REVIEW

Trans-18:1 Acid Content and Profile in Human Milk Lipids. Critical Survey of Data in Connection with Analytical Methods

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ABSTRACT: This study presents an in-depth, critical survey of the current knowledge about *trans*-18:1 acid content and profile in human milk lipids. Emphasis is placed on the analytical methods employed to quantitate *trans*-18:1 acids, most of which lead to imprecise quantitative data. It is demonstrated that data obtained by single gas–liquid chromatography (GLC) on polar capillary columns are underestimates by 25–40%. Several experiments indicate that the total content of *trans*-18:1 acids in human milk is directly related to the quantities ingested the previous day(s), provided no gross weight loss occurs during breast-milk feeding. Equations have been proposed to describe this relationship, and apparently the percentage of *trans*-18:1 isomers, relative to total fatty acids, is approximately three-fourths the quantity (in g) ingested by lactating mothers. That is, the determination of the *trans*-18:1 acid percentage in human milk is a convenient means to estimate *trans*-18:1 acid consumption by corresponding populations. Adapted methods (i.e., silver-ion thin-layer chromatography, coupled with GLC on long polar capillary columns) allow accurate quantitation of most individual *trans*-18:1 acids, more particularly of the *trans*-Δ16 isomer. This determination, along with a knowledge of the distribution of individual isomers in ruminant fats and partially hydrogenated oils, is a convenient means to estimate the relative contribution of these two dietary sources to the distribution of individual *trans*-18:1 isomers in human milk lipids. A comparison of human milk and infant formulas is made with regard to *trans*-18:1 acid content and profile. Important differences are noted between data from European countries and from North America.

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Humans do not synthesize *trans* fatty acids, and their presence in tissue and secretion (particularly milk) lipids is necessarily linked to their presence in the diet. *Tran*s fatty acids may originate from three possible dietary sources; ruminant fats (milk, meat, adipose tissue), partially hydrogenated oils (margarines, shortenings, cooking fats), and deodorized oils (particularly α-linolenic acid-containing oils, i.e., soybean and rapeseed oils). With the exception of the latter source, the principal *trans* components are 18:1 isomers. *Trans*-polyunsaturated (18:2 and 18:3) acids are also widely distributed in our diets, but in low amounts. Although both categories of isomers are suspected as harmful components, here we will focus our attention on *trans*-18:1 acids only.

Several exhaustive reviews on the health effects of *trans* fatty acids have been published during the last decade (1–7), but whether *trans*-18:1 isomers in human milk may impair any biochemical or physiological functions in breast-fed infants has not been proven with certainty. However, owing to possible negative physiological effects as a result of *trans*-fatty acid consumption in adults, there is, in particular, cause for concern for infants. It is therefore of interest to summarize the results obtained so far on *trans*-fatty acid levels in human milk. The purpose of this review is not to add controversy to this subject but rather to provide an update of our knowledge on the fatty acid composition of human milk lipids, with specific emphasis on *trans*-18:1 isomers. We also attempt to provide information that allows conclusions to be made on the relative contribution of partially hydrogenated oils and ruminant fats to the content of *trans*-18:1 fatty acid positional isomers in human milk. The importance of an accurate determination of total *trans*-18:1 acids in milk lipids as a means to estimate the daily intake of such components is highlighted.

Some papers on *trans*-unsaturated fatty acids in human milk lipids that appeared during the period 1977–1985, which are also included in the present critical survey, were reviewed earlier by Jensen (8).

Before examining the literature, let us identify both the limitations and the advantages of the methods used for *trans*-18:1 acid determination. This topic has been extensively reviewed by Firestone and Sheppard (9) and more recently by Ratnayake (10). We shall summarize the current opinions of analysts in this field.

Although popular and easy to perform, infrared (IR) spectroscopy measurements (absorption at *ca.* 970 cm^{-1}) do not provide information on the type of *trans*-acid (chainlength, number and position of ethylenic bonds), and the technique yields at best a semiquantitative assessment for low *trans*-acid content (this particularly holds true for older data), owing to the difficulty of establishing an accurate baseline. Gas–liquid chromatography (GLC) of methyl esters, on either packed or capillary columns, has its own drawbacks, in that several

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trans-18:1 isomers (those with high Δ positions) are either masked by the generally dominating *cis*-Δ9 18:1 (oleic) acid or even elute after it, thus leading to underestimates of *trans*-18:1 acids. Claims of accurate results obtained by single GLC on so-called highly efficient columns are groundless. Great care should be exercised when considering data obtained by single-run GLC without other complementary procedures. Particular credence should be given to the combination of silver-ion thin-layer chromatography (Ag-TLC) and GLC because the overlaps between *trans* and *cis* isomers are eliminated. After Ag-TLC, individual *trans*-18:1 acids can be quantitated either by ozonolysis-based procedures or, better, by GLC on the longest available (100 m) highly polar capillary columns (e.g., CPSil-88 or SP-2560), because in the latter conditions, there is no loss of the shorter fragments, and there is no need to apply response factors to correct the unequal response of the flame-ionization detector. Moreover, identifications of individual isomers of *trans*-18:1 acids by direct GLC on relatively short capillary columns (30 m or less) were somewhat whimsical in the past because (i) *trans*-18:1 acids eluted under a single uneven peak with no marked summits, and (ii) authentic individual standards were not commercially available and would have probably overlapped for several of them.

