RELATIONS OF *TRANS* FATTY ACIDS, *CIS* N-6 AND N-3 FATTY ACIDS IN NEWBORN CORD PLASMA, MATERNAL PLASMA AND DIET

By

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ABSTRACT

Trans fatty acids (TFA) are found in partially hydrogenated oils used in margarines, and in processed foods. Concern has been raised that TFA may inhibit desaturation of 18:2n-6 and 18:3n-3 to 20:4n-6 and 22:6n-3, respectively, which are necessary for normal growth and development. The effect of diet-derived TFA on human growth, however, is unclear. This study estimated intakes and major sources of dietary TFA in 60 women at 28 and 35 weeks gestation using a food frequency questionnaire. Maternal blood was collected at 35 weeks gestation. Cord blood was collected at birth to assess the relation of maternal diet and plasma TFA on cord TFA, n-6 and n-3 fatty acids. The mean estimated TFA intake was 3.8 and 3.4 g/day at 28 and 35 weeks, respectively (1.3% of total energy). The major dietary source of TFA was baked foods. TFA were present in maternal and cord plasma triglycerides (TG), mean $\pm$ S.E., 4.0 $\pm$ 0.2 and 2.9 $\pm$ 0.2%, phospholipids (PL), 2.4 $\pm$ 0.1 and 0.7 $\pm$ 0.0%, and cholesteryl esters (CE), 1.6 $\pm$ 0.1 and 2.0 $\pm$ 0.1% of total fatty acids, respectively. Cord plasma TG TFA was inversely related to 22:6n-3 ($r = -0.36$) and cord plasma TG and CE TFA were inversely related to 20:4n-6 ($r = -0.27$). Cord plasma TG and CE TFA were inversely related to length of gestation ($r = -0.28$ and $r = -0.25$, respectively), whereas TG 20:4n-6 was positively related to length of gestation ($r = 0.5$) and infant birthweight ($r = 0.4$). Conjugated linoleic acid (CLA) was inversely related to length of gestation in TG ($r = -0.44$) and in cord plasma CE to length of gestation ($r = -0.52$), birthweight ($r = -0.35$) and infant length ($r = -0.35$). In summary, this study found a mean TFA intake of <4g/d, with baked foods representing the major food source of dietary TFA. Evidence of an inverse association between cord plasma TFA, length of gestation, and with 20:4n-6 and 22:6n-3 in some lipids, suggests the need for larger prospective studies to determine relations between maternal dietary fat quality and birth outcomes, considering potential confounding dietary and lifestyle variables.

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LIST OF ABBREVIATIONS

TC	total cholesterol
LDL-C	low-density lipoprotein cholesterol
HDL-C	high-density lipoprotein cholesterol
Lp(a)	lipoprotein (a)
LPL	lipoprotein lipase
TFA	trans fatty acids
CLA	conjugated linoleic acid
TG	triglycerides
PL	phospholipids
CE	cholesteryl esters
g	grams
kcal	calorie
FFQ	food frequency questionnaire
18:2n-6	linoleic acid
18:3n-3	alpha-linolenic acid
20:4n-6	arachidonic acid
20:5n-3	eicosapentaenoic acid
22:6n-3	docosahexaenoic acid

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1 Introduction

Trans fatty acids (TFA) are formed during the process of hydrogenating vegetable oils which converts naturally occurring *cis* fatty acid isomers into a variety of *trans* fatty acid isomers, as well as *cis* positional isomers and saturated fatty acids. The hydrogenation process improves the oxidative stability and shelf life of unsaturated vegetable oils. Hydrogenated and partially hydrogenated oils are used in margarines, shortenings, snack items and in a variety of baked and prepared foods made with these fats. Dietary *trans* fatty acids, however, have been shown to have adverse effects on certain risk factors for cardiovascular health, which include elevated low-density lipoproteins (LDL), decreased high-density lipoproteins (HDL) (Mensink 1990, Zock 1992), and elevated lipoprotein (a) (Lp (a)) (Aro 1997, Nestel 1992, Sundram 1997). It has also been suggested that dietary TFA may have adverse effects on fetal growth and development (Carlson 1997). Findings from studies using animals and human fibroblasts have suggested that TFA inhibit n-6 and n-3 fatty acid metabolism, specifically the desaturation of linoleic acid (18:2n-6) and linolenic acid (18:3n-3) to arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3), respectively (Zevenbergen 1988, Rosenthal 1984). Arachidonic acid (20:4n-6) is an important fatty acid in cell membranes, in cell signaling and as a precursor for synthesis of eicosanoids. Docosahexaenoic acid (22:6n-3) is also found in high levels in cell membranes, particularly the non-myelin membranes of the central nervous system, including the brain (Innis 1991). Both 20:4n-6 and 22:6n-3 are, therefore, important in fetal growth. At the present time there is a paucity of information on TFA and fetal development. Some evidence to show placental transfer of TFA has, however, been published. For example, Koletzko and Muller (1990) reported that levels of TFA in term infant cord and maternal blood were similar, suggesting transfer of TFA. Other studies have also reported similar levels of TFA in maternal and fetal plasma, supporting the hypothesis that TFA are transported from the mother to the fetus (Ayyagari 1996, Berghaus 1998, Billeaud 1998). Thus, if TFA have an inhibitory effect on n-6 and n-3 fatty acid metabolism, it seems possible that maternal dietary TFA, and subsequent fetal TFA exposure, may adversely affect the growth and development of the fetus. The amount of dietary TFA required to decrease plasma 20:4n-6 and 22:6n-3 to levels likely to have adverse effects on growth and development may be much greater than dietary TFA intakes. To date, however, no studies have quantified maternal dietary TFA intake or looked at the relations between maternal dietary TFA intake and fetal plasma levels of TFA. This study determined the level of TFA, n-6 and n-3 fatty acids in cord blood in relation to maternal TFA intake and maternal blood levels of TFA during gestation.

2 LITERATURE REVIEW

2.1 Definition and Classification of Fatty Acids

Fatty acids are carbon chains with a carboxyl group (COOH) at one end and a methyl group (CH_3) at the other end. The number of carbons in a fatty acid can range from 4 to up to 30 carbons or more. When only single bonds are present between adjacent carbons, the fatty acid is saturated. When one or more double bonds are present between two adjacent carbons, the fatty acid is unsaturated. A monounsaturated fatty acid contains one double bond in the carbon chain, whereas a polyunsaturated fatty acid contains more than one double bond in the carbon chain **(Figure 2.1)**.

Fatty acids are commonly referred to by the number of carbons present in the carbon chain, followed by a colon and a number indicating the number of unsaturated bonds. A saturated fatty acid, therefore, will have the number of carbons present in the carbon chain followed by a colon and a '0' indicating no unsaturated bonds. In unsaturated fatty acids, the number of unsaturated bonds is followed by an 'ϖ' or 'n' and a number to indicate at which carbon from the methyl end the first unsaturated bond is positioned. For example 18:2n-6 would indicate 18 carbons in the fatty acid with 2 double bonds, of which the first double bond would be 6 carbons from the methyl end of the fatty acids. The position of the double bond in an unsaturated fatty acid may also be indicated by the number of carbons away from the delta (Δ) end, also known as the carboxyl end of the fatty acid. For example $\Delta^9$18:1 would indicate a fatty acid with 18 carbons and one double bond 9 carbons from the carboxyl or Δ end. If there is more than one unsaturated bond in the carbon chain, the unsaturated bonds are separated by a methyl group. Methyl groups, however, do not separate unsaturated bonds of conjugated linoleic acid (CLA); rather the double bonds are adjacent to each other in the carbon chain (**Figure 2.1**). When the hydrogen atoms of a double bond are on the same side of the double bond, the bond is referred to as a *'cis'* bond. When the hydrogen atoms are on opposite sides of the double bond, the bond is referred to as a *'trans'* bond (**Figure 2.2**). For example, 18:2n-6, an 18 carbon fatty acid with 2 double bonds may have one double bond in the *cis* position and the second double bond in the *trans* position. These would be referred to as geometric isomers of 18:2n-6. Positional isomers of unsaturated fatty acids also occur, in which the double bond shifts from the original carbon position to another carbon in the fatty acid. *Trans* fatty acids are fatty acids that contain one or more *trans* bonds and may be geometrical or positional isomers of unsaturated fatty acids.

Figure 2.1 Saturated, monounsaturated and polyunsaturated fatty acids

Saturated fatty acid

$$CH_3 - CH_{2\,(16)} - COOH$$

Stearic acid, 18:0

Monounsaturated fatty acid

$$CH_3 - CH_{2\,(7)} - CH = CH - CH_{2\,(7)} - COOH$$

Oleic acid (18:1n-9 or Δ^{9}18:1)

Polyunsaturated fatty acid

$$CH_3 - CH_{2\,(4)} - CH = CH - CH_2 - CH = CH - CH_{2\,(7)} - COOH$$

Linoleic acid (18:2n-6 or $\Delta^{9,12}$18:2)

$$CH_3 - CH_{2\,(5)} - CH = CH - CH = CH - CH_{2\,(7)} - COOH$$

Conjugated linoleic acid (18:2n-7 or $\Delta^{9,11}$18:2)

Figure 2.2 ***Trans*** **and** ***cis*** **bonds**

Trans **bond** ***Cis*** **Bond**

2.2 Formation of *Trans* Fatty Acids

The major dietary source of TFA is hydrogenated and partially hydrogenated vegetable oils. Hydrogenated and partially hydrogenated oils are formed when a vegetable oil is mixed with hydrogen atoms under high temperature and pressure in the presence of a catalyst such as nickel. The hydrogen atoms react with the fatty acids at the *cis* bonds and with complete hydrogenation *cis* unsaturated bonds are changed to saturated bonds (Stender 1995). The initial binding of the catalyst to the double bond is not generally stable and may result in the formation of *trans* double bonds. As the hydrogenation process continues, the fatty acid profile of the vegetable oil changes. Once hydrogenated, vegetable oils that initially contained 18:1, 18:2n-6 and 18:3n-3 will have significant levels of *trans* 18:1, 18:2 and 18:3 isomers with lower levels of *cis* 18:2n-6 and 18:3n-3 than the original oil. *Trans* 18:1 (elaidic acid) and *trans* 18:2 are usually the major TFA formed during hydrogenation (Stender 1995). A variety of *trans* 18:1 and 18:2 isomers may be formed including positional isomers of *trans* 18:1 and 18:2 and geometrical isomers such as *cis, trans* 18:2, *trans, cis* 18:2 or *trans, trans* 18:2 isomers.

The formation of the *trans* double bond results in a linear and more rigid carbon chain that is energetically more favorable and less susceptible to oxidative degradation than its *cis* configuration (Stender 1995, Welch 1992). In addition, the melting point of TFA is lower than for *cis* fatty acids, but lower than for saturated fatty acids of equal carbon length. For example, the melting points of 18:0 (stearic acid), 18:1n-9 (oleic acid) and *trans* 18:1n-9 (elaidic acid) are 70°C, 44°C and 13°C, respectively. Because of the higher oxidative and thermal stability, food manufacturers use hydrogenated oils rather than *cis* polyunsaturated and monounsaturated oil as the major oil source in a variety of baked and prepared foods.

2.3 Dietary Sources of *Trans* Fatty Acids

An assessment of TFA availability in the United States (U.S.), reported that significant sources of TFA in the diet are margarines, spreads, food service and industrial fats and oils (Hunter 1991). Food service fats and oils were described by Hunter and Applewhite (1991) as oils used in deep-frying fish, chicken and french fries, while industrial fats and oils are those used in the production of salted snacks, cookies, crackers and par-fried French fries. The level of TFA in foods in the Canadian market, i.e. salted snacks, cookies and crackers are similar to the levels reported for similar foods in the U.S. (Carlson 1997, Ratnayake 1991). According to Hunter and Applewhite (1991), many margarines and spreads have been modified in the last decade to contain lower levels of TFA. These products, however, remain a significant dietary source of TFA. The mean total content of TFA in stick and tub margarine in the U.S. in the early 1990's was estimated at 27% and 17.3% of their total fat, respectively, (Carlson 1997) while in the same time period Canadian stick and tub margarine contained 34.2% and 20.4%, respectively (Ratnayake 1991).

In addition to hydrogenated fats and oils, meats, dairy products and to a lesser extent, certain plants also contribute TFA to the diet. Bacterial hydrogenation of fatty acids in the rumen of ruminants (cattle, goat and sheep), leads to the presence of TFA in both the animal body fat and their milk (Keweloh 1996). The content of TFA in the body fat of ruminants ranges from 4-11% of the total fatty acids. The TFA level in milk fat is dependent on the animal's diet and can range from 2% to 9% of total fatty acids, (Keweloh 1996). In contrast to hydrogenated oils and shortenings in which the major *trans* isomer is elaidic acid (*trans* 18:1n-9) (Etherton 1995) the major *trans* isomer present in milk, butter, beef fat is vaccenic acid (*trans* 18:1n-11) (Sommerfeld 1983).

Trans fatty acids are not generally found in plants. Small amounts of TFA, however, have been detected in some plant seed oils as well as in plant leaves. Six species of plants have been identified as sources of TFA, including pomegranates, peas, cabbage, lettuce, leeks and spinach. The most common *trans* isomer in plants is 3-*trans* hexadecaenoic acid (16:1,3t) (Sommerfeld 1983).

Human milk can also be a source of TFA, depending on the mother's diet. A case-control study using eight lactating women demonstrated that as hydrogenated fat increased in the diet, the level of TFA, specifically isomers of 18:1 and 18:2, increased in breast milk (Craig-Schmidt 1984). Chen et al (1995) measured the TFA levels in 198 Canadian human milk samples. The major *trans* isomer present in these Canadian human milk samples was 18:1 with a mean of 5.9 (range 0.1-15.4%) of total fatty acids. The mean total TFA of human milk samples reported by Chen et al (1995) was 7.2% (range 0.1-17.2%) of total fatty acids. Studies in Spain have reported mean TFA level in human breast milk of 1.23% total fatty acids (Boatella 1993). This is probably reflective of a low mean dietary TFA intake in Spain of 2.4 g/person/day, as reported in 1988 by the United Kingdom Nutritional Consultative Panel (San Juan 1996) than in Canada. Canadian data on TFA intake, however, has not been published.

2.4 Variation of *Trans* Fatty Acid Levels in Food Sources

There is considerable variability in the TFA content of foods containing hydrogenated and partially hydrogenated fats or oils. The variability of TFA in foods is evident not only between different foods such as crackers and cookies, but also within similar foods, such as two types of cookies. Enig et al (1984) reported a wide variation of TFA within a variety of similar foods in the United States. The average amount of TFA, as a percentage of total fatty acids in the food ranged from 0.2 to 23.6% in breads and rolls, 8.1 to 32.7% in breading mixes and fried crusts, 1.9 to 29.0% in crackers, from trace amounts to 35.1% in french-fried potatoes, and from trace amounts to 32.1% in pastries and pastry crusts. Ratnayake et al (1991) and Ratnayake et al (1993) also reported a wide variation within food categories, such as cereals (9.2-33.7%), cookies (7.6-38.7%), crackers (13.8-35.4%), muffins (16.5-24.2%), print margarines (26-50%) and tub margarines (0-36%) in Canada. The amount of TFA in a variety of foods purchased in Vancouver, British Columbia, Canada has also been analyzed. Foods analyzed included bread (whole wheat and white), breaded chicken, breakfast cereals, cookies, muffins, pie shells and tub margarines. The average amount of TFA as a percentage of total fatty acids within these food categories, as found by others (Enig 1984), also varied widely. The percentage of TFA ranged from; in breads 1.0-36.3%, breaded chicken 11.9-56.7%, breakfast cereals 0.2-24.3%, cookies 1.4-45.7%, muffins 1.7-36.2%, pie shells 1.9-45.6% and tub margarines 1.1-44.4% (Innis 1999).

There are several explanations for the variability in the *trans* fatty acid content of foods containing hydrogenated oils. First, the production of hydrogenated oils can result in a variable content of *trans* fatty acids. Temperature, gas pressure, type and amount of catalyst, and agitation may all affect the resulting *trans* fatty acid content of the oil . Second, food producers may use single hydrogenated, partially hydrogenated or non-hydrogenated fats or oils, or many possible combinations of both hydrogenated, partially hydrogenated and non-hydrogenated fats and oils to achieve the desired final product characteristics. Finally, the use of hydrogenated and non-hydrogenated fats and oils in food products can also be expected to vary with national and regional domestic availability, and costs of various edible oils.

2.5 Estimated Consumption of *Trans* Fatty Acids

Several methods have been employed to estimate the TFA intake in the U. S. and Europe (ASCN/AIN Task Force on Trans Fatty Acid, 1996). No data have been published to describe the TFA intake of Canadians. Due to the widespread cultural and ethnic diversity in the U.S. and Canada and the apparent similarities in the TFA content in the food supply, it is reasonable to consider that TFA intakes in Canada and the U.S. may be similar.

The amounts of TFA in the U.S. diet has been estimated using several methods, including food disappearance data, food availability data, diet records, food frequency questionnaires (FFQ), and adipose tissue fatty acid analysis (ASCN/AIN Task Force on Trans Fatty Acid, 1996). Estimations of dietary TFA intakes obtained from food disappearance and availability data have given the highest values for TFA intakes, with estimates ranging from 8.1 -12.8 g/day/capita. This represented approximately 10% of total fat intake, and 2-4% of total energy intake (Enig 1990, Hunter 1991). Several problems exist when using food disappearance and availability data to estimate dietary intakes that may result in an over-estimation of actual per capita TFA intake. These problems include foods used but not consumed, for example oil discarded post-frying, restaurant, catering and home food wastage, and the wide variation in dietary intakes among individuals (Carlson 1997). Estimates of TFA intakes based on diet records and FFQ tend to give lower estimates than estimates based on food disappearance and availability data. Estimates of TFA intake based on diet records and FFQ, however, seem to be the most consistent among studies. For example, mean estimates of TFA intakes using diet records or FFQ have ranged from 2.6 – 5.3 g/person/d, representing a mean 5-7% of total fat intake (Allison 1999, London 1991, Troisi 1992, van den Reek 1986). Errors that occur when obtaining estimates of intake from dietary records, however, include problems associated with memory, accuracy of recall or recording and estimating portion sizes (Gibson 1990). These errors may, therefore, result in lower estimates of dietary TFA than actual intakes of TFA.

Trans fatty acid intakes have also been estimated from analysis of TFA in adipose tissue fatty acids. The analysis of adipose tissue TFA reflects long-term (months to years) TFA intake (London 1991). Katan et al. (1986) and van Staveren et al (1986) measured TFA levels in subcutaneous fat of Dutch women. The latter studies (Katan 1986, van Staveren 1986), found that the *trans* 18:1 isomers were the most prevalent *trans* isomers in adipose tissue, and that the levels of *trans* 18:1 in adipose tissue correlated with dietary intake of *trans* 18:1 isomers. Similar findings were also reported for women in the U.S. (London 1991) and for Canadian adults (Chen 1995).

The choice of database used to analyze the TFA content of foods can be a considerable source of error in the estimates of TFA intakes among and within different studies. Some databases use TFA values of specific food brands whereas other databases use average values for a food using TFA values from more than one brand. This may result in two distinctly different values for the same food . As reported in Innis and Green (1999) the use of an average TFA value for a food or a food group rather than the actual value for the food eaten, can lead to gross underestimation or overestimation of the true TFA intake. Consequently, it is important to obtain information on the actual food brands consumed when assessing an individual's dietary TFA intake. If the TFA content of a food brand has not been determined, an alternative analyzed food with the same amount and type of fat can be used (Innis 1999). Another source of variation in estimating TFA intake is the time in which the dietary data is collected. A significant change in the restaurant and par-frying operations during 1989 and 1990 was predicted to increase the TFA availability to consumers. These operations began to substitute partially hydrogenated vegetable oils for edible tallow in their deep frying methods. This switch in oils was implemented to lower the saturated fatty acid content of deep fried or par-fried foods (Hunter 1991). Data collected and analyzed prior to the late 1980's may, therefore, give lower estimates of TFA intakes than data collected and analyzed following 1989.

2.6 *Trans* Fatty Acid Absorption and Metabolism

The absorption and metabolism of TFA has been reported to be similar to that of *cis* unsaturated fatty acids. Geometrical isomerization does not appear to alter the hydrolysis of fatty acids from triglycerides via lipoprotein lipase (Emken 1979). Once digested and absorbed, TFA may be oxidized for energy or acylated to triglycerides, phospholipids or cholesteryl esters. *Trans* fatty acids have been suggested to have an inhibitory effect on the Δ 6 and Δ 5-desaturase enzymes. The Δ 6 desaturase enzyme is responsible for the desaturation of linoleic acid (18:2n-6) to γ-linolenic acid (18:3n-6) and α-linolenic acid (18:3n-3) to 18:4n-3. A Δ 6 desaturation is also involved in the conversion of 24:4n-6 to 24:5n-6, and of 24:5n-3 to 24:6n-3. It has been suggested that the enzyme responsible for this latter desaturation may be different than the Δ 6 desaturase responsible for the conversion of 18:2n-6 to 18:3n-6 and of 18:3n-3 to

18:4n-3 (Sprecher 1995). The Δ 5 desaturase enzyme is responsible for the desaturation of 20:3n-6 to arachidonic acid (20:4n-6), and the desaturation of 20:4n-3 to eicosapentaenoic acid (20:5n-3).

Cook and Emken (1990) and Rosenthal and Doloresco (1984) reported that *trans* isomers were potent inhibitors of Δ 5-desaturation activity in glioma cells and human fetal skin fibroblasts, respectively. Rosenthal and Doloresco (1984) incubated radioactively labeled 20:3n-6 with *trans* isomers of 16:1, 18:1, 18:2 or 20:1 in media containing normal human skin fibroblasts. The cells were harvested at 1, 2, 4, 6, 24 and 48 hours post-incubation. The amount of radioactively labeled 20:3n-6 incorporated into cellular lipids and the amount of 20:3n-6 desaturated to 20:4n-6 was then determined. The lowest amounts of radiolabeled 20:4n-6 was present in the media containing *trans* 18:1 isomers, followed by the media containing *trans* 18:2 isomers. A dose-dependent inverse relation was seen between the levels of *trans* 18:1 isomers and the amount of 20:4n-6 present in the media. At levels of *trans* 18:1 isomers (0.6 μM), of one-fourth the concentration of 20:3n-6, significantly decreased levels of 20:4n-6 were present in the media when compared to cell incubated without *trans* 18:1 isomers. The effect of *trans* 18:2 isomers on synthesis of 20:4n-6 was minimal at concentrations up to ten times that of 20:3n-6. Rosenthal and Doloresco (1984), thus concluded that *trans* 18:1 isomers were more effective at inhibiting the Δ 5-desaturase enzyme than *trans* 18:2 isomers.

In a similar study by Cook and Emken (1990) glioma cells were incubated with radiolabeled 18:2n-6 and 18:3n-3 and *trans* isomers of 18:1 or 18:2 at 40-100 μM concentrations. Cell cultures containing 18:2n-6 and 18:3n-3 were harvested after 4 and 2 hours incubation, respectively. The amount of 18:2n-6 and 18:3n-3 incorporated into cell tissues and the products of desaturation and chain elongation, 20:3n-6, 20:4n-6 and 20:5n-3 were then determined. The glioma cells rapidly incorporated 18:2n-6 and 18:3n-3 into tissue phospholipids and triglycerides. Incubation with *trans* 18:1 isomers at 40 μM, decreased the percent 20:4n-6 formed from 18:2n-6 by 60% relative to the cells incubated with 18:2n-6 without *trans* 18:1 isomers. When glioma cells were incubated with *trans* 18:2 isomers, the formation of 20:4n-6, but not that of 20:3n-6, from 18:2n-6 was decreased, suggesting that *trans* 18:2 inhibits the Δ 5, but not the Δ 6-desaturase. Inclusion of *trans* 18:1 or 18:2 isomers in the media, however, had no effect on the metabolism of 18:3n-3. The results of this study suggest that *trans* isomers of 18:1 and 18:2 at concentrations of 40 μM have an inhibitory effect on the Δ 5 desaturation of 18:2n-6. The concentrations of 18:2n-6 and 18:3n-3, however, were not reported in the studies by Cook and Emken (1990). Thus, consideration of the relative amounts of 18:2n-6 and 18:3n-3, with respect to the effects TFA have on n-6 and n-3 fatty acid desaturation is not possible.

Extrapolation of the results of in vitro studies to the multi-organ system, or whole body, is difficult. For example, in vivo, a variety, not individual fatty acids are present, together with a variety of hormonal and dietary factors which can influence Δ 5 or Δ 6 desaturation. Further, intracellular concentrations of unesterified fatty acids for desaturation in the endoplasmic reticulum are not yet clear. If inhibition of Δ 5 or Δ 6 desaturase occurs in the presence of TFA, the extent of inhibition is likely to be determined in part to the relative concentrations of TFA and of the substrates 18:2n-6 and 18:3n-3. Further, the inhibitory effect of the desaturation of 18:2n-6, but not of 18:3n-3, suggests that 18:2n-6 competes for the desaturase enzyme over 18:3n-3. If inhibition of the desaturase enzyme occurs in the presence of TFA, lower levels of both

20:4n-6 and 22:6n-3 would be expected. The effect of inhibiting the desaturation of 18:2n-6 and 18:3n-3 to 20:4n-6 and 22:6n-3, respectively, to alter physiological function, however, is unknown. Several in vivo studies (Anderson 1975, Zevenbergen 1988) have examined the effect of varying dietary levels of TFA and 18:2n-6 on levels of 20:3n-6 and 20:4n-6 in rat liver. Anderson et al (1975) fed rats a diet containing 20% energy from fat with *trans* 18:2 isomers at levels of 1.2, 2, 5, 10 or 19%, and 18:2n-6 at 3% of total dietary fatty acids for six weeks. Fatty acid analysis was completed on total lipid extracts of liver tissue. The proportion of 20:4n-6 in the liver lipids decreased with increasing *trans* 18:2 isomers, specifically the 18:2 isomers with two *trans* bonds. This geometrical isomer, however, is produced in very small amounts in the diet, therefore, any effect, if present in humans is likely to be minimal.

Similarly, Zevenbergen et al (1988) fed rats a diet with 40% energy from fat containing 0.4, 0.8, 2, 3, 5, or 7.1% of energy from 18:2n-6, and 20% of energy from *trans* 18:1 and 18:2 isomers. The fatty acid composition of liver mitochondrial membrane lipids was measured after 13-14 weeks of dietary treatment. Rats fed 0.4, 0.8 and 2% 18:2n-6 had slightly lower 20:4n-6 levels than the rats fed a TFA-free diet, however, the differences were not statistically significant. No effect of TFA on 20:4n-6 levels was found in rats fed 5 or 7% energy from 18:2n-6. Despite lower levels of 20:4n-6 in the rats fed 2% or less energy from 18:2n-6, mitochondrial function, as measured by oxygen uptake and ATP synthesis, was not different from that of rats fed 5 and 7% energy from 18:2n-6. These findings suggest that *trans* 18:1 and 18:2 isomers have little effect on mitochondrial function even though *trans* 18:1 and 18:2 isomers may decrease 20:4n-6 in membrane phospholipids.

Lawson et al (1983) studied the effect of feeding pregnant rats and their newborn pups with a diet containing approximately 42% energy from total fat with or without TFA. *Trans* 18:1 in the control diet and the TFA diet accounted for 2.4% and 33.2% of total fatty acids, respectively. The level of 20:4n-6 in the newborn pup liver phospholipid was 35% lower in the group born to rats fed a diet with TFA than pups born rats fed a diet with no TFA. The level of 18:2n-6, 18:3n-3, 18:3n-6 and 20:3n-6, however, were higher in the TFA group compared to the control group. The level of liver phospholipid 20:5n-3, was also higher in the TFA group than in the control group despite equal dietary amounts of 18:3n-3 in both groups. This suggests that rather than inhibiting the Δ 5 desaturase enzyme TFA favored the desaturation of 18:3n-3 rather than 18:2n-6.

In summary, the in vitro studies of Cook and Emken (1990) indicates that the *trans* isomers of both 18:1 and 18:2 can decrease production of 20:4n-6 from 20:3n-6. Rosenthal and Doloresco (1984), however, reported that *trans* 18:1, but not *trans* 18:2 isomers decreased synthesis of 20:4n-6 from18:2n-6 by cultured human fibroblasts. Studies with rats have reported that diets containing *trans, trans* 18:2 result in lower liver total lipid 20:4n-6 (Anderson 1975). Similarly, others have found lower liver phospholipid 20:4n-6 in rats fed diets containing a mixture of *trans* 18:1 and 18:2 (Zevenbergen 1988). The relevance of the findings by Anderson et al (1975), Cook and Emken (1990) and Zevenbergen et al (1988) to humans, is unclear.

2.7 Fatty Acids and Growth and Development

Arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) are important fatty acids with many functions. Arachidonic acid is required as an integral component of cell membranes and is involved in cell signaling, a mechanism that stimulates the production of certain proteins. In addition to cell membrane functions, 20:4n-6 is also important as a precursor to eicosanoids, a group of compounds that have a variety of functions, for example in inflammation, immune response and blood clotting. Docosahexaenoic acid, like 20:4n-6, is also important as a component of cell membranes, particularly within parts of the retina and the brain (Innis 1991). Because of the importance of these fatty acids in cell structure and function, it would be expected that an increase in both 20:4n-6 and 22:6n-3 would be present in situations of growth, such as during gestation.

Several studies have examined the relations between 20:4n-6 and 22:6n-3 in newborns and birth outcomes, such as length of gestation and birthweight. Foreman van Drongelen et al (1995) reported a positive relation between arterial cord vessel wall 20:4n-6 and length of gestation in pre-term infants. Similarly, Koletzko and Braun (1991) and Leaf et al (1992) reported a positive relation between birthweight and umbilical cord plasma 20:4n-6 in pre-term infants. Leaf et al (1992) also reported an association between 22:6n-3 and birthweight, head circumference and length of gestation in preterm infants. Woltil et al (1998) suggested that cord red blood cell 20:4n-6 was representative of in utero growth, rather than postnatal growth because lower levels of 20:4n-6 was present in term and small for gestational age infants than in pre-term infants. This, however, may suggest that certain fatty acids, such as 20:4n-6, are transferred at higher amounts by the placenta at certain periods within gestation rather than continuously throughout gestation. For example, 20:4n-6 is a constituent of cell membranes and, therefore, important during phases of growth when cell membranes are being made.

Several authors have reported a higher percentage of 20:4n-6 and 22:6n-3 in cord plasma than in maternal plasma (Al 1995, Otto 1997) suggesting that these fatty acids are either transported to or produced by the fetus. Although Δ 5 and Δ 6 desaturase activity has been detected in human fetal liver tissue (Rodriguez 1998), the relative significance of these enzymes to 20:4n-6 and 22:6n-3 produced in the fetus is not known. The fetus may, however, obtain 20:4n-6 and 22:6n-3 from the mother and therefore desaturase activity in the fetal liver may not contribute significantly to levels of 20:4-6 and 22:6n-3 in the fetus.

In addition to the importance of 20:4n-6 and 22:6n-3 in the developing fetus, 20:4n-6 and 20:5n-3, the precursor to 22:6n-3 are precursors to biologically active compounds called eicosanoids. The eicosanoids produced by 20:4n-6 are involved in a variety of functions such as vasoconstriction, platelet aggregation and more recently have been suggested to have a role as a promoter of parturition (Olsen, 1991). In contrast, the eicosanoids produced by 20:5n-3 are less involved in vasoconstriction and platelet aggregation, therefore, causing diminished platelet aggregation and prolonged bleeding. A study was conducted on women from the Faroese Islands because they tended to have longer pregnancies and infants with higher birthweights than women in Denmark. Olsen et al (1991) suggested that the higher birthweights and longer lengths of gestation in the Faroese women were due to higher intakes of marine fats such as whale and fish. Marine fats are rich sources of n-3 fatty acids, such as 20:5n-3 and 22:6n-3. It was speculated that n-3 fatty acids would decrease the production of the prostaglandins that are involved in parturition, specifically the prostaglandins produced by 20:4n-6. The shift in the balance of prostaglandins produced would thereby prolong gestation and subsequently increase birthweight. Olsen et al (1992) reported that

Danish women randomized to a fish oil supplement had a mean gestation of 283 days and infant birthweight of 3571 g, while women who received an olive oil supplement had a slightly lower mean gestation of 279 days and infant birthweight of 3445 g. The women who received a placebo had a mean gestation of 282 days and infant birthweight of 3504 g. Although there was a significant difference in mean duration of gestation between the fish oil and olive group, the infants born to women in both treatment groups and the control group were born within the parameters of a term pregnancy (38-42 weeks). An important observation, however, was that 12% of women receiving fish oil (n=32), 8% of women receiving olive oil (n=11) and 12% of women in the control group (n=16) had a duration of gestation longer than term (>42 weeks). A longer duration of gestation provides more time for the fetus to grow, but is also associated with decreased amniotic fluid levels and increased complications for delivery. Therefore, a duration of gestation greater than term (>42 weeks) does not necessarily benefit the fetus or mother and may potentially create more problems than benefits.

Inhibition of the Δ 5 and Δ 6 desaturation of 18:2n-6 and 18:3n-3 by TFA can be expected to result in an inverse relation between the levels of 20:4n-6, 20:5n-3 and 22:6n-3 and the levels of TFA in the newborn. This relation, however, assumes that the mother's diet is devoid of dietary sources of 20:4n-6 and 22:6n-3 such as meat, eggs and fish and that a similar inhibition of 20:4n-6 and 22:6n-3 synthesis occurs in the mother. Several investigators have examined the relations between TFA and 20:4n-6 and 22:6n-3 in newborns and children aged 1 –15 years (Decsi 1995, Koletzko 1992). The percent TFA in cholesteryl ester (CE) but not phospholipid (PL) showed an inverse correlation with the percent 20:4n-6 ($r = -0.38$, $p<0.05$) and 22:6n-3 ($r = -0.53$, $p<0.01$)

in 29 newborn premature infants (Koletzko 1992). In contrast, Decsi and Koletzko (1995), reported a significant inverse relation between *trans* 18:1 isomers and 20:4n-6, and between total TFA and 20:4n-6 in plasma PL but not CE of 53 healthy children (Desci 1995). Koletzko (1992) reported an inverse relation between birthweight and *trans* 18:1 isomers in plasma CE ($r = -0.40$, $p<0.05$) and plasma PL ($r = -0.42$, $p<0.01$) but not in plasma triglyceride (TG) of pre-mature infants. No statistically significant relations between the length of gestation and plasma TFA, however, have been reported in the literature. The statistical relation between TFA in plasma PL and CE and birthweight, however, can not be interpreted as evidence of a cause and effect relation. The absence of an inverse relation between TFA and 20:4n-6 in plasma PL (Koletzko 1992) provides no support for a hypothesis that TFA inhibits the synthesis of 20:4n-6. No studies on the possible associations between TFA in plasma TG, PL or CE and birthweight have been reported on term infants.

