# Inverse association between *trans* isomeric and long-chain polyunsaturated fatty acids in cord blood lipids of full-term infants[1–3]

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**ABSTRACT**

**Background:** Previous studies showed significant inverse correlations between values of *trans* isomeric and long-chain polyunsaturated fatty acids in plasma lipids of preterm infants and healthy children aged 1–15 y.

**Objective:** We sought to evaluate the same correlations in full-term infants at birth.

**Design:** We studied healthy full-term infants ($n$ = 42) born after normal pregnancies and deliveries. All infants had a family history of atopy (both parents or one of the parents and a sibling had atopic symptoms). The fatty acid composition of venous cord blood lipids was determined by high-resolution capillary gas-liquid chromatography.

**Results:** The mean (±SEM) sum of *trans* fatty acids was 0.49 ± 0.02% by wt in phospholipids, 2.47 ± 0.20% by wt in cholesterol esters, 1.73 ± 0.09% by wt in triacylglycerols, and 1.59 ± 0.07% by wt in nonesterified fatty acids. Linear correlation analysis showed significant inverse correlations between the sum of *trans* fatty acids and both arachidonic acid and docosahexaenoic acid in phospholipids ($r$ = –0.56, $P$ < 0.001, and $r$ = –0.48, $P$ = 0.01, respectively), cholesterol esters ($r$ = –0.52, $P$ < 0.001, and $r$ = –0.39, $P$ = 0.018, respectively), and nonesterified fatty acids ($r$ = –0.41, $P$ = 0.007, and $r$ = –0.41, $P$ = 0.006, respectively).

**Conclusion:** Because *trans* fatty acids in the fetal circulation must originate from the maternal diet, our results indicate that maternal exposure to *trans* fatty acids may represent a previously neglected variable that inversely influences long-chain polyunsaturated fatty acid status in full-term infants at birth. *Am J Clin Nutr* 2001;74:364–8.

**KEY WORDS** Arachidonic acid, cord blood, docosahexaenoic acid, full-term infant, long-chain polyunsaturated fatty acid, *trans* fatty acid, maternal diet, maternal nutrition, prenatal nutrition, pregnancy

## INTRODUCTION

In human diets, *trans* fatty acids are found in the meat and milk of ruminant animals, but the major sources of *trans* fatty acids are partially hydrogenated vegetable and fish oils. In North America, *trans* fatty acid intakes of pregnant and breast-feeding women were estimated to be 6.4 g/d in the United States (1) and 6.9 g/d in Canada (2). In the United Kingdom, *trans* fatty acid intakes were reported to be ≈5.6 g/d in men and 4.0 g/d in women (3). However, many studies consistently reported wide interindividual variation in dietary exposure to *trans* fatty acids (1–5).

The potential of *trans* fatty acids to induce atherogenic changes in plasma cholesterol profiles was shown convincingly (6, 7). In addition, the ability of *trans* fatty acids to interfere with essential fatty acid metabolism (8, 9) may be of practical importance in pediatrics. Evidence of this interference in humans includes significant inverse correlations between plasma values of *trans* isomeric and long-chain polyunsaturated fatty acids, both in preterm infants (10) and in healthy children aged 1–15 y (11).

Because long-chain polyunsaturated fatty acids play an important role in early human development (12–15), interference with their availability caused by exposure to *trans* fatty acids is of special concern during the perinatal period. Indeed, an expert panel on *trans* fatty acids and early development said that "Additional research is needed to determine...whether the conversion in infants of linoleic acid and α-linolenic acid to arachidonic acid and docosahexaenoic acid, respectively, is influenced by concentrations of *trans* fatty acids in tissue lipids or diet…" (1). In the present article, we report data on the associations between *trans* isomeric and long-chain polyunsaturated fatty acids in the cord blood lipids of full-term infants with an atopic trait.