TOTAL CONTENT OF *TRANS*-18:1 ISOMERS AND DEPENDENCE ON THE DIET

Since the early study in 1961 by Kaufmann *et al*. (11), who analyzed *trans* acids by IR spectroscopy, it has been known that human milk lipids may contain such geometrical isomers and that their presence in the milk is linked to their presence in the mothers' diet. To demonstrate this, lactating German women were switched from a diet that contained *trans* isomers to a diet devoid of *trans* isomers (without ruminant fats and partially hydrogenated vegetable oils). This led to a rapid decline in the content of the *trans* acids, and they finally completely disappeared within a few days. Because the *trans*-fatty acid content measurements were performed by IR spectroscopy, no data concerning individual isomers (chainlength, number of ethylenic bonds) were given. Later, Picciano and Perkins (12) confirmed the presence of *trans* acids in milk from American women by GLC analysis on a packed OV-275 column, and these authors established that these isomers had the structure 18:1. For three subjects, the *trans*-18:1 acid content was in the range of 2.1–4.0% of the total fatty acids. These values were not different from those determined by IR spectroscopy by Kaufmann *et al*. (11). Harzer *et al*. (13), on the other hand, reported that *trans*-18:1 acids were "not detectable" in the milk from German and English women as determined by GLC on a column packed with Silar-10C. In retrospect, the failure to detect *trans*-18:1 acids was undoubtedly linked to the inability of the column to separate the *trans*- from the *cis*-18:1 isomers, at least under the conditions used by the authors, rather than to their absence from milk lipids.

Later, Aitchison *et al*. (14) quantitated the *trans*-18:1 acids in milk from 11 American women, and they also checked their content in the diets. Analyses were performed by GLC. Although subjects were different in the percentages of *trans*-18:1 acids in their milk, for most women, but not all, the *trans* acid percentages in the milk were apparently correlated to the percentages of *trans* acids in the diets of the previous day. The range for *trans*-18:1 acids in milk lipids was 2.0–4.5%, whereas that in the dietary fats was 1.0–8.3%. *Trans* monoenes with shorter chains (14:1 and 16:1) were tentatively identified, but emphasis was put on probable interferences of these acids with odd-chain saturated acids when GLC analyses were performed on a packed Apolar-10C column. Similar values were reported a few years later by Clark *et al*. (15) for isolated triacylglycerols in the milk from 11 lactating American women. In this last study, cholesterol esters were also shown to contain *trans*-18:1 isomers, and *trans*-16:1 acids (less than 0.4% of total fatty acids) were tentatively identified through GLC analysis on a packed OV-275 column. Hundrieser *et al.* (16) studied the triacylglycerols, phosphatidylcholine, and phosphatidylethanolamine of milk from 10 American mothers (second week of lactation) with regard to both their total *trans*-18:1 acid content and their intramolecular distribution. The average *trans*-18:1 content of the triacylglycerols was 3.4% (range from 1.1 to 5.7%) and was greater in the *sn*-1,3 positions. The proportions of *trans*-18:1 acids averaged 2.5% in phosphatidylcholine and 3.7% in phosphatidylethanolamine, with slightly greater proportions in the *sn*-2 positions for both kinds of phospholipids. A combination of analyses on packed OV-275 and SP-2330 columns was used in this study. Finley *et al*. (17), also using a packed OV-275 column, could separate, in 70% of 242 samples of milk lipids from 57 American women, the *trans*- from the *cis*-18:1 acids. A mean value of 3.7 ± 2.3% *trans*-18:1 acid was established, and the sum of the percentages of *trans*-16:1 and *trans*-18:1 acids was significantly correlated with percentage margarine consumption. However, no formulas were given.

The dependence of the *trans* acid content of human milk on the mothers *trans* acid intake was confirmed and formalized by Craig-Schmidt *et al*. (18) in 1984. Though these authors were apparently not aware of the study by Kaufmann *et al*. (11), their experimental design was about the same, except that they analyzed *trans* acids by capillary GLC (30-m SP-2330 column) instead of by IR spectroscopy. For eight American women who ate a self-chosen diet, at the beginning of the experiment a content of 4.8% of *trans*-18:1 acids was determined. This value is similar to that reported more recently by Dotson *et al*. (19) for 30 milk samples collected in the United States (4.72%). However, these authors used a Supelcowax 10 (polyethylene-glycol) capillary column (30 m) without Ag-TLC fractionation. Such a stationary phase is rarely employed for the quantitation of *trans*-18:1 isomers. According to a study (20) with a similar stationary phase (Omegawax) and butyl esters instead of methyl esters, the *cis* and *trans* forms of each individual octadecenoic isomer (from butterfat) were reported to elute together. Consequently, we

suspect an overlap and confusion between *trans*-Δ11-18:1 and *cis*-Δ11-18:1 isomers, which is generally separated from the *cis*-Δ9-18:1 acid by capillary GLC on 30-m columns coated with this kind of stationary phase. So, the representativeness of the data reported by Dotson *et al*. (19) is questionable.

In the experiment of Craig-Schmidt *et al*. (18), one group of lactating mothers was provided with a diet poor in *trans* acids (equal to or less than 1% of total fatty acids, mainly coming from milk and butter), while the other group was provided with menus that included visible fats, such as margarines, hydrogenated soybean oil and shortenings (*trans*-18:1 acids relative to total dietary fatty acids in the range of 7.7–17.1%). After 6 d on these diets, milk from women of the first group had a content of *trans*-18:1 acids that had steadily declined to a residual value of *ca*. 2%, whereas those from the second group showed milk lipids with a *trans*-18:1 acid content that had approximately doubled (*ca*. 9% of milk total fatty acids).

From their data, Craig-Schmidt *et al*. (18) concluded that the amount of *trans*-18:1 acids in the milk was positively and linearly correlated with the *trans*-18:1 acid proportion in the previous day's diet. An equation was established between these two parameters: $Y = 1.49 + 0.42X$, where Y and X represent the percentages of *trans*-18:1 in total fatty acids from milk and from the diet of the previous day, respectively. *Trans*-18:1 acids were quantitated with a 30-m SP-2330 capillary column, which is unable to completely separate *trans*- from *cis*-18:1 isomers, and quantitative data were surely underestimates. Provided the same multiplicative error was made on X and Y, the equation remains valid and may be of practical interest for predictive calculations.

Anyway, the studies by Kaufmann *et al*. (11), Aitchison *et al*. (14), and Craig-Schmidt *et al*. (18) demonstrated that the *trans*-18:1 acid content of human milk is directly dependent on the quantity consumed in the previous days, although other parameters may interfere with the deposition of these isomers in milk lipids.