It should be noted that data reported on the relations between TFA and 20:4n-6 and 22:6n-3 are inconsistent and have been conducted primarily by one group of researchers in Germany. To establish whether the significant findings reported by Koletzko (1992), and Desci (1995) can be generalized to other groups of individuals, similar studies need to be conducted in other regions of the world.

2.8 Health Concerns About *Trans* Fatty Acids

2.8.1 Cardiovascular Disease

It has been suggested, based on epidemiological studies, that the development of certain diseases, including heart disease, may begin in utero (Barker 1998). Plasma cholesterol levels are typically low in the human fetus, but levels rapidly increase following birth (Van Biervleit 1986). It is not known whether TFA in the fetus plasma affect cholesterol levels in the fetus or following birth. In adults, elevated low-density lipoprotein cholesterol (LDL-C) is considered a risk factor for heart disease (Etherton 1995). Dietary TFA intakes have been positively correlated with plasma LDL-C, leading to the suggestion that dietary TFA may be associated with an increased risk of cardiovascular disease (Katan 1995). Epidemiological studies have identified a positive relation between hydrogenated fat intake and incidence of cardiovascular disease (Booyens 1988, Kromhout 1995). Although a relation between dietary TFA intake and incidence in heart disease has been reported (Booyens 1988, Kromhout 1995), the results of several case-control studies (Aro 1995, Bolton-Smith 1996, Roberts 1995) have found no relations between TFA intake and cardiovascular disease. Roberts et al (1995) found no relation between 18:1 or 18:2 *trans* isomers in adipose tissue and the incidence of sudden cardiac death. Similarly Aro et al (1995) found no relation between plasma *trans* 18:1 isomers and the incidence of acute myocardial infarction in European men. Bolton-Smith et al (1996) also reported no relation of TFA intake with diagnosed heart disease despite an inverse relation between TFA intake and high-density lipoprotein cholesterol (HDL-C).

The relations between TFA intake and incidence of cardiovascular disease may be due in part to the effect TFA have on plasma cholesterol levels, specifically on increasing LDL-C and lowering HDL-C, and possibly through raising Lp(a). A number of studies have reported that replacing dietary *cis* unsaturated fatty acids with TFA, either as *trans* 18:1n-9 (elaidic acid), or as a variety of *trans* isomers resulted in increased plasma total cholesterol (TC) and LDL-C (Judd 1994, Laine 1982, Lichtenstein 1993, Zock 1992). Several investigators have reported that TFA lowered HDL-C in addition to elevating TC and LDL-C in comparison to dietary *cis* unsaturated fatty acids (Mensink 1990, Zock 1992). Others have reported that when saturated fat is replaced with TFA, there was a decrease in HDL-C with no effect on LDL-C (Aro 1997) or an increase in LDL-C (Sundram 1997).

The mechanism by which TFA alters cholesterol profile, specifically HDL-C may involve cholesteryl ester transfer protein (CETP), a protein that transfers cholesterol from HDL-C to LDL-C and very low-density cholesterol (VLDL-C). Khosla et al (1997) demonstrated that the CETP activity of 11 normo-cholesterolemic cebus monkeys fed a diet rich in elaidic acid (*trans*-18:1n-9) was higher than the CETP activity of the monkeys fed a diet rich in saturated fatty acids. Similarly, an increase in CETP activity was seen in humans who were fed an elaidic-rich diet (Abbey and Nestel 1994). Aro et al (1997), however, reported no significant effect of dietary TFA compared to dietary saturated fat on CETP activity in a group of 80 healthy adults. In the study by Aro et al (1997) TFA contributed 8.7% of energy whereas Abbey and Nestel (1994) used 7% of energy as *trans* 18:1n-9. Possibly, an increase in CETP activity may be more likely to occur with a diet containing *trans* 18:1n-9, rather than a mixture of *trans* isomers.

Another possible mechanism to explain the effect TFA have on HDL-C is an alteration in lecithin: cholesterol acyltransferase (LCAT) activity. The LCAT enzyme catalyzes the transfer of a fatty acid from the 2 position of phosphatidylcholine to free cholesterol to form cholesteryl ester (CE). The LCAT enzyme is able to bind to HDL particles and after activation by apo-A-I, its action increases the amount of CE in HDL-C. The addition of LCAT, isolated from human or rat plasma, to substrates containing *trans* isomers resulted in a 30-50% lower LCAT activity when compared to the LCAT activity with *cis* unsaturated fatty acid isomers (Subbaiah 1998). This suggests that

LCAT prefers *cis* rather than *trans* fatty acid isomers as a substrate, however, levels of TFA in plasma PL are low with dietary TFA appearing in plasma TG. There are no published data on the levels of TFA in the 2-position of plasma as HDL phosphatidylcholine, i.e. the LCAT substrate. Thus, interpretation of the results of the in vitro studies of Subbaiah et al (1998) with respect to the effects of dietary TFA on plasma cholesterol is difficult. In summary, the available data does not allow a conclusion on the mechanisms involved in altering plasma lipoprotein cholesterol levels when TFA-rich diets are consumed.

More recently, interest has been focused on the possible effect of TFA on Lp(a), an LDL-C particle linked to apoprotein(a). Lp(a) has been found to be a strong risk factor for developing cardiovascular disease (Mackinnon 1997). It should be noted that Lp(a) has been described as a genetically determined risk factor for atherosclerotic vascular disease that remains constant in any given individual, irrespective of diet and exercise (Mackinnon 1997). Sundram et al (1997) recently reported elevated serum Lp(a) levels when a diet with 7% of energy from TFA, of which 39% TFA were *trans* 18:1n-9, was fed to a group of Malayasians. Aro et al (1997) and Nestel et al (1992) also reported elevated serum Lp(a) in humans fed TFA either as 8.7% or 7% of total energy, with 22 and 27% of TFA as *trans* 18:1n-9, respectively. Clevidence et al (1997), however, reported no effect of TFA, either at 3.8% or 6.6% of energy, on Lp(a) plasma levels. The dietary TFA were again a mixture of *trans* isomers, but the percent *trans* 18:1n-9 was not reported. The findings reported by Sundram et al (1997), Aro et al (1997) and Nestel et al (1992) are the first to show a negative effect of diet, specifically TFA, on Lp(a) levels in humans.

In summary, *trans* 18:1 has been the most common *trans* isomer used when studying the effect of *trans* isomers on plasma cholesterol levels. Although *trans* 18:1n-9 is the most prevalent *trans* isomer in the diet, many other mono and polyunsaturated *trans* isomers are also present in foods containing partially hydrogenated oils and shortenings. It is possible that plasma lipoprotein cholesterol levels may be affected by only certain *trans* isomers or perhaps by a certain concentration of one or more individual *trans* isomers, for example isomers of 18:2. With respect to human studies, this is further compounded by the differences in the amounts and proportion of TFA in partially hydrogenated oils and shortenings produced from different oils and by different manufacturers. For example, partially hydrogenated canola oil which has about 53% 18:1n-9, 21% 18:2n-6 and 11% 18:3n-3 will have different amounts and isomers of TFA from partially hydrogenated soybean oil which has 23% 18:1n-9, 54% 18:2n-6 and 8%18:3n-3.

2.8.2 Cancer

A link between TFA and cancer has also been postulated (Bakker 1997, Kohlmeier 1997, Petrek 1997) but is not well established. Most studies in this area have explored a possible relation between TFA intake and breast cancer. Petrek et al (1997) measured adipose tissue fatty acids in 161 American women presenting with an early-stage of breast cancer. The level of TFA in adipose tissue was inversely associated with positive lymph node status. In contrast, *cis* 18:1n-9 and saturated fatty acids were positively associated with positive lymph node status, an indicator of metastasis. Several studies in Europe have explored the possible relation between TFA and breast cancer in post-menopausal women. In one study, a significant relation between TFA in adipose tissue and incidence of cancer of the breast (r = -0.76) was found (Bakker 1997). Kohlmeier et al (1997) measured adipose fatty acids in 209 post-menopausal women in Europe who had been recently diagnosed with breast cancer. The mean levels of *trans* 18:1 and total TFA were higher in the women with breast cancer, compared with women with no history of breast cancer. Despite the finding of a positive relation between TFA in adipose tissue and breast-cancer, in several epidemiological studies (Bakker 1997, Kohlmeier 1997, Petrek 1997) it can not be concluded that dietary TFA have a direct effect on the cause or progression of cancer. Women with breast cancer may consume high levels of dietary TFA, but whether this has any significance to the development or progression of breast cancer can not be inferred from the results of epidemiological studies.

2.9 Transfer of Fatty Acids to the Fetus

The placenta is an organ that has a wide range of functions including the transfer of respiratory gases, fluid, waste products and nutrients to and from the fetus (Page 1993). Fatty acids are able to enter the placenta as free fatty acids via the placental lipoprotein lipase and the fatty acid-binding protein present in the maternal facing membranes of the placenta (Campbell 1995a, Campbell 1995b, Coleman 1989). The placental lipoprotein lipase (LPL) is responsible for the hydrolysis of maternal plasma triglycerides releasing free fatty acids and monoglycerides. The free fatty acids are then able to bind to a fatty acid-binding protein for transport into the placenta. The placental LPL activity, however, does not explain how and if fatty acids from maternal plasma PL and CE enter the placenta. Phospholipase A2, an enzyme that hydrolyzes fatty acids from the second carbon of the phospholipid glycerol backbone, has been detected in human placental tissue (Jendryczko 1989, Jendryczko 1990). Higher levels of phospholipase A2 enzyme have been associated with pre-eclampsia and onset of labour, however, the role of this enzyme in hydrolyzing fatty acids, from phospholipid, for entry into the placenta, is unknown.

The human is unable to synthesize certain nutrients such as 18:2n-6 and 18:3n-3, therefore, the fetus is dependent on the mother's diet or tissues for these fatty acids. A longitudinal study examining the n-6 and n-3 fatty acid status of women during normal pregnancy has reported that the percent 18:2n-6 of the fetal cord PL was correlated to the mother's plasma PL 18:2n-6 ($r = 0.52$, $p<0.01$) (Al 1995). No relation between maternal and cord plasma PL 18:3n-3, however, was reported. The level of 18:2n-6 in the fetal and maternal PL was approximately 7 and 22% of total fatty acids, respectively. An alteration in maternal dietary fat intake, either during or prior to pregnancy could theoretically modify the fatty acid pool available for transfer to the fetus. For example, if a women started consuming flaxseed oil, which contains about 54% 18:3n-3, several months prior to pregnancy, she would have more 18:3n-3 available for transfer to the fetus than if she had consumed a vegetable oil low in 18:3n-3. Connor et al (1996) supplemented pregnant women with fish oil containing 22:6n-3 and then measured the levels of plasma n-3 fatty acids in both the mother and newborn cord plasma. The percent 22:6n-3 in the cord plasma of the supplemented and control groups were 5.05% and 3.47% of total fatty acids, respectively. The percent 22:6n-3 in the maternal plasma, of the supplement and control groups at birth, were 2.35% and 1.47% of total fatty acids. The results of this study demonstrate that increasing the maternal intake of 22:6n-3 resulted in slightly higher levels of 22:6n-3 in the fetus. It has been suggested that the increased level of 20:4n-6 and 22:6n-3 in the fetus is a result of preferential, selective transfer by the placenta (Coleman 1995). This could explain why the level of 20:4n-6 and 22:6n-3 are lower in the maternal plasma than in cord plasma at birth (Hoving 1994, Innis 1991). No mechanisms to support selective transport of fatty acids across the placenta to the fetus, however, have been reported.

Other fatty acids, specifically *trans* fatty acids have been reported in cord plasma at birth (Ayyagari 1996, Koletzko 1990, Koletzko 1992) suggesting that TFA enter the placenta and eventually the fetal circulation via the maternal circulation. Several human studies have found similar levels of TFA in maternal and cord plasma, confirming placental transfer of TFA (Berghaus 1998, Hoving1994, Koletzko 1992). There are no published data concerning possible relations between maternal diet, maternal plasma and cord plasma levels of TFA. The purpose of this study, therefore, was to examine whether there was a relation between the level of TFA in maternal diet, maternal plasma and cord plasma at birth.

3 STUDY OVERVIEW

This research was conducted as two consecutive studies. Study One was a cross-sectional study using a convenience sample of pregnant women in early stage, normal labor in the Low Risk Labor and Delivery Unit at the BC Women's Hospital. Study Two was a prospective study with a sample of pregnant women from the Lower Mainland, recruited between 22 and 24 weeks gestation from pre-admission records provided by the BC Women's Hospital.

4 PURPOSE

The purpose of Study One was to determine the range of TFA present in cord plasma. Study Two was designed to determine the amounts of TFA in the maternal diet, the level of TFA in maternal and cord plasma, and whether variability in levels of TFA in cord plasma can be explained by levels of TFA in the maternal diet and plasma.

4.1 Objectives

- To determine the range of *trans* fatty acids and *cis* n-6 and n-3 polyunsaturated fatty acids in newborn cord plasma.
- To determine if *trans* fatty acids in newborn cord plasma are inversely related to *cis* n-6 and n-3 polyunsaturated fatty acids and birth outcome measures (length of gestation, birthweight, length and head circumference).
- To determine the range of TFA and *cis* n-6 and n-3 polyunsaturated fatty acids in maternal plasma.
- To estimate dietary TFA intake in pregnant women during the second and third trimester and to identify the major sources of dietary TFA.

- To identify relations between maternal TFA intake and level of TFA in newborn cord plasma.

4.2 Hypotheses

- *Trans* fatty acids are not well transported across the placenta, therefore, the range of TFA in cord plasma will be less than the range of TFA in maternal plasma.
- The range of TFA in maternal plasma is higher than the range of TFA in cord plasma
- There is a positive relation between the amount of TFA in the maternal diet and the level of TFA in cord plasma.
- There is no relation between the level of TFA and *cis* n-6 and n-3 fatty acids in cord plasma, or between TFA and birth outcome measures.
- The major dietary source of TFA is from invisible fats, such as those present in baked, processed and convenience foods.

5 ETHICS

The study protocol and procedures for both studies were approved by the University of British Columbia Clinical Research Ethics Board and the British Columbia Women's Hospital Research Coordinating Committee.

6 SUBJECTS AND METHODS

6.1 Study One

The purpose of Study One was to determine the range of TFA in cord plasma. This study provided data for Study Two, a larger study, to determine whether maternal dietary TFA intake and the range of TFA in maternal plasma are related to the range of TFA in cord plasma.

6.1.1. Participant Selection Criteria

Eligible participants for this study were women delivering full-term infants (37-42 weeks), 20-40 years in age, in normal, early stage labor in the Low Risk Labor and Delivery Unit at the British Columbia Women's Hospital. Women known to have a history of tuberculosis, HIV/AIDS, peri-natal infection, substance abuse or conditions known to affect lipid metabolism, such as maternal diabetes, were excluded. Women delivering more than one fetus or delivering a fetus with known or suspected problems were also excluded.

6.1.2. Participant Recruitment

Participants for this study were recruited between January and June 1997 in the Low Risk Labor and Delivery Unit at the British Columbia Women's Hospital. After giving informed consent (Appendix 1), the participants were asked to complete a brief questionnaire (Appendix 2). A self-addressed, stamped envelope was provided to those participants who chose to complete the questionnaire at a later time.

6.1.3. Study Questionnaire

The questionnaire used in Study One was designed to collect information on each woman's age, highest education level attained, gross annual family income, smoking habits, dietary practices, frequency of fast food and commercial baked goods consumption, and type(s) of fat used in baking and as a table spread. In addition, length of gestation, infant birthweight and gender were collected after deliver of the infant from the medical chart.

6.1.4. Blood Collection

A coded 3 mL EDTA vacutainer was given to each participant's nurse for collection of a newborn cord blood sample. The participant's nurse or physician collected the blood samples after the newborn cord was clamped. The blood samples were then placed in a –4°C refrigerator in the Low Risk Delivery Unit. Blood samples were transported on ice to the BC Research Center for Child and Maternal Health and centrifuged at 2000 g for 15 minutes. The plasma was separated from the red blood cells and frozen at –80°C.

6.1.5. Birth Outcome Measures

The length of gestation, determined using the date of the last normal menstrual period was recorded from the participant's medical chart. The infant gender and birthweight were also recorded from the participant's medical chart.

6.2 Study Two

The purpose of Study Two was to estimate dietary intakes of TFA in pregnancy, to determine the level of TFA in maternal and cord plasma, to determine if differences in levels of TFA in cord plasma can be explained by maternal diet and plasma TFA, and to determine if levels of TFA are inversely related to *cis* n-6 and n-3 polyunsaturated fatty acids in maternal and cord blood.

6.2.1. Participant Selection Criteria

Eligible participants for this study were low-risk pregnant women between 20-40 years of age who had registered to deliver their infants at the British Columbia's Women's Hospital with expected dates of delivery between June 1 and July 19, 1998. Exclusion criteria included diabetes of any origin, a known pre-pregnancy history of high blood cholesterol and triglycerides, and chronic or communicable diseases including HIV/AIDS, hepatitis or tuberculosis. Women who had used elicit drugs or consumed three or more alcoholic drinks per week during pregnancy, or expecting more than one child were also excluded.

6.2.2. Participant Recruitment

The Health Records Department generated a list of 804 pregnant women, who had registered to deliver their infants at the British Columbia's Women's Hospital between June 1 and July 19, 1998. This list was used to develop a mailing list of low-risk pregnant women between 20-40 years of age who lived in Vancouver, West Vancouver, North Vancouver, Burnaby or Richmond. Low-risk pregnancy, age and mailing address were provided on the pre-registration list. Women with a multiple gestation, previous pregnancy complications, previous pre-term delivery, poor obstetrical history including hyper-emesis, abnormal weight and congenital anomalies, psychological and social problems, street drug and alcohol use, cardiac disease, renal disease, diabetes, epilepsy, respiratory conditions, lupus or other rheumatoid conditions, cholestasis, HIV/AIDS, and other medical or surgical problems were not included on the mailing list.

A letter describing the study, the participant's role and the selection criteria was sent to 486 women between their 22^{nd}-24^{th} week of gestation (Appendix 3). Within two weeks of mailing the letters, a follow-up telephone call was made. During the telephone call, the women who were interested in participating were asked to confirm their eligibility, as described in the selection criteria of the recruitment letter, and the requirements of the study were explained.

6.2.3. Participant Interviews

Face-to face interviews were scheduled with each study participant at both 28 and 35 weeks gestation either at the participant's home, or at the British Columbia's Women's Hospital. The participants were asked to read and sign an informed consent (Appendix 4) at the first interview. After signing the informed consent, the participants then completed a socio-demographic and health questionnaire (Appendix 5). After completing the socio-demographic questionnaire, a food frequency questionnaire (FFQ) was administered to each participant (Appendix 6). This questionnaire was administered to each participant at 28 weeks and again at 35 weeks gestation. At the second interview, a 3 mL venous blood sample was also collected at the Outpatient Laboratory of the British Columbia's Women's Hospital.

6.2.4. Study Questionnaires

Questionnaires in Study Two were designed to collect information on the socio-demographic background, health and diet of each participant. The socio-demographic and health questionnaire included questions on the participant's age, ethnicity, highest level of education attained, gross annual family income, number of individuals residing in the household that were dependent on the family income, height, weight, weight history, pregnancy history, smoking habits, alcohol consumption during pregnancy, vitamin and mineral supplements used during pregnancy, as wells as any medications taken during pregnancy, dietary practices and infant feeding plans (breast-feeding vs. formula feeding).

For the purpose of collecting information on fat and energy consumption of the participants, a FFQ was developed using an unpublished FFQ that had been designed to measure total fat intake and dietary sources of fat. A FFQ was chosen rather than a 3-day food record to estimate TFA intake because a longer time period could be covered in which a variety of foods containing TFA could be consumed. The unpublished questionnaire was modified to include all known sources of dietary TFA and to include questions on brand names and methods of food preparation. Known food sources of TFA were identified from detailed fatty acid analysis of over 250 foods previously conducted in our laboratory, and from previous publications on sources of TFA in Canadian and U.S. foods. In addition, frequency of consumption and usual portion size was included on the FFQ. The FFQ contained 106 items, the completeness of foods listed on the FFQ was then validated using data from 24 hour dietary recalls and three day food records completed by 24 lactating women in the Okanogan Valley. Any additional foods containing TFA identified on these records were added to the FFQ.

The FFQ was administered in a face to face interview with each participant at each of 28 and 35 weeks gestation. Participants were asked to indicate their average total intake during the past 30 days, either on a daily, weekly or monthly basis for each food and beverage item listed in the FFQ. In addition, the participants were asked to give the usual brand or brands of each food and beverage consumed. Food pictures and plastic food models were used to help each participant determine usual portion sizes of the foods and beverages consumed.

6.2.5. Blood Collection

A 10 ml maternal venous blood sample was collected from each participant during their 35th week of gestation at the British Columbia Women's Hospital laboratory, by a registered phlebotomist. Blood samples were then transported on ice to the BC Research Center for Child and Maternal Health and centrifuged at 2000 g for 15 minutes. The plasma was separated from the red blood cells and frozen at –80°C.

A research study protocol sheet, describing the procedure for collecting the newborn cord blood sample, was attached to each participant's admission sheet in the BC Women's Hospital Admitting area. A box of 3 mL EDTA vacutainers was placed in the Low Risk Delivery Unit. The participant's nurse or physician collected the blood sample after the newborn cord was clamped. The blood samples were then placed in a –4°C refrigerator in the Low Risk Delivery Unit. Blood samples were transported on ice to the BC Research Center for Child and Maternal Health and centrifuged at 2000 g for 15 minutes. The plasma was separated from the red blood cells and frozen at –80°C.

6.2.6. Birth Outcome Measures

The length of gestation, determined by the first day of the last normal menstrual period, was recorded from the participant's medical chart. Infant gender, birthweight, length and head circumference were also recorded from the participant's medical chart.

7 DATA ANALYSIS

7.1 Demographic and Health Data Handling

Data from the questionnaire in Study One were entered into a computer spreadsheet. Numerical values were assigned to all categorical variables such as highest level of education attained, gross annual family income, consumption of fried fast foods, consumption of commercial baked goods, smoking habits, infant gender, baking practices, type of baking fat used, type of fat used as a table spread, dietary practices and food avoidances. Continuous variables such as maternal age, length of gestation and birthweight were entered as the value provided by the participant or recorded from the participant's medical chart.

Data from the socio-demographic and health questionnaire and birth outcome measures in Study Two were entered into a second computer spreadsheet. Numerical values were assigned to all categorical variables such as ethnicity, highest level of education attained, gross annual family income, smoking habits, alcohol consumption, supplement and medication use, dietary practices, whether the woman was planning to breast or formula feed the infant and infant gender. Continuous variables such as maternal age (calculated from their date of birth), number of individuals in the household dependent on the gross annual family income, height, present weight, pre-pregnancy weight, number of previous pregnancies, number of live births, number of miscarriages, number of alcoholic drinks consumed weekly, length of gestation, infant birthweight, length and head circumference were entered as provided by the participant or recorded from the medical chart.

7.2 Analysis of the Food Frequency Questionnaire

The dietary intakes provided in the FFQ were converted to daily intake values based on the number of servings and frequency of consumption, i.e. daily, weekly or monthly. For example, if a participant consumed four ounces of orange juice six times per week, their daily intake of orange juice would be calculated by multiplying the serving size (4 oz) by the frequency of consumption (6 days) and dividing by the time period covered (7 days), resulting in an average intake of 3.4 oz of orange juice per day. The daily intake values were then entered into a computer nutrient database (Food Processor II version 7, 1997, ESHA Research, Salem, Oregon).

The Food Processor database was modified to include nutrient information for all foods and beverages containing dietary fat which were consumed by the study participants. The fatty acid composition of 250 different foods and beverages analyzed in this laboratory were included in the updated database. If a specific brand consumed by a participant was not present in the updated database, values were extrapolated from the closest brand of food or beverage product from the 250 items analyzed in this laboratory, based on label information on the type and amount of fat in the product.

Once all foods were entered into the computer nutrient database, all dietary sources of TFA were identified for each participant. The dietary sources of TFA were then categorized into one of fifteen food groups. These food groups were baked foods, fast foods, margarine and shortenings, snack foods, breads, cereal/waffles/pancakes, pasta and other grain products, dairy products, peanut butter, meat, fish, oil/sauces/dressings, fruits and vegetables, chocolate/candy/icing, and soup. These food groups were developed using a modified list of previously identified food groups containing TFA (Enig 1990, Hunter 1991). The contribution of food groups to the total

TFA intake of the participants was calculated using three methods. First, the mean percent contribution of each food group to the total TFA intake was calculated for the study population. Second, for each food group, the mean TFA intake was calculated using only those participants who consumed foods within that food group, that is, for example, the mean TFA intake from margarine was calculated for only those participants who consumed margarine. Finally, the top five individual foods contributing to each participant's TFA intake were determined, and then each classified into one of the fifteen above food groups. Foods within a food group were then summed and the food groups ranked in order by their contribution to total TFA intake.

7.3 Fatty Acid Analysis

Total lipids were extracted from 400 μL plasma aliquot using chloroform-methanol-saline (6/3/1 v/v/v) (Folch, 1957). The phospholipid (PL), triglyceride (TG) and cholesteryl ester (CE) fractions were then separated by thin layer chromatography (TLC) using silica plates (Whatman PK6F Silica Gel 60 Å, 20 x 20 cm, 500 μm) with a solvent system of hexane-diethyl ether-glacial acetic acid (60/40/2 v/v/v) (Hrboticky 1990). Authentic TLC standards containing TG and CE were used on each TLC plate to identify the TG and CE bands. The PL bands, which remain at the origin of the TLC plate were then scraped into tubes. The fatty acids in the PL were converted to methyl esters using 1 mL BF_3 and 2 mL hexane, at 100°C for 15 minutes. The bands on the TLC plate corresponding to TG and CE were visualized under ultra-violet light and the silica containing the bands scraped into labeled glass tubes. The TG and CE were extracted from the silica using chloroform/methanol. The fatty acids in the TG and CE were then converted to methyl esters using 1 mL BF_3-benzene-methanol (25/25/55, v/v/v) at 100°C for 30 minutes, and 1 mL BF_3-benzene-methanol (35/30/55 v/v/v) at 100°C for 45 minutes, respectively. The fatty acid methyl esters were then separated and quantified by gas liquid chromatography (GLC) (Varian 3400, Georgetown, Ontario). The GLC separation was achieved using 100 m x 0.25 mm non-bonded, fused silica capillary SP2560 columns (Supelco, Bellefonte, Pennsylvania). Helium was used as the carrier gas at a column flow of 1 mL/min and an inlet pressure of 55 pounds per square inch. The inlet splitter was set at 10 to 1. Samples were injected at 100°C and the oven temperature programmed to remain at 100°C for 2 minutes, then increase to 190°C at 5°C/minute, stabilize for 20 minutes, rise to 255°C at 1°C/minute and held for 2 minutes. The column was then heated to 245°C at 25°C/minute and held for 22 minutes prior to returning to 100°C and stabilization for the injection of the next sample. A 15 minute stabilization period at 100°C, was set prior to each sample injection. The injectors and detectors were set at 240°C and 260°C, respectively. Fatty acid methyl esters were identified by comparison of retention time with that of authentic standards. The identification of fatty acids for this study did not include mass spectrometry of the samples.

For the purpose of this research, the identified fatty acids were grouped as saturated fatty acids, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, 24:0; monounsaturated fatty acids, 16:1, 18:1, 20:1, 22:1; polyunsaturated n-9 series, 20:3; n-6 series, 18:2, 18:3, 20:2, 20:3, 20:4, 22:4, 22:5; n-3 series, 18:3, 20:5, 22:5, 22:6; and *trans* isomers of 18:1 and 18:2. With the availability of new conjugated linoleic acid (CLA) isomer standards, CLA was identified in maternal plasma and the newborn cord plasma in Study Two, but not Study One.

Peak area counts and retention times were recorded by a GLC data system (IBM computer system using Varian "STAR" software, Georgetown, Ontario). Peak area counts for each identified fatty acid were entered into a computer spreadsheet and the relative percents of each fatty acid were calculated. The response of the detector to equivalent weights of fatty acids across the range of C14 to C22 was similar. The variance in the flame ionization detector response across the C14 to C22 did not at any time exceed 5% and was within the range of inter-assay reproducibility. The consistency of the flame ionization detector response was checked by injecting a calibration mixture containing equivalent amounts of saturated fatty acids 14:0 through to 22:0, as well as known authentic saturated, monounsaturated and polyunsaturated fatty acid methyl ester standard mixtures.

The use of sodium methoxide, as a methylating agent, has been suggested to be possibly superior to BF_3 (Kramer 1997). The latter suggestion was due to concerns that BF_3 may artificially elevate *trans* isomers during the methylation procedure by converting some *cis* isomers to *trans* isomers. To address this issue, three randomly chosen plasma samples from Study One were extracted and methylated using 1mL BF_3-benzene-methanol (25/25/55, v/v/v) at 100°C for 30 minutes and 1 mL sodium methoxide at 50°C for 10 minutes. The analyses found no differences in the relative percent of any fatty acids, including TFA between the two methods. Thus, BF_3 was used as the methylating agent in all fatty acid analyses in both Study One and Two.

7.4 Statistical Analysis

All data from Study One and Two were analyzed using the Statistical Package for the Social Science (SPSS Inc. version 7.5 for Windows, Chicago, Illinois).

Descriptive statistics were obtained for dietary energy and fat intakes, birth outcome measures, maternal plasma fatty acids and cord plasma fatty acids. Frequency counts were calculated for all categorical variables. When variables were not normally distributed, the data were transformed using the natural log, square roots or squared values. Non-parametric statistics were performed on non-normally distributed data.

A paired sample t-test was used to compare means of total energy and fat intakes in the second and third trimester. Pearson correlation coefficients were calculated to determine potential relations between:

- total energy and fat intakes in the second trimester and total energy and fat intakes in the third trimester
- maternal TFA intake and TFA in maternal plasma TG, PL and CE
- maternal TFA intake and TFA in cord plasma TG, PL and CE
- TFA in maternal plasma TG, PL and CE and *cis* n-6 and n-3 polyunsaturated fatty acids
- TFA in cord plasma TG, PL and CE and *cis* n-6 and n-3 polyunsaturated fatty acids
- birth outcome measures (length of gestation, birthweight, infant length) and cord plasma fatty acids.
- maternal plasma TFA, *cis* n-6 and n-3 polyunsaturated fatty acids in TG, PL and CE and cord plasma TFA, *cis* n-6 and n-3 polyunsaturated fatty acids in TG, PL and CE

Results with a probability less than 0.05 were considered statistically significant.

8 RESULTS

8.1 Participant Recruitment and Characteristics

8.1.1. Study One

A total of 33 women were approached for participation in Study One. Of the 33 approached, 24 (72.7%) agreed to participate. Of these 24 women, 16 completed the socio-demographic and health questionnaire. The mean age of the women who participated in Study One was 32.6 years (range 18-40) (**Table 8.1**). Over 68% of women (n=11) had completed post-secondary education. Most of the women (56.3%) had a gross family income greater than $50,000, only one woman had an annual family income less than $20,000. The average length of gestation and birthweight were 40.5 (range 38-42) weeks and 3625.5 (3045-4460) g, respectively (**Table 8.2**). Fourteen of the infants were female and 10 were male.

One woman smoked during her pregnancy. None of the women followed a special diet during pregnancy, e.g. vegetarian. Three women indicated that they had avoided certain foods during pregnancy including red meat, offal, wheat, eggs and seafood. One woman had an allergy to onions and one woman was lactose intolerant. Nine women (56.3%) consumed commercial baked foods, such as muffins and cookies, one to two times per week and thirteen women (81.3%) consumed fast foods one to two times per week. The type of fat used by women as a table spread was butter, n=8, margarine, n=4, butter and/or margarine, n=2 and none, n=1. Six women baked on a weekly basis of which, one woman used butter, two women used vegetable oil and three women used a combination of butter, margarine and/or oil in their baking.

Table 8.1 Characteristics of women in Study One[1]

	n	%
Age (yr)		
< 24.0	1	6.3
≥ 25 < 30	1	6.3
≥ 30 < 35	11	68.8
≥ 35 < 40	3	18.8
Education Completed		
< Secondary School	2	12.5
Secondary School	3	18.8
College/Technical School	5	31.3
Undergraduate Degree	4	25.0
Graduate Degree	2	12.5
Gross Annual Family Income		
$10,000 < $20,000	1	6.3
≥ $20,000 < $35,000	2	12.5
≥ $35,000 < $50,000	3	18.8
≥ $50,000 < $75,000	3	18.8
≥ $75,000	6	37.5
Did not report income	1	6.3

[1] Of the 24 women who participated, only 16 women completed the socio-demographic and health questionnaire

Table 8.2 Birth outcome measures in Study One[1]

	n=24	%
Birthweight (g)		
≥ 3000 < 3500	11	45.8
≥ 3500 < 4000	8	33.3
≥ 4000	5	20.8
Mean (range)	3625.5 (3045-4460)	
Infant Gender		
Female	14	58.3
Male	10	41.7

[1] The mean length of gestation was 40.5 wks, study eligibility criteria was 38-42 weeks

8.1.2 Study Two

Of 504 letters mailed, two letters were returned marked undeliverable. A follow-up telephone call was made to all women with the exception of those women who had called the Research Center upon receiving the letter (n=19). Follow-up phone calls were made to 483 women, of whom 33 women could not be reached due to incorrect or out of service phone numbers (**Table 8.3**). A further 65 women did not speak or understand English. Reasons for not participating in the study included time constraints, acute illness, other study commitments (n=18), or study ineligibility due to gestational diabetes, drug use, miscarriage, or a change in hospital (n=15). Of the 151 eligible women contacted, 70 women agreed to participate in the study and 60 women were interviewed at 28 weeks. The remaining 10 women who agreed to but did not participate, declined due to time constraints or illness. All but three of the 60 women completed the study, and one woman joined the study at 35 weeks gestation.