## SUBJECTS AND METHODS

### Subjects

We studied 42 singleton full-term infants with a mean (±SEM) birth weight of 3461 ± 53 g and a mean birth length of 51.8 ± 0.3 cm. The infants were enrolled in an Austrian nutritional trial investigating the effects of different feeding regimens on the prevention of allergy in high-risk infants. All the infants had a positive family history of atopy; both parents or one of the parents and a sibling had atopic symptoms. Maternal age ranged from 22 to 35 y (mean: 28.2 ± 0.5 y). None of the mothers

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**TABLE 1**
Concentrations of selected fatty acids in cord blood lipids of 42 full-term infants[1]

| | Phospholipids | Cholesterol esters | Triacylglycerols | Nonesterified fatty acids |
|---|---|---|---|---|
| | | *% by wt* | | |
| Sum of saturated fatty acids | 52.70 ± 0.58 | 28.79 ± 0.96 | 41.40 ± 0.81 | 58.76 ± 0.97 |
| Sum of *cis* monounsaturated fatty acids | 14.34 ± 0.27 | 39.42 ± 0.69 | 37.97 ± 0.84 | 21.92 ± 0.68 |
| Sum of *trans* fatty acids | 0.49 ± 0.02 | 2.47 ± 0.20 | 1.73 ± 0.09 | 1.59 ± 0.07 |
| n−6 PUFAs | | | | |
| 18:2n−6 | 6.06 ± 0.20 | 16.10 ± 0.74 | 11.32 ± 0.48 | 9.14 ± 0.43 |
| 20:4n−6 | 13.82 ± 0.36 | 9.47 ± 0.54 | 2.68 ± 0.20 | 4.44 ± 0.30 |
| Ratio of 20:4n−6 to 18:2n−6 | 2.37 ± 0.10 | 0.60 ± 0.03 | 0.25 ± 0.02 | 0.53 ± 0.05 |
| Sum of n−6 long-chain PUFAs | 20.88 ± 0.50 | 11.26 ± 0.59 | 5.43 ± 0.31 | 6.61 ± 0.39 |
| n−3 PUFAs | | | | |
| 18:3n−3 | 0.03 ± 0.01 | 0.16 ± 0.03 | 0.24 ± 0.03 | 0.27 ± 0.02 |
| 22:6n−3 | 4.42 ± 0.22 | 0.68 ± 0.05 | 0.80 ± 0.08 | 1.12 ± 0.10 |
| Ratio of 22:6n−3 to 18:3n−3 | 302 ± 67 | 8.65 ± 0.85 | 13.54 ± 8.20 | 5.88 ± 0.69 |
| Sum of n−3 long-chain PUFAs | 5.03 ± 0.23 | 0.97 ± 0.07 | 1.22 ± 0.15 | 1.31 ± 0.10 |

[1] $\bar{x}$ ± SEM. Sum of saturated fatty acids: 12:0 + 14:0 + 16:0 + 17:0 + 18:0 + 20:0 + 22:0 + 24:0; sum of *cis* monounsaturated fatty acids: 14:1n−5 + 16:1n−7 + 18:1n−9 + 18:1n−7 + 20:1n−9 + 22:1n−9 + 24:1n−9; sum of *trans* fatty acids: *t*-16:1 + *t*-18:1 + *ct, tc, tt*-18:2; sum of n−6 long-chain polyunsaturated fatty acids (PUFAs): 20:2n−6 + 20:3n−6 + 20:4n−6 + 22:4n−6 + 22:5n−6; sum of n−3 long-chain PUFAs: 20:3n−3 + 20:5n−3 + 22:5n−3 + 22:6n−3.

followed any self-restrictive diet. The pregnancy and delivery were normal in all cases. About two-thirds of the mothers (27 of 42) decided to breast-feed.

## Methods

Venous cord blood was collected into tubes containing EDTA (1 g/L) and was centrifuged immediately (1500 × *g* for 5 min at room temperature). Blood plasma was stored at −20°C until analyzed. Blood samples were transported frozen to the laboratory in Hungary; all samples were thawed only once.