Chappell *et al*. (21) also observed through a cross-over study that the *trans* fatty acid content of milk (almost exclusively 18:1 acids) was a function of recent maternal *trans* acid intake. However, these authors showed that the relation between *trans* acid intake and their deposition in milk lipids was not as simple as initially thought. In this study, there was an evident dependence of the *trans*-18:1 acid proportion in milk fatty acids on weight loss by lactating mothers. At the beginning of the experiment, the milk from mothers who presented a weight loss of 4–7 kg contained almost twice as much *trans* acids (3.5%) as that of mothers with no or smaller weight loss (0–2 kg) (*ca*. 2.0%). This trend was also apparent when the diet of the volunteers was supplied with a margarine that contained 35% *trans*-18:1 acids, although the *trans* acid content of milk was increased (up to 7% for the large weight-loss group and 4% for the small weight-loss group). Because there is a mobilization of triacylglycerols from adipose tissue during weight loss, Chappell *et al*. (21) concluded that the levels of *trans* acids in human milk should thus certainly reflect short- and also long-term maternal diets. When mothers returned from diets with a high *trans*-18:1 acid intake to diets with a medium intake (diets including margarines with 13% *trans*-18:1 acids), the contents of *trans*-18:1 acids in their milk decreased and tended to reach, for each group, levels similar to those observed at the beginning of the cross-over experiment. The *trans* acid content of milk lipids was also apparently dependent on the gestational age and lactational stage. In addition to the *trans*-Δ9 isomer, which was by far the major *trans* component, Chappell *et al*. (21) also tentatively identified (by their retention times, but without authentic standards) the *trans*-Δ11 and -Δ12 isomers at levels less than or equal to 0.1% of total fatty acids. On the other hand, they apparently failed to detect *trans*-14:1 and *trans*-16:1 acids.

The influence (direct or indirect) of the *trans*-18:1 acid amount in the diet on its proportion in milk lipids is also illustrated by a few studies for some countries with obviously differing *trans*-18:1 acid consumption levels. For example, Koletzko *et al*. (22) compared the fatty acid compositions (by capillary GLC on a 50-m CPSil-88 column) of milk lipids from lactating Nigerian and German women and found a higher mean content for the second population (3.1%; $n = 15$) than for the first one (0.9%; $n = 10$). Nigerian women evidently consumed less milk-based products or partially hydrogenated fats than German women. Koletzko *et al*. (22) also mentioned in their list of fatty acids the presence of *trans*-14:1 and *trans*-16:1 isomers in the milk of both populations, but it is likely that these components were overlapped with branched uneven fatty acids (branched 15:0 and 17:0 acids, respectively). More recently, the same laboratory (23) followed the fatty acid composition of human milk (from German women) during the first month after term or preterm delivery. There were apparently no differences between the two subpopulations with regard to total *trans* fatty acids (determined by single GLC runs on a 50-m CPSil-88 capillary column). The *trans* isomers ranged from 1 to 1.5%, which was 1/2 to 1/3 of that in the initial study (22). This would indicate that the German mothers' diets were quite different in the two studies (hospital vs. home diets?). The milk from rural African women, living in two Sudanese villages, contained $0.4 \pm 0.2\%$ *trans*-18:1 acids, as determined by direct analyses on a CPSil-88 capillary column, with a range of 0.1 to 1.1% ($n = 77$) (24). According to the description of the diets consumed by the participants, the major *trans*-18:1 acid sources were cow and goat milk. Hydrogenated oils were apparently not consumed. Consequently, the main *trans*-18:1 isomer in milk fat should be the Δ11 and not the Δ9 isomer as arbitrarily and erroneously reported in Reference 24.

A low level of *trans*-18:1 acids (1.0%; $n = 38$) was also determined by capillary GLC (50-m CPSil-88 column) by Boatella *et al*. (25) in human milk collected from Spanish women. These results are not surprising when compared to those of Koletzko *et al*. (22) for German women. In Germany, the consumption of all milk-derived products corresponds to

about 370 kg/person/yr of full-fat milk (3.7% fat, corresponding to 1.3 g *trans*-18:1 acid per day), whereas the corresponding value for Spain is only 140 kg/person/yr (0.5 g *trans*-18:1 acid per day) (26). A similar disparity between the two countries is observed in the consumption of margarines; the individual consumption of such foods in Germany and Spain is 7.7 and 2.1 kg/yr, respectively. It is thus clear from these studies that the *trans*-18:1 acid percentage in human milk depends in some way on the quantity consumed. With respect to the consumption of margarines, Boatella *et al*. (25) could additionally find a significant difference in total *trans*-18:1 acid levels in human milk between consumers (1.2%) and nonconsumers (0.6%), but no correlations were established.

The limitations concerning the accuracy of methods, and particularly of GLC used alone to determine total *trans*-18:1 acids in human milk, apply to all studies already cited. Consequently, the contents of *trans*-18:1 isomers reported in these studies have to be considered with caution, being undoubtedly underestimates (by *ca.* 25–40%, see following), and are merely of qualitative interest. Moreover, no data concerning the distribution profile of individual *trans*-18:1 isomers were given.

INDIVIDUAL ISOMERS AND DEPENDENCE ON THE *TRANS*-18:1 ACID SOURCES

Detailed profiles of *trans*-18:1 isomers in human milk lipids have only recently been established unambiguously. In a study of major analytical importance, Chen *et al*. (27) used a combination of Ag-TLC and capillary GLC on a 100-m SP-2560 capillary column to quantitate the *trans* content of 198 samples of Canadian human milk. Moreover, the *trans*-18:1 acids isolated by Ag-TLC were further cleaved by BF_3/CH_3OH oxidative ozonolysis, and the resulting dimethyl esters were separated by GLC. This procedure allowed the identification and quantitation of all individual *trans*-18:1 isomers. Such results had never been reported before. By this combination of techniques, the presence of all *trans*-18:1 isomers with ethylenic bonds between positions Δ7 and Δ16 was unambiguously demonstrated. *Trans* fatty acids were found in all samples analyzed. The major *trans* group was *trans*-18:1 acids, which ranged from 0.1 to 15.4%, with a mean value of 5.9 ± 2.5% of total fatty acids. A range of similar amplitude for total *trans*-fatty acids was reported almost 20 yr earlier by Beare-Rogers and Nera (28) for Canadian women. Later, the same laboratory (29) specified that the *trans* fatty acids occurred mostly in the 18:1 fraction, with differences between lactating mothers living at home (higher content) or in hospitals (lower content).