The majority of women in the study (96.6%) were between 25 and 40 years of age, with a mean age of 32.5 years (range 20-40 years). Forty-nine women were Caucasian (80.3%), 10 were Asian (16.4%), 1 was First Nations (1.6%) and 1 was

Black (1.6%) (**Table 8.4**). Most women (n=52) had completed post-secondary education that included college/technical school, undergraduate and graduate university degrees. Thirty-nine women (63.9%) had a gross annual family income of more than $50,000. Over 90% (n=57) of the women had a total of two or three persons living in their household, including themselves, who were dependent on the family income.

About half of the women who participated (52.5%) had a pre-pregnancy body mass index between 20.0 and 25.0 with a mean of 23.5 (range 17.9-30.7). The average height of the women was 164.2 cm (**Table 8.5**) and the average pre-pregnancy weight was 63.3 kg. Most of the women gained between 7-11 kg (68.4%), from the beginning of their pregnancy to 35 weeks gestation. The heights and weights were self-reported by the participants. Over 50% (n=34) of the women had had a previous pregnancy and 34% (n=24) of women had had at least one previous live birth (**Table 8.6**). Over 95% of participants (n=44) had a full-term pregnancy, with an average length of gestation of 39.9 wks (range 36-42 wks) and birthweight of 3524 g (2035-4810 g). Thirty-two infants had a length at birth between 50-55 cm and 24 infants had a head circumference at birth between 35-37 cm. Of the 46 infants, 23 were female and 23 were male.

Twelve of the women (19.7%) followed a lacto-ovo vegetarian, or semi-vegetarian diet and one woman was allergic to dairy products, wheat products and nuts. Over 80.3% of the women who participated in the study were taking a supplement intended for use during pregnancy (prenatal supplement) at 28 weeks gestation and 9.8% were taking a regular adult multi-vitamin and mineral supplement at 28 weeks gestation (**Table 8.7**). In addition, 9.8% were taking iron, 11.5% were taking folic acid, and 11.5% were taking calcium supplements. None of the woman who participated in the study smoked and 4 women consumed alcohol during their pregnancy.

Table 8.3 Participant recruitment results for Study Two

	n	%
Letters mailed to participants	504	100.0
Individuals contacted by telephone who were unable to communicate with interviewer	67	13.3
Individuals with telephone numbers not in service	33	6.5
Individuals telephoned, but no answer	230	45.6
Individuals successfully contacted by telephone	174	34.5
Individuals successfully contacted who did not met eligibility criteria[1]	23	13.2
Individuals successfully contacted who declined with no reason given	63	36.2
Individuals successfully contacted who declined because of other commitments	18	10.3
Individuals successfully contacted who agreed to participate	70	40.2
Individuals successfully contacted who attended the first appointment	60	85.7
Individuals successfully contacted who attended the second appointment	58	82.9

[1] Eligibility criteria included: registered to deliver their infant at British Columbia's Women's Hospital, 20-40 years of age, singleton pregnancy, no drug use and fewer than 3 alcoholic drinks per week during pregnancy, no tuberculosis, HIV/AIDS, hepatitis, diabetes and no known pre-pregnancy history of high blood cholesterol or triglycerides.

Table 8.4 Socio-demographic characteristics of women in Study Two

	n =61	%
Race		
Caucasian	49	80.3
Asian	10	16.4
Other	2	3.3
Age (yr)		
< 25.0	2	3.3
≥ 25 < 30	19	31.1
≥ 30 < 35	19	31.1
≥ 35 < 40	21	34.4
Education Completed		
High School	9	14.8
College/Technical School	14	23.0
Undergraduate Degree	31	50.8
Graduate Degree	7	11.5
Gross Annual Family Income		
< $20,000	7	11.5
≥ $20,000 ≤ $50,000	15	24.6
> $50,000	39	63.9
Number of Individuals Dependent on Family Income [1]		
1	4	6.6
2	32	52.5
3	21	34.4
≥ 4	4	6.5

[1] Number of individuals residing in the household who were dependent on the family income, including the participant, spouse, children, any extended family or other persons

Table 8.5 Characteristics of women in Study Two[1]

Height (cm), n=61	164.2 ± 0.9 (146.7-177.1)
Pre-pregnancy weight (kg), n=61	63.3 ± 3.0 (40.0-90.0)
Weight at 28 weeks gestation (kg), n=60	71.8 ± 2.9 (50.9-94.5)
Weight at 35 weeks gestation (kg), n=57	75.9 ± 3.0 (54.5-100.0)
Pregnancy weight gain (kg), n=57	12.3 ± 1.3 (-0.08-22.7)

[1] Values reported are means ± SE (range) and were self-reported

Table 8.6 Birth outcomes of infants born to women in Study Two[1]

	n	% of participants
Previous Pregnancies, n=61		
0	27	44.3
1	17	27.9
2	11	18.0
≥3	6	9.8
Previous Live Births, n=61		
0	37	60.7
1	19	31.1
2	5	3.3
Length of gestation (wk) n=46		
< 38	2	4.3
≥ 38 ≤ 42	44	95.7
> 42	0	0.0
Mean (range)	40 (36-42)	
Birthweight (g), n=46		
<2500	1	2.2
≥ 2500 < 3000	6	13.0
≥ 3000 < 3500	13	28.3
≥ 3500 < 4000	15	32.6
≥ 4000	11	23.9
Mean (range)	3523 (2035-4810)	
Infant Length (cm), n=44		
≥ 45 < 50	6	13.6
≥ 50 < 55	32	72.7
≥ 55.0	6	13.6
Mean (range)	52 (46-57)	
Head Circumference (cm), n=45		
≥ 33 < 35	18	40.0
≥ 35 < 37	24	53.3
≥ 37 < 39	3	6.7
Mean (range)	35 (31-39)	
Gender, n=46		
Male	23	50.0
Female	23	50.0

[1] Collected, where recorded, from the participant's medical chart record

Table 8.7 Diet and health characteristics of participants in Study Two

	n =61	%
Diet		
Lacto-ovo-vegetarian	4	6.6
Semi-vegetarian[1]	8	13.1
Other[2]	1	1.6
No diet restrictions	46	75.4
Vitamin/Mineral Supplements[3]		
Prenatal	49	80.3
Folic acid	7	11.5
Calcium	7	11.5
Iron	6	9.8
Multi-mineral/vitamin	6	9.8
B vitamins	4	6.6
Vitamin E	3	4.9
Smokers	0	0
Alcohol Intake/Week[4]		
0 drinks	57	93.4
> 0 ≤ 1 drink	4	6.6
> 1 drink	0	0

[1] Included milk, milk products, eggs, poultry and/or fish
[2] Wheat and dairy free diet
[3] Women were allowed to check more than one supplement
[4] Alcohol consumption during pregnancy, 1 drink = 1 bottle of beer/cider, 4 ounces wine or 1 ounce spirits

8.2 Estimated Energy and Dietary Fat Intakes

The mean daily energy intake in the second trimester was 2691.8 (range 1251-5775) kcal/d and in the third trimester was 2404.8 (range 1246-3697) kcal/d (**Table 8.8**). The average daily energy intake in the third trimester was about 300 kcal/d lower than in the second trimester.

The mean daily fat intake was 85.8 and 73.9 g/d in the second and third trimester, respectively, contributing less than 30% of energy in both trimesters. Total fat intake ranged from 37.5-200.8 g/d in the second trimester and from 22.4-124.3 g/d in the third trimester (**Table 8.8**). Thirty-eight and 35 women consumed between 50 and 100 g/d of total fat in the second and third trimester, respectively (**Table 8.9**).

The mean daily dietary TFA intake was 3.8 and 3.4 g/d in the second and third trimester, respectively, contributing approximately 1% of total energy in both trimesters. The TFA intake ranged from 0.7-11.3 g/d in the second trimester and from range 0.9-9.6 g/d in the third trimester. Twenty-seven and 31 women consumed between 0 and 3 g/d of TFA in the second and third trimester, respectively.

The mean daily polyunsaturated fatty acid intake was 13.6 and 12.2 g/d in the second and third trimester, respectively, contributing about 4.5% of energy in both trimesters. The polyunsaturated fatty acid intake ranged from 5.8-40.0 g/d in the second trimester and from 2.9-23.3 g/person/d in the third trimester. Thirty-one and 33 women consumed between 10 and 20 g/d polyunsaturated fatty acids in the second and third trimester, respectively.

The mean daily monounsaturated fatty acid intake was 31.5 and 26.4 g/d in the second and third trimester, respectively, contributing about 10% of energy in both trimesters. The monounsaturated fatty acid intake ranged from 13.9-78 g/d in the second trimester and from 3.7-53.3 g/d in the third trimester. Thirty-eight and 40 women consumed between 20 and 40 g/d monounsaturated fatty acids in the second and third trimester, respectively.

The mean daily saturated fatty acid intake in the first and second trimester was 29.7 and 26.3 g, respectively, contributing 9.8% of energy in both trimesters. The saturated fatty acid intake ranged from 11.0-67.8 g/d in the second trimester and from

7.3-51.4 g/d in the third trimester. Thirty-four and 31 women consumed between 20 and 40 g/d saturated fatty acids in the second and third trimester, respectively.

The total energy intake and total fat intakes of individual women at 28 weeks gestation were significantly related to the total energy and total fat intakes at 35 weeks gestation. The correlation coefficient for energy was 0.63, total fat was 0.69, TFA was 0.54, polyunsaturated fatty acids was 0.61, monounsaturated fatty acids was 0.64 and saturated fatty acids was 0.7 (**Table 8.8**). The *trans* fatty acid intake (g/d) was significantly related to the total fat intake (g/d) in the second trimester, $r = 0.49$, $p<0.05$ and in the third trimester, $r = 0.57$, $p<0.05$ (**Figure 8.1**). Similarly, TFA and total fat intake as a percent of total energy, were also significantly related at both 28 and 35 weeks gestation, at $r = 0.10$, $p<0.05$ and $r = 0.26$, $p<0.05$, respectively (**Figure 8.2**).

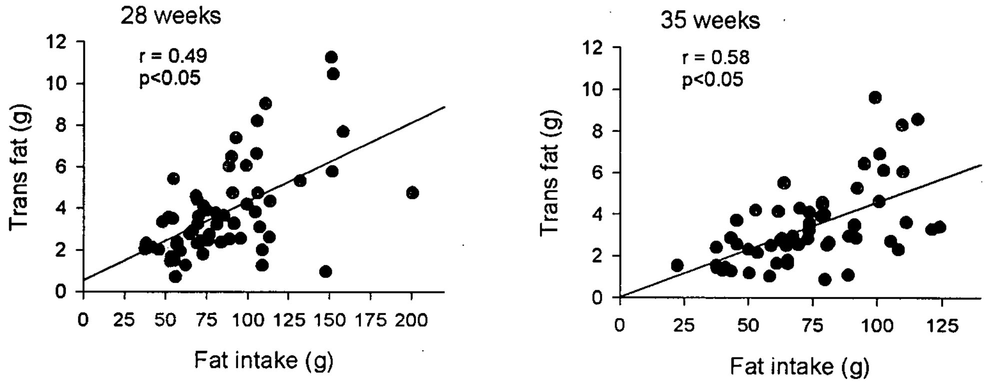


Figure 8.1 Scatter plots of estimated dietary *trans* fatty acid and total fat intakes (g/d) of women at 28 and 35 weeks gestation, n=57

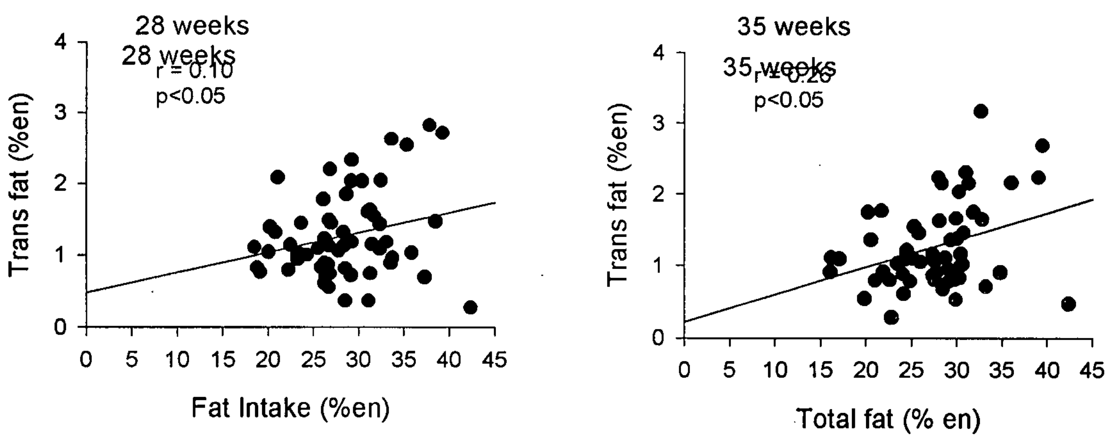


Figure 8.2 Scatter plots of estimated dietary *trans* fatty acid and total fat intakes as percent total energy (%en) of women at 28 and 35 weeks gestation, n=57[1]

[1] The percent total energy of total fat and TFA were calculated by multiplying the total fat and TFA intake (g) by 9kcal/g and dividing by total energy (kcal)

Table 8.8 Estimated dietary *trans*, polyunsaturated, monounsaturated and saturated fatty acids, total fat and energy intakes as determined by a food frequency questionnaire at 28 and 35 weeks gestation[1]

	Gestation Week		
	28 *n=60*	35 *n=57*	r
Energy (kcal/d)	2691.8 ± 101.6 (1250.7-5774.5)	2404.8 ± 82.6 (1245.6-3696.9)[3]	0.63 <0.001
Total Fat (g/d)	85.8 ± 4.3 (37.5-200.8)	73.9 ± 3.2 (22.4-124.3)[3]	0.69 <0.001
% Energy from total fat	28.3 ± 0.7 (18.5-42.4)	27.5 ± 0.7 (16.1-42.4)	
Trans fatty acids (g/d)	3.8 ± 0.3 (0.7-11.3)	3.4 ± 0.3 (0.9-9.6)	0.54[2] <0.001
% Energy from *trans* fatty acids	1.3 ± 0.1 (0.3-2.8)	1.3 ± 0.1 (0.3-3.2)	
Polyunsaturated fatty acids (g/d)	13.6 ± 0.9 (5.8-40.4)	12.2 ± 0.6 (2.9-23.3)[3]	0.61 <0.001
% Energy from polyunsaturated fatty acids	4.5 ± 0.2 (2.4-8.1)	4.6 ± 0.2 (2.0-11.9)	
Monounsaturated fatty acids (g/d)	31.5 ± 1.8 (13.9-78.0)	26.4 ± 1.3 (3.7-53.3)[3]	0.64 <0.001
% Energy from monounsaturated fatty acids	10.4 ± 0.4(6.5-22.4)	9.8 ± 0.3 (2.7-17.6)	
Saturated fatty acids (g/d)	29.7 ± 1.6 (11.0-67.8)	26.3 ± 1.3 (7.3-51.4)[3]	0.75 <0.001
% Energy from saturated fatty acids	9.8 ± 0.3 (5.3-14.8)	9.8 ± 0.3 (4.5-14.6)	

[1] Values are reported as mean ± SE (range)
[2] Pearson correlation coefficients of intakes at 28 and 35 weeks
[3] Significantly different from dietary intakes at 28 weeks, $p < 0.05$

Table 8.9 Distribution of women by quartiles of estimated *trans*, polyunsaturated, monounsaturated and saturated fatty acids and total fat intakes(g/d) as determined by a food frequency questionnaire

	Quartiles			
	1	**2**	**3**	**4**
Total fat (g/d)	***0 < 50***	***50 < 100***	***100 < 150***	***150 < 200***
2nd trimester[1]	5	38	12	5
3rd trimester[2]	11	35	11	0
Trans fatty acids (g/d)	***0 < 3***	***3 < 6***	***6 < 9***	***9 < 12***
2nd trimester	27	23	7	3
3rd trimester	31	19	6	1
Polyunsaturated fatty acids (g/d)	***0 < 10***	***10 < 20***	***20 < 30***	***30 < 40***
2nd trimester	20	31	8	1
3rd trimester	19	33	5	0
Monounsaturated fatty acids (g/d)	***0 < 20***	***20 < 40***	***40 < 60***	***60 < 80***
2nd trimester	10	38	9	3
3rd trimester	14	40	3	0
Saturated fatty acids (g/d)	***0 < 20***	***20 < 40***	***40 < 60***	***60 < 80***
2nd trimester	16	34	9	1
3rd trimester	17	31	9	0

[1] n=60 at 28 weeks gestation
[2] n=57 at 35 weeks gestation

8.3 Dietary Sources and Contribution of *Trans* Fatty Acids by Food Groups

Baked foods were the major source of TFA in the women's diets, contributing over 30% of total dietary TFA in the second and third trimester (**Figures 8.3 and 8.4**). Most of the women consumed baked foods in the second (n=58/60) and third trimester (n=57/57) providing a mean 1.3 and 1.1 g of total dietary TFA, respectively (**Table 8.10**). The top five ranked foods, classified by food group, were all baked foods (**Figures 8.5 and 8.6**). Snack foods and breads each contributed 11% of total dietary TFA in the second trimester and 9 and 10%, respectively, of the total dietary TFA in the third trimester. Snack foods were consumed by more women in the second trimester (n=58/60) than in the third trimester (n=49/57), and provided an average of 0.4 g of dietary TFA in both trimesters of those women who consumed snack foods. Fast foods contributed approximately 11 and 13% of total dietary TFA in the second and third trimester, respectively. Forty-five women consumed fast foods in the second trimester, which contributed an average of 0.5 g of dietary TFA to the diet of those women who consumed fast foods. In the third trimester 46 women consumed fast foods also contributing an average of 0.5 g of dietary TFA. Less significant contributors to the total dietary TFA intake in the second and third trimester were margarines and shortenings (7 and 9%, respectively), dairy products (6 and 7%, respectively), breakfast cereals (5 and 6%, respectively), pasta and other grains such as stuffing and bread crumbs (4 and 6%, respectively), meats (3%), oils and sauces (3%), peanut butter (3 and 2%, respectively) and chocolate and candies (2 and 0%, respectively).

Margarines and shortenings contributed 0.5 and 0.6 g dietary TFA to the diet of those eating these fats in the second (n=32/60) and third trimester (n=29/57), respectively. Of note, 28 women did not use margarines or shortenings as an added fat, e.g. as a spread on bread or with vegetables, in either the second or third trimester. Breads contributed 0.4 g of dietary TFA to the diet of women who consumed bread in both the second (n=58/60) and third trimester (n=51/57). Cereals, including waffles and pancakes, provided an average of 0.3 g of dietary TFA to the diet of women who consumed these foods in the second (n=33/60) and third trimester (n=36/57). Pasta and other grains such as bread crumbs and stuffing contributed an average of 0.3 and 0.4 g of dietary TFA to the diet of women who consumed these foods in the second (n=32/60) and third trimester (n=27/57), respectively. The majority of women consumed dairy products, contributing an average of 0.2 g of dietary TFA in the second (n=59/60), and third trimester (n=56/57). Twenty-seven and 25 women consumed peanut butter, contributing 0.2 g of dietary TFA in both second and third trimester, respectively. Meats, e.g. beef, contributed an average of 0.2 g of dietary TFA in both second (n=43/60) and third trimester (n=36/57). Most women consumed oils and sauces such as mayonnaise and salad dressings in the second (n=52/60) and third trimester (n=50/57). Oils and sauces contributed an average of 0.2 and 0.1g of dietary TFA, to the diet of women who consumed oils and sauces in the second and third trimester, respectively. Battered fish contributed 0.2 and 0.1 g of dietary TFA to the diet of a small number of women in the second (n=8/60) and third trimester (n=10/57), respectively. Fruits and vegetables, such as fruit leather and frozen potato products, contributed an average of 0.2 g of dietary TFA to the diet of women who consumed these products in the second (n=17/60) and third trimester (n=10/57), respectively. Chocolates and candy contributed about 0.1 g of dietary TFA to the diet of women who consumed chocolate and candy in the second trimester (n=31/60) and less than 0.1 g in the third trimester (n=16/57). Canned and packaged soups contributed 0.1 g of dietary TFA in both the second (n=11/60) and third trimester (n=6/57) but again were eaten by relatively few of the women.

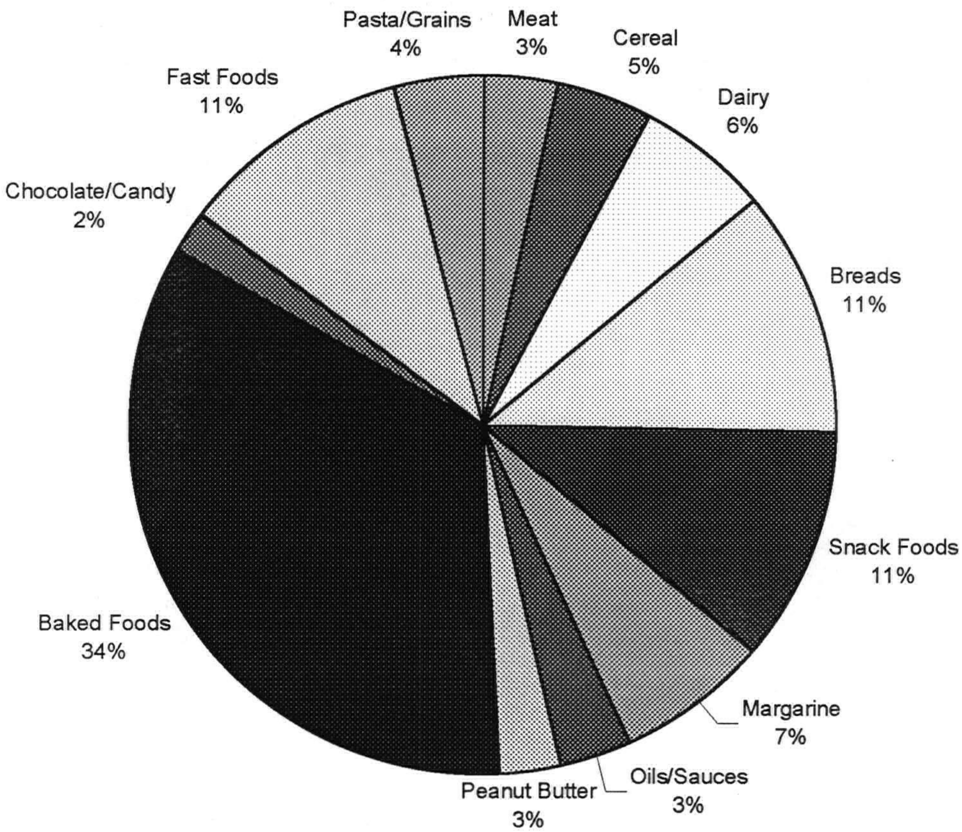


Figure 8.3 Estimated contribution of *trans* fatty acids by food groups to the total *trans* fatty acid intake of women in their second trimester, n=60[1]

[1]The mean percent contribution of each food group to the total TFA intake was calculated for all 60 women

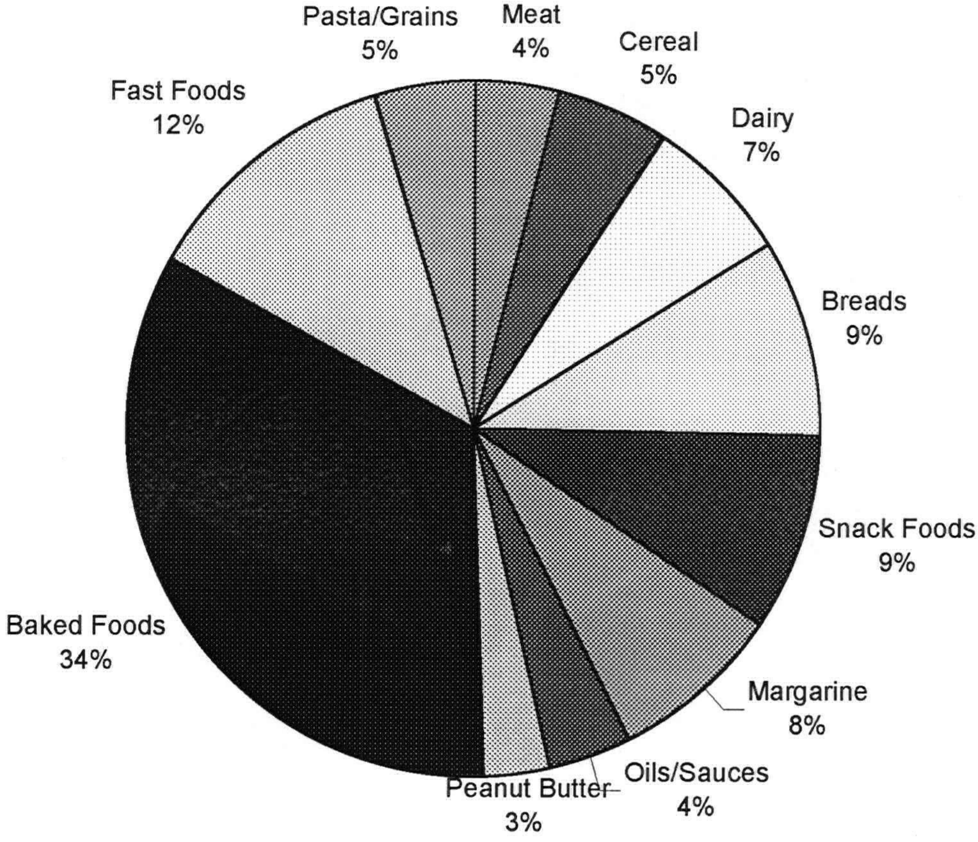


Figure 8.4 Estimated contribution of *trans* fatty acids by food groups to the total *trans* fatty acids women in their third trimester, n=57[1]

[1]The mean percent contribution of each food group to the total TFA intake was calculated using all 57 women

Table 8.10 Quantity of *trans* fatty acids consumed by food group in the second and third trimester[1]

Food Group	2nd Trimester	3rd Trimester
	(g)	
Baked Foods[2]	1.34 ± 0.02 (0.05-6.43) *n=58*	1.09 ± 0.11 (0.01-3.84) *n=57*
Fast Foods	0.54 ± 0.01 (0.02-6.66) *n=45*	0.55 ± 0.08 (0.01-4.06) *n=46*
Margarine	0.50 ± 0.01 (0.01-2.46) *n=32*	0.60 ± 0.17 (0.02-3.64) *n=29*
Snack Foods[3]	0.44 ± 0.01 (0.01-2.79) *n=56*	0.35 ± 0.04 (0.01-1.11) *n=49*
Breads	0.44 ± 0.01 (0.02-2.41) *n=58*	0.36 ± 0.05 (0.01-1.40) *n=51*
Cereal/Waffles/Pancakes	0.32 ± 0.01 (0.01-1.32) *n=33*	0.30 ± 0.06 (0.01-1.21) *n=36*
Pasta and other Grain Products[4]	0.30 ± 0.01 (0.01-5.13) *n=32*	0.41 ± 0.17 (0.01-4.06) *n=27*
Dairy Products	0.24 ± 0.00 (0.02-0.75) *n=59*	0.22 ± 0.02 (0.01-0.66) *n=56*
Peanut Butter	0.23 ± 0.00 (0.01-1.43) *n=27*	0.15 ± 0.03 (0.01-0.49) *n=25*
Meat	0.17 ± 0.00 (0.01-0.79) *n=43*	0.16 ± 0.02 (0.03-0.55) *n=36*
Fish	0.15 ± 0.00 (0.06-0.37) *n=8*	0.09 ± 0.01 (0.03-0.19) *n=10*
Oil/Sauces/Dressings	0.15 ± 0.00 (0.01-1.11) *n=52*	0.12 ± 0.04 (0.01-1.47) *n=50*
Fruits and Vegetables[5]	0.14 ± 0.00 (0.01-0.75) *n=17*	0.16 ± 0.06 (0.01-0.55) *n=10*
Chocolate/Candy/Icing	0.13 ± 0.00 (0.01-0.96) *n=31*	0.04 ± 0.01 (0.01-0.13) *n=16*
Soup	0.10 ± 0.00 (0.01-0.19) *n=11*	0.10 ± 0.02 (0.01-0.17) *n=6*

[1] Values are expressed as mean ± SE (range) for women consuming the food group and were obtained from food frequency data
[2] Includes cookies, cakes, pies, muffins, scones, croissants, pastries
[3] Includes potato, corn and taco chips, popcorn, crackers, cereal, granola bars
[4] Includes pasta, breadcrumbs, croutons, taco shells
[5] includes fruit leather and frozen potato products

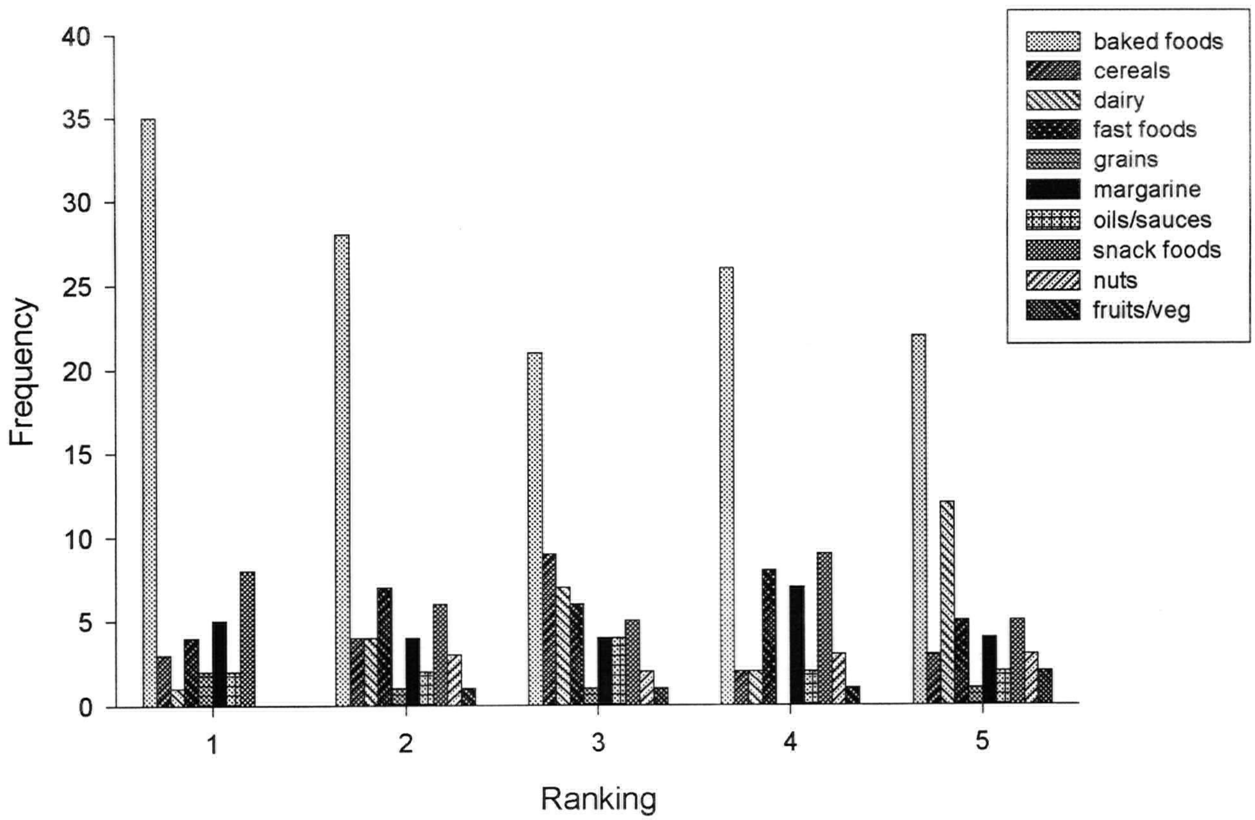


Figure 8.5 Top five individual foods classified by food groups that contributed to *trans* fatty acid intake of women at 28 weeks gestation by rank order n=60[1]

[1]The top five foods that provided the highest amount of dietary TFA (g), to each woman's total TFA intake, were ranked 1 to 5 and were classified by food group. The frequency represents the number of women who consumed a food containing TFA, categorized by food group.