Plasma lipids were extracted from 0.25 mL plasma with chloroform and methanol (16) after addition of internal standards (phosphatidylcholine-, cholesterol-, and triacylglycerol-derived and free pentadecanoic acids, 15:0). Lipid classes were separated on silica gel plates (no. 5721; Merck, Darmstadt, Germany) with one 8-cm run of hexane, diethylether, chloroform, and acetic acid (70:20:10:1, by vol). Fatty acids in plasma phospholipids, cholesterol esters, triacylglycerols, and nonesterified fatty acids were transesterified with methanol and hydrochloric acid (17). Fatty acid methyl esters were determined by high-resolution capillary gas-liquid chromatography with a Finnigan 9001 chromatograph (Finnigan/Tremetrics Inc, Austin, TX) with split injection (1:15) and a flame ionization detector. A 40-m cyanopropyl column (DB-23; J & W Scientific, Folsom, CA) was used. The temperature program was as follows: an initial temperature of 100°C for 0.1 min, followed by a temperature increase of 40°C/min up to 180°C, a 1-min isotherm period, a temperature increase of 2°C/min up to 200°C, a 1-min isotherm period, a temperature increase of 10°C/min up to 240°C, and a 9.9-min isotherm period. The constant linear velocity was 0.3 m/s (referred to 100°C).

Identification of fatty acids was confirmed by comparison with authentic standards (NuChek Prep, Elysian, MN). Fatty acid results were expressed as % by wt of 31 fatty acids detected with chain lengths between 12 and 24 carbon atoms (**Table 1**). Because *trans* hexadecenoic acid (*t*-16:1), *trans* octadecenoic acid (*t*-18:1), and *trans* octadecadienoic acid (*ct, tc, tt*-18:2) were detected in all samples, the value calculated by summing these *trans* fatty acids — termed for the purposes of this paper as sum of *trans* fatty acids — was entered into the correlation analysis. A typical chromatogram with identification marks for *t*-16:1, *t*-18:1, and *ct, tc, tt*-18:2 is shown in **Figure 1.** We used the STATISTICAL PACKAGE FOR SOCIAL SCIENCES FOR WINDOWS, release 7.5 (SPSS Inc, Chicago) for the statistical analyses. Linear correlation coefficients were considered statistically significant at $P < 0.05$.

## RESULTS

Values of selected fatty acids in plasma lipid classes are shown in Table 1. Values of n−6 long-chain polyunsaturated fatty acids were highest in phospholipids and lowest in triacylglycerols, whereas values of n−3 long-chain polyunsaturated fatty acids were highest in phospholipids and lowest in cholesterol esters. The percentage of *trans* fatty acids was highest in cholesterol esters and lowest in phospholipids.

The linear correlation coefficients between the sum of *trans* fatty acids and the major polyunsaturated fatty acids and product-to-substrate ratios of long-chain polyunsaturated fatty acid biosynthesis (arachidonic acid:linoleic acid and docosahexaenoic acid:α-linolenic acid) are shown in **Table 2**. The sum of *trans* fatty acids was significantly and inversely associated with values of both arachidonic and docosahexaenoic acids in phospholipids, cholesterol esters, and nonesterified fatty acids. In cholesterol esters, the sum of *trans* fatty acids was significantly and inversely associated with the parent n−6 polyunsaturated fatty acid, linoleic acid. The sum of *trans* fatty acids was significantly and inversely related to the ratio of arachidonic acid to linoleic acid in phospholipids and to the ratio of docosahexaenoic acid to α-linolenic acid in cholesterol esters (Table 2). There was a significant inverse association between the sum of *trans* fatty acids and the sum of n−3 plus n−6 long-chain polyunsaturated fatty acids in phospholipids, cholesterol esters, and nonesterified fatty acids (**Figure 2**). There were no correlations between *trans* fatty acid values and infant birth weight or birth length.