In contrast to data previously published by other authors, who did not use Ag-TLC to separate *cis*- and *trans*-18:1 acids, which otherwise overlap during GLC on all known capillary columns, even on the longest ones, and who consequently published underestimates, the data by Chen *et al*. (27) should be considered as precise and accurate. Because these authors determined the *trans*-18:1 acid content of milk lipids both by simple direct GLC and by Ag-TLC coupled with GLC in a great number of samples, one can calculate from their data a correction factor of 1.26 that should be applied to results obtained simply by direct GLC (column length of 50 m or less) alone to calculate the true amount of total *trans*-18:1 acids in human milk. Nearly the same value (1.25) was calculated for *trans*-18:1 acids from beef meat lipids analyzed on a 50-m CPSil-88 capillary column (30). Sampugna *et al*. (31) observed with partially hydrogenated vegetable oils that this factor varied in the range 1.15–1.33 for a 15-m SP-2340 capillary column, depending on the percentage of *trans*-18:1 acids in the total observed octadecenoic acids. On a 50-m CPSil-88 capillary column, which appears to be one of the most popular columns for the separation of *trans*-18:1 acids, the *trans*-Δ5 to *trans*-Δ11 isomers elute before the leading edge of the generally major *cis*-9 18:1 acid, whereas all other *trans* isomers with ethylenic bonds between Δ12 and Δ16 elute after this point, in admixture with *cis*-18:1 acids (32). An apparently similar overlap would occur with 50-m columns coated with OV-275 or SP-2340 stationary phases (33). For 100-m SP-2560 columns, probably not operated under the best conditions to allow an optimal separation of *trans*- and *cis*-18:1 isomers (34,35), the *trans*-Δ12 isomer is only on the verge of separating from the *cis*-9 18:1 acid; and its accurate quantitation (as that of other individual *trans* isomers with lower Δ positions: they appear only as shoulders or small bumps on the general envelope of the *trans*-18:1 acid peak) is doubtful. This does not mean that a 100-m SP-2560 column is less efficient than the 100-m CP-Sil 88 column: under optimal conditions, and provided the *trans*-18:1 acids are first isolated by argentation chromatography, both columns give comparable results (Molkentin, J., and D. Precht, unpublished observations; 36).

From a large and representative number of analyses of both ruminant fats and partially hydrogenated oils, where Ag-TLC was used (reviewed in Ref. 32), the following proportions of *trans*-18:1 isomers (relative to total *trans*-18:1 acids), which are not taken into account during a single-run GLC analysis on 50-m columns, can be calculated: cow milk fat, 35–45% (depending on the feed; average, 40%); beef meat fat, 24–33%; beef tallow, 26%. The corresponding value for partially hydrogenated vegetable oils would average *ca*. 25%, but it is likely that this value depends on the nature of the oil and on the hydrogenation conditions. The correction factor for *trans*-18:1 acids in human milk lipids (and even more so for any human tissue) thus depends on the respective proportions of ruminant milk fats and partially hydrogenated vegetable oils in the diet, which are lower in North America (*ca*. 1.25) than in several European countries (from 1.33 when partially hydrogenated vegetable oils dominate to 1.55 when cow milk fat is prominent). As already discussed, these correction factors should apply to data obtained with most capillary columns coated with cyanoalkyl polysiloxane stationary phases with lengths from 30 to 100 m.

Using the minimal correction factor 1.30 and the data from Craig-Schmidt *et al*. (18) for American women leads to a bet-

ter estimate of the mean *trans*-18:1 acid content, *ca*. 6.2% in milk lipids, which is not very different from the corresponding value for Canadian women. This corrected figure is corroborated by data from Sampugna and Teter [cited in (37)], who found 6.6 ± 0.7% of *trans*-18:1 acids (range, 1.4–13.3%). In a personal communication, Sampugna stated that this average value was established by direct GLC on a 30-m SP-2380 column, with application of a correction factor to raw analytical data for *cis*- and *trans*-18:1 acids. Milks from 29 free-living women on representative American diets were analyzed. The average content of *trans*-16:1 isomers was estimated to be 0.15%, but possible interferences with other fatty acids were not checked. Chen *et al*. (27) reported a mean *trans*-16:1 acid content of 0.18%, both by direct GLC and by Ag-TLC/GLC.

Comparing qualitatively the distribution profile of *trans*-18:1 isomers in Canadian women's milk with those in partially hydrogenated soybean and canola oils and in cow milk fat, Chen *et al*. (27) deduced that the major dietary sources of *trans*-18:1 isomers were partially hydrogenated vegetable oils, whereas contribution from dairy products was comparatively minor. Identical conclusions were drawn by the same group from the distribution profile of *trans*-18:1 isomers (established after their fractionation by Ag-TLC and ozonolysis) in adipose tissue from 12 Canadian volunteers (38). However, the mean content of *trans*-18:1 acids in adipose tissue was lower than in milk lipids and was apparently (though not statistically) different depending on the location of the adipose tissue (abdomen, 4.9 ± 0.8%; thigh, 3.8 ± 0.7%; $n = 12$ in both cases). A similar deduction was made by Ohlrogge *et al*. (39), who compared the distribution profile of individual *trans*-18:1 isomers from adipose tissue of U.S. inhabitants with that of partially hydrogenated oils and of cow milk fat after isolation of the *trans* monoenoic acid fraction and ozonolysis.