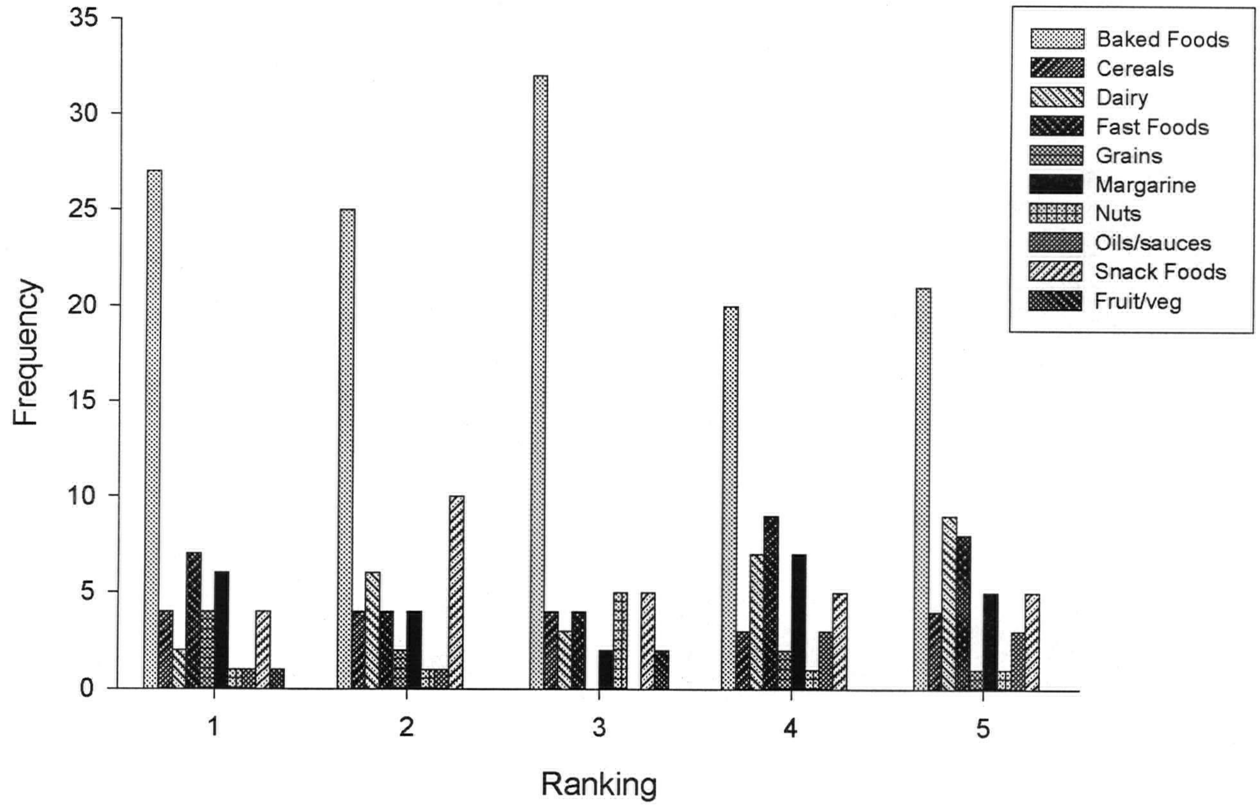


Figure 8.6 Top five individual foods classified by food groups which contributed to *trans* fatty acid intake of women at 35 weeks gestation by rank order, n=57[1]

[1]The top five foods that provided the highest amount of dietary TFA (g), to each woman's total TFA intake, were ranked 1 to 5 and were classified by food group. The frequency represents the number of women who consumed a food containing TFA, categorized by food group.

8.4 Maternal Plasma Fatty Acids

8.4.1 Triglyceride

The mean total *trans* fatty acids in maternal plasma TG was 3.98 (range 1.26-7.90)% of total fatty acids (**Table 8.11**). *Trans* 18:1 and 18:2 were the predominant *trans* isomers and represented a mean of 2.07 (range 0.65-5.06)% and 1.91 (range 0.40-3.27)% of plasma TG total fatty acids, respectively. Polyunsaturated fatty acids represented a mean of 20.11 (range 13.26-33.64)% of total fatty acids in maternal plasma TG. *Cis* 18:2n-6 was the predominant polyunsaturated fatty acid in maternal plasma TG, and represented a mean of 13.65 (range 7.75-26.16)% of total fatty acids. *Cis* 18:3n-3 was present at a mean of 1.21 (range 0.42-3.75)% of total fatty acids and the remaining *cis* polyunsaturated fatty acids, 18:3n-6, 20:2n-6, 20:3n-6, 20:3n-9, 20:4n-6, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3 and CLA were each present at less than 1.0% of total fatty acids. Monounsaturated fatty acids represented a mean 42.89 (range 33.85-48.54)% of total fatty acids in maternal plasma TG. The predominant monounsaturated fatty acids were the *cis* isomers of 18:1 and 16:1 and represented a mean of 35.61 (range 26.97-43.90)% and 4.28 (range 1.48-10.07)% of total fatty acids, respectively. The remaining monounsaturated fatty acids, 14:1, 20:1, 22:1 and 24:1, each represented less than 1.0% of total fatty acids in maternal plasma TG. Saturated fatty acids represented a mean of 37.00 (range 26.00-47.60)% of total fatty acids in maternal plasma TG. Palmitic acid (16:0) was the predominant saturated fatty acid in TG, and represented a mean of 29.51 (range 19.92-38.07)% of total fatty acids followed by 18:0 and 14:0 with a mean of 4.12 (2.53-9.25)% and 2.77 (range 0.96-4.62)% of total fatty acids, respectively. The remaining saturated fatty acids, 12:0, 20:0, 22:0 and 24:0, were each present at less than 1.0% of total fatty acids.

8.4.2 Phospholipid

The mean total *trans* fatty acids in maternal plasma PL was 2.37 (range 1.13-4.47)% of total fatty acids (**Table 8.11**). *Trans* 18:1 and 18:2 were the predominant *trans* isomers present, with a mean of 1.37 (range 0.58-3.01)% and 1.00 (range 0.35-1.69)% of total fatty acids, respectively. Polyunsaturated fatty acids represented a mean of 42.99 (range 38.76-47.31)% of total fatty acids in maternal plasma PL. *Cis* 18:2n-6 was the predominant polyunsaturated fatty acid in maternal plasma PL, and represented a mean of 20.81 (range 13.59-27.24)% of total fatty acids. *Cis* 20:3n-6, 20:4n-6 and 22:6n-3 were present at a mean of 3.78 (range 2.35-4.98)%, 8.73 (range 5.55-11.76)%, and 5.04 (range 2.84-8.11)% of total fatty acids, respectively. The remaining *cis* polyunsaturated fatty acids, 18:3n-3, 18:3n-6, 20:2n-6, 20:3n-9, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3 and CLA were each present at less than 1.0% of total fatty acids. Monounsaturated fatty acids represented a mean of 15.21 (range 11.89-18.67)% of total fatty acids in maternal plasma PL. The predominant monounsaturated fatty acids were the *cis* isomers of 18:1 and 16:1 and represented a mean of 12.28 (range 9.10-14.53)% and 1.05 (range 0.58-2.41)% of total fatty acids, respectively. The remaining monounsaturated fatty acids, 14:1, 20:1, 22:1 and 24:1, each represented less than 1.0% of total fatty acids in maternal plasma PL. Saturated fatty acids represented a mean of 41.84 (range 38.90-41.84)% of total fatty acids in maternal plasma PL. Palmitic acid (16:0) was the predominant saturated fatty acid in PL and represented a mean of 30.61 (range 27.94-30.61)% of total fatty acids. Stearic acid (18:0) represented a mean of 10.40 (range 9.37-11.81)% of total fatty acids, respectively. The remaining saturated fatty acids, 12:0, 14:0, 20:0, 22:0 and 24:0 were each present at less than 1.0% of total fatty acids.

8.4.3 Cholesteryl ester

The mean total *trans* fatty acids in maternal plasma CE was 1.57 (range 0.64-3.70)% of total fatty acids (**Table 8.11**). *Trans* 18:1 and 18:2 were the predominant *trans* isomers identified with a mean 0.64 (range 0.18-1.71)% and 0.92 (range 0.32-2.60)% of total fatty acids, respectively. Polyunsaturated fatty acids represented a mean of 53.27 (range 44.34-66.05)% of total fatty acids in maternal plasma CE. *Cis* 18:2n-6 was the predominant polyunsaturated fatty acid in maternal plasma CE, and represented a mean of 42.44 (range 33.19-56.73)% of total fatty acids. *Cis* 20:4n-6 and 18:3n3 were present at a mean of 5.31 (range 2.48-8.37)% and 1.02 (range 0.05-2.62) % of total fatty acids, respectively. The remaining *cis* polyunsaturated fatty acids, 18:3n-6, 20:2n-6, 20:3n-6, 20:3n-9, 20:5n-3, 22:4n-6, 22:5n-6, 22:5n-3, 22:6n-3 and CLA were each present at less than 1.0% of total fatty acids. Monounsaturated fatty acids represented a mean of 27.89 (range 19.34-36.59)% of total fatty acids in maternal plasma CE. The predominant monounsaturated fatty acids were the *cis* isomers of 18:1 and 16:1 and represented a mean of 22.24 (range 16.66-27.14)% and 4.41(range 2.03-11.25)% of total fatty acids, respectively. The remaining monounsaturated fatty acids, 14:1, 20:1, 22:1 and 24:1 each accounted for less than 1.0% of total fatty acids in maternal plasma CE. Saturated fatty acids represented a mean of 18.85 (range 12.97-28.82)% of total fatty acids in maternal plasma CE. Palmitic acid (16:0) was the predominant saturated fatty acid in CE and represented a mean of 13.79 (range 10.62-16.77)% of total fatty acids followed by 18:0 and 14:0 with a mean 3.29 (range 0.87-9.70)% and 1.15 (range 0.04-1.71)% of total fatty acids, respectively. The remaining saturated fatty acids, 12:0, 20:0, 22:0 and 24:0, were each present at less than 1.0% of total fatty acids.

Table 8.11 Fatty acid composition of maternal plasma triglyceride (TG), phospholipid (PL) and cholesteryl ester (CE) at 35 weeks gestation (g/100g)[1]

Fatty Acid	TG	PL	CE
14:0	2.77 ± 0.12 (0.96-4.62)	0.39 ± 0.01 (0.18-0.64)	1.15 ± 0.04 (0.04-1.71)
16:0	29.51 ± 0.51 (19.92-38.07)	30.61 ± 0.15 (27.94-30.61)	13.79 ± 0.20 (10.62-16.77)
18:0	4.12 ± 0.14 (2.53-9.25)	10.40 ± 0.08 (9.37-11.81)	3.29 ± 0.33 (0.87-9.70)
20:0	0.15 ± 0.01 (0.03-0.32)	0.09 ± 0.00 (0.06-0.14)	0.15 ± 0.02 (0.02-0.54)
22:0	0.12 ± 0.01 (0.05-0.30)	0.23 ± 0.01 (0.08-0.49)	0.17 ± 0.02 (0.02-0.59)
cis 16:1	4.28 ± 0.18 (1.48-10.07)	1.05 ± 0.16 (0.58-2.41)	4.41 ± 0.21 (2.03-11.25)
cis 18:1	35.61 ± 0.48 (26.97-43.90)	12.28 ± 0.15 (9.10-14.53)	22.24 ± 0.45 (16.66-27.14)
cis 20:1	0.58 ± 0.02 (0.34-1.08)	0.20 ± 0.01 (0.12-0.36)	0.29 ± 0.03 (0.01-1.35)
cis 22:1	0.16 ± 0.01 (0.06-0.44)	0.09 ± 0.01 (0.02-0.28)	0.18 ± 0.02 (0.02-0.81)
cis 18:2n6	13.65 ± 0.49 (7.75-26.16)	20.81 ± 0.40 (13.59-27.24)	42.44 ± 0.68 (33.19-56.73)
cis 18:3n6	0.14 ± 0.01 (0.04-0.37)	0.06 ± 0.00 (0.00-0.12)	0.51 ± 0.03 (0.17-1.27)
cis 18:3n3	1.21 ± 0.08 (0.42-3.75)	0.36 ± 0.02 (0.17-0.75)	1.02 ± 0.05 (0.05-2.62)
cis 20:2n6	0.29 ± 0.01 (0.14-0.50)	0.49 ± 0.01 (0.30-0.72)	0.09 ± 0.01 (0.01-0.24)
cis 20:3n6	0.25 ± 0.01 (0.16-0.44)	3.78 ± 0.07 (2.35-4.98)	0.77 ± 0.01 (0.49-1.03)
cis 20:3n9	0.12 ± 0.01 (0.03-0.29)	0.17 ± 0.01 (0.06-0.51)	0.10 ± 0.01 (0.03-0.27)
cis 20:4n6	0.88 ± 0.04 (0.47-2.12)	8.73 ± 0.17 (5.55-11.76)	5.31 ± 0.14 (2.48-8.37)
cis 20:5n3	0.10 ± 0.01 (0.02-0.42)	0.52 ± 0.03 (0.11-1.64)	0.58 ± 0.05 (0.13-1.64)
cis 22:4n6	0.13 ± 0.01 (0.06-0.26)	0.39 ± 0.01 (0.18-0.75)	0.04 ± 0.01 (0.00-0.61)
cis 22:5n6	0.16 ± 0.01 (0.04-0.38)	0.57 ± 0.03 (0.19-1.27)	0.06 ± 0.01 (0.00-0.17)
cis 22:5n3	0.13 ± 0.01 (0.04-0.37)	0.71 ± 0.02 (0.24-1.11)	0.07 ± 0.02 (0.00-0.86)
cis 22:6n3	0.53 ± 0.04 (0.23-1.40)	5.04 ± 0.17 (2.84-8.11)	0.69 ± 0.03 (0.05-1.37)
CLA[2]	0.59 ± 0.03 (0.23-1.41)	0.32 ± 0.01 (0.10-0.58)	0.61 ± 0.06 (0.12-1.82)
trans 18:1[3]	2.07 ± 0.12 (0.65-5.06)	1.37 ± 0.07 (0.58-3.01)	0.64 ± 0.06 (0.18-1.71)
trans 18:2[3]	1.91 ± 0.09 (0.40-3.27)	1.00 ± 0.04 (0.35-1.69)	0.92 ± 0.04 (0.32-2.60)
Total *trans*[3]	3.98 ± 0.21 (1.26-7.90)	2.37 ± 0.10 (1.13-4.47)	1.57 ± 0.10 (0.64-3.70)
Saturates[4]	37.00 ± 0.64 (26.00-47.60)	41.84 ± 0.14 (38.90-41.84)	18.85 ± 0.56 (12.97-28.82)
MUFA[5]	42.89 ± 0.43 (33.85-48.54)	15.21 ± 0.23 (11.89-18.67)	27.89 ± 0.52 (19.34-36.59)
PUFA[6]	20.11 ± 0.52 (13.26-33.64)	42.99 ± 0.23 (38.76-47.31)	53.27 ± 0.63 (44.34-66.05)

[1] Data presented are means ± SE (range), n=58
[2] CLA = conjugated linoleic acids
[3] Includes all *trans* and *cis-trans* geometric and positional isomers
[4] Sum of all saturates, including 12:0 and 24:0
[5] Sum of all *cis* monounsaturates, including 14:1 and 24:1
[6] Sum of all *cis* polyunsaturates

8.5 Relations Between *Trans* Fatty Acids and *Cis* n-6 and n-3 Fatty Acids in Maternal Plasma

The maternal plasma TG percent TFA showed a significant inverse relation with *cis* 18:2n-6 ($p<0.05$) and *cis* 18:3n-3 ($p<0.05$) (**Figure 8.7 and Table 8.12**). There was no significant relation between the percent TFA in the maternal plasma TG and *cis* 20:4n-6 and 22:6n-3. The maternal plasma PL percent TFA showed no significant relation to the percent *cis* 18:2n-6, 18:3n-3, 20:4n-6 or 22:6n-3. The maternal plasma CE percent TFA, however, showed a significant inverse relation with *cis* 18:2n-6 ($p<0.01$), *cis* 20:4n-6 ($p<0.05$), and *cis* 22:6n-3 ($p<0.05$) and a positive relation with *cis* 18:3n-3 ($p<0.01$) (**Figure 8.9**).

Table 8.12 Relations between *trans* fatty acids and selected *cis* n-6 and n-3 fatty acids in maternal plasma at 35 weeks gestation and in newborn cord plasma at birth

	Maternal plasma *n=58*	Cord Plasma *n=70*
	(r)	
Triglyceride		
Cis 18:2n-6	-0.53[1]	-0.43[1]
Cis 18:3n-3	-0.41[1]	-0.12 (n.s.)
cis 20:4n-6	-0.08 (n.s.)[2]	-0.06 (n.s.)
cis 22:6n-3	-0.17 (n.s.)	-0.36[1]
Phospholipid		
cis 18:2n-6	-0.19 (n.s.)	-0.05 (n.s.)
cis 18:3n-3	-0.10 (n.s.)	0.14 (n.s.)
cis 20:4n-6	-0.03 (n.s.)	0.06 (n.s.)
cis 22:6n-3	-0.11 (n.s.)	-0.11 (n.s.)
Cholesteryl ester		
cis 18:2n-6	-0.68[1]	-0.35[1]
cis 18:3n-3	0.36[1]	0.07 (n.s.)
cis 20:4n-6	-0.28[3]	-0.27[3]
cis 22:6n-3	-0.32[3]	-0.12 (n.s.)

[1] $p<0.01$
[2] Not statistically significant
[3] $p<0.05$

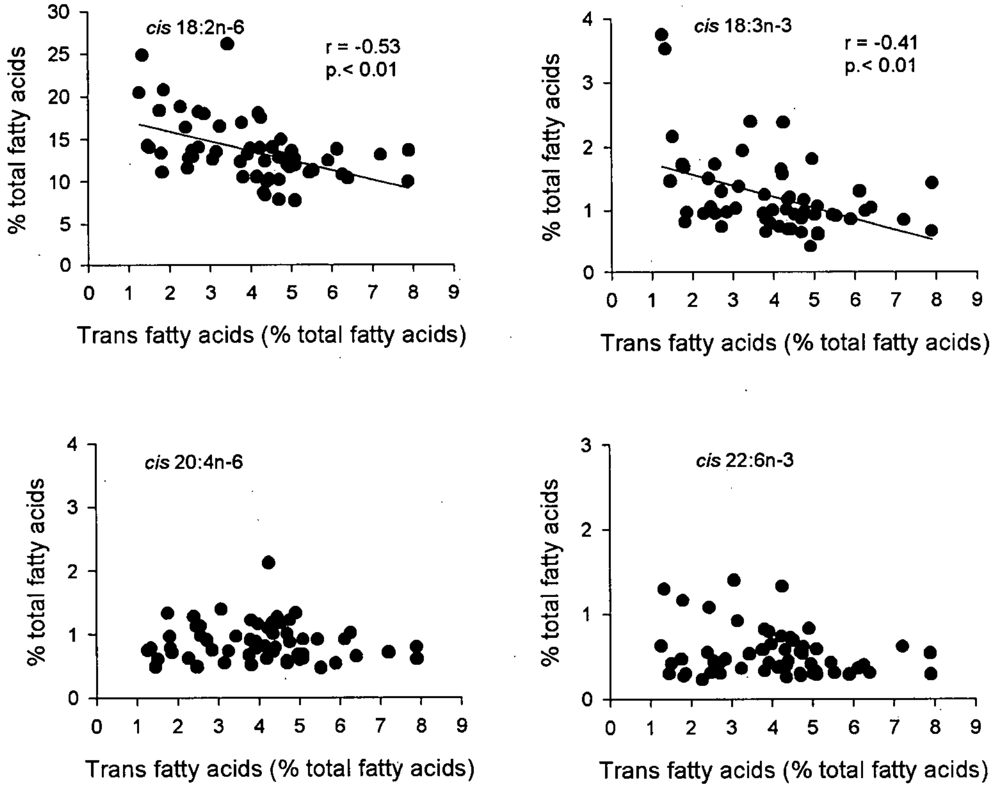


Figure 8.7 Scatter plots of selected *cis* n-6, n-3 and *trans* fatty acids in maternal plasma triglyceride at 35 weeks gestation, n=58

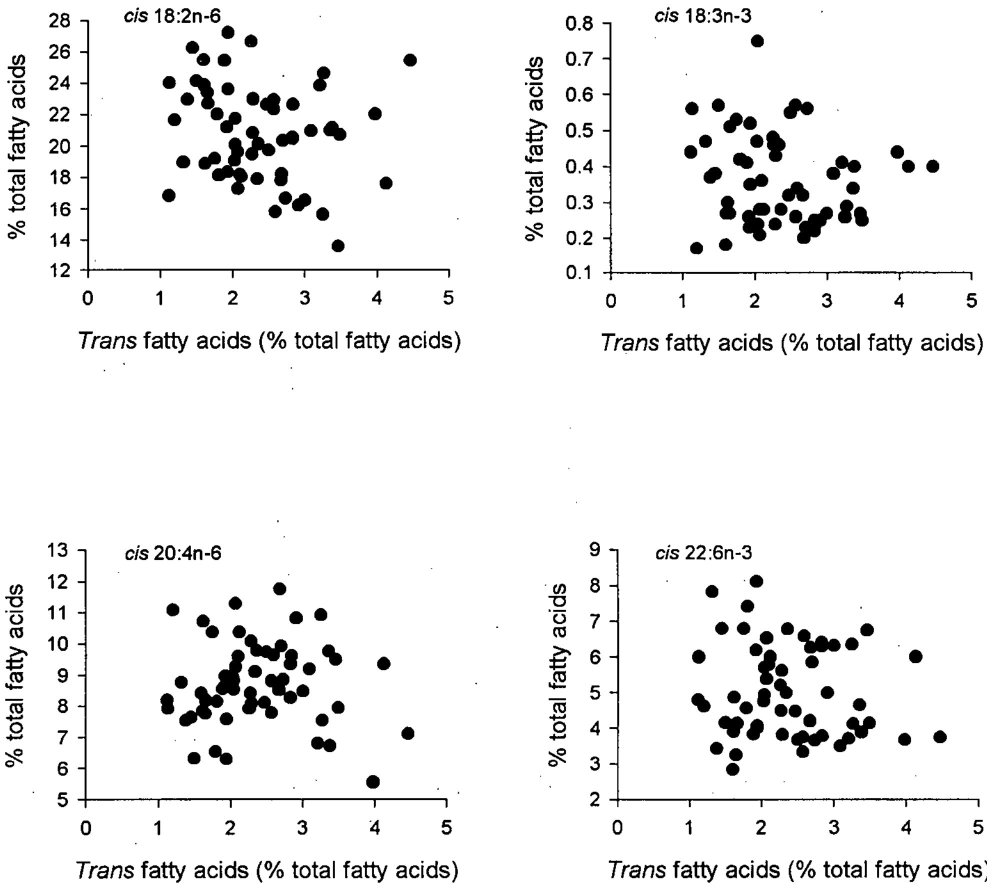


Figure 8.8 Scatter plots of selected *cis* n-6, n-3 and *trans* fatty acids in maternal plasma phospholipid at 35 weeks gestation, n=58[1]

[1] No statistically significant relations were found.

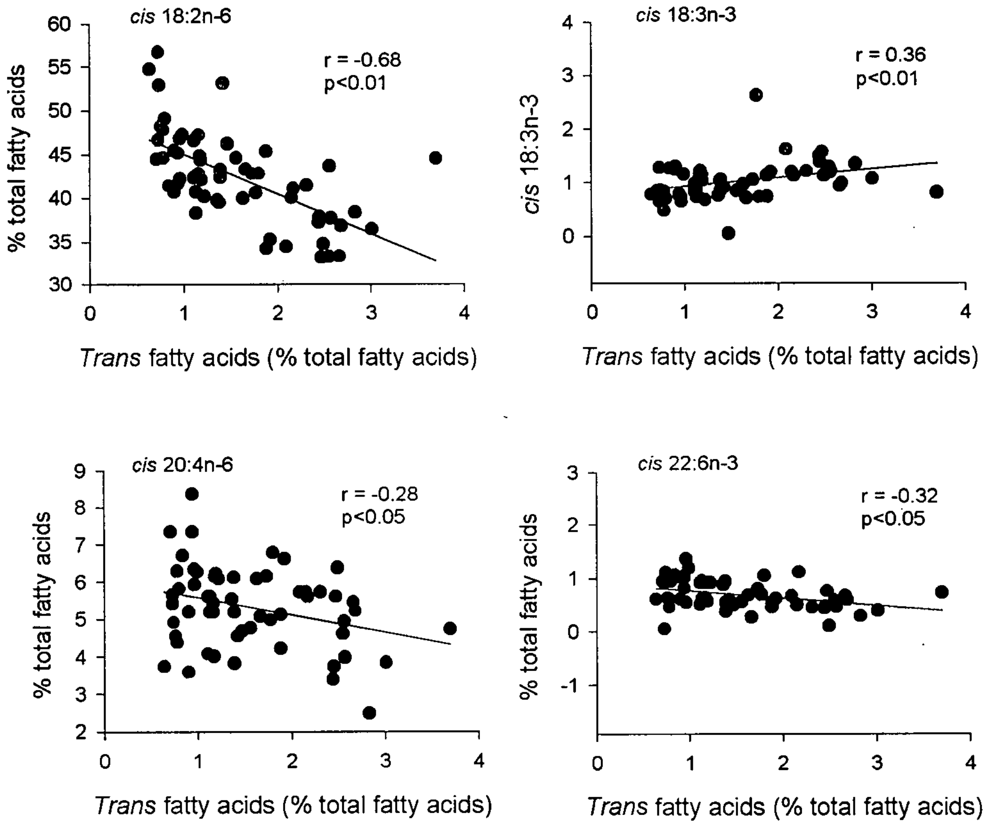


Figure 8.9 Scatter plots of selected *cis* n-6, n-3 and *trans* fatty acids in maternal plasma cholesteryl ester at 35 weeks gestation, n=58

8.6 Cord Plasma Fatty Acids

8.6.1 Triglyceride

The mean total *trans* fatty acids in cord plasma TG was 2.87 (range 0.64-12.79)% of total fatty acids (**Table 8.13**). *Trans* 18:1 and 18:2 were the predominant *trans* isomers, and represented a mean of 1.63 (range 0.06-9.61)% and 1.23 (range 0.12-3.18)% of total fatty acids, respectively. Polyunsaturated fatty acids represented a mean of 22.62 (range 8.39-31.34)% of total fatty acids in cord plasma TG. *Cis* 18:2n-6 was the predominant polyunsaturated fatty acid in cord plasma TG, and represented a mean of 10.09 (range 2.93-17.00)% of total fatty acids. *Cis* 20:4n-6 and 22:6n-3 were present at a mean 3.58 (range 1.04-7.26)% and 2.77 (range 0.49-5.91)% of total fatty acids. The remaining *cis* polyunsaturated fatty acids 18:3n-3, 18:3n-6, 20:2n-6, 20:3n-6, 20:3n-9, 20:5n-3, 22:4n-6, 22:5n-6 and 22:5n-3 were each present at less than 1.0% of total fatty acids. Monounsaturated fatty acids represented a mean of 41.58 (range 33.63-61.98)% of total fatty acids in cord plasma TG. The predominant monounsaturated fatty acids were the *cis* isomers of 18:1 and 16:1, and represented a mean of 33.23 (range 25.34-54.64)% and 5.56 (range 2.46-9.29)% of total fatty acids, respectively. The remaining monounsaturated fatty acids, 14:1, 20:1, 22:1 and 24:1, each represented less than 1.0% of total fatty acids in cord plasma TG. Saturated fatty acids represented a mean of 35.80 (range 29.63-45.96)% of total fatty acids in cord plasma TG. Palmitic acid (16:0) was the predominant saturated fatty acid in TG and represented a mean of 25.73 (range 18.70-31.30)% of total fatty acids followed by 18:0 and 14:0 with a mean 7.00 (3.95-12.74)% and 1.69 (range 0.60-3.88)% of total fatty acids, respectively. The remaining saturated fatty acids, 12:0, 20:0, 22:0 and 24:0, were each present at less than 1.0% of total fatty acids.

8.6.2 Phospholipid

The mean total *trans* fatty acids in cord plasma PL was 0.67 (range 0.11-1.33)% of total fatty acids (**Table 8.13**). *Trans* 18:1 and 18:2 were the predominant *trans* isomers present with a mean of 0.40 (range 0.04-0.97)% and 0.27 (range 0.03-0.52)% of total fatty acids, respectively. Polyunsaturated fatty acids represented a mean of 42.51 (range 38.22-45.52)% of total fatty acids in cord plasma PL. *Cis* 20:4n-6 was the predominant polyunsaturated fatty acid in cord plasma PL, and represented a mean of 17.71 (range 13.79-23.04)% of total fatty acids. *Cis* 22:6n-3, 18:2n-6 and 20:3n-6 were present at a mean 7.65 (range 4.35-11.67)%, 7.52 (range 5.04-10.66)%, and 5.72 (range 2.55-9.96)% of total fatty acids, respectively. The remaining *cis* polyunsaturated fatty acids, 18:3n-3, 18:3n-6, 20:2n-6, 20:3n-9, 20:5n-3, 22:4n-6, 22:5n-6 and 22:5n-3, were each present at less than 1.0% of total fatty acids. Monounsaturated fatty acids represented a mean of 12.62 (range 9.99-17.38)% of total fatty acids in cord plasma PL. The predominant monounsaturated fatty acids were the *cis* isomers of 18:1 and 16:1, and represented a mean of 11.19 (range 9.20-15.49)% and 1.01 (range 0.64-1.70)% of total fatty acids, respectively. The remaining monounsaturated fatty acids, 14:1, 20:1, 22:1 and 24:1, each represented less than 1.0% of total fatty acids in cord plasma PL. Saturated fatty acids represented a mean of 44.68 (range 42.86-46.18)% of total fatty acids in cord plasma PL. Palmitic acid (16:0) was the predominant saturated fatty acid in PL and represented a mean of 28.82 (range 26.10-31.00)% of total fatty acids followed by 18:0 with a mean 14.94 (range 13.04-16.80)% of total fatty acids, respectively. The remaining saturated fatty acids, 12:0, 14:0, 20:0, 22:0 and 24:0, were each present at less than 1.0% of total fatty acids.

8.6.3 Cholesteryl ester

The mean total *trans* fatty acids in cord plasma CE was 2.04 (range 0.72-4.30)% of total fatty acids (**Table 8.11**). *Trans* 18:1 and 18:2 were the predominant *trans* isomers present, with a mean of 1.00 (range 0.11-2.94)% and 1.04 (range 0.13-3.10)% of total fatty acids, respectively. Polyunsaturated fatty acids represented a mean of 33.19 (range 19.21-42.35)% of total fatty acids in maternal plasma CE. *Cis* 18:2n-6 and 20:4n-6 were the predominant polyunsaturated fatty acid in maternal plasma CE, and represented a mean of 15.60 (range 8.97-22.72)% and 11.79 (range 2.05-17.40)% of total fatty acids, respectively. *Cis* 22:6n-3 and 20:3n-6 were present at a mean 1.44 (range 0.55-3.14) and 1.34 (range 0.40-2.51)% of total fatty acids, respectively. The remaining *cis* polyunsaturated fatty acids, 18:3n-6, 18:3n-3, 20:2n-6, 20:3n-9, 20:5n-3, 22:4n-6, 22:5n-6 and 22:5n-3 were each present at less than 1.0% of total fatty acids. Monounsaturated fatty acids represented a mean of 38.39 (range 29.58-56.03)% of total fatty acids in cord plasma CE. The predominant monounsaturated fatty acids were the *cis* isomers of 18:1 and 16:1, and represented a mean of 30.00 (range 22.30-48.90)% and 6.94 (range 2.71-11.16)% of total fatty acids, respectively. The remaining monounsaturated fatty acids, 14:1, 20:1, 22:1 and 24:1, each represented less than 1.0% of total fatty acids in cord plasma CE. Saturated fatty acids represented a mean of 28.41 (range 24.52-35.05)% of total fatty acids in cord plasma CE. Palmitic acid (16:0) was the predominant saturated fatty acid in CE and represented a mean of 20.66 (range 16.55-25.49)% of total fatty acids followed by 18:0 and 14:0 with a mean 5.26 (range 2.71-8.89)% and 1.06 (range 0.51-3.38)% of total fatty acids, respectively. The remaining saturated fatty acids, 12:0, 20:0, 22:0 and 24:0, were each present at less than 1.0% of total fatty acids.

8.7 Relations Between Cord Plasma *Trans* Fatty Acids and *Cis* n-6 and n-3 Fatty Acids

The cord plasma TG percent TFA showed a significant inverse correlation to the percent *cis* 18:2n-6 ($p<0.01$) and *cis* 22:6n-3 ($p<0.01$) (**Table 8.12 and Figure 8.10**). The cord plasma PL percent TFA showed no significant relation to the percent *cis* 18:2n-6, 18:3n-3, 20:4n-6 or 22:6n-3. The cord plasma CE percent TFA, however, showed a significant inverse relation to the percent *cis* 18:2n-6 ($p<0.01$) and *cis* 20:4n-6 ($p<0.05$). (**Figure 8.11**).

