Results: In Germany. The fatty acid composition of the milk samples was inversely correlated with linoleic acid (P < 0.0001), arachidonic acid (P < 0.0001), docosahexaenoic acid (P < 0.0001), and docosahexaenoic acid and polyunsaturated fatty acids.

Conclusions: The data obtained in the present study suggest that the availability of 18-carbon trans isomeric fatty acids may be inversely related to the availability of long-chain polyunsaturated fatty acids in mature human milk. Am J Clin Nutr 2007;85:1320–6.

INTRODUCTION

Unsaturated fatty acids (FAs) containing double bonds in trans stereoisomeric configuration have been attributed to untoward nutritional effects. From a pediatric perspective, it is not their atherogenic effect but the potential interference of trans FAs with the availability of long-chain polyunsaturated FAs (LC-PUFAs) that is, PUFAs with greater than 20 carbon atoms, that deserve special attention (1). Significant inverse correlations were found between TFA and LC-PUFA in ≥5 human studies: in cord blood lipids both in healthy full-term infants (2) and in full-term infants with an atopic trait (3), in cord vessel wall lipids in healthy full-term infants (4), in plasma lipids in young preterm infants (5), and in plasma lipids in children (6). Because PUFAs are considered important for neurodevelopment in infancy (7,8), the potentially untoward effect of exposure to trans isomeric FAs has also received increased attention within the pediatric community (9).

LC-PUFA concentrations in infantile plasma lipids (10), erythrocyte membranes (11), and cerebral tissues (12) are largely determined by the dietary intake of preformed LC-PUFAs. Human milk is the principal source of LC-PUFAs for most healthy, full-term infants; hence, LC-PUFA content of human milk is an important concern. The question of the potential interference of TFAs with LC-PUFAs in human milk has been addressed in only a few studies and yielded controversial results (13–15). However, the putative association of the presence of TFAs to LC-PUFA supply through human milk is of great practical relevance. Although the human metabolism is able to elongate and desaturate ingested trans isomeric FAs into longer-chain and more unsaturated metabolites (16), it is unable to de novo synthesize TFA isomers. Hence, TFAs detected in human milk must have their origin in the maternal diet. Consequently, maternal TFA exposure might influence LC-PUFA supply to the breastfed infant. The aim of this study was to investigate the relation between TFAs and LC-PUFAs in mature human milk within the context of a large population-based birth cohort study.

SUBJECTS AND METHODS

Study design and study population

Women who came to the Department of Gynecology and Obstetrics at the University of Ulm between November 2000 and November 2001 for the delivery of their babies were invited to participate in the study. In Germany at the time of the study, women stayed in the hospital for ≈5 d after delivery; recruitment was done during this time. During the time of recruitment, the

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Department of Gynecology and Obstetrics at the University of Ulm was the only large department of obstetrics in the study area; the Department of Gynecology and Obstetrics at the University of Ulm was the only large department of obstetrics in the study area; Ulm was the only large department of obstetrics in the study area; Department of Gynecology and Obstetrics at the University of Ulm was the only large department of obstetrics in the study area.

To obtain a birth cohort of healthy and mature babies, we excluded women with a baby of <32 gestational weeks, a baby of <2500 g birth weight, or a baby transferred to inpatient pediatric care immediately after delivery. Furthermore, we excluded women with no understanding of the German, Turkish, or Russian language and all women who left the hospital immediately after birth. Overall, 1066 families could be included into this study (67% of all 1593 eligible families who fulfilled the inclusion criteria).

Participation was voluntary and informed consent was obtained in each case. The study was approved by the ethics boards of the University of Ulm and of the physicians’ boards of the states of Baden-Württemberg and Bavaria.

Data collection

All mothers underwent standardized interviews conducted by trained interviewers during hospitalization after delivery. Interviews included detailed questions about housing and living conditions, lifestyle factors, medical history, and health status during pregnancy. A standardized form was used to collect anthropometric data before and during pregnancy from the mothers’ pregnancy health charts.