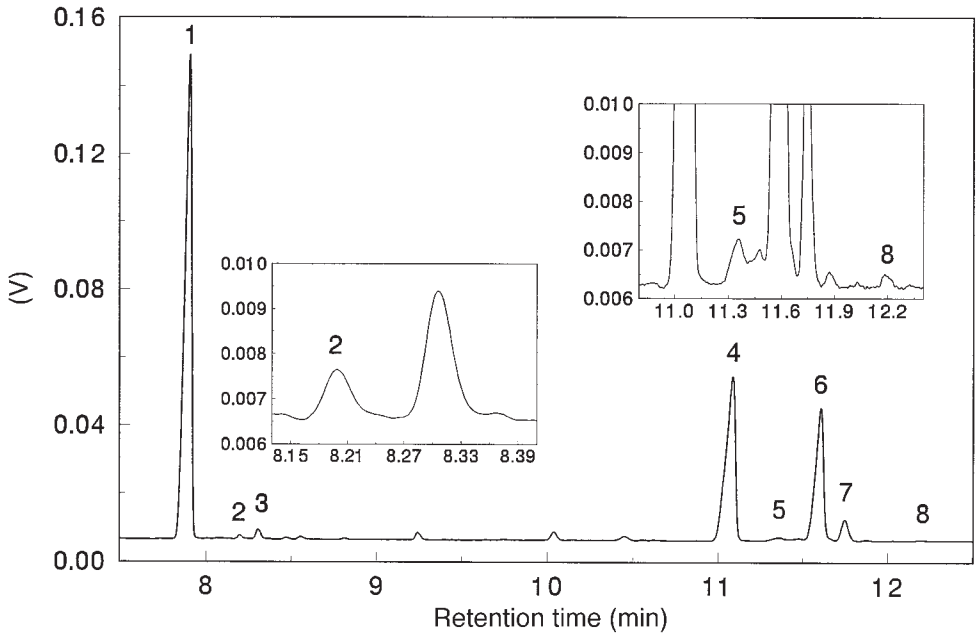


**FIGURE 1.** A typical chromatogram of cord blood plasma phospholipids. The numbers over the peaks identify selected fatty acids as follows: 1, 16:0; 2, *t*-16:1; 3, 16:1n−7; 4, 18:0; 5, *t*-18:1; 6, 18:1n−9; 7, 18:1n−7; and 8, *ct, tc, tt*-18:2. The insets show with greater magnification the *trans* fatty acids included when calculating the sum of *trans* fatty acids (*t*-16:1 + *t*-18:1 + *ct, tc, tt*-18:2).

## DISCUSSION

The most notable findings of the present study are the significant inverse associations between *trans* isomeric and long-chain polyunsaturated fatty acids in cord blood lipids at the end of apparently normal human pregnancies. Because humans do not synthesize *trans* fatty acids, the *trans* fatty acids detected in cord blood lipids must have originated from the maternal diet. Moreover, it is reasonable to assume that high *trans* fatty acid concentrations in cord blood indicate high maternal dietary intakes of *trans* fatty acids.

Therefore, the data obtained in the present study raise the possibility that maternal exposure to *trans* fatty acids may be inversely related to long-chain polyunsaturated fatty acid status in full-term infants at birth. Because long-chain polyunsaturated fatty acids are important for early postnatal visual and cognitive development (12–15), factors that reduce the availability of long-chain polyunsaturated fatty acids at birth are of serious concern. The potential importance of the inverse association between *trans* fatty acids and long-chain polyunsaturated fatty acid status at birth is supported further by the recent observation that for docosahexaenoic acid and arachidonic acid, status at birth is one of the major determinants of postnatal changes in these fatty acids (18).

The cord blood *trans* fatty acid values measured in the present study were well within the range reported in the literature (19–22). For example, in 1994 one of us reported the total *trans* fatty acid concentrations in cord blood phospholipids (0.69%), cholesterol esters (2.02%), and triacylglycerols (1.91%) of German neonates, and these values were similar to those measured in Austrian newborns in the present study. However, correlations between *trans* isomeric and long-chain polyunsaturated fatty

**TABLE 2**
Linear correlation coefficients (*r*) between percentage contributions (% by wt) of the sum of *trans* fatty acids and the major polyunsaturated fatty acids and product-to-substrate ratios for 20:4n−6 and 22:6n−3 biosynthesis in cord blood lipids of 42 full-term infants[1]