Table 8.13 Fatty acid composition in cord plasma triglyceride (TG), phospholipid (PL) and cholesteryl ester (CE) of term gestation infants at birth (g/100g)[1]

Fatty Acid	TG	PL	CE
12:0	0.22 ± 0.03 (0.00-0.92)	0.02 ± 0.00 (0.00-0.08)	0.32 ± 0.03 (0.00-1.19)
14:0	1.69 ± 0.07 (0.60-3.88)	0.24 ± 0.01 (0.16-0.51)	1.06 ± 0.05 (0.51-3.38)
16:0	25.73 ± 0.31 (18.70-31.30)	28.82 ± 0.12 (26.10-31.00)	20.66 ± 0.17 (16.55-25.49)
18:0	7.00 ± 0.22 (3.95-12.74)	14.94 ± 0.09 (13.04-16.80)	5.26 ± 0.15 (2.71-8.89)
20:0	0.36 ± 0.03 (0.11-1.22)	0.16 ± 0.00 (0.10-0.30)	0.34 ± 0.04 (0.08-2.50)
22:0	0.53 ± 0.03 (0.12-1.22)	0.30 ± 0.01 (0.08-0.57)	0.52 ± 0.03 (0.03-1.48)
24:0	0.28 ± 0.01 (0.00-0.68)	0.19 ± 0.01 (0.07-0.29)	0.25 ± 0.02 (0.03-0.78)
cis 16:1	5.56 ± 0.17 (2.46-9.29)	1.01 ± 0.02 (0.64-1.70)	6.94 ± 0.19 (2.71-11.16)
cis 18:1	33.23 ± 0.74 (25.34-54.64)	11.19 ± 0.15 (9.20-15.49)	30.00 ± 0.56 (22.30-48.90)
cis 20:1	0.34 ± 0.03 (0.02-1.06)	0.07 ± 0.00 (0.02-0.13)	0.08 ± 0.02 (0.00-0.70)
cis 22:1-n9	0.83 ± 0.10 (0.00-3.54)	0.08 ± 0.01 (0.00-0.26)	0.37 ± 0.05 (0.00-2.07)
cis 18:2n6	10.09 ± 0.35 (2.93-17.00)	7.52 ± 0.15 (5.04-10.66)	15.60 ± 0.35 (8.97-22.72)
cis 18:3n6	0.37 ± 0.02 (0.13-0.87)	0.07 ± 0.00 (0.02-0.20)	0.61 ± 0.03 (0.08-1.27)
cis 18:3n3	0.48 ± 0.02 (0.16-0.96)	0.05 ± 0.00 (0.02-0.18)	0.15 ± 0.01 (0.00-0.48)
cis 20:2n6	0.30 ± 0.02 (0.14-0.91)	0.38 ± 0.01 (0.24-0.54)	0.10 ± 0.01 (0.00-0.49)
cis 20:3n6	0.86 ± 0.03 (0.28-1.44)	5.72 ± 0.13 (2.55-9.96)	1.34 ± 0.04 (0.40-2.51)
cis 20:3n9	0.55 ± 0.02 (0.21-0.99)	0.60 ± 0.04 (0.11-1.97)	0.28 ± 0.02 (0.04-0.88)
cis 20:4n6	3.58 ± 0.16 (1.04-7.26)	17.71 ± 0.24 (13.79-23.04)	11.79 ± 0.37 (2.05-17.40)
cis 20:5n3	0.30 ± 0.02 (0.03-1.33)	0.38 ± 0.02 (0.06-1.47)	0.33 ± 0.03 (0.09-1.46)
cis 22:4n6	0.62 ± 0.03 (0.16-1.36)	0.74 ± 0.02 (0.47-1.16)	0.03 ± 0.01 (0.00-0.57)
cis 22:5n6	0.93 ± 0.04 (0.36-1.64)	0.86 ± 0.04 (0.26-1.68)	0.13 ± 0.01 (0.00-0.90)
cis 22:5n3	0.52 ± 0.03 (0.18-1.37)	0.64 ± 0.03 (0.20-1.38)	0.36 ± 0.07 (0.00-3.11)
cis 22:6n3	2.77 ± 0.15 (0.49-5.91)	7.65 ± 0.20 (4.35-11.67)	1.44 ± 0.07 (0.55-3.14)
Trans 18:1[2]	1.63 ± 0.15 (0.06-9.61)	0.40 ± 0.02 (0.04-0.97)	1.00 ± 0.08 (0.11-2.94)
Trans 18:2[2]	1.23 ± 0.07 (0.12-3.18)	0.27 ± 0.01 (0.03-0.52)	1.04 ± 0.05 (0.13-3.10)
Total *trans*[2]	2.87 ± 0.19 (0.64-12.79)	0.67 ± 0.03 (0.11-1.33)	2.04 ± 0.11 (0.72-4.30)
Saturates[3]	35.80 ± 0.40 (29.63-45.96)	44.68 ± 0.11 (42.86-46.18)	28.41 ± 0.30 (24.52-35.05)
MUFA[4]	41.58 ± 0.71 (33.63-61.98)	12.62 ± 0.18 (9.99-17.38)	38.39 ± 0.60 (29.58-56.03)
PUFA[5]	22.62 ± 0.61 (8.39-31.34)	42.51 ± 0.19 (38.22-45.52)	33.19 ± 0.61 (19.21-42.35)

[1] Data are presented as means ± S.E. (range), n=70
[2] includes all *trans* and *cis-trans* geometric and positional isomers
[3] sum of all saturates

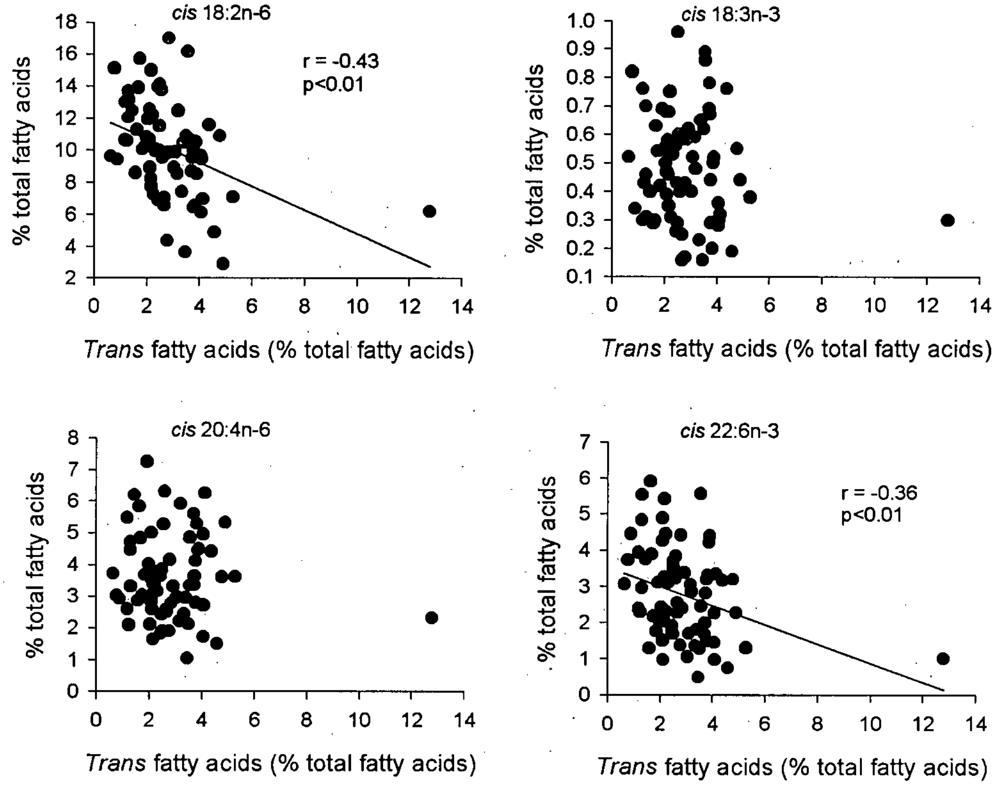


Figure 8.10 Scatter plots of selected *cis* n-6, n-3 fatty and *trans* fatty acids in cord plasma triglyceride at birth, n=70

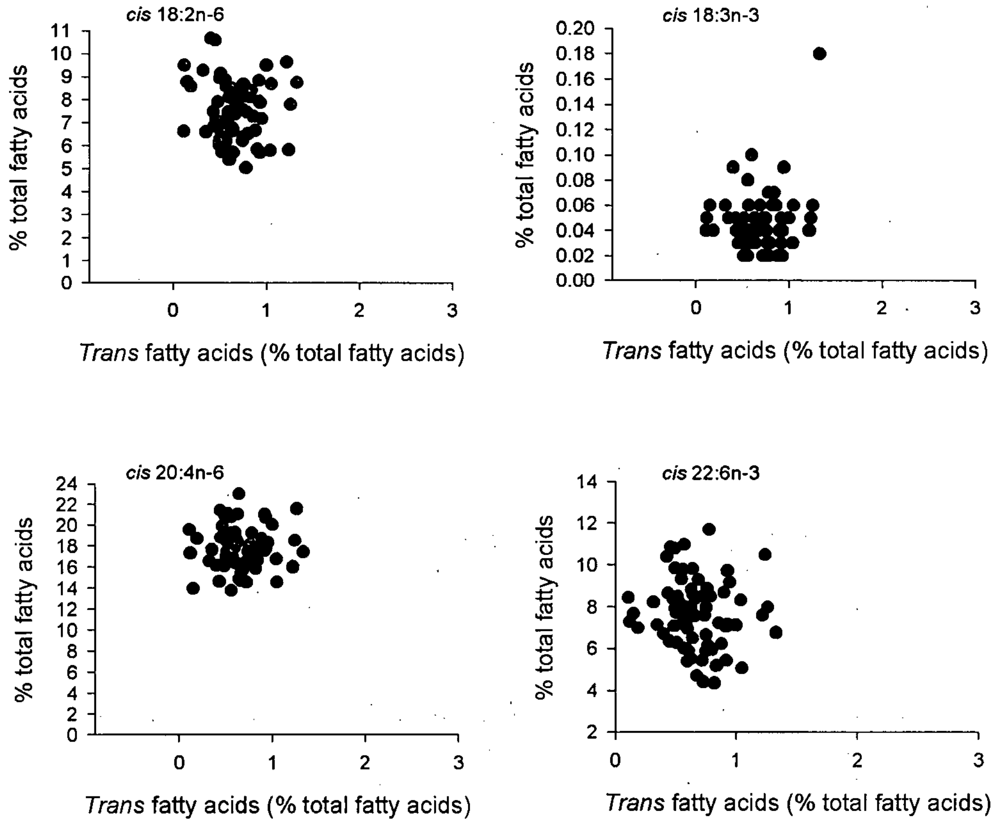


Figure 8.11 Scatter plots of selected *cis* n-6, n-3 and *trans* fatty acids in cord plasma phospholipid at birth, n=70[1]

[1] No statistically significant relations were found.

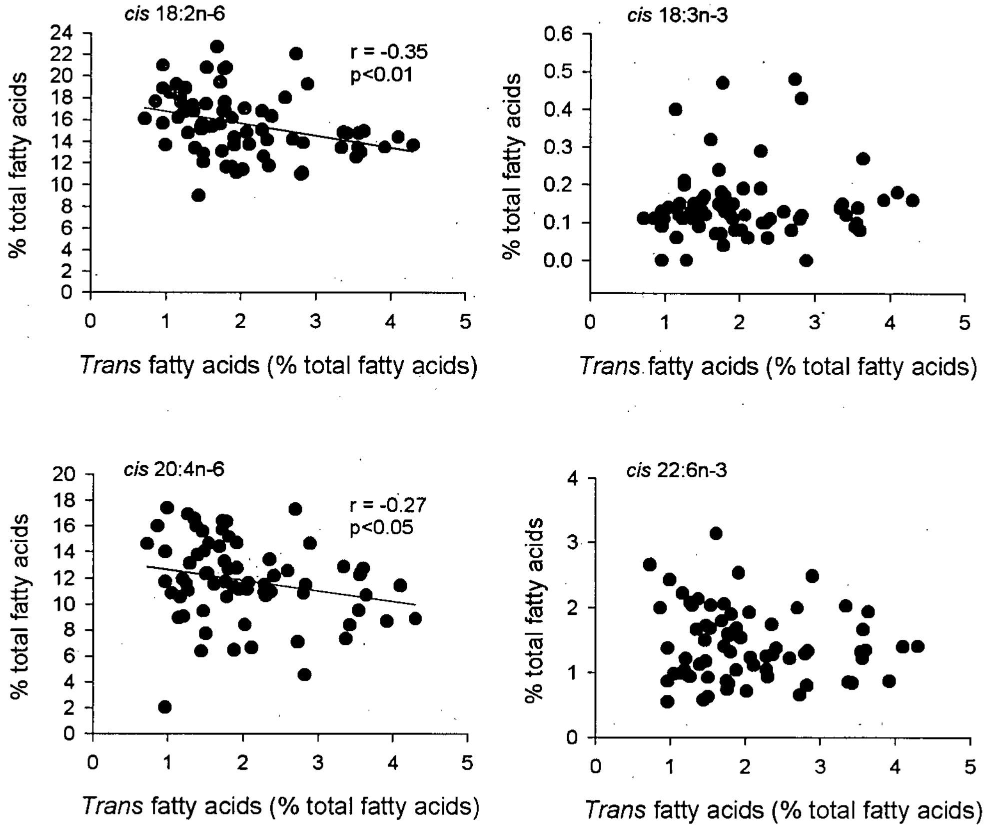


Figure 8.12 Scatter plots of selected *cis* n-6, n-3 and *trans* fatty acids in cord plasma cholesteryl ester at birth, n=70

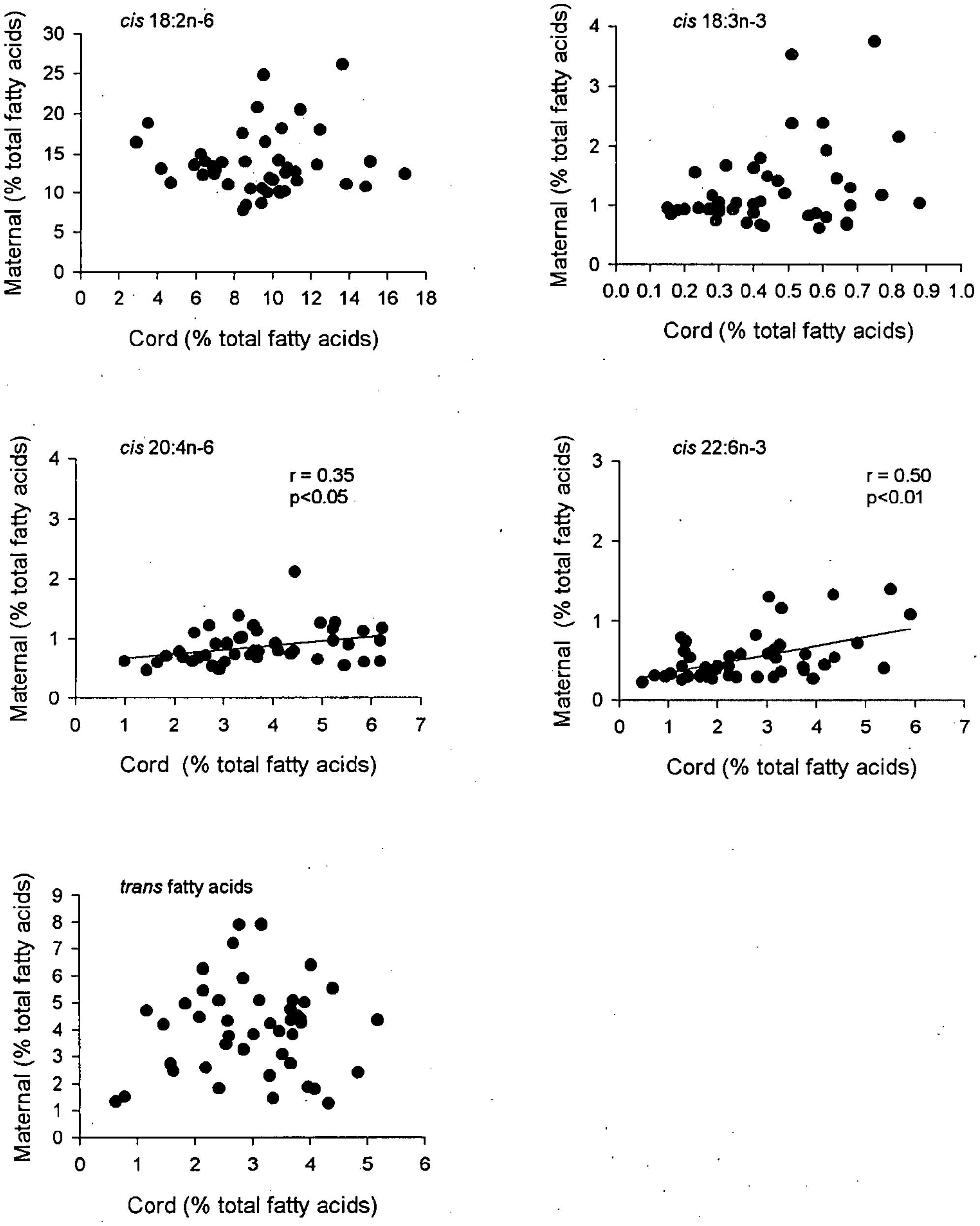
8.8 Relations Between Selected Fatty Acids in Maternal and Cord Plasma

A significant, positive relation was present between the level of *cis* 20:4n-6 in maternal and cord plasma TG (r = 0.35) and between the level of *cis* 22:6n-3 in maternal and cord plasma TG (r = 0.50) (**Table 8.14 and Figure 8.12**). Significant associations were also present between the percent of each of *cis* 18:2n-6 (r = 0.51), 20:4n-6 (r = 0.48) and 22:6n-3 (r = 0.48) in maternal and cord plasma PL as well as between total *trans* isomers in maternal and cord plasma PL (r = 0.53) (**Figure 8.13**). A significant association was also present between the levels of *cis* 18:2n-6 (r = 0.36) and 18:3n-3 (r = 0.33) in maternal and cord plasma in CE (**Figure 8.14**). No other statistically significant relations were present between maternal and cord plasma 18:2n-6, 18:3n-3, 20:4n-6 and 22:6n-3 in TG, PL or CE.

Table 8.14 Relations between *trans* fatty acids, *cis* n-6 and n-3 fatty acids in maternal plasma at 35 weeks gestation and cord plasma lipids at birth[1]

	Triglyceride *n=46*	Phospholipid *n=46*	Cholesteryl ester *n=46*
18:2n-6	0.04	0.51[3]	0.36[2]
18:3n-3	0.22	-0.23	0.33[2]
20:4n-6	0.35[2]	0.48[3]	0.21
22:6n-3	0.50[3]	0.48[3]	0.26
total *trans* isomers	0.07	0.53[3]	-0.02

[1] Values given are Pearson correlations (r)
[2] $p<0.05$
[3] $p<0.01$



[1] not statistically significant

Figure 8.13 Scatter plots of selected fatty acids in triglyceride in maternal plasma at 35 weeks gestation and cord plasma at birth, n=46

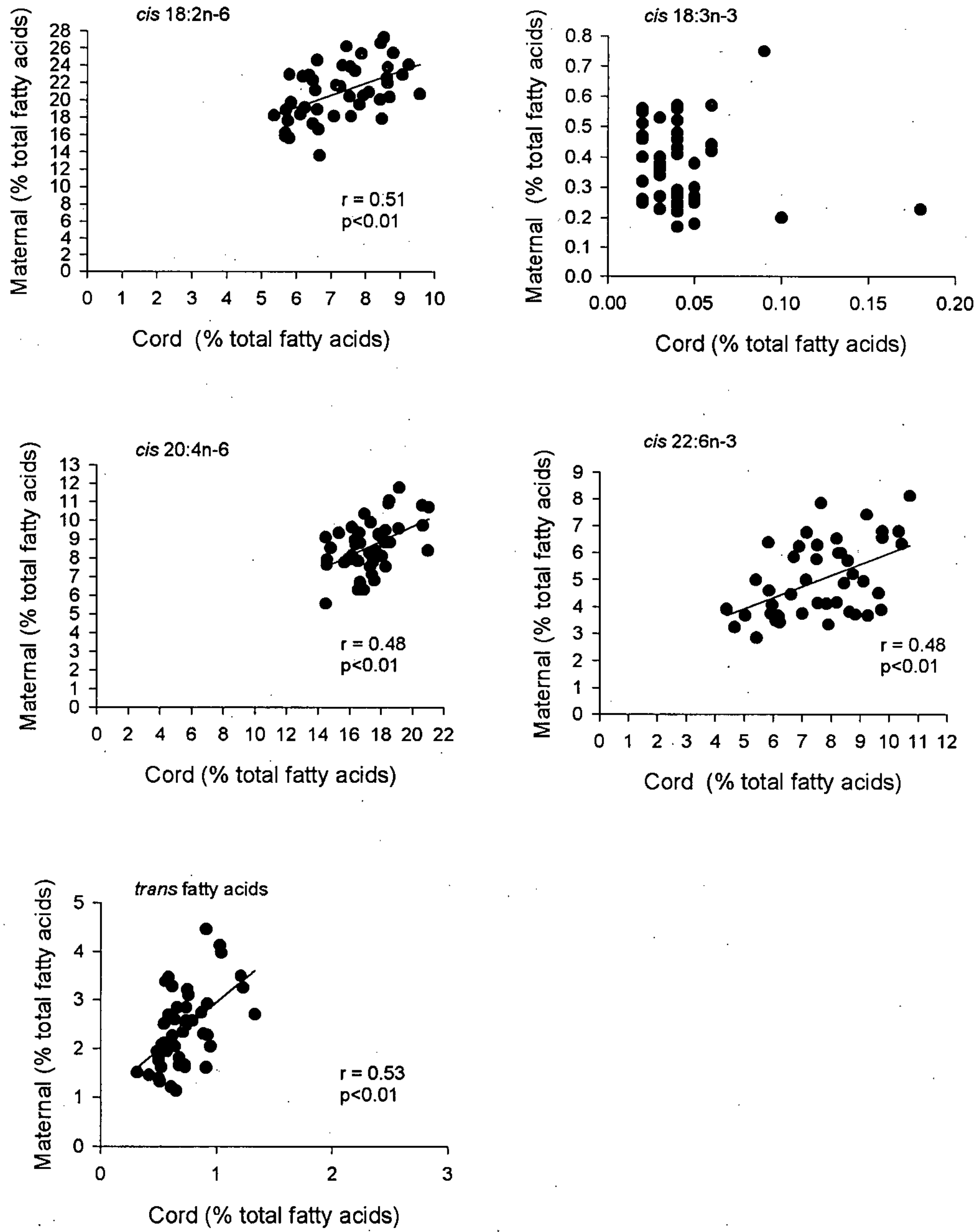


Figure 8.14 Scatter plots of selected fatty acids in phospholipid in maternal plasma at 35 weeks gestation and cord plasma at birth, n=46

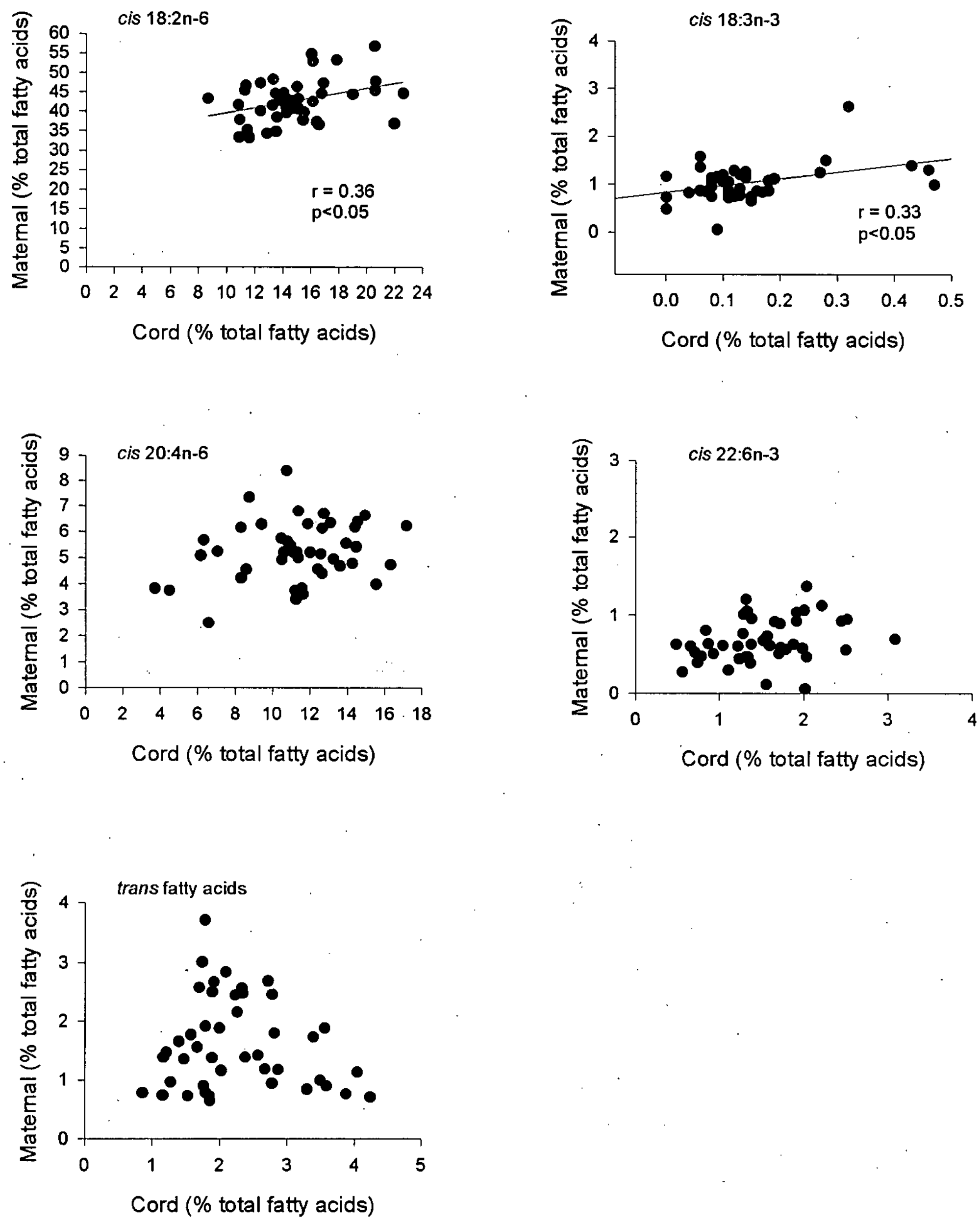


Figure 8.15 Scatter plots of selected fatty acids in cholesteryl ester in maternal plasma at 35 weeks gestation and cord plasma at birth n=46

8.9 Relations Between *Trans* Fatty Acid Intake and Level of *Trans* Fatty Acids in Maternal Plasma Lipids

8.9.1 Triglyceride

A significant association was present between the dietary TFA intake and the level of TFA in maternal plasma TG at 28 weeks gestation (r = 0.40) and at 35 weeks gestation (r = 0.31) (**Table 8.15**). When these relations were adjusted for total energy intake, the relations were still significant, r = 0.45 and 0.33 at 28 and 35 weeks, respectively.

8.9.2 Phospholipid

A significant association was present between the dietary TFA intake and the level of TFA in maternal plasma PL at both 28 weeks gestation (r = 0.36) and at 35 weeks gestation (r = 0.35). When these relations were adjusted for total energy intake, the relations were still significant, r = 0.40 and 0.39 at 28 and 35 weeks, respectively.

8.9.3 Cholesteryl ester

As for the plasma triglyceride and phospholipid, a significant association was present between the dietary TFA intake and level of TFA in maternal plasma CE at 28 weeks gestation, r = 0.26. However, when the intake of TFA was adjusted for total energy intake, the relation between TFA intake and cord plasma CE TFA was statistically significant (r = 0.34, $p<0.05$).

8.10 Relations Between *Trans* Fatty Acid Intake and Level of *Trans* Fatty Acids in Cord Plasma Lipids

A significant association was present between the dietary TFA intake at 28 weeks gestation and level of TFA in cord plasma PL (r = 0.31) both without and with adjustment for total energy intake (r = 0.38). No significant association was present between the dietary intake of TFA at 35 weeks gestation and level of TFA in cord plasma PL. However, when the intake of TFA was adjusted for total energy a statistically significant relation (r = 0.36, $p<0.05$) was present. There were no other statistically signification relations present between dietary intake of TFA without or with adjustment for energy intake, at either 28 or 35 weeks gestation and the cord plasma TG, PL or CE level of TFA.

Table 8.15 Relations between *trans* fatty acid (TFA) intake at 28 and 35 weeks gestation and *trans* fatty acids in maternal plasma at 35 weeks gestation and cord plasma at birth[1]

Plasma lipid	TFA Intake (g)		TFA Intake (g) adjusted for energy	
	28 weeks	35 weeks	28 weeks	35 weeks
Triglyceride				
Maternal	0.39[2]	0.31[3]	0.45[2]	0.33[3]
Cord	0.08	0.02	0.14	0.05
Phospholipid				
Maternal	0.36[2]	0.35[2]	0.39[2]	0.39[2]
Cord	0.31[3]	0.21	0.38[3]	0.36[3]
Cholesteryl ester				
Maternal	0.26[3]	0.24	0.26	0.34[3]
Cord	0.08	0.03	0.20	0.06

[1] Pearson correlation coefficients, r
[2] $p<0.01$
[3] $p<0.05$

8.11 Dietary *Trans* Fatty Acid Intake and Birth Outcomes

There were no statistically significant relations between the maternal dietary TFA intakes and infant birthweight, length, head circumference or length of gestation.

8.12 Relations Between Cord Plasma Lipid Fatty Acids and Birth Outcomes

8.12.1 Triglyceride

A significant relation was present between the length of gestation and *cis* 20:4n-6 ($r = 0.40$, $p<0.01$) and the total *trans* isomers ($r = -0.28$, $p<0.05$) in cord plasma TG (**Table 8.16**). A significant inverse relation was also present between the isomers of CLA in cord plasma TG and the length of gestation ($r = -0.44$, $p<0.01$) (**Figure 8.16**), between *cis* 20:4n-6 and infant birthweight ($r = 0.37$, $p<0.01$) (**Figure 8.17**), and between *cis* 20:4n-6 ($r = 0.52$, $p<0.01$) and *cis* 20:5n-3 ($r = 0.37$, $p<0.05$) and infant length (**Figure 8.18**). No significant relations were found between infant head circumference and the cord plasma TG levels of TFA, CLA, *cis* n-6 or n-3 fatty acids (**Figure 8.19**).

Table 8.16 Relations between selected fatty acids in cord plasma triglyceride at birth and birth outcomes

Birth Outcome	Fatty acid	*n=46*[1]	*n=70*[2]
Length of gestation (wk)	18:2n-6	0.27	0.23
	20:4n-6	0.50[3]	0.40[3]
	18:3n-3	0.13	0.13
	20:5n-3	0.27	-0.20
	22:6n-3	0.23	0.09
	20:5n-3/20:4n-6	0.00	-0.06
	Total *trans*	-0.19	-0.28[4]
	CLA[5]	-0.44[3]	---
Birth weight (g)	18:2n-6	-0.03	-0.07
	20:4n-6	0.40[3]	0.37[3]
	18:3n-3	0.12	0.09
	20:5n-3	0.22	0.17
	22:6n-3	0.10	-0.03
	20:5n-3/20:4n-6	0.04	-0.03
	Total *trans*	-0.03	0.06
	CLA	-0.21	---
Infant length (cm)	18:2n-6	0.07	---
	20:4n-6	0.52[3]	---
	18:3n-3	0.25	---
	20:5n-3	0.37[4]	---
	22:6n-3	0.27	---
	20:5n-3/20:4n-6	0.16	---
	Total *trans*	-0.03	---
	CLA	-0.24	---
Head circumference (cm)	18:2n-6	-.042	---
	20:4n-6	.181	---
	18:3n-3	.062	---
	20:5n-3	.040	---
	22:6n-3	.068	---
	20:5n-3/20:4n-6	-.094	---
	Total *trans*	-.034	---
	CLA	-.183	---

[1] Cord plasma from Study Two
[2] Cord plasma from Study One and Two
[3] $p<0.01$
[4] $p<0.05$
[5] Conjugated linoleic acid

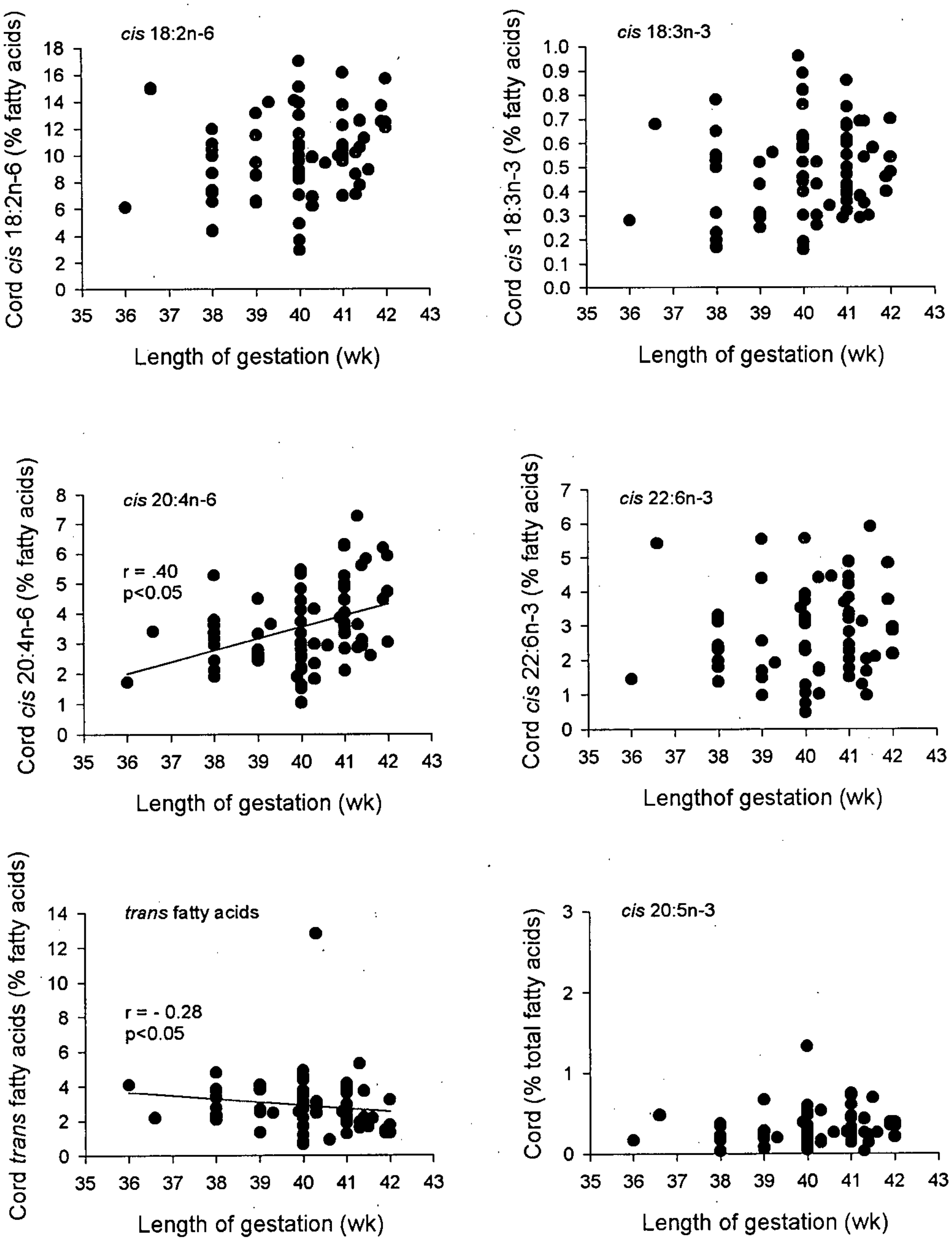


Figure 8.16 Scatter plots of length of gestation and selected fatty acids in cord plasma triglyceride at birth, n=70