The interview was also offered in Turkish and Russian if this was the mother’s native language. All mothers included at baseline were contacted by telephone 6 wk postpartum and were asked whether they were breastfeeding at this time: 1024 mothers (90%) were successfully contacted again, 786 (76.7%) were still breastfeeding their infants, and 769 milk samples (97.8%) could be collected. A trained nurse visited all women who were still breastfeeding and collected 10 mL human milk, which was immediately cooled and was frozen at −80 °C within 24 h. In the vast majority of cases (90%) milk samples were collected by the trained nurse from both breasts by manual expression before feeding. In rare cases milk samples were collected by the mothers themselves or by means of a breast pump.

Analytic methods

Frozen milk samples were melted at 36 °C. For the FA analysis, lipids were extracted from 100 μL milk with HCl methanol after the addition of acetyl-chloride and the internal standard (pentadecanoic acid, 15:0). FA methyl esters were measured by high-resolution capillary gas-liquid chromatography with the use of a Finnigan 9001 chromatograph (Finnigan/Tremetrics Inc, Austin, TX) with split injection (1:15) and a flame ionization detector. A 60-m cyanopropyl column (DB-23; J&W Scientific, Folsom, CA) was used. The temperature program was as follows: an initial temperature of 50 °C for 0.1 min, followed by a temperature increase of 50 °C/min up to 173 °C, a 10-min isotherm period, a temperature increase of 25 °C/min up to 221 °C, an 8-min isotherm period, a temperature increase of 10 °C/min up to 250 °C, and a 24.62-min isotherm period.

Peak identification was confirmed by comparison with authentic mixtures of weighed FA methyl esters (GLC-463: NuChek Prep, Elysian, MN; and Supelco 37 FAME Mix: Supelco, Bellefonte, PA). Individual FA response factors determined from these weighed standards were used to calculate the percentage by weight for individual FAs from the percentage of area for individual FAs from the percentage of area under the curve. Data are reported for 28 FAs detected with chain lengths between 10 and 24 carbon atoms. Sum of the trans hexadecenoic acid (16:1t), trans octadecenoic acid (18:1t), and trans octadecadienoic acid (18:2t) was entered into the correlation analysis as sum of the TFAs.

Statistical analysis

We first carried out descriptive analyses about sociodemographic and anthropometric characteristics of the mothers, and we described the principal FAs in milk according to maternal nationality and country of birth. We used the Kruskal-Wallis test and the chi-square test for detecting differences between groups. Correlations between concentrations of each of the TFAs (16:1t, 18:1t, and 18:2t) as well as their sum and the other main FAs were determined by Spearman’s and partial Spearman’s correlation coefficients, respectively. All analyses were performed with the use of the SAS STATISTICAL SOFTWARE (version 8,
When comparing *trans* isomeric and PUFAs in milk samples, we found significantly lower 16:1*ω* values in the Turkish mothers than in the 3 other groups (Table 2). Total n-6 PUFA and linoleic acid (18:2*ω*6) values were highest in the Turkish mothers than for the mothers in the other groups (*P < 0.0001*). No statistically significant differences were seen among the 4 groups in n-3 PUFAs.

The values of 16:1*ω* showed weak, albeit significant, positive correlations with dihomo-γ-linolenic acid (20:3*ω*6), arachidonic acid (20:4*ω*6), and α-linolenic acid (18:3*ω*3) (Table 3). In contrast, we found clear, statistically significant negative
TABLE 3
Correlation analysis between trans isomeric and polyunsaturated fatty acids in human milk samples (n = 769) obtained at the 6th week of lactation.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>16:1r</th>
<th>Spearman $\rho$</th>
<th>$P$</th>
<th>18:1r</th>
<th>Spearman $\rho$</th>
<th>$P$</th>
<th>18:2r</th>
<th>Spearman $\rho$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2n−6</td>
<td>0.02</td>
<td>0.69</td>
<td></td>
<td>−0.32</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.33</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>18:3n−6</td>
<td>−0.05</td>
<td>0.20</td>
<td></td>
<td>−0.36</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.017</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>20:2n−6</td>
<td>+0.07</td>
<td>0.06</td>
<td></td>
<td>−0.51</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.44</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>20:3n−6</td>
<td>0.10</td>
<td>0.006</td>
<td></td>
<td>−0.55</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.39</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>20:4n−6</td>
<td>0.14</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.60</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.47</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>22:4n−6</td>
<td>−0.004</td>
<td>0.91</td>
<td></td>
<td>−0.57</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.34</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>18:3n−3</td>
<td>0.08</td>
<td>0.021</td>
<td></td>
<td>−0.35</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.27</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>20:3n−3</td>
<td>0.02</td>
<td>0.53</td>
<td></td>
<td>−0.42</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.29</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>20:5n−3</td>
<td>0.02</td>
<td>0.53</td>
<td></td>
<td>−0.43</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.25</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>22:5n−3</td>
<td>0.002</td>
<td>0.95</td>
<td></td>
<td>−0.53</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.33</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
<tr>
<td>22:6n−3</td>
<td>0.07</td>
<td>0.063</td>
<td></td>
<td>−0.51</td>
<td>&lt; 0.0001</td>
<td></td>
<td>−0.33</td>
<td>&lt; 0.0001</td>
<td></td>
</tr>
</tbody>
</table>

