Content and Distribution of *trans*-18:1 Acids in Ruminant Milk and Meat Fats. Their Importance in European Diets and Their Effect on Human Milk

Robert L. Wolff*

ISTAB, Laboratoire de Lipochimie Alimentaire, Université Bordeaux 1, Talence, France

ABSTRACT: The *trans*-18:1 acid content and distribution in fats from ewe and goat milk, beef meat and tallow were determined by a combination of capillary gas–liquid chromatography and argentation thin-layer chromatography of fatty acid isopropyl esters. The *trans* isomers account for 4.5 ± 1.1% of total fatty acids in ewe milk fat (seven samples) and 2.7 ± 0.9% in goat milk fat (eight samples). In both species, as in cow, the main isomer is vaccenic (*trans*-11 18:1) acid. The distribution profile of *trans*-18:1 acids is similar among the three species. The contribution of ewe and goat milk fat to the daily intake of *trans*-18:1 acids was estimated for people from southern countries of the European Economic Community (EEC): France, Italy, Greece, Spain, and Portugal. It is practically negligible for most of these countries, but in Greece, ewe and goat milk fat contribute *ca.* 45% of the daily consumption of *trans*-18:1 acids from all dairy products (0.63 g/person/day for a total of 1.34 g/person/day). The *trans*-18:1 acid contents of beef meat fat (ten retail cuts, lean part) and tallow (two samples) are 2.0 ± 0.9% and 4.6%, respectively, of total fatty acids (animals slaughtered in winter). Here too, the main isomer is vaccenic acid. Other *trans* isomers have a distribution pattern similar to that of milk fat. Beef meat fat contributes less than one-tenth of milk fat to the *trans*-18:1 acid consumed. The daily per capita intake of *trans*-18:1 acids from ruminant fats is 1.3–1.8 g for people from most countries of the EEC, Spain and Portugal being exceptions (*ca.* 0.8 g/person/day). In France, the respective contributions of ruminant fats and margarines to the daily consumption of *trans*-18:1 acids are 1.7 and 1.1 g/person/day (60 and 40% of total, respectively). These proportions, based on consumption data, were confirmed by the analysis of fat from milk of French women (ten subjects). The mean content of *trans*-18:1 acids in human milk is 2.0 ± 0.6%, with vaccenic acid being the major isomer. Based on the relative levels of the *trans*-16 18:1 isomer, we could confirm that milk fat is responsible for the major part of the daily intake of *trans*-18:1 acids by French people. The daily individual intake of *trans*-18:1 isomers from both ruminant fats and margarines for the twelve EEC countries varies from 1.5 g in Spain to 5.8 g in Denmark, showing a well-marked gradient from the southwest to the northeast of the EEC.

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In a recent study (1), the *trans*-18:1 acid content of butterfat was reinvestigated by a combination of capillary gas–liquid chromatography (GLC) and argentation thin-layer chromatography (Ag–TLC) of fatty acid isopropyl esters. This was done because literature data concerning *trans* fatty acid contents conflict. Natural *trans*-18:1 isomers are present not only in cow milk fat, but more generally in all ruminant milk and meat fats as a result of biohydrogenation by rumen bacteria of dietary polyunsaturated fatty acids. In the present study, we extend our previous analyses to other natural edible sources of *trans* acids that may not be negligible in the diet of European people and for which literature data are practically unavailable.

Among these sources are ewe and goat milks. Their production may be considered to be of marginal quantitative importance when compared to that of cow milk at a world level (1–2%) (2). However, it is not negligible in those countries where climatic and geographic conditions are not well suited to cattle-rearing. For example, 46% of the European caprine livestock is concentrated in Greece (2), where the number of cows for milk production is only 1% of cows in the European Economic Community (EEC) (3). For those countries where the consumption of dairy products from bovine origin is low, and the consumption of ovine and caprine products is comparatively high, the data for dietary *trans*-18:1 acid intake that were recently published (1) must be corrected to take into account the consumption of ewe and goat milk fat. In the EEC, the production (and consumption) of ewe and goat milk is practically limited to the four countries near the Mediter-