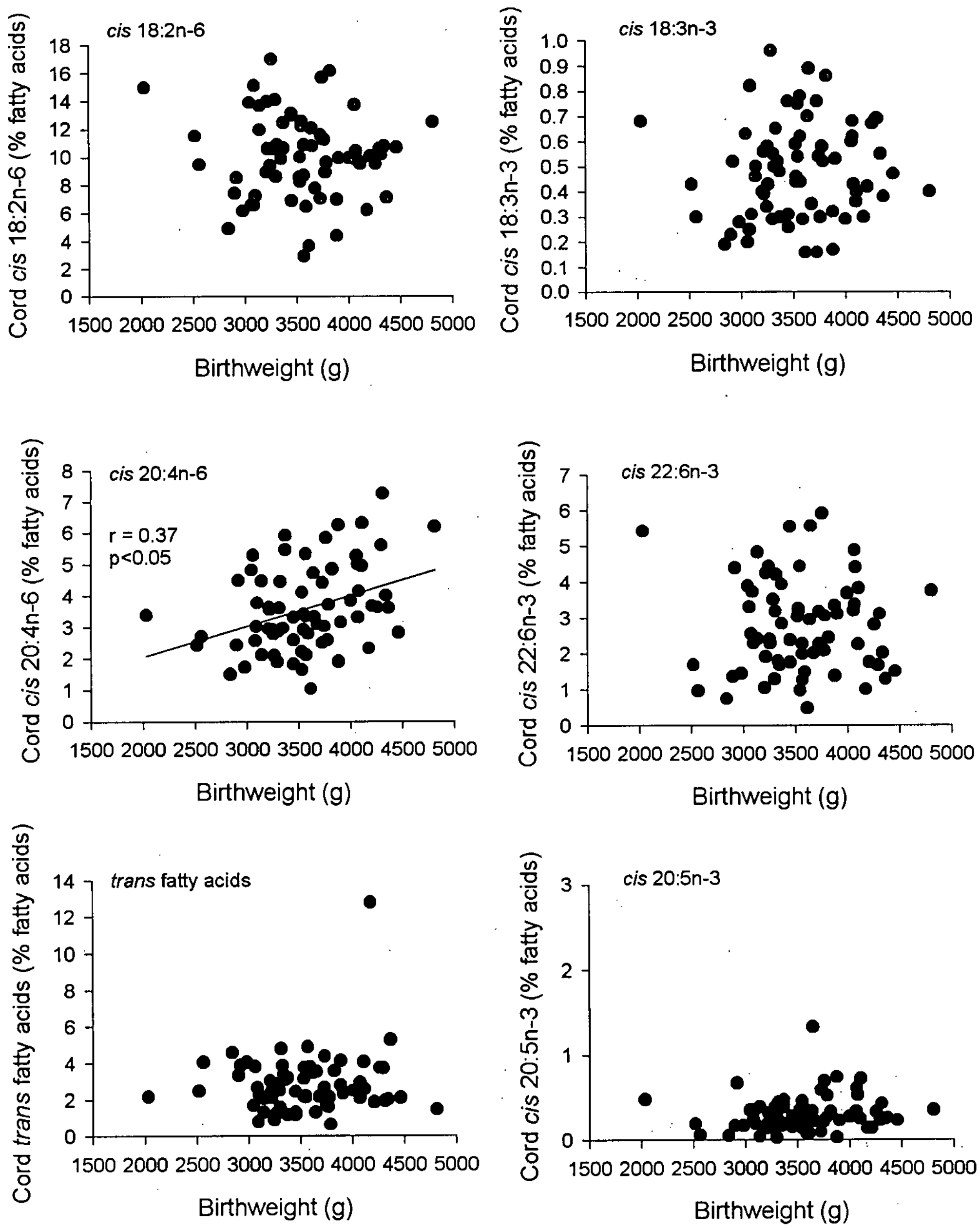


Figure 8.17 Scatter plots of infant birthweight and selected fatty acids in cord plasma triglyceride at birth, n=70

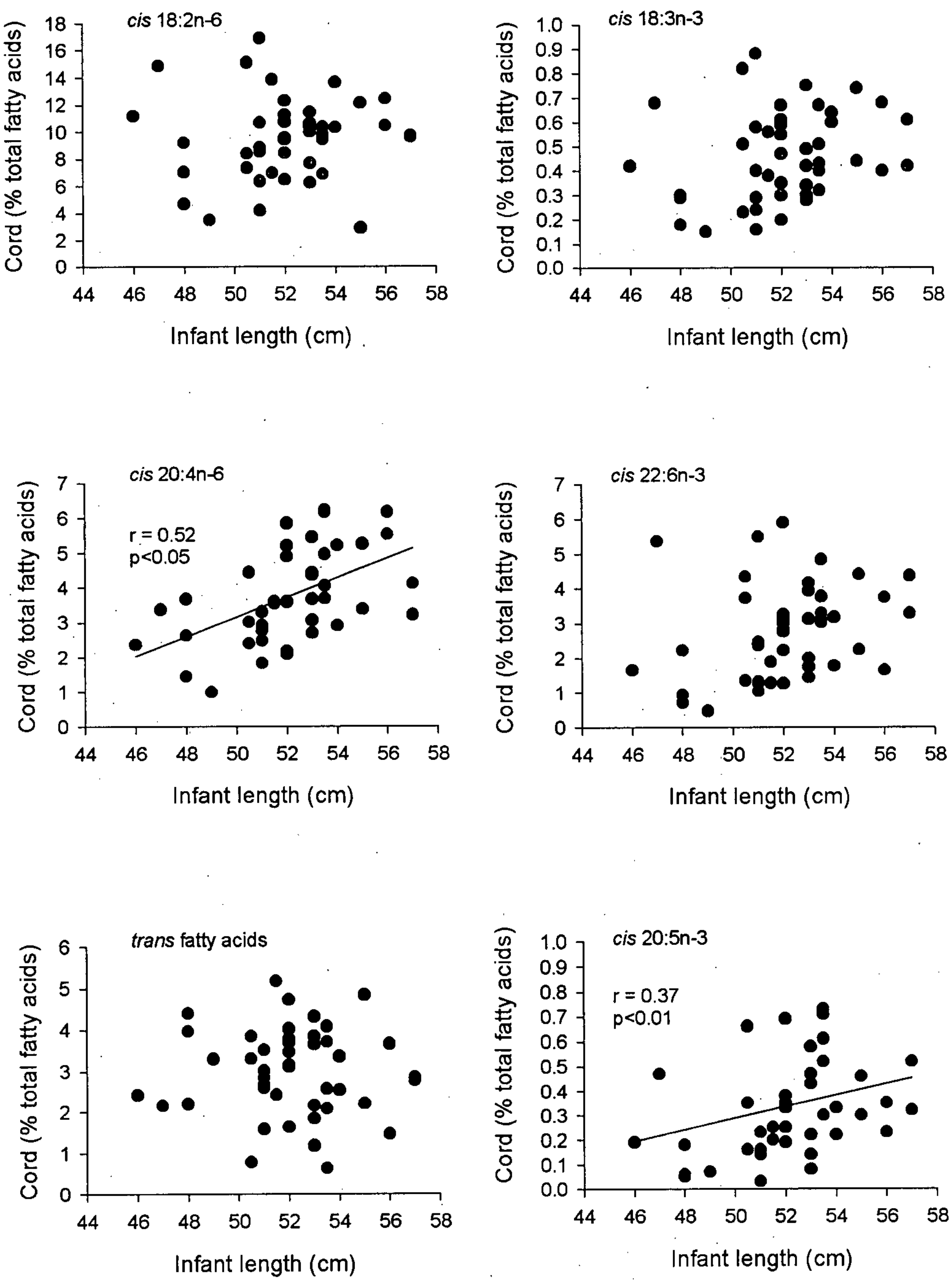


Figure 8.18 Scatter plots of infant length and selected fatty acids in cord plasma triglyceride at birth, n=44

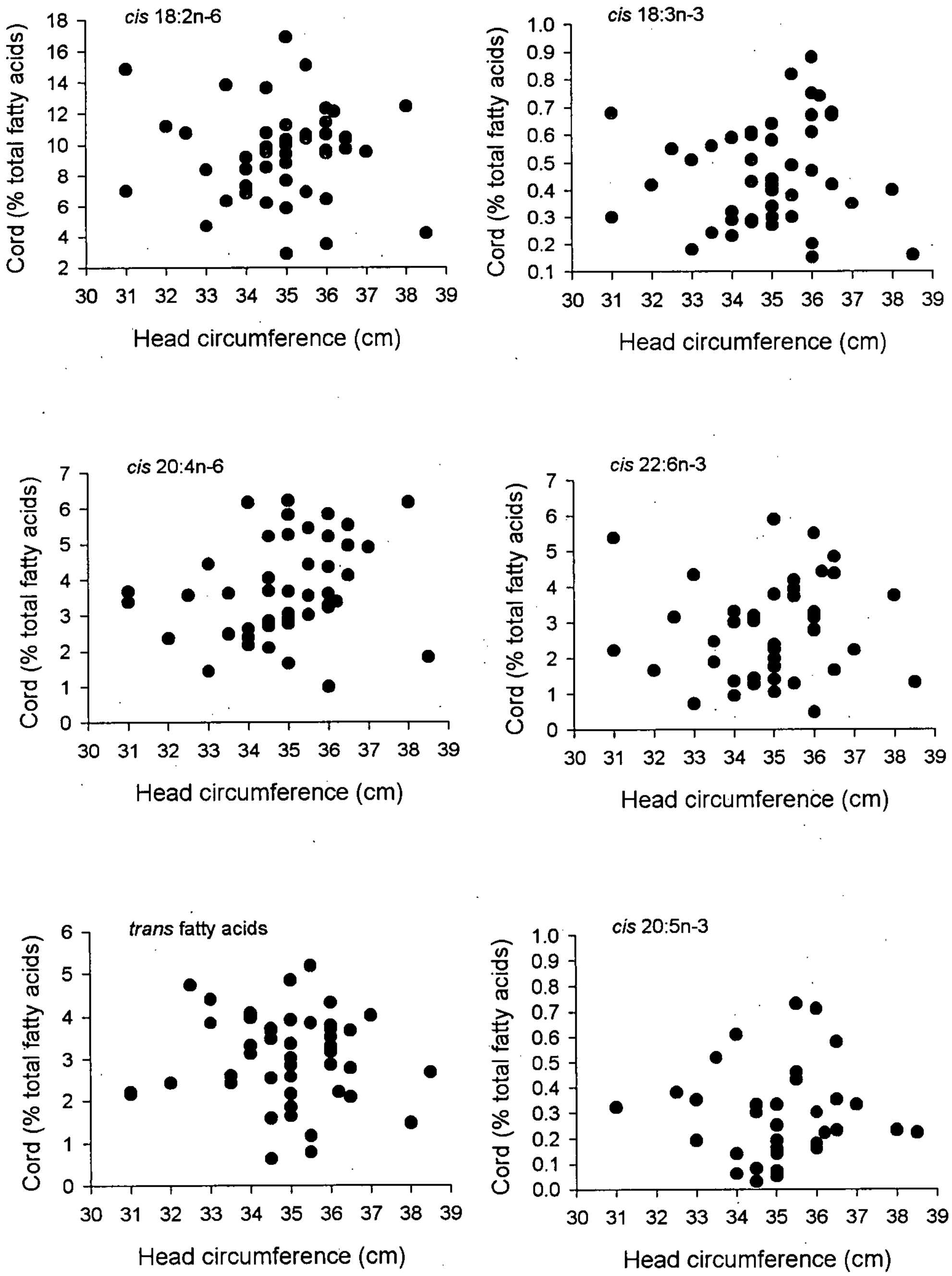


Figure 8.19 Scatter plots of infant head circumference and selected fatty acids in cord plasma triglyceride at birth, n=45[1]

[1] No statistically significant relations were found.

8.12.2 Phospholipid

A significant inverse relation was found between the cord plasma PL *cis* 18:2n-6 and length of gestation (r = -0.25, p<0.05) (**Figure 8.20),** infant birthweight (r = -0.39, p<0.01) (**Figure 8.21**) and infant length at birth (r = - 0.38, p<0.05) (**Figure 8.22 and Table 8.17**). No significant relations were found between the infant head circumference and the cord plasma PL level of TFA, CLA, *cis* n-6 or n-3 fatty acids (**Figure 8.23**).

8.12.3. Cholesteryl ester

A significant relation was found between the length of gestation and the cord plasma CE *cis* 20:4n-6 (r = 0.33, p<0.01) and total *trans* isomers (r = -0.25, p<0.05) (**Table 8.18 and Figure 8.24**). A significant relation was also found between the cord plasma CE *cis* 20:4n-6 and infant birthweight (r = 0.26, p<0.01) (**Figure 8.25**) and infant length (r = 0.43, p<0.01), as well as between *cis* 22:6n-3 and infant length (r = 0.31, p<0.05) (**Figure 8.26**). A significant inverse relation was found between the cord plasma CE isomers of CLA and length of gestation (r = -0.52, p<0.01) infant birthweight (r = -0.35, p<0.05) and infant length (r = -0.35, p<0.05) (**Figure 8.28**). No significant relations were present between the infant head circumference and the cord plasma CE level of TFA, CLA, *cis* n-6 or n-3 fatty acids (**Figure 8.27**).

Table 8.17 Relations between selected fatty acids in cord plasma phospholipid at birth and birth outcomes

Birth Outcome	Fatty acid	*n=46*[1]	*n=70*[2]
Length of gestation (wk)	18:2n-6	-0.28	-0.25[3]
	20:4n-6	-0.04	0.03
	18:3n-3	-0.15	-0.04
	20:5n-3	-0.04	-0.14
	22:6n-3	0.14	0.19
	20:5n-3/20:4n-6	-0.03	-0.12
	Total *trans*	-0.09	-0.16
	CLA[4]	0.08	---
Birth weight (g)	18:2n-6	-0.45[5]	-0.39[3]
	20:4n-6	0.00	0.02
	18:3n-3	-0.12	0.01
	20:5n-3	0.07	-0.05
	22:6n-3	0.14	0.13
	20:5n-3/20:4n-6	0.05	-0.01
	Total *trans*	-0.10	-0.02
	CLA	0.15	---
Infant length (cm)	18:2n-6	-0.38[1]	---
	20:4n-6	0.03	---
	18:3n-3	-0.17	---
	20:5n-3	0.16	---
	22:6n-3	0.22	---
	20:5n-3/20:4n-6	0.08	---
	Total *trans*	-0.11	---
	CLA	0.17	---
Head Circumference (cm)	18:2n-6	-0.17	---
	20:4n-6	-0.03	---
	18:3n-3	0.05	---
	20:5n-3	0.01	---
	22:6n-3	-0.15	---
	20:5n-3/20:4n-6	-0.04	---
	Total *trans*	-0.08	---
	CLA	-0.18	---

[1] Cord plasma from Study Two
[2] Cord plasma from Study One and Two
[3] $p<0.05$
[4] Conjugated linoleic acid
[5] $p<0.01$

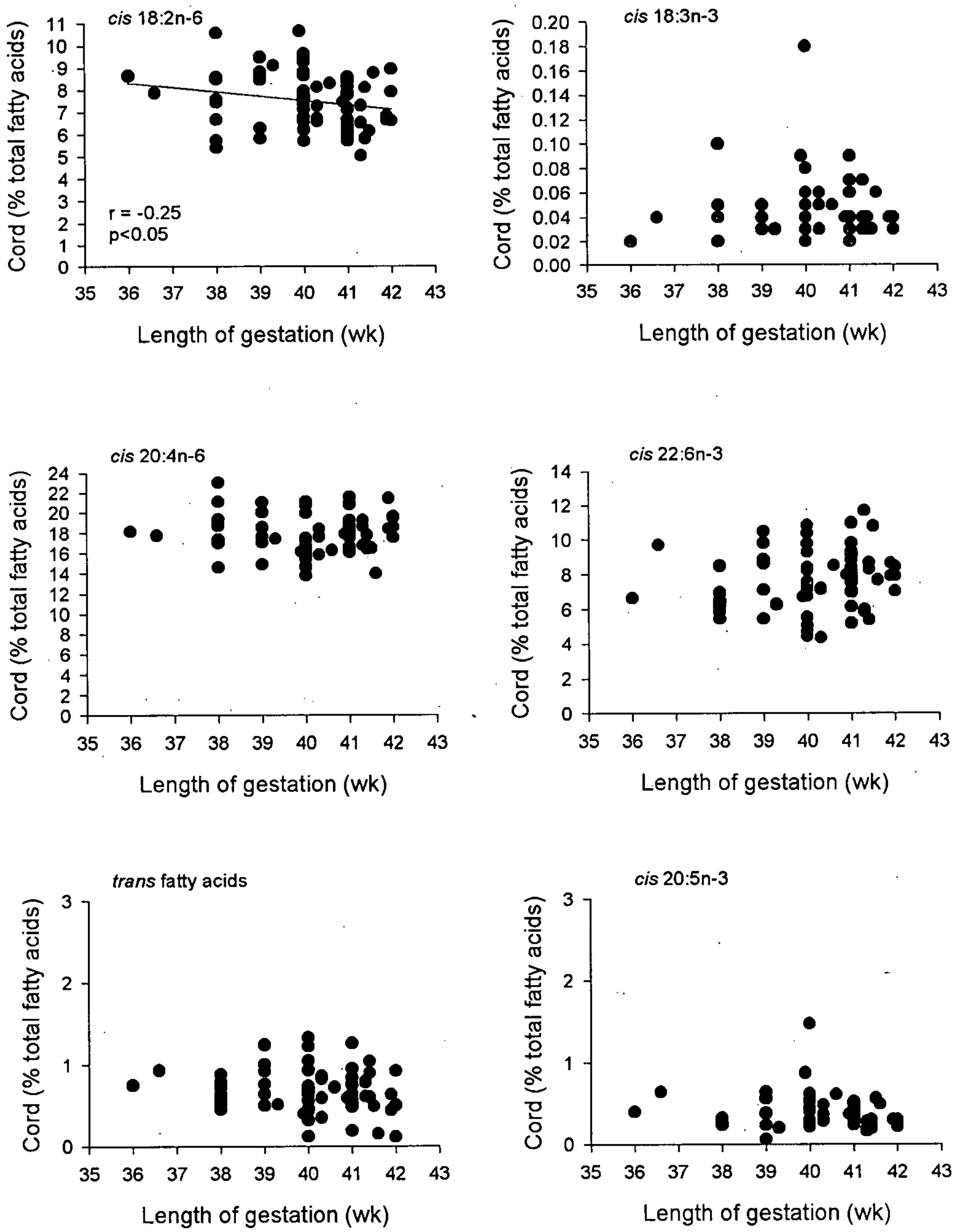


Figure 8.20 Scatter plots of length of gestation and selected fatty acids in cord plasma phospholipid at birth, n=70

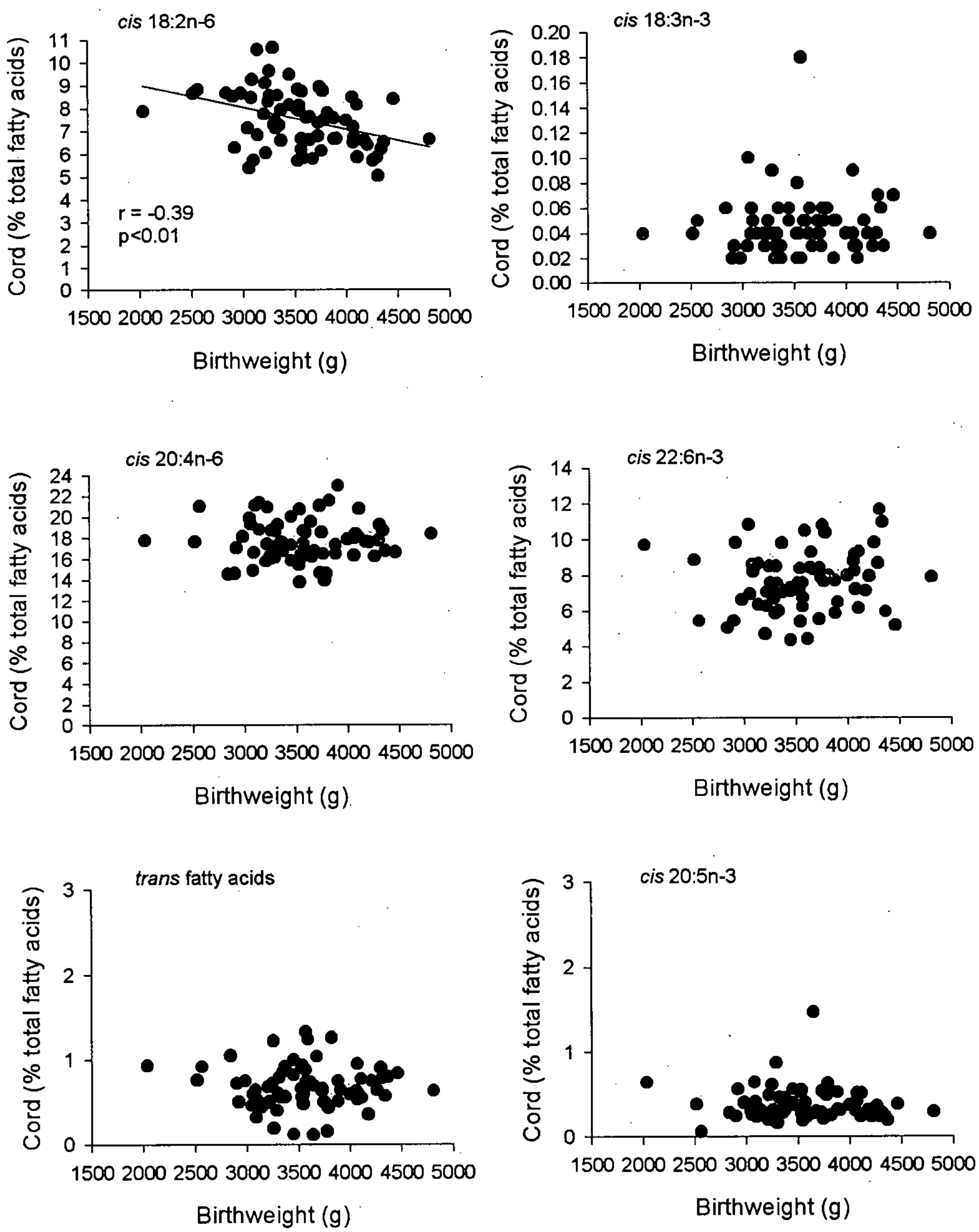


Figure 8.21 Scatter plots of infant birthweight and selected fatty acids in cord plasma phospholipid at birth, n=70

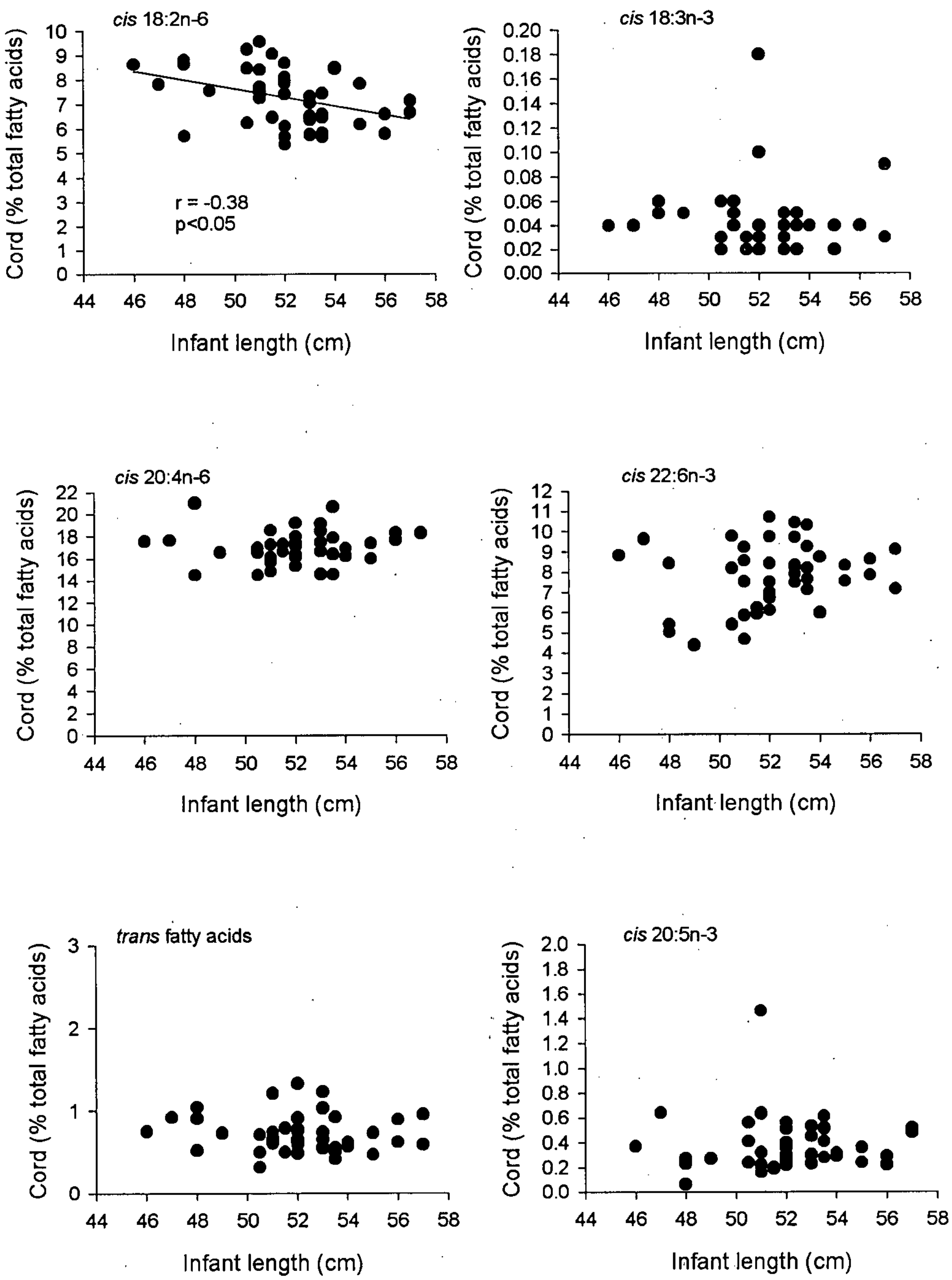


Figure 8.22 Scatter plots of infant length and selected fatty acids in cord plasma phospholipid at birth, n=44

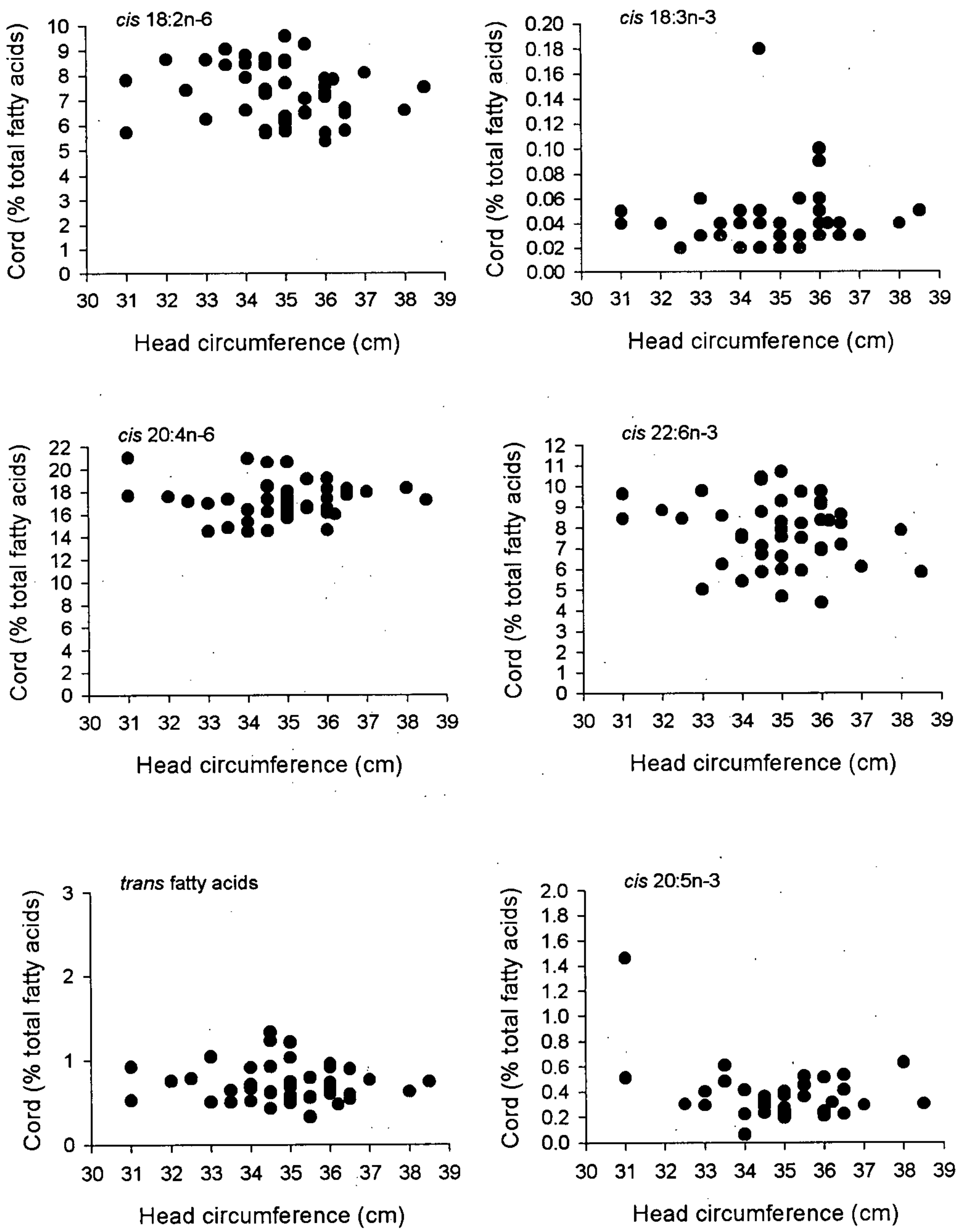


Figure 8.23 Scatter plots of infant head circumference and selected fatty acids in cord plasma phospholipid at birth, n=45[1]

[1] No statistically significant relations were found.