However, these observations for North American people should not be generalized to other populations. Wolff (30) and Chardigny *et al*. (40) analyzed the milk lipids from 10 French women (region of Dijon), with a combination of Ag-TLC and capillary GLC (50-m CPSil-88 column). Several distinctive features for French women, as compared to North American women, were found. First, the *trans*-18:1 acid content in milk lipids was relatively low (2.0 ± 0.6%). Second, the major isomer was *trans*-11 18:1 acid, although all isomers with ethylenic bonds between Δ6 and Δ16 were identified. Authentic standards were used to identify individual isomers. The overall distribution pattern of *trans*-18:1 acids resembled more that of ruminant fats than that of partially hydrogenated oils, although these processed oils contributed to a significant increase in the relative proportions of Δ6 to Δ10 *trans* isomers as compared to the typical profile of ruminant fats. It is thus clear from this study (30) and that of Chen *et al*. (27) that the *trans*-18:1 acid profile depends on the respective proportions of partially hydrogenated oils and ruminant fats in the diet.

Wolff (30) tried to formalize this conclusion by noting that the *trans*-Δ16 18:1 isomer, which is particularly well isolated on chromatograms after Ag-TLC separation of the *trans*-monoenoic acid fraction, is high in milk fat (8.1% of total *trans*-18:1 isomers, a value based on the analysis of 60 butter samples collected in all seasons) but low in partially hydrogenated oils (initially estimated from literature data, 0.5%). Combining these data, he proposed an equation to link the relative contributions of these two dietary sources and the percentage of *trans*-16 18:1 acid relative to total *trans*-18:1 isomers: $8.1X + 0.5Y = Z$, where X and Y are, respectively, the proportions of milk fat and partially hydrogenated oils in the diet, and Z is the percentage of the *trans*-Δ16 18:1 isomer relative to total *trans*-18:1 isomers in human milk lipids (with $X + Y = 1$). Later, after having accumulated more experimental data for both kinds of fat (41,42) and having included data for ruminant meat fat (30), a slightly modified equation was proposed: $7.8X + 0.8Y = Z$ (36). When applied to the milk of French women, for whom the mean relative proportion of the *trans*-Δ16 18:1 isomer (Z) is known (4.9%), X and Y are, respectively 60 (ruminant fats) and 40% (partially hydrogenated vegetable oils). These values were identical to those established from consumption data (30). However, the validity of this equation relies on the hypothesis that there is no selectivity in the metabolism of individual *trans*-18:1 isomers between their initial ingestion and their final deposition in milk lipids, or their storage in adipose tissues and their further mobilization for milk lipid synthesis. Although probable, this remains to be experimentally proven.

Wolff (30) also emphasized the significance of the content of *trans*-18:1 acids in human milk in relation to *trans*-18:1 acid consumption. Using selected literature data available for a few countries (Spain, France, Germany, and the United States), and applying the correction factor deduced from data of Chen *et al*. (27) when necessary, he could construct a linear relationship between these two parameters, described by the equation $Y = 0.76X + 0.20$, where Y is the percentage of *trans*-18:1 acids in total human milk fatty acids and X the mean individual daily intake of such isomers (in g). This equation indicates that the percentage of *trans*-18:1 acids in human milk total fatty acids is approximately three-fourths the quantity ingested. However, we believe that this equation is of limited application. It should apply only to populations and not to individuals because several physiological parameters may influence the fatty acid composition of milk [see, for example, Chappell *et al*. (21)]. Specifically, in the United States, the data of Craig-Schmidt *et al*. (18) (corrected: 6%) and Sampugna and Teter (cited in Ref. 37) (6.6%) would correspond to a mean daily intake of 7.7 to 8.5 g of *trans*-18:1 acids, which is well within the range 7.6–12.8 g established from other parameters (particularly on food consumption and disappearance data) (6). If one considers that the mean intake of *trans*-18:1 acids from ruminant fats is 0.8–1.3 g/d in the United States (6), the proportion of *trans*-18:1 acids from partially hydrogenated oils would be 85–90% of the total *trans*-18:1 acid intake. The same would also hold true for Canada.

Combe *et al*. (43), who used the same analytical procedures (Ag-TLC/GLC) and the same 50-m CPSil-88 capillary column as Wolff (30), confirmed the low level of *trans*-18:1

acids in the milk of 16 French women from the region of Bordeaux. The mean content of *trans*-18:1 acids was 1.6 ± 0.8% of total fatty acids. N. Combe (personal communication) also confirmed the predominance of vaccenic acid among *trans*-18:1 acids in the milk lipids from French women, and its proportion was fairly well correlated to dairy fat consumption (43). It was also noted that the *trans*-Δ9 18:1 isomer (elaidic acid) was positively correlated to hydrogenated vegetable fat consumption. However, the quantitation of elaidic acid under such chromatographic conditions (50-m CPSil-88 column) is questionable because this acid is poorly resolved from the adjoining Δ6-8 and Δ10 isomers (44). Most probably, the so-called elaidic acid included some of the latter isomers, that are indeed higher in partially hydrogenated fats than in cow milk fat. Only when using a 100-m column is it possible to suitably isolate the *trans* 9-18:1 isomer from adjacent peaks (44). Additionally, Combe *et al*. (43) isolated by Ag-TLC a *trans* 16:1 acid fraction along with the *trans*-18:1 isomers, which accounted for less than 0.1% of total fatty acids. This was at variance with the data of Koletzko *et al*. (22) for German women (0.5%), but in this case, the identification was only tentative (single GLC runs, without Ag-TLC fractionation), and interferences with other fatty acids may have occurred, leading to a misidentification of the *trans*-16:1 acid (in fact a group of several isomers). Entressangles (45) indicated a mean *trans*-18:1 acid content of 1.8 ± 0.9% (range, 0.3–3.6%) in the milk lipids of 27 women from the region of Bordeaux. Most interesting, this proportion is the same as in the adipose tissue of six women from the same region (45). The *trans*-Δ11 18:1 acid is reported to be the prominent isomer in milk and adipose tissue. It represents *ca*. 33% of total *trans*-18:1 acids in both cases (45). When considering the sum of *trans*-Δ10 plus *trans*-Δ11, which are not easily separated on 50-m capillary columns, it represented 46% in Entressangles' report for women in the region of Bordeaux, whereas Wolff established earlier a value of 49% for women in the region of Dijon (30). These data, obtained independently (but with the same methods) for women from two different locations in France, are quite homogenous, and a mean value for the *trans*-18:1 acid content in human milk from French women of 1.9% [range, 0.3–4.4%; $n = 37$ (40,45)] appears quite sound. On average, it is estimated that 60–63% of these isomers comes from ruminant fats and 37–40% from partially hydrogenated oils (41,45).