*Address correspondence at ISTAB, Laboratoire de Lipochimie Alimentaire, Université Bordeaux 1, Allee des Facultes, 33405 Talence Cedex, France.

ranean Sea (France, Greece, Spain, and Italy) and to Portugal (2). Although the presence of *trans*-18:1 acids in ewe and goat milk has been recognized for a long time, data in the literature concerning their content or distribution are limited and unsuitable for quantitative evaluations. Most recent reviews on *trans* fatty acids, originating from North American authors (4), do not explicitly mention these foods as potential dietary sources of *trans* isomers for humans, probably because these products are not usually consumed by the majority of American people. In the present study, using the same analytical procedures as for cow butterfat (1), we established the *trans*-18:1 acid content of ewe and goat milk fat. For this study, seven kinds of ewe cheeses and eight kinds of goat cheeses were examined.

Beef meat fat is also a potential source of *trans*-18:1 acids. In contrast to ewe and goat milk fats, it is frequently cited in literature reviews on *trans* fatty acids. This is explained by the fact that almost everyone, except vegetarians, eat beef meat in industrialized countries. Some studies have indicated that beef fat should contribute minimally (5) to the daily intake of *trans* isomers, but no assessments were made. Another edible source of beef fat is tallow. This fat is mainly used in food industries, and seldom for household deep frying in a few countries. Although some data concerning the *trans* content of beef meat fat and tallow are available in the literature, we thought it was necessary to reanalyze these products by the same procedures as used for ruminant milk fats (GLC coupled with Ag–TLC) to avoid any interfering variations linked to differences in analytical methods [e.g., infrared (IR) absorption at 970 cm^{-1} vs. GLC or GLC coupled with Ag–TLC; see Ref. 1 for discussion]. With this objective in mind, we determined the *trans*-octadecenoic acid content of the fat from ten different beef retail cuts and two samples of beef tallow. However, it should be emphasized that these samples were from cattle slaughtered in winter. Because it is known that the *trans* content in cow milk fat varies with the feed and accordingly with the season (1), there is some doubt as to whether the level of *trans*-18:1 acids in winter meat fat or tallow can be extended to samples processed in other seasons.

Human tissues also contain *trans*-18:1 acids that originate from ruminant fats or partially hydrogenated oils in the diet (6). However, biological samples of human origin are not easily available, with the exception of blood or milk. To get access to the effect of dietary *trans*-18:1 acids on human lipids in the particular case of the French population, which eats more *trans*-18:1 acids from milk fat than from margarines (1) and thus does not fit the American model (6), we analyzed human milk lipids. For this purpose, samples of milk lipids from ten subjects were used. The results presented here are part of an in-depth collaborative study of *trans* mono- and polyunsaturated acids in human milk that will be published elsewhere.

Because the capillary column we used (CP Sil 88; Chrompack, Middelburg, The Netherlands) is highly efficient, a partial insight in the distribution of *trans*-18:1 acids can be obtained by simply analyzing these isomers by GLC under optimal conditions with no other complementary analytical techniques (1). However, in our previous work on *trans*-18:1 acids in butterfat, the identification of individual peaks was tentative (1). In the present study, the use of individual synthetic *trans*-18:1 isomers allowed us to confirm these identifications. Consequently, we also describe the distribution of *trans*-18:1 acids in the fats analyzed.

Combining our experimental results with those available in the literature, when necessary, together with consumption data, allows estimation of the daily per capita intake of *trans*-18:1 acids from natural sources by populations of the twelve EEC countries. Our assessments are based on three parameters: the daily intake of a given category of food, its fat content, and the *trans*-18:1 acid content in the fat. In some cases, these parameters are fine-tuned, but in others they are only estimates.

EXPERIMENTAL PROCEDURES

Samples and chemicals. Eight different kinds of goat cheese and seven different kinds of ewe cheese were purchased in local supermarkets and groceries during October and November 1993. The samples were produced in France except for one sample of ewe cheese that was imported from Spain. Ten beef retail cuts, each from a different anatomical part of the animal, were purchased in January 1994 from several local butchers' stores to ensure that the samples did not come from a single animal. Beef tallow samples were from animals slaughtered in March and April 1994 and were kindly donated by C. Foures (Soprarga, Saint-Denis, France).