| | Phospholipids | Cholesterol esters | Triacylglycerols | Nonesterified fatty acids |
|---|---|---|---|---|
| n−6 PUFAs | | | | |
| 18:2n−6 | −0.02 | −0.61[2] | −0.25 | −0.27 |
| 20:4n−6 | −0.56[2] | −0.52[2] | −0.01 | −0.41[3] |
| Sum of n−6 long-chain PUFAs | −0.48[3] | −0.49[3] | 0.17 | −0.33[4] |
| Ratio of 20:4n−6 to 18:2n−6 | −0.37[4] | −0.15 | 0.14 | −0.19 |
| n−3 PUFAs | | | | |
| 18:3n−3 | 0.24 | 0.22 | 0.03 | −0.20 |
| 22:6n−3 | −0.48[3] | −0.36[4] | −0.23 | −0.41[3] |
| Sum of n−3 long-chain PUFAs | −0.47[3] | −0.03 | −0.01 | −0.41[3] |
| Ratio of 22:6n−3 to 18:3n−3 | −0.28 | −0.39[4] | 0.07 | 0.03 |

[1] Sum of n−6 long-chain polyunsaturated fatty acids (PUFAs): 20:2n−6 + 20:3n−6 + 20:4n−6 + 22:4n−6 + 22:5n−6; sum of n−3 long-chain PUFAs: 20:3n−3 + 20:5n−3 + 22:5n−3 + 22:6n−3.
[2] $P < 0.001$.
[3] $P < 0.01$.
[4] $P < 0.05$.