$^a$At the calculation of partial Spearman $\rho$ correlation coefficients, the fatty acid data were adjusted for nationality and country of birth of the mothers.

DISCUSSION

In this large population-based study that included 769 milk samples collected at the 6th week of lactation from mothers participating in a birth cohort in Germany, we found clear inverse correlations between 18:1r and all of the n−3 and n−6 PUFAs studied. Similarly, clear, statistically significant negative correlations were observed between 18:2r and the n−3 and n−6 PUFAs, except for $\gamma$-linolenic acid (18:3n−6).

The sum of TFAs correlated negatively to the n−6 essential FA, 18:2n−6, and its longer-chain metabolites, 20:3n−6 and 20:4n−6 (Figure 1), in a statistically significant way. Similarly, statistically significant negative correlations were observed between the sum of TFAs and the values of the n−3 essential FA, 18:3n−3, and its longer-chain metabolites, eicosapentaenoic acid (20:5n−3) and docosahexaenoic acid (22:6n−3) (Figure 2). The sum of TFAs correlated inversely to the sum of n−6 PUFAs and the sum of n−6 LC-PUFAs in a statistically significant way (Figure 3). The negative association proved to be stronger between the sum of TFAs and n−6 LC-PUFAs than between the sum of TFAs and n−6 PUFAs (Figure 3).

FIGURE 1. Correlations between the sum of trans fatty acids and linoleic acid (18:2n−6), dihomo-$\gamma$-linolenic acid (20:3n−6), and arachidonic acid (20:4n−6) in human milk samples (n = 769) obtained at the 6th week of lactation. (For the calculation of partial Spearman $\rho$ correlation coefficients, the fatty acid data were adjusted for nationality and country of birth of the mothers.)

FIGURE 2. Partial Spearman $\rho$ correlation between the sum of trans fatty acids and each of the respective LC-PUFAs.

FIGURE 3. Partial Spearman $\rho$ correlation between the sum of trans fatty acids and each of the respective n-3 PUFAs.
TFA values were similar to those reported for German human milk samples studied previously by others (20, 21).

The main finding of the present study is the statistically significant inverse correlations between 18:1t and 18:2t, on the one hand, and various n-6 and n-3 PUFAs, including 18:2n-6, 20:4n-6, 18:3n-3, and 22:6n-3, on the other hand. Inverse correlations between TFAs and 18:2n-6 and 18:3n-3 were reported together with the lack of correlation between TFAs and 20:4n-6 and 22:6n-3 in the studies of Ratnayake and Chen (13) and Innis and King (14). In contrast, no correlation was found between TFAs and any n-6 or n-3 PUFAs in the recent study of Mosley et al (15). To the best of our knowledge, no inverse correlations were reported between TFAs and 20:4n-6 and 22:6n-3 in human milk so far.

The significant inverse correlations between 18-carbon TFAs and PUFAs together with the lack of any negative correlation between 16-carbon trans isomers and PUFAs in the present study are in close agreement with our previous data obtained by studying the FA composition of umbilical cord blood vessel wall lipids in a sizeable cohort (n = 308) of women living in the Netherlands (4). In umbilical cord artery wall lipids, C16:1 trans isomers and PUFAs in the present study were inversely related to 18:2n-6, 20:4n-6, 18:3n-3, and 22:6n-3. In contrast, values of C18:1t were inversely related to 20:4n-6 and 22:6n-3. A similar pattern was found in umbilical vein wall lipids.