J. Molkentin and D. Precht (unpublished results) investigated the milk lipids of eight German women (region of Berlin) by combined Ag-TLC/GLC with a 100-m capillary column (CPSil-88). As for the extensive analyses of cow milk fats (46–49) and partially hydrogenated vegetable oils (46,48,49), this highly polar and efficient column shows a good resolution of all positional isomers, spanning from *trans*-Δ5 to *trans*-Δ16, except for the groups *trans*-Δ6-8 and *trans*-Δ13-14 (after their separation by Ag-TLC). An example of the resolutions obtained is given in Figure 1, which compares the *trans*-18:1 acid chromatographic pattern obtained from human milk lipids with those obtained from representative samples of cow milk fat and margarine. All fractions were isolated by Ag-TLC prior to GLC analysis. The content of *trans*-18:1 acids in German human milk lipids ranged from 1.4 to 4.7% with a mean value of 2.6 ± 0.9%, which is slightly higher than for French women (30,40,43,45), but less than the mean value reported by Koletzko *et al*. (22) for another group of German women (determined by direct GLC).

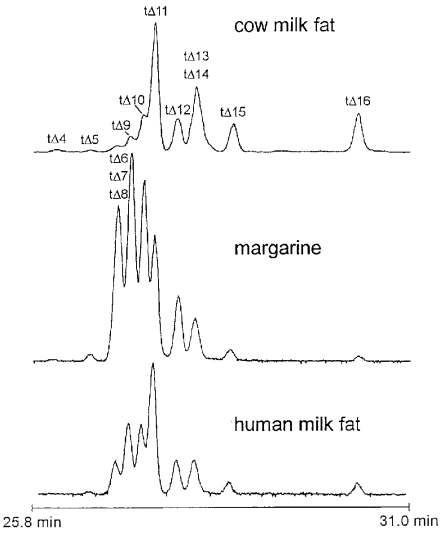

FIG. 1. Partial chromatograms of *trans*-18:1 acid methyl esters isolated by silver-ion thin-layer chromatography from human milk lipids in comparison to representative samples of margarine and cow milk fat. Analyses on a 100-m CPSil-88 fused-silica capillary column (Chrompack, Middelburg, The Netherlands) operated at 160°C with helium as a carrier gas (inlet pressure, 160 kPa).

The following mean absolute contents of individual *trans*-18:1 isomers (weight% of total fatty acids), as well as their percentages relative to their total (values given in parentheses), were found: Δ5 = 0.01% (0.47), Δ6-8 = 0.29% (11.10), Δ9 = 0.48% (18.26), Δ10 = 0.40% (14.89), Δ11 = 0.62% (23.55), Δ12 = 0.25% (9.63), Δ13-14 = 0.32% (12.71), Δ15 = 0.09% (3.48), and Δ16 = 0.14% (5.91). Vaccenic acid was the major isomer, though significantly less abundant than in cow milk fat. On the other hand, the Δ6 to Δ10 isomers were higher than in cow milk fat, and this can only be explained by the consumption of partially hydrogenated vegetable oils, in which these isomers are more abundant than in milk fat. On average, the isomeric distribution in these German human milks was intermediate between the mean pattern of a representative number ($n = 62$) of German margarines and shortenings (7) and the distribution in cow milk fats, as can be seen from Figure 1 as well.

Regarding Wolff's equation for calculating the origin of

trans fatty acids in French women's milk lipids as described above, slight changes must be introduced for German women, leading to $9.8X + 1.1Y = Z$. The value 9.8 is derived from the analyses of 1756 cow milk fat samples, taking into account all seasonal variations (7), and the value 1.1 from the analyses of 62 German margarines and shortenings (7,49). With the percentage of *trans*-Δ16 18:1 isomer in human milk lipids (relative to total *trans*-18:1 acids) $Z = 5.9$, the average intake of *trans*-18:1 acids from ruminant milk fats (X) and partially hydrogenated vegetable oils (Y) for German women is calculated to be 55 and 45%, respectively. Calculations based on consumption data for German women with differentiation of various groups of food items, published in 1994 by Steinhart and Pfalzgraf (50), resulted in exactly the same ratio, for a total daily intake of 3.4 (females) or 4.1 (males) g/person. In France, the corresponding estimate for women would be 2.6 g/person/d (males and females together, 2.8 g person/d) (41). For the two countries, it can be deduced that the ratio percentage of *trans*-18:1 acid intake in milk lipids/d/person would be around 0.75. Comparing the consumption data and the values for human milk lipids in France and Germany indicates that the differences between German and French populations with regard to the *trans*-18:1 acid consumption are minimal, less than 1 g/person/d. This is of considerable importance regarding a possible relation between *trans* fatty acid intake and risk of coronary heart disease: in Germany, the death rate for ischemic heart diseases among male subjects is more than twice that in France (30).

TRANS-18:1 ACIDS IN INFANT FORMULAS

To our knowledge, and with the exception of the brief report by Picciano and Perkins (12) on a nonrepresentative number of U.S. infant formulas, five comprehensive studies have been conducted on human milk substitute lipids: in the United States (51), in Germany (52), in Spain (53), in France (54), and in Canada (55). In some of these studies (51,54,55), emphasis was also placed on *trans* isomers of essential polyunsaturated acids, mainly originating from the addition of deodorized oils to the formulas. *Trans*-18:1 acids were quantitated in German, Spanish, French, and Canadian artificial milks (52–55), but not in formulas from the United States (51). In the French and Canadian studies (54,55), the combination Ag-TLC/GLC (50-m CPSil-88 capillary column) was used, whereas German and Spanish formula fatty acids were analyzed by single GLC on a 50-m CPSil-88 column (52,53).