Table 8.18 Relations between selected fatty acids in cord plasma cholesteryl ester at birth and birth outcomes

Birth Outcome	Fatty acid	*n=46*[1]	*n=70*[2]
Length of gestation (wk)	18:2n-6	0.25	0.22
	20:4n-6	0.35[3]	0.33[4]
	18:3n-3	-0.17	-0.21
	20:5n-3	0.05	-0.06
	22:6n-3	0.19	0.18
	20:5n-3/20:4n-6	-0.14	-0.21
	Total *trans*	-0.26	-0.25[3]
	CLA[5]	-0.52[4]	---
Birth weight (g)	18:2n-6	-0.09	-0.09
	20:4n-6	0.47[4]	0.26[4]
	18:3n-3	-0.34[3]	-0.19
	20:5n-3	-0.10	-0.15
	22:6n-3	0.20	0.18
	20:5n-3/20:4n-6	-0.10	-0.19
	Total *trans*	-0.22	-0.19
	CLA	-0.35[3]	---
Infant length (cm)	18:2n-6	0.01	---
	20:4n-6	0.43[4]	---
	18:3n-3	-0.14	---
	20:5n-3	0.11	---
	22:6n-3	0.31[3]	---
	20:5n-3/20:4n-6	0.13	---
	Total *trans*	-0.13	---
	CLA	-0.35[3]	---
Head Circumference (cm)	18:2n-6	-.084	---
	20:4n-6	-.021	---
	18:3n-3	-.118	---
	20:5n-3	-.268	---
	22:6n-3	-.073	---
	20:5n-3/20:4n-6	-.145	---
	Total *trans*	-.202	---
	CLA	-.167	---

[1] Cord plasma from Study Two
[2] Cord plasma from Study One and Two
[3] p<0.05
[4] Conjugated linoleic acid
[5] p<0.01

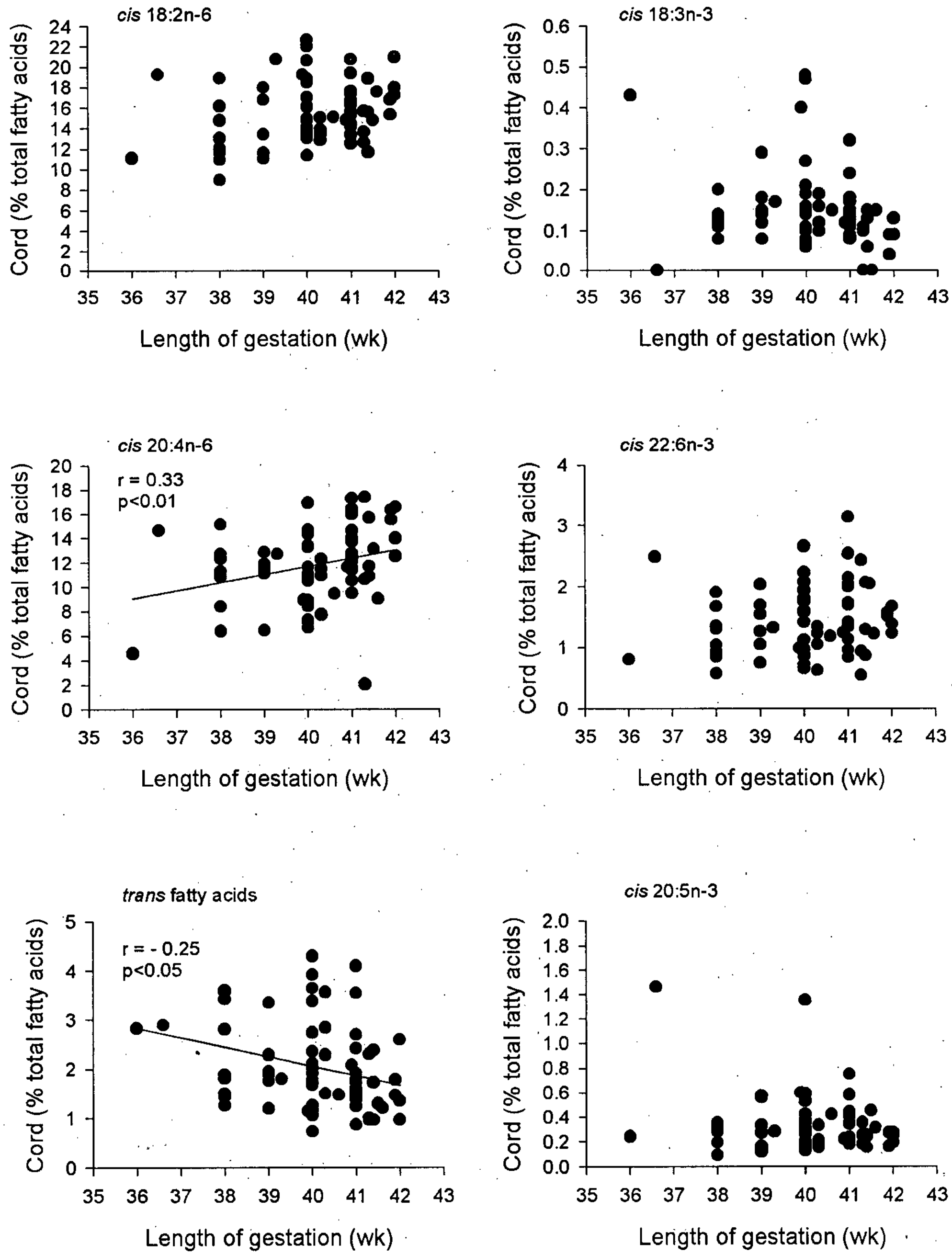


Figure 8.24 Scatter plots of length of gestation and selected fatty acids in cord plasma cholesteryl ester, n=70

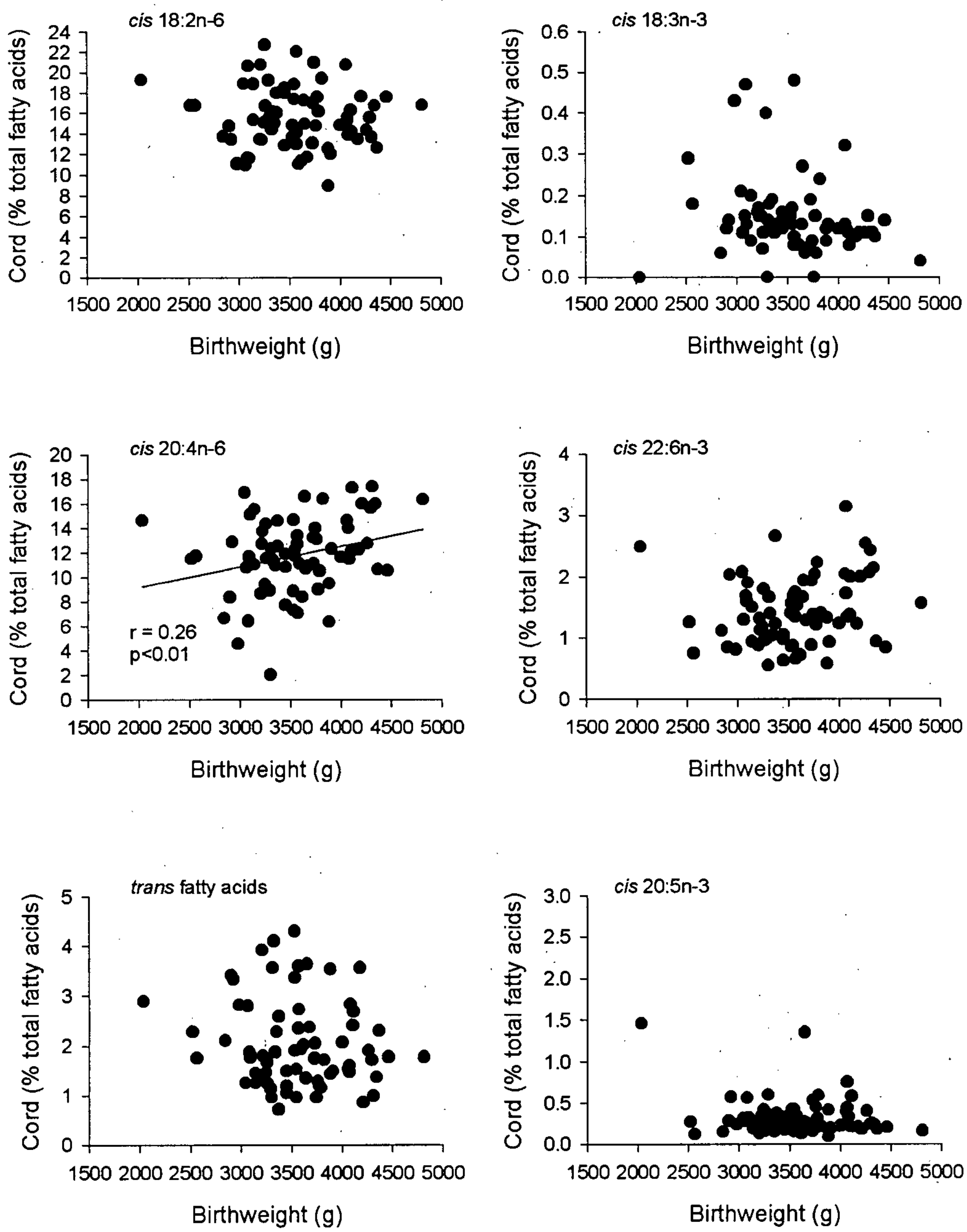


Figure 8.25 Scatter plots of infant birthweight and selected fatty acids in cord plasma cholesteryl ester, n=70

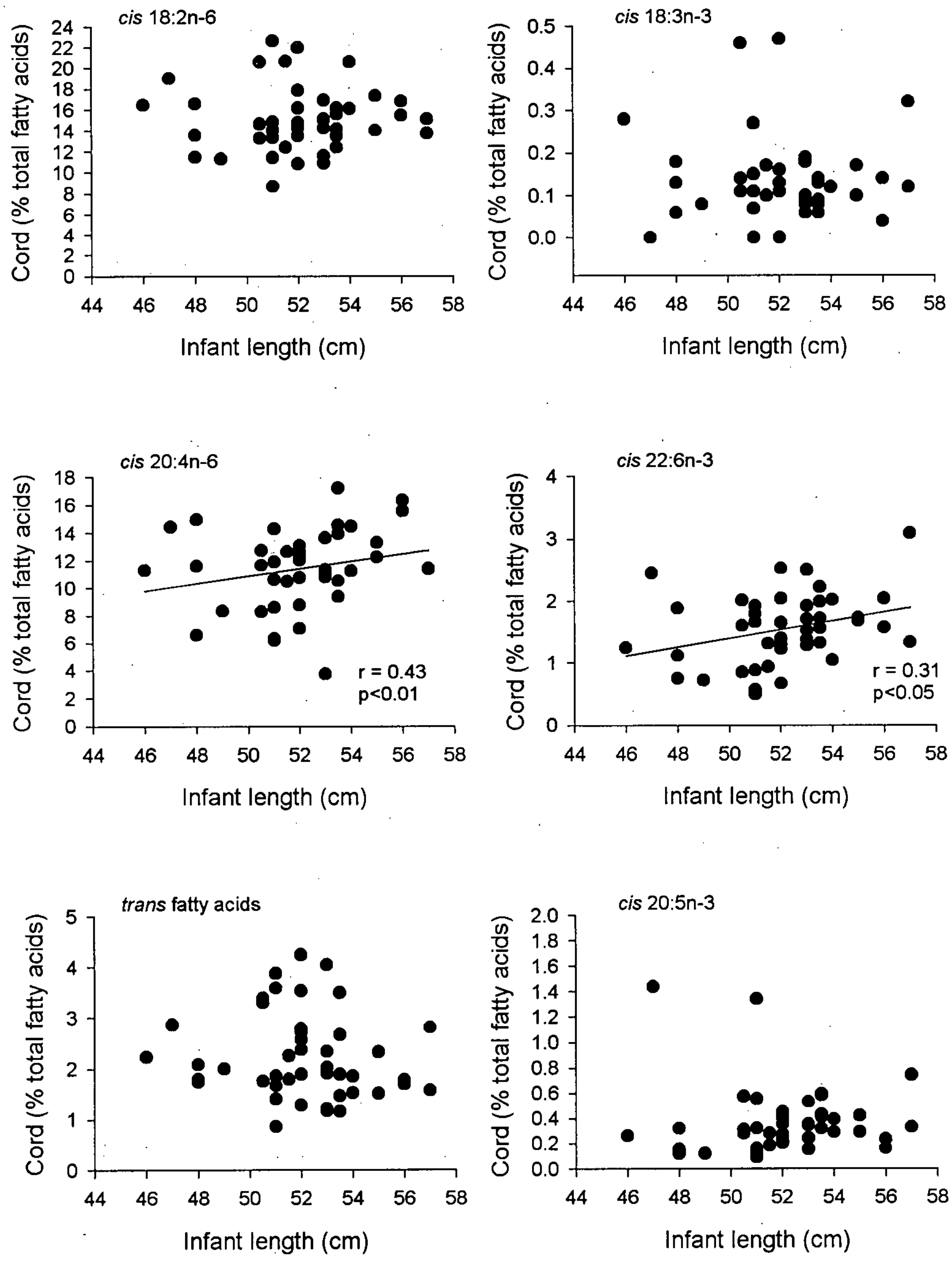


Figure 8.26 Scatter plots of infant length and selected fatty acids in cord plasma cholesteryl ester at birth, n=44

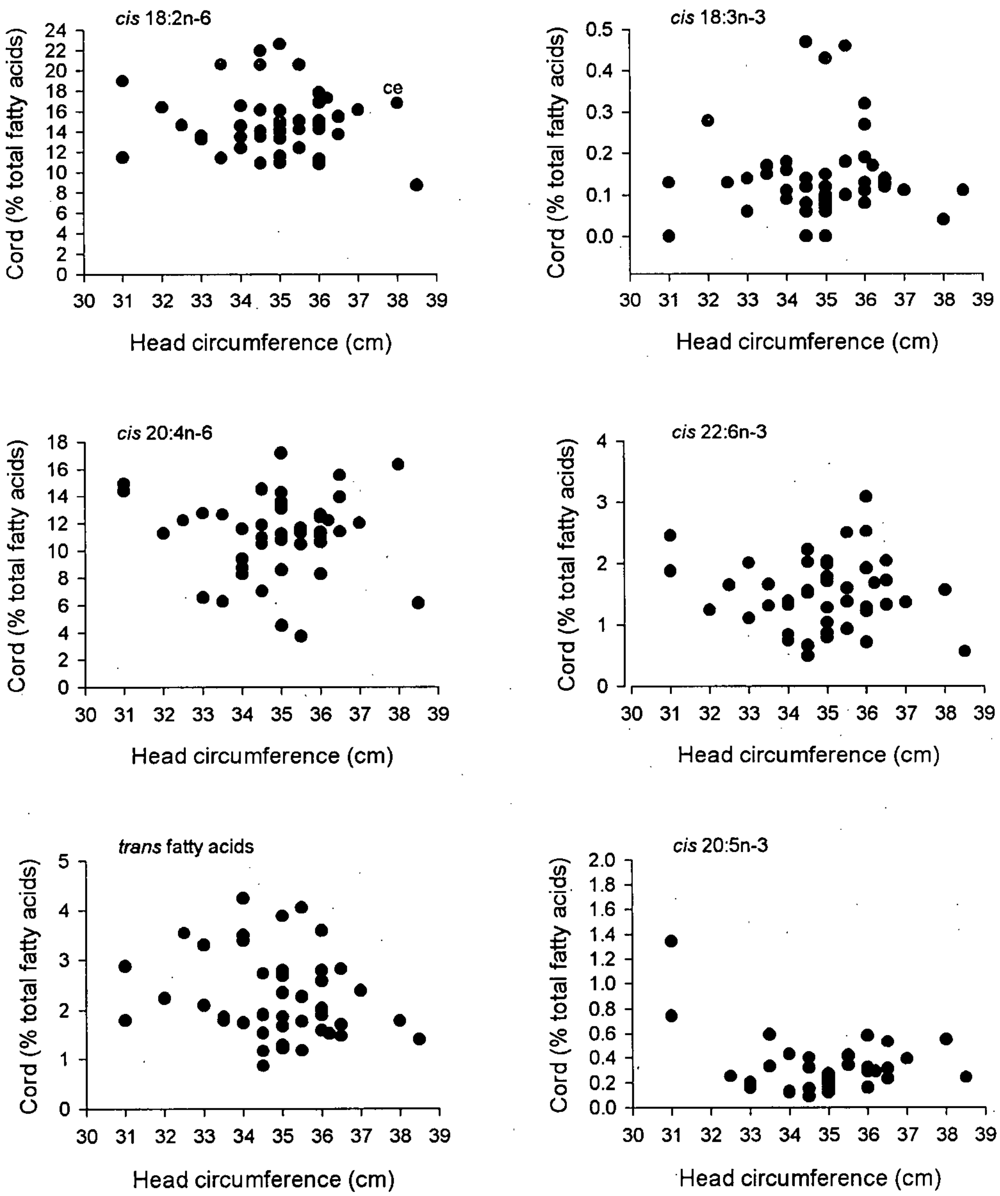


Figure 8.27 Scatter plots of head circumference and selected fatty acids in cord plasma cholesteryl ester at birth, n=46[1]

[1] No statistically significant relations were found.

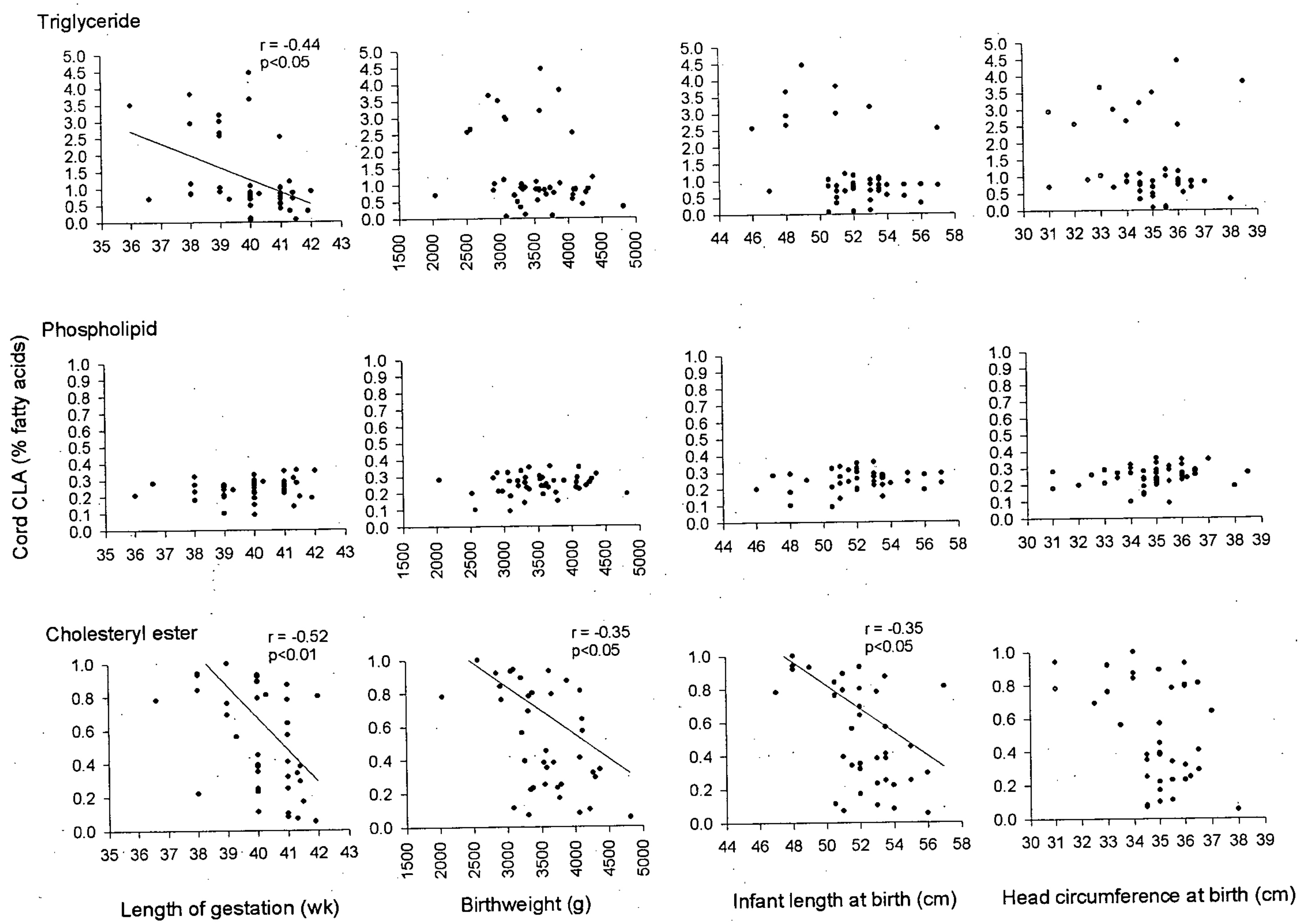


Figure 8.28 Scatter plots of birth outcome measures and conjugated linoleic acid (CLA) in cord plasma at birth

DISCUSSION

9.1 Dietary *Trans* Fatty Acid Intakes

The results of the study have shown dietary TFA intake of pregnant women was about 3-4 g/person/d in both the second and third trimester. The mean (median) intakes of TFA were 3.8 (3.3) and 3.4 (2.9) g/person/d, respectively, representing approximately 1.3% of total energy in both trimesters. The estimates of TFA intake obtained here are lower than reported by Ayyagari et al (1996) for 43 pregnant women in the U.S, with an estimated median of 5 g TFA/person/d. However, despite the lower median intakes of the women in the study here, than that of Ayyagari et al (1996), the range of TFA intakes (range 0.7-11.7 g/person/d) in the study here compare well with those of Ayyagari et al (1996) (range 1.3-14.7 g/person/d). The estimated TFA intake for the pregnant women from Vancouver studied here are, however, similar to information on the TFA intakes of non-pregnant women in the U.S. both as absolute intakes (g/d), and as a percent total energy intake. For example, Garland et al (1998) estimated TFA intakes of 2.8 g/person/d, representing approximately 1.4% of total energy, for 140 non-pregnant nurses (mean age = 52), using two FFQ collected between 1986 and 1987. Similarly, the mean TFA intake in a Boston-area study of 115 non-pregnant women (mean age = 60) was 3.4 g/person/d, representing approximately 1.7% of energy (London 1991). More recently, Allison et al (1999) reported a mean dietary TFA intake of 4.6 g/person/d (10^{th} percentile = 1.6 g/person/d, 90^{th} percentile = 8.0g/person/d) in women aged 20-49 in the U.S. The range of TFA intakes among individuals is wide, with low mean and median values, indicating that TFA intakes are not normally distributed in the population. Therefore, it is difficult to compare mean dietary TFA intakes here with the study of Allison et al (1999), Garland et al (1998) and London et al (1991), or with the median values reported by Ayyagari et al (1996).

One of the reasons why the results reported here differed from the results reported by Allison et al (1999), Garland et al (1998), London et al (1991) and Ayyagari et al (1996) may be due to methodological differences. For example, Allison et al (1999) used 3-day food records to estimate dietary TFA intake whereas Ayyagari et al (1996), Garland et al (1998) and London et al (1991) all used a FFQ to estimate TFA intakes. Food records tend to under-estimate usual dietary intakes while FFQ over-estimate usual dietary intakes (Gibson 1990). Allison et al (1999), however, reported higher TFA intakes using a 3-day food record than dietary TFA intakes found here and by Garland et al (1998) and London et al (1991) who both used a FFQ to estimate dietary TFA intakes. Allison et al (1999) used a different nutrient database to calculate TFA intake than Garland et al (1998) and London et al (1991), which is one possible explanation for the differences in dietary TFA intake.

Despite using the same FFQ to estimate dietary intakes, the lower TFA intakes found in non-pregnant women (Garland 1998, London 1991) than that found in pregnant women (Ayyagari 1996) could be explained, at least in part, by increased energy intakes associated with pregnancy. The lower estimated intake of TFA of pregnant women in the study here when compared to the study by Ayyagari et al (1996) could be explained by methodological differences such as the use of a FFQ versus a 3-day food record and the use of different nutrient databases, as well as differences in food supply and in eating habits of different populations.

Ayyagari et al (1996), Garland et al (1998) and London et al (1991) all used the Harvard/Willett FFQ to estimate TFA intakes. This questionnaire contains between 116 to 131 food items with standard portion sizes given and nine response categories for frequency of consumption (ranging from never or less than once per month to 6+ times per day). The Harvard/Willett FFQ was designed to assess average nutrient intake over the last year. This questionnaire asks the individual to indicate their consumption of vitamin and mineral supplements, dairy products, fruits, vegetables, eggs, meat, breads, cereals, starches, carbonated and other beverages, sweets, baked goods and miscellaneous foods such as dressings and sauces. There are also questions on type of fat used in baking and as a table spread, consumption of visible fat on meat, fried food consumption, amount of sugar used in beverages, usual oil used in cooking, and usual cold breakfast cereal consumed. At the end of the FFQ, participants were asked to indicate all other foods consumed at least once per week with the usual serving size and number of servings consumed per week. The Harvard/Willett FFQ uses standard portion sizes, collapses various foods into food categories and then assigns an average nutrient value for that category (Willett 1990). In contrast, the FFQ used in the study here was designed to include specific brand names and to provide an estimate of portion sizes using food models. Furthermore, *trans* fatty acid values for given foods were obtained from a 250-food TFA database (Innis 1999). Innis and Green (1999) caution against collapsing foods into broad food categories to estimate TFA intakes because of the considerable variation within a food category. For example, 6 types of white bread analyzed here were found to vary in TFA content from1.3 –34.9% of total fatty acids (Innis 1999).

It is also important to recognize that oils used in the hydrogenation process differ in Canada and U.S. In Canada, canola oil is the most common oil used, whereas in the U.S., soybean oil is more prominent. Canola oil contains predominately monounsaturated fatty acids, with approximately 53% *cis* 18:1n-9, 21% *cis* 18:2n-6 and 11% *cis* 18:3n-3 whereas soybean oil contains approximately 23% *cis* 18:1n-9, 54% 18:2n-6 and 8% 18:3n-3 (Laposata 1998). When these oils are hydrogenated, the amount of TFA and the *trans* isomer content and composition will differ because of the difference in the initial fatty acid profile. When canola oil, a good source of monounsaturated fatty acids, primarily *cis* 18:1n-9, is partially hydrogenated, isomers of *trans* 18:1 may be formed. When soy oil, which contains mostly polyunsaturated fatty acids, specifically *cis* 18:2n-6, is partially hydrogenated both *trans* 18:1 and 18:2 isomers may be formed. The amount of *trans* isomers formed, however, is dependent on the temperature, gas pressure, type and amount of catalyst used and the length of time the hydrogenation process continues. Using different oils for hydrogenation can, therefore, result in a variety of *trans* isomers and may possibly affect the amount of TFA formed and the amount of TFA consumed. This is an important consideration when comparing TFA intakes from Canada and the U.S. In addition, dietary habits and intakes may differ between the U.S. and Canada and may further explain the differences between the results reported here and those of Ayyagari et al (1996). It is also possible that increased public awareness of the possible role of dietary fat in certain chronic diseases has resulted in changes in dietary fat intakes in the last several years.

9.2 Dietary Fat Intakes: Proportion of Energy From Total, *Trans*, Polyunsaturated, Monounsaturated and Saturated Fat

The mean energy intake of the participants in the present study was 2692 kcal (range 1251-5775 kcal) in the second trimester and 2405 kcal (range 1246-3697 kcal) in the third trimester. The decrease in mean energy intake from the second to the third trimester is consistent with previous studies that have estimated energy intake throughout pregnancy. Beal (1971) and Haste et al (1990) both reported a decrease in energy intake of pregnant women in the U.S. in their third trimester compared to energy intakes in their second trimester of pregnancy.

The total fat intake of women in the second and third trimesters of pregnancy, in this study, ranged from 38-201 and 22-124 g/person/day, respectively. The mean fat intake was 86 g in the second trimester and 74 g in the third trimester. Similar results were reported by Al et al (1996) in a study of the fat intake of 176 Dutch women using a food-frequency questionnaire in the second and third trimesters. Al et al (1996) found fat intakes of 88 and 87 g/person/day in the second and third trimester, respectively. Studies from the southwest region of England and Spain have also reported mean fat intakes of 70 and 101 g/person/day in the second and third trimester, respectively (Ortega 1996, Rogers 1998). The study in England by Rogers and Emmett (1998) used a food frequency questionnaire with standard portion sizes to estimate nutrient intakes of 11,923 women. Ortega et al (1996) used a 5-day food record to obtain diet information on 292 Spanish women. Rogers and Emmett (1998) reported a mean dietary fat intake of women in their third trimester (32 weeks gestation) of 70.4 g, similar to the mean fat intake of 74 g/person/d in the third trimester found in the present study. The percent energy from fat, however, reported by Rogers and Emmett (1998) was 34.4%, whereas in the study here, the percent energy from fat was less than 30% in both the second and third trimesters. The higher fat intakes in England may be explained by differences in food choices and diet between England and Vancouver, but may involve differences in the methods used to estimate fat intake. In the study here, a quantitative FFQ was designed to not only measure the frequency of food consumption, but also to measure typical portion sizes. Rogers and Emmett (1998) also used a FFQ, but rather than measuring portion size, standard portion sizes were used. This possibly may have over-estimated the mean fat intake. In contrast to Rogers and Emmett (1998), the mean fat intake reported by Ortega et al (1996) of 101 g/d for women in Spain, representing about 40% of daily energy from fat, was substantially higher than the mean fat intake found in the study here or by Rogers and Emmett (1998) for women in England. In addition to the cultural and food supply differences, the use of a five-day food record in the study by Ortega et al (1996) could possibly have provided an over-estimation of the true mean fat intake. However, dietary records usually under-estimate rather than over-estimate dietary intakes.

In the study here, fat accounted for about 28% of total energy intake both in the second and third trimester. Health and Welfare Canada (Committee 1990) and the American Heart Association (Krauss 1996) have recommended a decrease in total fat intake to 30% of total energy. Despite a decrease in the mean fat intakes in the third trimester, the mean percentage of dietary energy from fat was the same in the second and third trimester. These results indicate a proportional decrease in the total energy and fat intakes from the second to third trimester. A decrease in fat intake, in late pregnancy, related to a proportional reduction in the total energy intake, has been reported by other investigators (Beal 1971, Haste 1990). The daily intakes of saturated, polyunsaturated and monounsaturated fat in this study were 30, 14 and 32g/d in the second trimester, and 26, 12 and 26g/d in the third trimester in this study. The proportion of energy as saturated, monounsaturated and polyunsaturated fat was constant at 9%, 5% and 10% in both trimesters, respectively. The American Heart

Association has also recommended that saturated fatty acid intake should not exceed 10% of total energy intake and to increase polyunsaturated fatty acids to no more than 10% of total energy (Krauss 1996). The women in the study reported here met current recommendations not only for total fat but also for saturated and polyunsaturated fatty acid intakes.

The lack of change in the percent of energy as saturated, polyunsaturated and monounsaturated fat from the second to third trimester suggests a decrease in total fat intake rather than a change in the type of fat consumed. Consistent with this interpretation, the energy and nutrient intakes of individual women in the second trimester show a strong, significant correlation to the intakes in the third trimester, $r = 0.54$ for TFA, $r = 0.61$ for polyunsaturated fat, $r = 0.64$ for monounsaturated fat and $r = 0.75$ for saturated fat. These statistically significant relations give further evidence that the energy distribution of fat from macronutrients and the food sources of fat of the women in this study were fairly consistent from the second to third trimester. The high correlation coefficients further suggest a high degree of reproducibility of the questionnaire. Relations between dietary fat intakes of pregnant women in the second and third trimester by Al et al (1996) have been reported to be similar with an $r = 0.61$, 0.58, 0.62 and 0.66 for total fat, saturated fat, monounsaturated fat and polyunsaturated fat, respectively.

9.3 Dietary Sources of *Trans* Fatty Acids

In recent years health professionals have advised the public to decrease total fat intake and to choose fats and oils containing polyunsaturated and monounsaturated fat rather than saturated fat, in order to lessen their risk for heart disease. Saturated fats such as butter and lard or tropical (palm/coconut) vegetable oils can be replaced with vegetable oil margarines and shortenings, or in some foods and cooking, with liquid vegetable oils. Most solid and semi-solid margarines and vegetable shortenings, however, contain significant amounts of *trans* fatty acids. Therefore, a switch from butter or other saturated animal and vegetable fats to margarines and shortenings may decrease the proportion of saturated fat from one's diet while increasing the proportion of TFA. When decreasing total fat intake, consumers often choose products perceived as low fat with the intent of increasing carbohydrates, and thus decreasing fat intake. However, many products, for example baked foods, contain hydrogenated and/or partially hydrogenated fat, therefore TFA. Although products such as breads contain relatively small amounts of hydrogenated fat these foods are consumed frequently. As a result, they can contribute significant amounts of TFA to an individual's total TFA intake.

The five most important sources of TFA in the diet of the women in the study here were baked foods, fast foods, breads, snack foods and margarines (**Figures 5.3 and 5.4**). These foods contributed over 70% of the estimate total TFA intake. In the study here, baked foods was the most frequent food category for each of the first, second, third, fourth and fifth-ranked contributors to the average TFA intake of the participants in the present study. This result clearly shows that a variety of baked foods containing hydrogenated fats were being consumed regularly. Further, these results show that although margarines may contain the highest amounts of TFA, margarines represents a relatively low proportion of the daily fat intake. Consequently, margarines are not the main contributors of an individual's TFA intake. Because margarines are a visible fat, a low contribution of margarines to the total fat intake would be expected, particularly if individuals are attempting to decrease total fat intake in an attempt to meet the dietary recommendations of 30% energy from fat. Similarly, in the study by Garland et al (1998) the five foods most predictive of the dietary TFA intake were store bought cookies (r=0.46), pastry (r=0.46), margarine (r=0.41), french fries (r=0.36) and crackers (r=0.24). Together these foods accounted for a cumulative r of 0.89. When correlated with a biochemical marker, fat aspirate TFA content, the five most predictive foods were: brownies (r=0.32), pastry (r=0.28), margarine (r=0.26), chocolate (r=0.22) and frozen potato products (r=0.20).

Most of the women in the present study (>95%) consumed baked foods which provided a mean of about 1.2 g TFA/person/d for those women who ate these foods. Data compiled by the US Department of Agriculture found that cakes and related baked goods provided only 0.3 g of TFA/person/day . The latter data, however, were estimated between 1989 and 1991, using three-day food records of individuals. One acknowledged problem of food records is that they are known to under-estimate actual food intakes and are not representative of the variety of foods consumed by individuals (Gibson 1990). The present study, in contrast, used a FFQ to estimate usual dietary intakes covering a one-month period in 1998. Using a FFQ, rather than a food record, may over-estimate rather than under-estimate usual dietary intakes because of the time period it spans and the number of foods the FFQ contains. A wide variety of foods can be collected using a FFQ in comparison to a three-day food record because over 30 days it is more likely that an individual will consume a wider variety of foods than over 3 days (Gibson 1990).

9.4 Maternal Plasma Fatty Acids

9.4.1 Relations Among Selected Fatty Acids in Maternal Plasma

The total *trans* isomers were present in maternal plasma TG, PL and CE at a mean of 4.0 (range 1.3-7.9), 2.4 (range1.1-4.5) and 1.6 (range 0.6-3.7) % of total fatty acids, respectively. No published data are available on the plasma TFA levels of women during pregnancy. Several studies, however, have measured plasma TFA in women immediately following delivery of their infant. Berghaus et al (1998) measured maternal plasma fatty acids in 41 German women after delivery. All births were between 38 and 42 weeks gestation and the median age of the women was 30 years. The median and interquartile range of TFA in TG, PL, CE and free fatty acids were 0.83 (0.63), 0.42 (0.32), and 0.28 (0.38), respectively. A limitation of this study is that a 50 m column was used in the gas chromatograph for separation and identification of fatty acids. For complete separation of fatty acid isomers, particularly the separation of *cis* and *trans* isomers, a 100 m column is recommended (Ratnayake 1995).

In a preliminary report, Ayyagari et al (1996) noted median values for *trans* 18:1 isomers in maternal TG and PL of 2.57 (range 1.0-7.8) and 1.44 (range 0.4-2.6) % of total fatty acids, respectively. Preliminary data from France (Billeaud 1998) estimated plasma CE TFA of 0.38% of total fatty acids in pregnant women.

A significant inverse relation was found between TFA and 18:2n-6 and 18:3n-3 in maternal plasma TG in the study here. This relation may suggest that participants who consumed higher amounts of TFA consumed less 18:2n-6 and 18:3n-3 than participants who consumed lower amounts of TFA. In addition, dietary sources of TFA are relatively poor sources of 18:2n-6 and 18:3n-3 and, therefore, as the intake of TFA increases, the intake of 18:2n-6 and 18:3n-3 may decrease. The plasma CE TFA was inversely related to plasma CE18:2n-6, 20:4n-6 and 22:6n-3. These relations may be a result of an effect of TFA on cholesterol metabolism, specifically on LCAT or CETP activity.