The inverse association between TFAs and LC-PUFAs seen in the present study may be explained by the effect of TFAs to impair the synthesis of LC-PUFAs from 18:2n-6 and 18:3n-3 to their longer-chain metabolites. In vivo studies on rodent tissues (22) and on human fibroblast cultures (23) clearly showed that various TFAs inhibit the desaturation and chain-elongation of 18:2n-6 and 18:3n-3 to their longer-chain metabolites. In vivo studies in rats also showed an impairment of 18:2n-6 and 18:3n-3 metabolism with exposure to various TFAs (24–28). In a more recent study, high exposure to TFAs inhibited the incorporation of LC-PUFAs into the phospholipids of arterial cells in a porcine model (29). The putative effect of 18-carbon TFAs to inhibit the conversion of essential FAs to their longer-chain metabolites may explain the stronger negative associations between TFAs and 20:4n-6 and
When looking at the results of this study, the following limitations have to be considered. First, there was considerable interindividual variability in both TFA and PUFA values among human milk samples in this study, and a wide variability of data always gives rise to the suspicion of some threshold effect within a relation. However, wide variability of TFA values is a common finding in studies on human milk; for example, a 172-fold interindividual variability of total TFA values in human milk was reported for a group of Canadian women (13). Furthermore, median total TFA values in the present study were not higher but were >3 times lower than the average values reported in previous studies on the relation of TFAs to LC-PUFAs in human milk (13–15). Second, only 3 trans isomers were quantified in the present study, whereas at least 16 different TFAs can be detected by sophisticated methods in human milk (15). Although the most prevalent TFA both in the human diet (30–32) and in human milk (18, 33) is 18:1t, more detailed analysis of various trans isomers might show further associations between individual TFAs and LC-PUFAs in human milk. Third, the present observational study is unable to establish a causative relation between high TFA and low LC-PUFA values in human milk. As a note of caution, it is possible that TFAs detected in the present study are only indicators of some hitherto undefined dietary or other factor that decreases LC-PUFA content in human milk. Fourth, the lack of data on the dietary habits of the mothers studies may also represent serious limitation of the interpretation of the results obtained in the present study.

Nevertheless, the present study also has several strengths. First, we studied a sizeable group of lactating women; indeed, the number of samples investigated in the present study was nearly twice as high as the cumulative number of samples investigated in the 3 previous studies that reported associations between TFAs and PUFAs in human milk (13–15). Second, we studied a relatively homogenous cohort of lactating women who were recruited at delivery and who all donated milk samples 6 wk after delivery. To control further for potential sources of systematic variability among lactating women, FA data were adjusted for nationality and country of birth before further analysis. Third, milk samples were obtained by health professionals in a standardized way (see Data collection), and FA analysis was carried out in a laboratory with considerable experience both in analyzing TFAs (3–4) and human milk FA composition (34–37).

In summary, we report a statistically significant inverse relation of 18-carbon TFAs to LC-PUFAs in human milk studies in a large birth cohort study. Because TFAs in human milk must originate from the maternal diet, and LC-PUFAs in human milk are important for the neurodevelopment of the infant, our results suggest that decreased maternal dietary intake of TFAs with 18 carbons may enhance the LC-PUFA supply in human milk. Moreover, the data obtained in the present study support the concept that TFAs should be regarded as independent variables in studies investigating the supply of LC-PUFAs in infants.

The authors’ responsibilities were as follows—ES and TD: designed the study, organized the laboratory analysis, performed the main analysis, designed the data analysis, and drafted and revised the manuscript; GB and CB: designed the study, organized the laboratory analysis, and performed the main analysis; DR and MW: organized the recruitment of mothers, collected the data, designed the data analysis, and drafted and revised the manuscript; HB: organized the recruitment of mothers and collected data. All authors contributed to the final wording of the manuscript. TD is the guarantor for the report and had full responsibility for the decision to submit the manuscript for publication. None of the authors had a financial or personal conflict of interest related to the content of this study.

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