Ten samples of lipids (50 mg each) that were extracted from human milk were provided by Dr. J.-M. Chardigny (INRA, Dijon, France). Individual synthetic positional isomers of *trans*-18:1 acids with double bonds between positions 5 and 15 were generous gifts from Dr. L. Svensson (Kabi Pharmacia, Stockholm, Sweden). Authentic fatty acid standards were purchased from Sigma Chemical Company (St. Louis, MO).

Determination of the fat content in cheeses and meat. Cheese fat was extracted mainly according to Wolff and Castera-Rossignol (7). About 5 g of the sample was dispersed with an Ultraturrax in 10 mL isopropanol. Sufficient anhydrous Na_2SO_4 was added, followed by 15 mL hexane. The mixture was dispersed a second time. The suspension was then filtered on a column (2 cm i.d.) equipped with a sealed-in coarse fritted disk protected by a paper disk and containing a lower layer of anhydrous Na_2SO_4 separated from an upper layer of Celite 545 (*ca.* 2 cm height each) by a second paper disk. The lipid solution was eluted with hexane/isopropanol (2:1, vol/vol) and collected in a 100-mL volumetric flask. After evaporation of the solvents, cheese fat was weighed.

All visible fat and connective tissues were removed from beef meat cuts, and the lean portions (about 50 g) were homogenized in a household electric meat grinder. An aliquot of the resulting homogenate (*ca.* 7.5 g) was dispersed first in

50 mL methanol with the Ultraturrax and then in 100 mL chloroform. The suspension was filtered on paper in a separatory funnel, and the residue on the filter was rinsed with 20 mL of chloroform/methanol (2:1, vol/vol). To the clear filtrate was added 35 mL of an aqueous 0.9% KCl solution (8), and the mixture was thoroughly shaken and allowed to separate. The lower phase was drained, the solvents were evaporated and the fat was weighed.

Preparation of fatty acid isopropyl esters (FAIPE). To avoid the loss of volatile free fatty acids present in cheeses, the following procedure, which does not need any solvent evaporation step, was used. Portions of cheese containing 650–700 mg of fat were dispersed in isopropanol and hexane in the presence of anhydrous Na_2SO_4 as described above. An aliquot (2.5 mL) of the suspension was withdrawn with an all-glass syringe and filtered into a Teflon-lined screw-capping tube through a disposable microfiltration unit (9). To the clear filtrates were added 1.8 mL isopropanol and 0.25 mL concentrated H_2SO_4. The tubes were tightly capped and vigorously shaken, and the reaction was allowed to proceed at 100°C for 1 h (10). At the end of the reaction, the tubes were cooled, and distilled water (5 mL) was added. The tubes were vortexed for *ca.* 30 s and allowed to stand for 1 min. The upper phase was withdrawn and replaced by an equal volume of hexane. After vortexing and standing a second time, the upper phase was withdrawn and pooled with the first one. A third extraction was performed in the same manner (10). Beef meat fat was dissolved in chloroform to obtain solutions with a concentration of 50 mg/mL. One mL of these solutions was pipetted in Teflon-lined screw-capped tubes, and the solvent was removed under a stream of N_2. Beef tallow was melted at 60°C and homogenized, and two drops of fat were used for transesterification. To the tubes were added 1.5 mL hexane, 2.8 mL isopropanol, and 0.25 mL concentrated H_2SO_4. The reaction and the extraction of FAIPE were realized as described above. Human milk lipids, individual synthetic *trans*-18:1 acids, and authentic fatty acid standards were transformed into isopropyl esters in a similar manner.