The TFA intake in the second and third trimester was significantly and positively associated with maternal plasma TG, PL and CE TFA. These significant relations between dietary intake and plasma TFA were increased when the values for TFA intake were adjusted for total energy intake. It was anticipated that TFA intake estimated in the third trimester would relate to the plasma level of TFA because the blood sample was taken at the same time that the third trimester FFQ was administered. Previous studies have reported positive relations between TFA intake and blood levels of TFA in TG, PL and CE (Mensink 1995, Seppanen-Laakso 1996, Zock 1997). Mensink and Hornstra (1995) studied the effect of replacing the usual sources of fat in the Dutch diet (animal and hydrogenated fat) with palm oil. All 40 participants consumed a control diet, containing usual Dutch fat sources, for three weeks. The participants were then randomly divided into two groups, one group receiving the control diet and the other group receiving the palm oil diet for six weeks. This was followed by a washout period of three weeks, and then the dietary regimen was crossed-over. The proportion of *trans* 18:1n-9 in serum TG and in platelet PL decreased during the consumption of the palm oil diet and increased when the control diet, containing hydrogenated oil was consumed. The proportion of *trans* 18:1n-9 in the diet was positively related to the level of TFA in serum TG ($r = 0.41$, $p<0.05$) after the first three weeks of consuming the control diet. The authors concluded that the level of TFA in serum TG reflects the dietary intake of TFA over the previous three weeks.

The level of elaidic and *trans*-vaccenic acids in plasma PL have also been considered as indicators of dietary intake of *trans* 18:1 isomers (Seppanen-Laakso

1996). A study of 46 Finnish men and women examined the changes in plasma PL fatty acids when consumers who ate margarine were switched to butter and vice versa for three to six weeks. When butter consumers switched to margarine they consumed on average 3.7 g TFA/day, representing 4.3% of total fat and 1.7% of total energy. After three weeks of consuming margarine, the individuals who usually consumed butter had similar TFA levels to the individuals who usually consumed margarine. These findings show that the levels of PL elaidic and *trans*-vaccenic acids are reliable indicators for 18:1 *trans* fatty acid intake.

Two cross-over studies by Zock et al (1997) have measured the effects of modifying dietary fat content on plasma fatty acids. In the first study, 25 men and 34 women consumed a diet rich in monounsaturated fat, TFA, or saturated fat for three weeks each in random order. In the second study, 26 men and 30 women consumed a diet in which 18:2n-6 was replaced with *trans* 18:1 or stearic acid. Blood was sampled on the 21st day of each diet period. The results of these studies showed that the level of TFA in CE increased when participants consumed a TFA-rich diet and decreased when the TFA-rich diet was replaced with a diet rich in monounsaturated fat or saturated fat. In addition to this finding, the authors concluded that the proportions of TFA increased to approximately the same level in all subjects, but an associated decrease in 18:2n-6 was not seen in all individuals when a diet rich in 18:2n-6 was replaced with TFA.

The studies by Zock et al (1997), Seppanen-Laakso et al (1996) and Mensink and Hornstra (1995) confirm that when a diet containing TFA is consumed, TFA are present in plasma or serum TG, PL and CE. Because TFA are not synthesized in vivo, the presence of TFA in plasma lipid can be used as evidence of dietary TFA intake. The findings of Zock et al (1997), Seppanen-Laakso et al (1996) and Mensink and

Hornstra (1995) suggest that plasma TFA reflect the last three weeks of dietary intake. Because of this, it seemed probable that the TFA levels in the 35 week blood sample taken in the study here may not have correlated with the FFQ data on TFA intake at 26 weeks gestation. Positive associations, however, were found between TFA intake during the second trimester and plasma TFA measured in the third trimester. This can reasonably be explained by the similar intake of TFA in the second and third trimester.

9.4.2. Relations Between Selected Fatty Acids in Maternal Plasma and Dietary *Trans* Fatty Acid Intake

The correlation between dietary TFA intake and plasma PL TFA at 28 weeks (r = 0.36, n=60) and at 35 weeks (r = 0.35, n=58) are similar to the preliminary findings of Ayyagari et al (1996). Ayyagari et al (1996) reported a positive relation between dietary TFA intake and PL *trans* 18:1 isomers (r = 0.4), with no correlation between PL total TFA (*trans* 18:1 and 18:2) and dietary TFA intake.

9.5 Cord Plasma Fatty Acids

9.5.1 Relations Among Selected Fatty Acids in Cord Plasma

The results of this study show the presence of TFA in the plasma of infants at birth, and demonstrate considerable variability in the plasma TFA levels among infants. The range of TFA in cord plasma was, for TG 0.6-12.8; for PL 0.1-1.3; and for CE 0.7-4.3% total fatty acids. TFA were present in highest levels in the TG, with mean levels of 2.6% fatty acids, when compared to PL (0.7%) and CE (2.0%). The *trans* 18:1 isomers represented 1.6% of total fatty acids in the TG. The *trans* 18:1 isomers represented a mean of 0.4 and 1.0% fatty acids in plasma PL and CE, respectively. Similarly, a preliminary report by Ayyagari et al (1996), has noted that *trans* 18:1 isomers represented 1.2% and 0.5% fatty acids in cord blood TG and PL, respectively, of newborn term infants in the U.S. Studies in Germany, however, have noted mean levels of about 1.6, 1.1 and 2.0% TFA in plasma TG, PL and CE, respectively, of 4-day old premature infants (Koletzko 1992).

The study reported here found no statistically significant relation between TFA and *cis* 18:2n-6, 18:3n-3, 20:4n-6 or 22:6n-3 in cord plasma PL, or *cis* 18:2n-6, 18:3n-3 and 20:4n-6 in TG or *cis* 18:2n-6, 18:3n-3 and 22:6n-3 in CE. An inverse relation was found between TFA and *cis* 22:6n-3 in TG, and between TFA and *cis* 20:4n-6 in CE. Similarly, Koletkzo (1992) found no inverse relations between TFA and 18:2n-6, 18:3n-3, 20:4n-6 or 22:6n-3 in cord plasma PL of premature infants, but did report a significant inverse relation between TFA and *cis* 22:6n-3 in TG, and between TFA and *cis* 20:4n-6 in CE. The inverse association between TFA and *cis* 22:6n-3, but not *cis* 18:3n-3 in TG suggests that the association of high TFA with low *cis* 22:6n-3 is not explained by low intakes of *cis* 18:3n-3. However, cord plasma *cis* 18:3n-3 levels are very low in all infants less than 1.0% fatty acids therefore a significant relation would be difficult to find.

Some studies have suggested TFA may inhibit desaturation of 18:2n-6 and 18:3n-3 (Anderson 1975, Beyers 1991, Cook 1990, Rosenthal 1984). Further, Carlson et al (1997) suggested that TFA could interfere with the final steps in the formation of 22:6n-3 , which occurs in the peroxisome (Sprecher 1995). The inverse relation between TFA and 22:6n-3 in cord blood in TG in the study reported here is consistent with this suggestion. However, differences in maternal intakes of 18:3n-3 or 22:6n-3 among women with high versus low dietary TFA intakes, or other dietary, genetic, or environmental factors could also explain these relations. In view of the current interest in the importance of 22:6n-3 in fetal and infant growth and development these findings suggest the need for further investigation. However, no inverse relations were found here between TFA and 22:6n-3 in cord plasma PL or CE, although the cord plasma CE TFA was inversely related to 20:4n-6. If TFA have the ability to inhibit the desaturase enzymes, a decrease in 22:6n-3 and in 20:4n-6 would be expected in all lipid classes. Phospholipids (PL) are critical components of the cell membrane structure and, therefore, in cell growth, it would be expected that PL fatty acids such as 20:4n-6 and 22:6n-3 would be positively related with growth. No associations between PL fatty acids including 22:6n-3 and 20:4n-6, and birth outcomes were present in cord plasma. It is possible, however, that the sample size in this study was too small to detect significant effects. Further, all but 2 newborns were born at term, between 37-42 weeks gestation.

The study reported also shows TFA are incorporated into CE, with isomers of 18:2 representing 50% of all *trans* fatty acids in maternal and cord plasma CE. This pattern appears consistent with the preferential use of 18:2n-6 for acylation of plasma unesterified cholesterol via the lecithin:cholesteryl acyl transferase (LCAT) reaction in humans. Whether or not this has any significance to lipoprotein metabolism, particularly in relation to the increase in LDL and Lp(a), that may accompany diets high in TFA (Sundram 1997), may be worth further consideration.

Several studies have shown fetal plasma PL and TG are relatively enriched in 20:4n-6 and 22:6n-3 when compared to maternal plasma PL and TG (Hoving 1994, Koletzko 1990). This could reflect preferential uptake and/or release of 20:4n-6 and 22:6n-3 to the fetus by the placenta. Alternatively, the high levels of 20:4n-6 and 22:6n-3 in fetal plasma PL could reflect selection or synthesis from 18:2n-6 and 18:3n-3, retention and preferential acylation of 20:4n-6 and 22:6n-3 by the fetal liver during PL and TG synthesis.

9.5.2 Relations Between Selected Fatty Acids in Cord Plasma and Maternal Dietary *Trans* Fatty Acid Intake

The levels of TFA in the newborn cord plasma PL were positively associated with the maternal intake of TFA in both the second and third trimester. Ayyagari et al (1996) also reported a positive relation between maternal TFA intake and TFA in cord plasma PL. Ayyagari et al (1996), however, correlated dietary TFA with 18:1 *trans* isomers in cord plasma, rather than with the total *trans* isomers as was done in the present study. No other studies have reported a relation between maternal TFA intake and cord plasma TFA. Phospholipids are critical components of membranes, thus, high levels of TFA in PL could possibly alter the function of developing membranes. Whether the developing fetus of women with high intakes of TFA is adversely affected with respect to normal growth and development is not known.

In contrast to the PL, the present study found no relation between the maternal TFA intake and TFA in cord plasma CE or TG. The lack of relation between maternal TFA intake and cord plasma CE may be explained by the low concentration of cholesterol and CE in the fetal plasma. Cord plasma TG fatty acids reflect synthesized and secreted by the fetal liver. In contrast to postnatal life, the fetus does not obtain fatty acids from the gut but rather the fetus receives unesterified (free) fatty acids through the placental vein with de novo synthesis of fatty acids also contributing to the fetal TF pool. The hepatic fatty acids can then be oxidized for energy, or packaged into TG for secretion to plasma. Rodriguez et al (1998) reported significant Δ 5 and Δ6 desaturase activity in 16 fetal liver samples from legally approved abortions conducted between 17 and 35 weeks gestation. These enzymes are required to produce 20:4n-6 and 22:6n-3 from 18:2n-6 and 18:3n-3, respectively. This suggests that the fetal liver is capable of synthesizing long chain n-6 and n-3 fatty acids. The fetus is in an anabolic state and favoring the production of adipose tissue, muscle, and skeletal growth. Fatty acids from the placenta may be used for triglyceride synthesis and accumulation in adipose, and for oxidation for energy by skeletal muscle. Most information suggests placental transfer of fatty acids is low relative to the de novo synthesis of fatty acids from glucose in the fetal liver. This may explain the lack of a relation between maternal TFA intake and TFA in cord plasma TG. The dietary TFA intake of the women between 35 weeks gestation and parturition, when the cord blood sample was collected, might also have differed. It is also possible that changes in the TG pool may occur towards the end of pregnancy due to decrease intakes, and/or during labor due to the potential of a fasting state.

9.6 Relations Between Selected Fatty Acids in Maternal and Cord Plasma

The mean (median) percent TFA in maternal plasma TG was 4.0% (4.2%), about 30% higher than in cord plasma TG of 2.9% (2.6%). Similarly, the mean (median) percent TFA in maternal plasma PL was 2.4% (2.3%), approximately 1.5 times higher than in cord plasma PL of 0.7% (0.6%). The median percent TFA in maternal and cord plasma TG and PL at 0.8% (maternal TG), 0.5% (cord TG), 0.4% (maternal PL) and 0.2% (cord PL) in the study by Berghaus et al (1998) were lower than the TFA values found in the study here. In contrast the mean (median) percent TFA in the cord plasma was higher than in the maternal plasma; CE TFA 2.0% (1.8%) and 1.6% (1.3%) for total maternal and cord plasma, respectively. Similarly, Berghaus et al (1998) reported a higher percentage of TFA in cord plasma CE (0.8%) than in maternal CE (0.3%). Koletzko and Muller (1990) also measured in maternal and cord plasma TFA but used total plasma lipid (TG, PL and CE fractions) rather than separated lipid classes. The mean percent of TFA in the maternal plasma total lipid was 1.99% of total fatty acids, about 18% higher than the 1.66% TFA in cord plasma total lipid. The results of our study and those of Berghaus et al (1998) and Koletzko and Muller (1990) clearly indicates that TFA cross the placenta to the fetus. In maternal plasma TG and PL, the *trans* 18:1 isomers were the predominant *trans* isomers representing over 50% of the *trans* isomers. In cord plasma CE, however, *trans* 18:2 isomers represented 50% of total TFA; in maternal plasma CE *trans* 18:2 represented about 60% of total TFA. Whether the accumulation of TFA in plasma CE, specifically *trans* 18:2 isomers, has any adverse effect on cholesterol metabolism in the fetus is not known.

The levels of essential fatty acids, 18:2n-6 and 18:3n-3 were lower in the newborn cord plasma TG, PL and CE than in maternal blood at 35 weeks of gestation in the study here **(Table 8.11 and 8.13)**. In contrast, the level of 20:4n-6 and 22:6n-3 values in newborn cord plasma TG, PL and CE were higher than in the maternal blood at 35 weeks of gestation. Higher proportions of 20:4n-6 and 22:6n-3 and lower 18:2n-6 in newborn than maternal blood has been documented in many previous studies including studies in the U.S. (Reece 1997), Germany (Berghaus 1998, Koletzko 1992), the Netherlands (Hoving 1994), Britain (Hendrickse 1985) and Japan (Young 1997). The higher level of 20:4n-6 and 22:6n-3 in the fetus than the mother is consistent with the higher need of the fetus for the long chain polyunsaturated n-6 and n-3 fatty acids for growth and development.

9.7 Relations Between Cord Plasma Fatty Acids and Birth Outcomes

The study here found a significant, positive relation between TG and CE 20:4n-6 and length of gestation, birthweight and infant length. Arachidonic acid (20:4n-6) is found in phospholipids throughout the body and is required in growth and development. Hoving et al (1994) also reported a gestational age-dependent increase in 20:4n-6 in a study of 503 cord blood TG and CE samples collected from spontaneous deliveries in the Caribbean. However, Rodriguez et al (1998) reported that the amount of 20:4n-6 in 16 fetal liver microsomal PL remained relatively stable, regardless of length of gestation, while 22:6n-3 increased and was positively correlated with length of gestation and fetal weight. Because liver PL were measured in the study by Rodriguez et al (1998), rather than plasma PL, it is difficult to compare the results from Rodriguez et al (1998) with the results of Hoving (1994) and the study here.

The studies here found a significant positive relation between 22:6n-3 and infant length at birth. van Houwelingen et al (1995) also found this relation in a study of 86 umbilical plasma samples obtained by trans-abdominal puncture during ongoing pregnancies. It is reasonable to expect a significant positive relation between 22:6n-3 and infant length because 22:6n-3 was positively related to length of gestation. The longer the gestation, the more time the fetus has to grow in both weight and length.

Koletzko (1992) reported an inverse relation between TFA and birthweight in cord plasma PL and CE of 29 premature infants. This relation was not present in our study with term infants; however, there was an inverse association between TG and CE TFA and length of gestation of the 70 newborns studied here.

The study here also found inverse relations between cord plasma PL 18:2n-6 and length of gestation, birthweight and infant length. Possibly, the inverse relation between 18:2n-6 and each of length of gestation, birthweight and infant length is explained by increased utilization of 18:2n-6 for 20:4n-6 synthesis with progressing gestation.

The study here provides the first data on conjugated linoleic acid (CLA) in cord plasma lipids. The TG and CE CLA were inversely related to the length of gestation and CE CLA was inversely related to birthweight and infant length. Conjugated linoleic acid has been found to inhibit tumor growth during the post-initiation phase of mammary carcinogenesis Conjugated linoleic acid has been suggested to have anticarcinogenic properties because of its antioxidant properties or alterations in eicosanoid metabolism, however, the mechanism by which CLA inhibits tumor growth is not known (Cunningham 1997, Ip 1997). Conjugated linoleic acid is found in dairy products and in the meat of ruminant animals. There are many other fatty acids other than CLA, and also other nutrients common to these foods that may explain the inverse relation between CLA and birth outcome measures. However, further work on the relation of CLA to growth would seem important.

9.8 Conclusions and Future Directions

The major food source of TFA for the women in the study here was baked foods, which provided approximately one third of the mean total TFA intake of 3.8 and 3.4 g/person/d, respectively. Despite relatively low intakes of TFA, with mean intakes representing about 1% of total dietary energy, maternal TFA intakes adjusted for energy at 35 weeks gestation were related to the maternal plasma levels of TFA in CE (r =

0.34) and in TG (r = 0.33). In contrast, cord plasma CE and TG levels of TFA showed no significant relation to the maternal TFA intake. The level of TFA in cord plasma PL, however, was significantly related to the maternal TFA intakes adjusted for energy at 28 weeks (r = 0.38) and 35 weeks of gestation (r = 0.36). Because PL are important in all cell membranes, high maternal TFA intakes, could possibly have an effect on fetal membrane function and therefore on normal growth and development, however, all but 2 infants studied here were born term (37-42 weeks) and were of appropriate birthweights for gestational age.

Inverse relations between the level of TFA and 22:6n-3 in cord plasma TG, and between the level of TFA and 20:4n-6 in CE were found . Whether or not variability in 22:6n-3 and 20:4n-6 states at birth has any physiological significance to infant growth and development is not known. Many factors such as race, parity, maternal height, pre-pregnancy weight, weight gain during pregnancy and activity level are associated with birth outcome measures. In order to control for these environmental and genetic effects, studies with a much larger sample size than used in the study here are needed to determine whether the amounts of TFA in the food supply have any effect on birth outcome. Significant inverse associations were found, however, between the levels of CLA in cord plasma CE and length of gestation, birthweight and length, and between CLA in cord plasma TG and length of gestation. These statistical associations require further investigation to further elucidate whether CLA has a direct effect on fetal growth or pregnancy duration, or whether another fatty acid or nutrient present in dairy products, or some other lifestyle factor associated with high dairy product intake, is responsible for the relations.

The findings from the present study do not substantiate the hypothesis that dietary TFA have adverse effects on normal fetal growth and development. However, it is possible that the amounts of TFA consumed by the women in this study were too low for adverse effects to be evident, or that the generally high socio-economic and nutritional status of the women studied here compensated for any potential adverse effects. The results of this study, therefore, should not be generalized to all pregnant Canadian women, particularly those who may be in the highest quartile of TFA intake. Of note, only one mother in the study here had a TFA intake at 35 weeks gestation >9g/d, equivalent to about 3-4% of dietary energy. With respect to TFA intake, the eating habits of the women in the study here may not reflect usual eating habits of other socio-economic or ethnic groups, or of other women in other regions of Canada. For example, many of the women in the study here consumed a diet with a variety of fresh fruits and vegetables and included fish and seafood on a regular basis. Many of the women studied here also indicated that they consumed only organic fruits, vegetables and grains.

The results of this research suggest the need to consider the value of ingredient labeling on foods such as bakery items in grocery stores and in coffee shops with respect to fat content and composition. It is evident from the results of the study here that most of the dietary TFA intake was found in invisible fats and often in foods that the women in the study here may not have considered as contributing to fat or TFA intake. In addition to ingredient labeling, consumers need to be aware that many processed and prepared foods such as pie shells, bakery items, prepared pasta meals and granola bars contain TFA. It is important to educate the consumer to identify food sources of TFA on ingredient labels, by looking for ingredients such as partially hydrogenated fats and oils and vegetable shortenings on packaged foods. It is also important that the public understands the importance of n-6 and n-3 fatty acids in normal growth and development, particularly in children and pregnant women, and that foods containing TFA are often poor dietary sources of n-6 and n-3 fatty acids.

Future research should address whether diets containing TFA have lower n-6 and n-3 fatty acid levels than diets devoid of TFA. To understand whether TFA are likely to inhibit the Δ 5 and Δ 6-desaturation of 18:2n-6 and 18:3n-3 at usual TFA intakes, future studies will need to determine dietary n-6 and n-3 fatty acid intakes in addition to estimating dietary TFA intakes. A large, prospective study with pregnant women will be needed to determine whether TFA, CLA or other n-6 and n-3 fatty acids influence birth outcomes. This is necessary to control for all other known nutritional, environmental and genetic factors that contribute to birth outcome. Studies are also needed to understand the mechanism(s) that allow fatty acids to be transported to the fetus through placental transport. Finally, studies are needed to understand fatty acid metabolism within the fetus, including the significance of the fetal liver to make long chain polyunsaturated fatty acids and whether TFA have an effect on fetal cholesterol metabolism.

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APPENDIX 1

Informed consent: Study One

APPENDIX 2

Research study questionnaire: Study One

APPENDIX 2

ID No.__________

Initials __________

Fatty Acids in Infant Cord Blood
Research Study Questionnaire

Mother's age ______________

Gestational age _____________ (wk)

Smoker

Yes ______

No ______

Level of Education (highest level achieved)

<Secondary ______

Secondary ______

Post-Secondary

Community/Technical ______

Undergraduate ______

Graduate ______

Doctorate ______

Infant Gender

M ______

F ______

Infant Birthweight (g)

____

Gross Family Income

$0 < $10,000 ______

$10,000 < $20,000 ______

$20,000 < $35,000 ______

$35,000 < $50,000 ______

$50,000 < $75,000 ______

$75,000 or < ______

Nutrition

1. How often do you eat the following foods?

	<1x/wk	1-2x/wk	3-4x/wk	>4x/wk
- fried fast foods (french fries, onion ring, fish)	______	______	______	______
- commercial baked goods (muffins, cookies, donuts, crackers, cakes)	______	______	______	______

2. Do you regularly (weekly) do your own baking? Yes______ No______

If yes, what type of fat do you use in baking?

a) butter ______ d) vegetable oil ______

b) lard ______ e) stick margarine ______

c) shortening ______ f) tub margarine ______

3. What type of fat do you use as a table spread (i.e. on breads, buns, vegetables, pasta)?

____

4. Do you follow any particular diet (e.g. vegetarian)?

____

____

5. Do you purposefully avoid any foods (allergies, intolerances, dislikes)? If yes, what foods?

____

____

APPENDIX 3

Recruitment letter: Study Two

APPENDIX 4

Informed consent: Study Two

APPENDIX 5

Socio-demographic and health questionnaire: Study two

Fatty Acids in Maternal Diet, Maternal Blood and Infant Cord Blood

Socio-Demographic and Health Questionnaire

Date:____________ **ID#**________

Some of these questions are personal but very important to our study. Please answer questions as accurately as possible. Remember, your answers will be kept strictly confidential. Your name is not attached to any of these sheets.

Socio-Demographic Information

1. Date of Birth ( ) ( ) ( )
2. Country of birth ____________________
3. Mother's Ethnicity
   - ☐ White
   - ☐ First Nations
   - ☐ Black
   - ☐ Chinese
   - ☐ East Indian
   - ☐ Other Asian (please specifiy) ______________
   - ☐ Other (please specify) _________________

4. Father's Ethnicity

- ☐ White
- ☐ First Nations
- ☐ Black
- ☐ Chinese
- ☐ East Indian
- ☐ Other Asian (please specify) ________________
- ☐ Other (please specify) ____________________

5. What is the highest level of education you have completed?

- ☐ Less than high school
- ☐ High school
- ☐ Community College/Technical school
- ☐ Undergraduate degree
- ☐ Graduate degree

6. What is your usual occupation? ____

7. What is your gross family income?

- ☐ less than $20,000
- ☐ $20,000 - $50,000
- ☐ >$50,000

8. How many people are dependent on this income? ____________________

Health Information

The next set of questions ask about your personal health and lifestyle habits. These questions are very important to our clinical study. I would again like to remind you that all the information given is **strictly confidential**.

1. What is your height? _______ feet _______ inches
2. What was your pre-pregnancy weight? _______ lbs
3. What is your present weight? _______ lbs
4. What week of pregnancy are you at? _______ weeks
5. Is this your first pregnancy?

   ☐ Yes

   ☐ No

   If no, how many pregnancies have you had? ___________

   How many live births have you had? ___________

   Of these live births, how many were male? ___________

   Of these live births, were there any multiple pregnancies, i.e. twins?

   ☐ Yes☐ No

6. Have you smoked cigarettes during your pregnancy?

   ☐ No

   ☐ Yes

   If yes, what is the average number of cigarettes you smoke per week? ___________

7. Have you consumed alcoholic beverages during your pregnancy?

   ☐ No

   ☐ Yes

   If yes, what is the average number of drinks per week? ___________

   (1 drink = 1.5 ounces of spirits, whiskey, etc.; 12 oz beer; 5 oz wine)

8. During your pregnancy have you taken any vitamin and/or mineral supplement?

☐ No

☐ Yes

If yes:

1. supplement name ____________________________

   how often do you take them?______________________

   when did you start taking them? __________________

2. supplement name ____________________________

   how often do you take them?______________________

   when did you start taking them? __________________

(if more than two supplements were used, please use back side of page)

9. During your pregnancy have you taking any medications? (includes current use).

☐ No

☐ Yes

If yes:

1. medication______________________________ dose ___________________

   condition _____________________________ time period ___________________

2. medication______________________________ dose ___________________

   condition _____________________________ time period ___________________

3. medication______________________________ dose ___________________

   condition _____________________________ time period ___________________

   (if more than three medications were taken, please continue on the back side)

10. During your pregnancy did you follow any particular diet?

☐ No

☐ Yes

If yes what diet did you follow:

☐ Lacto-ovo vegetarian (eats all milk and milk products and eggs)

☐ Semi-vegetarian (eats all milk and milk products, eggs, poultry and fish)

☐ Vegan (avoids ALL animal products)

☐ Other (please specify) ______________________________

Thank you for taking the time to complete this questionnaire. Your cooperation is greatly appreciated!

APPENDIX 6

Food frequency questionnaire: Study Two

FOOD FREQUENCY QUESTIONNAIRE

(TO BE COMPLETED BY NUTRITIONIST)

Subject Code [ ]

Date [ ]

Week of Gestation [ ]

Date of Follow-up Visit Day [ ] Month [ ] Year [ ]

[ ] Day of week

CODE: ______

DAIRY PRODUCTS

	ITEM NAME		BRAND/HOMEMADE	AMOUNT		FREQUENCY	
1)	Milk (drinking) - cow (homo, 2%,1%, skim) goat, rice, soy, chocolate, hot choc	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
	(used in cereal)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
	(used in tea/coffee)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
	(used in cooking/ baking)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
2)	Cheese (hard) -cheddar, mozarella	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
	(soft) - brie/camembert, cottage, edam	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
3)	Processed Slices	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
4)	Cheese Spreads - Cheez Whiz -Country Crock	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT		FREQUENCY
5)	Yogurt	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
6)	Puddings	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
7)	Ice cream/ Frozen Yogurt	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
8)	Milkshakes/ Yogurt Shake	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
9)	Eggs - fried, scrambled - poached, deviled, boiled - omelettes, quiches	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
10)	Other Dairy Products - eggnog, Caresse fresh cheese	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

TABLE /COOKING FAT

1)	Margarine (for spreading)	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT	FREQUENCY
	(used in baking)	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
2)	Shortening	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
3)	Cooking Oils	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
4)	Salad dressings	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
5)	Mayonnaise Miracle Whip	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
6)	Peanut Butter - tahini, nut butters	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
7)	Coffee Whiteners (non dairy liquid, powder)	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
8)	Whipped Toppings - Cool Whip, NutriWhip - whipping cream	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT		FREQUENCY	
9)	Other Table/ Cooking Fats - cereal cream, cream cheese, sour cream	___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
BREADS/CEREALS AND BAKED GOODS							
1)	Bread (including pita/bagels) - corn, rye, sourdough - chapati, roti	___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
2)	Buns/Rolls (including hamburger/hotdog buns)	___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
3)	Breadsticks/Croutons	___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
4)	Cereals	___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month
		___	___ ☐ brand ☐ homemade	___	☐ oz/g ☐ cup/ml ☐ piece	___	☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT	FREQUENCY
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
5)	Wheat germ (used in/on foods)	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
6)	Pancakes/Waffles	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
7)	Muffins	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
8)	English Muffin	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
9)	Scones/Tea Biscuits	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
10)	Poptarts/Toaster Pastries	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
11)	Donuts/Fritters	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piecE	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
12)	Danish/pastries	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT		FREQUENCY
13)	Croissants	____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
14)	Cakes/Squares - brownies, cake rolls, cheesecake	____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
15)	Pies/Tarts	____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
16)	Cookies	____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
17)	Other Breads/Cereals and Baked Goods -cinnamon buns, wagon wheels fruit crisps, stuffing	____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month

SNACK ITEMS

1)	Chocolate Bars/ Chocolates/ Candies	____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
2)	Frostings/Icings	____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT	FREQUENCY		
3)	Potato Chips	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
4)	Corn/Taco Chips	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
5)	Other Chips - apple chips - banana chips	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
6)	Buttered Popcorn - microwave, air - movie	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
7)	Party snacks - Nuts & Bolts, pretzel - Crunch N' Munch	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
8)	Crackers	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
9)	Granola/Cereal Bars	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
10)	Nuts/Seeds - alone or in salads, baking	____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____	____ ☐ brand ☐ homemade	____	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT		FREQUENCY	
11)	Other snack items	______	______	______	☐ oz/g	____	☐ Day
	- Cheezies, Bugles		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month

COMBINATION FOODS/MEALS
(Purchased and Home-made)

1)	Prepared Sauces	______	______	______	☐ oz/g	____	☐ Day
	- Cheese, Hollandaise		☐ brand		☐ cup/ml		☐ Week
	Pesto, Bernaise		☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
2)	Prepared Pasta/Rice	______	______	______	☐ oz/g	____	☐ Day
	Dishes (bottled, canned, boxed)		☐ brand		☐ cup/ml		☐ Week
	- KD, Lipton's		☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
3)	Rice	______	______	______	☐ oz/g	____	☐ Day
	(other than mentioned in 2)		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
4)	Pasta	______	______	______	☐ oz/g	____	☐ Day
	(other than mentioned in 2)		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
5)	Seasoning/Coating Mixes	______	______	______	☐ oz/g	____	☐ Day
	- Shake n'Bake		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		______	______	______	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT		FREQUENCY
6)	Frozen Dinners	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
7)	Fries - french, New York	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
8)	Perogies - potato, cheese	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
9)	Tacos/Burritos Quesidias	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
10)	Pizza (including rolls/pops)	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
11)	Wieners/Frankfurters - beef, turkey, veggie, tofu, other	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
12)	Sausages - Polish, farmer, breakfast	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
13)	Luncheon Meats/Spreads - in sandwiches/subs	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT	FREQUENCY		
14)	Meat Pies	________	________	________	☐ oz/g	____	☐ Day
	Shepards Pie		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
15)	Hamburgers	________	________	________	☐ oz/g	____	☐ Day
	-beef, veggie,soy		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
16)	Fish sticks/	________	________	________	☐ oz/g	____	☐ Day
	pieces		☐ brand		☐ cup/ml		☐ Week
	frozen fast food		☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
17)	Fish	________	________	________	☐ oz/g	____	☐ Day
	(Baked/Steamed/BBQ etc)		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
18)	Chicken/Fish	________	________	________	☐ oz/g	____	☐ Day
	Burgers		☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
19)	Fried Chicken	________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
20)	Chicken Nuggets	________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
21)	Chicken Wings	________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month
		________	________	________	☐ oz/g	____	☐ Day
			☐ brand		☐ cup/ml		☐ Week
			☐ homemade		☐ piece		☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT		FREQUENCY
22)	Poultry (Baked/Roast/BBQ)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
23)	Beef (Baked/Roast/BBQ)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
24)	Pork (Baked/Roast/BBQ)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
25)	Lamb (Baked/Roast/BBQ)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
26)	Other meat -game, venison, rabbit - lobster, mussels	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
27)	Shrimp (fried/boiled) (cooked, canned)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
28)	Other seafood -scallops, clams -lobster, mussels	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
29)	Legumes, dry peas, beans, lentils (canned,cooked)	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT	FREQUENCY		
30)	Tofu	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Mon
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
SOUPS							
1)	Cream Soups	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
2)	Vegetable/Noodle Soups include cup of soups	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
3)	Instant Noodle Soups - Ichiban, Chow Mein	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
4)	Other Soups - borscht, miso	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
VEGETABLES (Canned, fresh, frozen)							
1)	Green/yellow beans Peas	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
2)	Broccoli, Brussel sprouts Cabbage	________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

CODE: ______

	ITEM NAME		BRAND/HOMEMADE	AMOUNT	FREQUENCY		
3)	Lettuce, Kale, Spinach	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
4)	Squash, Corn	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
5)	Potatoes - other than fries	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
6)	Vegetable juices	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
7)	Other Vegetables	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

FRUITS (Canned, fresh, frozen)

1)	Apples/applesauce	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	oz/g ☐ cup/ml ☐ piec	____	☐ Day ☐ Week ☐ Month
2)	Oranges, Grapefruits Lemons, Limes	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
3)	Pear, Plums, Peaches Nectarines	______	______ ☐ brand ☐ homemade	______	☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

CODE: ______

		ITEM NAME	BRAND/HOMEMADE	AMOUNT	FREQUENCY	
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
4)	Raisins, prunes, Dried fruit	____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
5)	Melons, Lychees	____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
6)	Bananas	____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
7)	Berries	____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
8)	Fruit cocktail/ Fruit salads	____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
9)	Fruit Juices	____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
10)	Other Fruits	____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month
		____________	____________ ☐ brand ☐ homemade	________☐ oz/g ☐ cup/ml ☐ piece	____	☐ Day ☐ Week ☐ Month

CODE: ______

		ITEM NAME	BRAND/HOMEMADE	AMOUNT	FREQUENCY
	BEVERAGES (Other than milk and juices)				
1)	Soft Drinks (regular)	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
2)	Drink Mixes (Iced teas, Kool-Aids)	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
3)	Other Beverages	________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month
		________	________ ☐ brand ☐ homemade	________ ☐ oz/g ☐ cup/ml ☐ piece	____ ☐ Day ☐ Week ☐ Month