0.00		0.00	357	0.47
755	2.49	596	1.96		0.00		0.00	134	0.44
980	1.40	788	1.13		0.00		0.00	355	0.51
492	2.05	310	1.29		0.00		0.00	106	0.44
522	1.51	356	1.03		0.00		0.00	177	0.51
994	2.38	1042	2.49	37	0.09		0.00	165	0.39
273	1.26	158	0.73		0.00		0.00	99	0.46
	1.89		1.48		0.01		0.00		0.46
	0.69		0.59		0.03		0.00		0.04

20:3n9		20:3n6		22:0		20:3n3		20:4n6	
Area	Area %		Area %	Area	Area %	Area	Area %	Area	Area %
	0.00	59	0.22		0.00		0.00	148	0.55
	0.00	107	0.33		0.00		0.00	236	0.73
	0.00	47	0.30		0.00		0.00	91	0.58
	0.00	77	0.35		0.00		0.00	136	0.63
	0.00	72	0.24		0.00		0.00	142	0.48
	0.00	60	0.28		0.00		0.00	144	0.66
	0.00	37	0.22		0.00		0.00	131	0.77
	0.00	120	0.33		0.00		0.00	209	0.57
	0.00	62	0.32		0.00		0.00	111	0.58
	0.00	143	0.35		0.00		0.00	231	0.57
	0.00	44	0.31		0.00		0.00	77	0.53
	0.00		0.30		0.00		0.00		0.60
	0.00		0.05		0.00		0.00		0.08
	0.00	185	0.30		0.00		0.00	388	0.62
	0.00	83	0.27		0.00		0.00	190	0.61
	0.00	135	0.35		0.00		0.00	236	0.62
	0.00	134	0.18		0.00		0.00	380	0.50
	0.00	68	0.22		0.00		0.00	165	0.54
	0.00	144	0.21		0.00		0.00	343	0.49
	0.00	75	0.31		0.00		0.00	170	0.71
	0.00	84	0.24		0.00		0.00	151	0.44
	0.00	145	0.35		0.00		0.00	313	0.75
	0.00	74	0.34		0.00		0.00	133	0.61
	0.00		0.28		0.00		0.00		0.59
	0.00		0.06		0.00		0.00		0.09

22:1n11		22:1n9		20:4n3		23:0		20:5n3c	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00	62	0.23		0.00		0.00		0.00
	0.00	241	0.74		0.00		0.00	32	0.10
	0.00	537	3.42		0.00		0.00	29	0.18
	0.00	39	0.18		0.00		0.00		0.00
	0.00	197	0.66		0.00		0.00		0.00
	0.00	359	1.66		0.00		0.00		0.00
	0.00	170	1.00		0.00		0.00		0.00
	0.00	60	0.16		0.00		0.00	36	0.10
	0.00	106	0.55		0.00		0.00		0.00
	0.00	227	0.56		0.00		0.00	38	0.09
	0.00	103	0.71		0.00		0.00		0.00
	0.00		0.90		0.00		0.00		0.04
	0.00		0.89		0.00		0.00		0.06
	0.00	224	0.36		0.00		0.00	47	0.08
	0.00	1316	4.24		0.00		0.00	51	0.16
	0.00	225	0.59		0.00		0.00	45	0.12
	0.00	2019	2.68		0.00		0.00	83	0.11
	0.00	672	2.21		0.00		0.00	35	0.12
	0.00	172	0.25		0.00		0.00	55	0.08
	0.00	219	0.91		0.00		0.00	29	0.12
	0.00	169	0.49		0.00		0.00		0.00
	0.00	1769	4.23		0.00		0.00	75	0.18
	0.00	28	0.13		0.00		0.00		0.00
	0.00		1.61		0.00		0.00		0.10
	0.00		1.54		0.00		0.00		0.06

22:2n6		22:3n9		22:3n6		24:0		22:4n6	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00	41	0.13
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00	35	0.10
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00	36	0.09
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00		0.03
	0.00		0.00		0.00		0.00		0.05
	0.00		0.00		0.00		0.00	73	0.12
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00	35	0.09
	0.00		0.00		0.00		0.00	53	0.07
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00	53	0.08
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00	27	0.08
	0.00		0.00		0.00		0.00	43	0.10
	0.00		0.00		0.00		0.00		0.00
	0.00		0.00		0.00		0.00		0.05
	0.00		0.00		0.00		0.00		0.05