For Germany, 25 samples were shown to contain *trans*-18:1 acids in the range from nondetectable amounts to 3.5% (52). *Trans*-14:1 and -16:1 isomers were also tentatively identified in many samples. For Spain (28 samples), the *trans*-18:1 acid range was between 0.04 and 3.15% (53). Infant formulas from this country, including whole cow's milk as an ingredient, had higher values of *trans* isomers (average value, 1.8%) than formulas manufactured with skimmed milk (1%). In France, the infant formulas also are frequently based on cow's milk, and thus, the *trans*-18:1 acid content is variable, within the range 0.2–4.3% (average, 2%) (54), depending on the relative proportion of cow's milk in the formula, its status (whole milk or skimmed milk), and the collecting season (*trans*-18:1 acids are low in winter, high in spring). Generally, the *trans*-18:1 isomer distribution profile is identical to that of cow milk fat (15 out of the 20 French samples analyzed), with vaccenic acid being the major isomer; and other isomers spanning from positions Δ5 to Δ16 are present in lesser amounts (54).

In Canada and the United States, formulation habits are completely different, and cow milk fat is generally not used (51,55,56). The maximum *trans*-18:1 acid content that was found in 24 Canadian formulas, either liquid or powdered, reached only 2.3% (mean, 0.7 ± 0.2%) of total fatty acids (55), which is considerably less than in most Canadian human milk samples (27,28). The origin of these isomers might correspond to the addition of slightly hydrogenated oils to the formulas. For the United States, only old and ambiguous data are available (56). For 12 formulas, and with only one exception (a U.S. Department of Agriculture formula), IR spectroscopy revealed the presence of "low levels" of *trans* isomers (range 0.8–2.0%) that apparently could not be quantitated with a 50-m Silar-10C capillary column (56). In Canada, the percentage of total *trans*-18:1 acids in infant formula lipids is similar to that of *trans*-polyunsaturated (18:2n-6 and 18:3n-3) acids (*ca.* 0.7–0.9%), which are formed during deodorization of oils (57,58). This should also occur in the U.S., though it was shown that data reported by O'Keefe *et al.* (51) for liquid infant formulas from this country were overestimates, due to an overlap of the *cis*-11-20:1 acid with the *trans*-9,*cis*-12,*cis*-15-18:3 isomer during GLC (59).

In a few studies, the total *trans* acids (including 16:1, 18:1, and 18:2 geometrical isomers) in infant formulas also were reported, but the detailed compositions of individual isomers were not given. Moreover, the data were obtained by single direct GLC analyses, with an unavoidable underestimate of the *trans*-18:1 isomers and probable misidentification of isomeric 16:1 and 18:2 acids. For 20 samples of Spanish infant formulas, an average content of 2.3 ± 1.1% of *trans* acids was determined (60), whereas Danish formulas, frequently based on cow's milk, contained 2.0 ± 1.3% of such isomers ($n = 13$; range, 0.3–4.2%) (61).

In summary, our survey shows that, with regard to their *trans*-18:1 acid content, human milk and infant formula lipids are rather similar in several European countries, whereas in North America, infant formulas have a considerably lower content of *trans*-18:1 acids than human milk.

DISCUSSION

The *trans*-18:1 acid content of human milk lipids largely depends on the availability of these isomers in the diets of the previous day(s). Their distribution profile is a combination of partially hydrogenated vegetable oils and ruminant fats, and it varies with the relative intake of foods from each origin. According to Ratnayake (62, and personal communication),

TABLE 1
***Trans*-18:1 Acid Contents in Lipids from Human Milk[a]**

Trans-18:1 acid content[a]	Min[a]	Max[a]	*n*[a]	Method[b]	Reliability of data[c]	Year	Country	Individual isomers[d]	Reference
	1[e]	4[e]	5	IR	No	1961	Germany	No	11
3.1	2.1	4.0	3	GLC/P	No	1977	U.S.A	No	12
4.64	1.00	8.27	11	GLC/P (6.4)	No	1977	U.S.A	No	14
4.48			11	GLC/P	No	1980	U.S.A	No	15
3.38	1.1	5.7	10	GLC/P	No	1983	U.S.A	No	16
4.76			8	GLC/C (30)	No	1984	U.S.A	No	18
3.67			242	GLC/P	No	1985	U.S.A	No	17
	0.5	4.5	14	GLC/C (30)	No	1985	Canada	No	21
3.12	1.47	4.38	15	GLC/C (50)	No	1988	Germany	No	22
0.86	0.52	4.94	10	GLC/C (50)	No	1991	Nigeria	No	22
1.0			38	GLC/C (50)	No	1993	Spain	No	25
0.4	0.1	1.1	77	GLC/C (50?)	No	1995	Sudan	No	24
1.99	1.20	3.17	10	Ag-TLC-GC/C (50)	Yes	1995	France	Yes	30,40
1.8	0.3	3.6	27	Ag-TLC-GLC/C (50)	Yes	1995	France	Yes	45
5.9	0.1	15.4	198	Ag-TLC-GLC/C (100)	Yes	1995	Canada	Yes	27
6.6	1.4	13.3	29	GLC/C (30; c.f.)	Yes	1995	U.S.A.	No	—[f]
2.6	1.4	4.7	8	Ag-TLC-GLC/C (100)	Yes	1995	Germany	Yes	—[g]

[a]Mean value; weight percentage relative to total fatty acids. Min, minimal content, Max; maximal content; *n*, number of samples.
[b]IR, absorption of infrared radiation at 970 cm^{-1}; GLC, gas-liquid chromatography; P, packed; C, capillary (the length is specified); c.f., correction factor; Ag-TLC, isolation of the *trans*-monoenoic acids by argentation thin-layer chromatography prior to GLC.
[c]Reliable results with application of a correction factor or using Ag-TLC to compensate overlaps of *cis*- and *trans*-18:1 isomers that occur during GLC.
[d]Separation and identification of all or most individual isomers.
[e]Total *trans*-fatty acid contents.
[f]Sampugna, J., personal communication.
[g]Precht, D., and J. Molkentin, unpublished results.