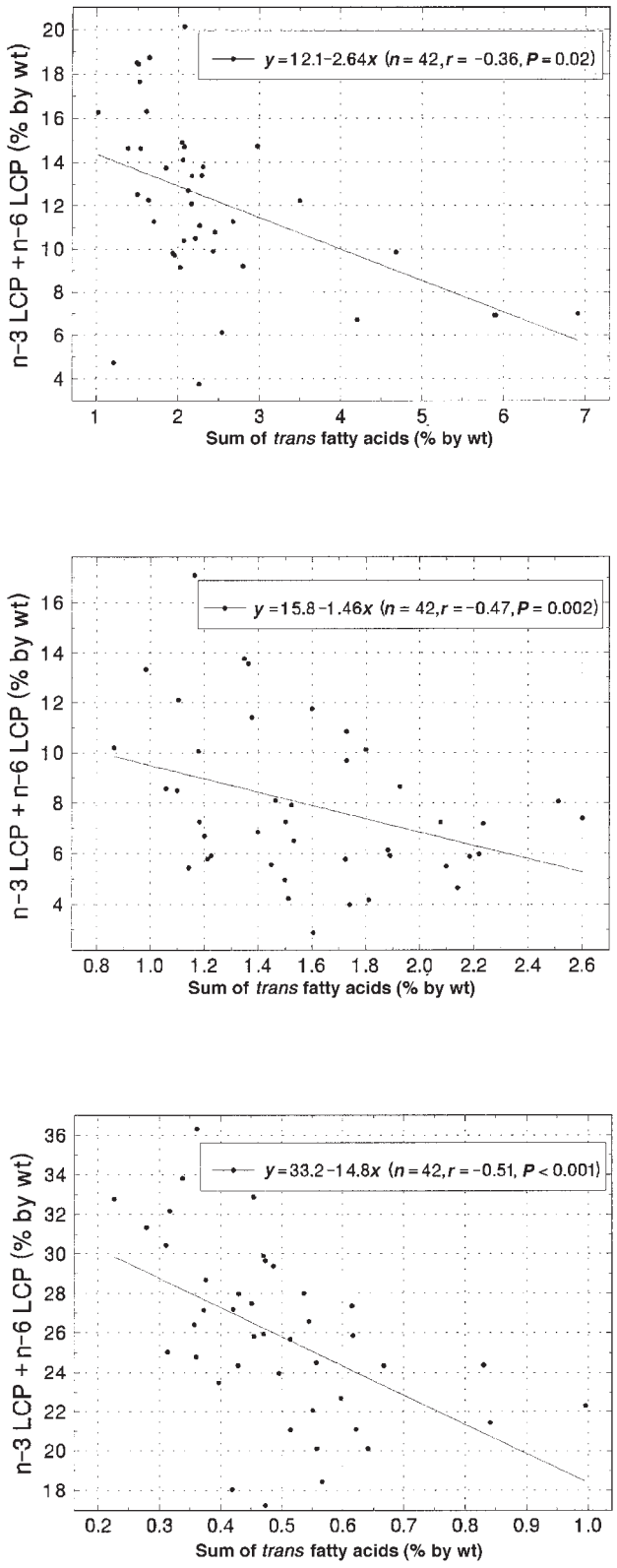


**FIGURE 2.** Relations between the sum of *trans* fatty acids (*t*-16:1 + *t*-18:1 + *ct, tc, tt*-18:2) and the sum of n−3 plus n−6 long-chain polyunsaturated fatty acids (LCP; 20:3n−3 + 20:5n−3 + 22:5n−3 + 22:6n−3 + 20:2n−6 + 20:3n−6 + 20:4n−6 + 22:4n−6 + 22:5n−6) in nonesterified fatty acids (top), cholesterol esters (middle), and phospholipids (bottom) in venous cord blood of 42 full-term infants.

acids were not reported in the aforementioned studies describing cord blood *trans* fatty acids (19–22).

Three previous reports indicated interference of *trans* fatty acids with the availability of long-chain polyunsaturated fatty acids in humans. First, there were significant inverse correlations between total *trans* fatty acids and both arachidonic and docosahexaenoic acids, but not linoleic and α-linolenic acids, in plasma cholesterol esters and triacylglycerols of 29 premature infants on day 4 of life (10). *trans* Fatty acids were also significantly and inversely correlated with birth weight but not with gestational age in that study (10). Second, *trans* fatty acids were significantly and inversely correlated with arachidonic acid but not with linoleic acid in plasma phospholipids of 53 healthy children aged 1–15 y (11). Third, a significant inverse relation was found between plasma triacylglycerol *t*-18:1 and plasma phospholipid docosahexaenoic acid values in cord blood of 50 full-term infants in a study that was reported in abstract form (23). In the same study, a strong association was found between maternal *trans* fatty acid intake and both maternal and infant *trans* fatty acid status at delivery (24).

The inhibiting effect of *trans* fatty acids on the enzymatic conversion of linoleic acid to arachidonic acid was documented in numerous studies conducted in animal models and in human fibroblasts in vitro (reviewed in 25). More recently, in pregnant rats, high dietary intakes of *trans* fatty acids were associated with diminished $\triangle^6$-fatty acid desaturase activity in liver microsomes and decreased contribution of n−3 long-chain polyunsaturated fatty acids to fetal plasma lipids in the offspring (26). In the present study, *trans* fatty acids were inversely related not only to long-chain polyunsaturated fatty acids and the product-to-substrate ratios of their biosynthesis but also to the parent n−6 essential fatty acid, linoleic acid, in cholesterol esters. Therefore, the present study did not determine whether the observed inverse association between *trans* isomeric and long-chain polyunsaturated fatty acids resulted from an interaction between *trans* fatty acid intake and essential fatty acid intake, or an inhibitory effect of *trans* isomers on long-chain polyunsaturated fatty acid biosynthesis, or a combination of these factors.

Did the atopic trait of the infants studied influence the inverse association between *trans* isomeric and long-chain polyunsaturated fatty acids? Higher prevalences of childhood asthma and allergy were associated with higher *trans* fatty acid intakes in an extensive epidemiologic study that compared 55 populations (27). In a different study of umbilical cord serum phospholipids, significantly higher concentrations of long-chain polyunsaturated fatty acids were found in infants whose mothers had allergies than in infants whose mothers did not (28). These data do not seem to suggest an atopy-related inverse association between *trans* isomeric and long-chain polyunsaturated fatty acids, and we are not aware of any studies that measured these 2 groups of fatty acids simultaneously in atopy.

In summary, we report a significant inverse correlation between concentrations of *trans* isomeric fatty acids and long-chain polyunsaturated fatty acids in venous cord blood lipids of full-term infants. Although the available data do not allow us to reach any firm conclusion on the nutritional safety of *trans* fatty acids in the diets of pregnant women, the results reported here suggest that maternal *trans* fatty acid intake may be inversely associated with infant long-chain polyunsaturated fatty acid status at birth. Further prospective studies and reevaluation of exist-

ing databases on the fatty acid composition of cord blood lipids should contribute to a better understanding of the nutritional role of *trans* fatty acids during pregnancy.

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