24:1		22:5n6		22:5n3		22:6n3		Others	
Area	Area %	Area	Area %	Area	Area %	Area	Area %	Area	Area %
26	0.10		0.00		0.00	43	0.16	263	0.98
90	0.28		0.00		0.00	81	0.25	458	1.41
64	0.41		0.00		0.00	36	0.23	325	2.07
	0.00		0.00		0.00	63	0.29	218	1.00
40	0.13		0.00		0.00	40	0.13	255	0.86
52	0.24		0.00		0.00	42	0.19	307	1.42
34	0.20		0.00		0.00	33	0.19	165	0.97
29	0.08		0.00		0.00	48	0.13	478	1.30
53	0.28		0.00		0.00	34	0.18	160	0.83
115	0.28		0.00		0.00	72	0.18	603	1.49
39	0.27		0.00		0.00	29	0.20	104	0.72
	0.21		0.00		0.00		0.19		1.19
	0.11		0.00		0.00		0.05		0.38
72	0.12		0.00		0.00	152	0.24	892	1.43
225	0.72		0.00		0.00	55	0.18	534	1.72
74	0.19		0.00		0.00	72	0.19	635	1.66
265	0.35		0.00		0.00	103	0.14	1784	2.36
113	0.37		0.00		0.00	43	0.14	479	1.58
74	0.11		0.00		0.00	138	0.20	964	1.38
70	0.29		0.00		0.00	89	0.37	267	1.11
62	0.18		0.00		0.00	43	0.12	310	0.90
212	0.51		0.00		0.00	120	0.29	748	1.79
	0.00		0.00		0.00	37	0.17	241	1.11
	0.28		0.00		0.00		0.20		1.50
	0.20		0.00		0.00		0.07		0.40

Sum		SFA	MUFA	PUFA	n6	n3	n6/n3	PI	UI
Area	Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %	Area %
26747	100.00	36.74	47.05	15.23	12.86	2.37	5.42	19.41	34.64
32545	100.00	36.48	43.19	18.93	16.64	2.29	7.26	24.19	43.12
15707	100.00	39.24	42.41	16.28	12.66	3.62	3.49	22.42	38.70
21724	100.00	42.25	39.32	17.43	15.65	1.78	8.81	21.68	39.11
29822	100.00	43.95	40.36	14.84	12.71	2.14	5.95	18.57	33.41
21662	100.00	31.66	50.44	16.48	14.52	1.96	7.42	20.62	37.10
17052	100.00	38.43	46.56	14.05	12.22	1.83	6.68	18.21	32.25
36803	100.00	38.73	42.36	17.62	15.54	2.07	7.50	21.93	39.54
19229	100.00	40.43	44.23	14.50	13.00	1.50	8.65	18.01	32.52
40407	100.00	38.24	45.18	15.09	13.41	1.68	7.98	19.17	34.26
14425	100.00	39.83	43.94	15.50	13.98	1.53	9.16	19.00	34.50
	100.00	38.73	44.09	15.99	13.92	2.07	7.12	20.29	36.29
	0.00	3.06	3.01	1.43	1.40	0.56	1.59	1.92	3.31
62215	100.00	37.29	48.23	13.05	11.73	1.32	8.88	17.04	30.09
31039	100.00	37.33	44.25	16.70	12.97	3.73	3.48	22.77	39.47
38238	100.00	38.56	45.02	14.76	13.07	1.69	7.71	19.03	33.79
75442	100.00	33.53	47.88	16.23	13.96	2.27	6.15	20.45	36.68
30362	100.00	31.87	46.58	19.98	17.23	2.74	6.28	24.69	44.66
69814	100.00	39.20	46.99	12.43	10.75	1.68	6.40	16.20	28.63
24039	100.00	35.85	45.31	17.73	15.19	2.54	5.99	23.35	41.07
34480	100.00	40.61	43.07	15.41	13.78	1.64	8.41	18.70	34.12
41791	100.00	33.35	48.73	16.13	13.19	2.93	4.50	22.42	38.54
21705	100.00	40.42	43.58	14.88	13.45	1.43	9.42	18.39	33.27
	100.00	36.80	45.96	15.73	13.53	2.20	6.72	20.30	36.03
	0.00	2.91	1.90	2.08	1.68	0.74	1.80	2.73	4.74