it appears that, under normal circumstances, there is little mobilization or contribution of adipose tissue *trans*-fatty acids (and even of 18:2n-6 and 18:3n-3 acids), except during severe food restriction (dieting). Owing to limitations of the analytical methods, older literature data (practically all those published before 1995; Table 1) cannot be regarded as sufficiently accurate for quantitative considerations. Many of them are misleading, and there is a need for further studies with appropriate method combinations, i.e., Ag-TLC coupled with capillary GLC on very long and efficient columns. This combination is essential and should attract a following. Furthermore, this method, applied to human milk fatty acids, is an adequate means to resolve and quantitate most individual isomers [under specific thermal conditions, even the Δ13 and Δ14 isomers can be separated, but the Δ6 to Δ8 isomers (generally minor) remain unresolved (63,64)], with a particular interest in the *trans*-Δ16 18:1 acid. This allows rapid estimate of the quantity of *trans*-18:1 acids consumed the previous day(s), provided an accurate quantitation of this isomer is established for both ruminant and partially hydrogenated fats. Accurate data for all types of ruminant fats (milk, meat, and fatty tissues), based on large collections of samples, are available and have been reviewed recently (32). However, it should be stressed that experimental data for a few samples only are likely to be erroneous, owing to the large variations of the *trans*-18:1 isomer content and profile occurring in ruminant fats (i.e., seasonal variations of milk fatty acids). Our conclusions should also apply to human adipose tissue triacylglycerols. However, in this case, the *trans*-18:1 acid content and profile will be a reflection of long-term intake of such isomers.

The accurate analysis of *trans*-16:1 isomers is also of concern, because they have sometimes been implicated as a cause for the potential negative effects of *trans* acids (3). These isomers may be of dietary origin [principally ruminant fats (30,42,65–67) and partially hydrogenated fish oils (66)], but one can hypothesize that they may also originate from *trans*-18:1 isomers by chain shortening. Here too, the quantitation of *trans*-16:1 isomers by direct GLC without Ag-TLC fractionation lends itself to criticism. The major reason is that there is not a single isomer as frequently and erroneously reported. All *trans*-16:1 acids having their ethylenic bond between position Δ5 (or possibly Δ4) and position Δ14 or Δ15 occur in cow milk fat (65) and adipose tissues (67), and in partially hydrogenated fish oils (66), and thus most likely in human tissues. On either packed (14,68) or capillary columns [50 m (42) or 100 m (66)], the *trans*-16:1 isomers interfere with branched 17:0 acids (*iso* and *anteiso* forms) and even with 17:0 acid. In hydrogenated fish oils, part of the *trans*-16:1 isomers are also probably intermingled with some *cis*-16:1 isomers, in the same way as their higher homologues *trans*- and *cis*-18:1, -20:1, and -22:1 acids [see, for example, the highly complex chromatogram obtained with a 50-m Silar-10CP, and published as early as 1978 by Ojanperä (69) for a partially hydrogenated herring oil]. Most data on *trans*-16:1 isomers reported earlier in human tissues (essentially milk, serum, and adipose tissue; for a review, see Ref. 3) are thus probably inaccurate estimates and need reinvestigations with appropriate methods.

Concerning specifically infants, we are aware of only one study that described negative physiological effects of *trans*-

isomers, that of Koletzko (52), who observed that *trans*-fatty acid levels in newborn serum cholesterol esters were negatively correlated with the infants' birth weight, so that intrauterine growth might be impaired by these isomers. This observation was apparently confirmed by Jendryczko *et al*. (70) in a paper written in Polish, with no analytical details, where *trans*-fatty acids in mothers' plasma were reported to be inversely correlated with birth weights. Whether such an effect during the perinatal period may exist still remains to be proven. However that may be, it is easy to reduce the *trans*-18:1 acid level in milk by merely choosing menus low in ruminant fats and particularly in partially hydrogenated oils during both pregnancy and lactating periods.

Finally, the accurate quantitation of *trans*-18:1 acids in human milk (or adipose tissue) as an indicator of *trans*-18:1 acid consumption may have considerable consequences in the interpretation of epidemiological studies linking the intake of *trans* acids and the risk of coronary heart disease, such as that of Willett *et al*. (71) for the United States [and many others based on *trans*-18:1 acids in adipose tissues (3)]. In that study, the mean intake of *trans* fatty acids in the highest quintile was estimated at 5.7 g/person/d. According to Enig *et al.* (72), this maximum could in fact reach 28.7–38.7 g/person/d in the United States. Ratnayake and Chen (73) proposed an upper average value of 26.1 g/person/d for both male and female Canadian adults, with a possible maximum of 38.9 g/person/d (young male adults). Because Sampugna and Teter (cited in Ref. 37) [and also Craig-Schmidt *et al*. (18), after correction; see above] found similar data for the milk of U.S. women, it can be deduced that Canada and the United States are rather similar with regard to the daily individual intake of *trans*-18:1 acids. On the basis of data on human milk from Ratnayake and Chen (73), it can be estimated that only 40% of the participants in their study consumed less than 6 g/person/d of *trans*-18:1 acids (9% of total dietary fatty acids), with the bulk consuming 6–15 g/person/d. This is at variance with the values calculated by Willett *et al*. (71) from dietary questionnaires (range for the *trans*-fatty acid consumption by 100% of the 85,000 participants was 2.4–5.7 g/person/d). More generally, data for average *trans*-fatty acid consumption in the United States, based on food-frequency questionnaires from Willett's laboratory (2.6–3.8 g/person/d) are two to four times lower than estimates by all other authors (7–12 g/person/d) (data reviewed in Refs. 2,3,72), and roughly correspond to values estimated for most European countries, except perhaps Norway and the Netherlands (30,74). Provided data for France and Germany (which are particularly well documented; see above) can be justifiably extended to North America, the ratio of the intake of *trans*-18:1 acids (in g) on the percentage of the same components in milk total fatty acids (*ca*. 1.33) would indicate that the average consumption of *trans*-18:1 acids by lactating women in the United States and in Canada would lie in the range of 7.9–8.4 g/person/d [compare with estimates from Craig-Schmidt *et al*. (18), 7.8; Hunter and Applewhite (75), 8.1; and Ratnayake and Chen (73), 7.0] and certainly more for male subjects.

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