Separation of phospholipids and triglycerides from beef meat fat. Aliquots of total lipids from beef meat were fractionated by TLC on commercial precoated plates (DC-Vertigplatten Kieselgel H; Merck, Darmstadt, Germany). Total neutral lipids were separated as a whole from phospholipids by a short migration (*ca.* 4 cm) in the solvent mixture diethyl ether/acetone (60:20, vol/vol) (11). The plates were then briefly air-dried, and neutral lipids were fractionated with the solvents hexane/diethyl ether/acetic acid (90:10:1, vol/vol/vol) in the same direction. At the end of the chromatographic runs, the plates were sprayed with a 0.2% (wt/vol) ethanolic solution of 2′,7′-dichlorofluorescein. After examination of the plates under ultraviolet (UV) light, the bands corresponding to phospholipids (remaining at the origin) and triglycerides were scraped off, and the gel was transferred into Teflon-lined screw-capped tubes for further transmethylation of fatty acids.

Preparation of fatty acid methyl esters (FAME). To the gel containing phospholipids or triglycerides from meat fat was added 1.5 mL of a 12% solution of BF_3 in methanol (wt/vol) and 1 mL benzene (12). The transmethylation reaction was allowed to proceed for 1 h at 100°C. FAME were extracted twice with 2 mL hexane.

Fractionation of FAIPE by Ag–TLC. FAIPE were fractionated according to the number and geometry of double bonds by TLC on silica-gel plates impregnated with $AgNO_3$. The plates were prepared as described elsewhere (1). The developing solvent was the mixture hexane/diethyl ether/acetic acid (90:10:1, vol/vol/vol). At the end of the chromatographic runs, the plates were sprayed with the solution of 2′,7′-dichlorofluorescein and viewed under UV light. The bands corresponding to the saturated and *trans*-monoenoic acids were scraped off in an aluminum foil, and the gel from the two bands was transferred into the same test tube. To the gel were added successively 1.5 mL methanol, 2 mL hexane, and 1.5 mL of a 5% (wt/vol) aqueous solution of NaCl (1). Thorough mixing followed each addition. After standing for *ca.* 1 min, the hexane phase was withdrawn almost quantitatively and concentrated under a light stream of N_2. The residue was dissolved in a small volume of hexane for further GLC analyses. Palmitic and stearic acids were used as internal standards to calculate the content of *trans*-18:1 isomers (1).

GLC. Analyses of FAIPE and FAME by GLC were carried out on a Carlo Erba 4130 chromatograph fitted with a flame-ionization detector and a split injector and coupled to an LT 430 temperature programmer (Carlo Erba, Milano, Italy). Separations were performed on a CP Sil 88 fused-silica capillary column (50 m × 0.25 mm i.d., 0.20 μm film; Chrompack). For the separation of total FAIPE prepared with cheese fat or human milk lipids, the column was operated at 65°C for 6 min, the temperature was then increased at a rate of 5°C/min up to 185°C (200°C for FAIPE prepared with human milk lipids) and left at this point until the end of the analysis. When either saturated plus *trans*-monoenoic acids or individual synthetic *trans*-18:1 isomers were analyzed, the column was operated isothermally at 160°C. In all cases, the temperature of the detector and the injector was 250°C, and the inlet pressure of the carrier gas (helium) was 100 kPa. FAME prepared with triglycerides and phospholipids of meat fat were analyzed isothermally at 175°C with an inlet pressure of the carrier gas of 120 kPa. Quantitative analyses were performed with an SP 4290 integrator (Spectra Physics, San Jose, CA).

RESULTS AND DISCUSSION

Trans-18:1 acids in goat and ewe milk fat. In the present study, we assume that cheese fat is representative of milk fat, because milk fat globules are retained in the cheese matrix during cheese processing. Consequently, results obtained with cheese fats are extended to milk fats in our calculations. The fat content of goat cheeses varied from 12 to 30%, that of ewe cheeses from 23 to 36%. Their fatty acid compositions are presented in Table 1. Qualitatively, the fatty acids are the

TABLE 1
Fatty Acid Compositions as Weight Percentages of Total Fatty Acids of Fat from Goat and Ewe Cheeses Purchased in October–November

	Goat cheese			Ewe cheese		
Fatty acid	Mean ± SD (n = 8)[a]	Minimum values	Maximum values	Mean ± SD (n = 7)	Minimum values	Maximum values
4:0	2.37 ± 0.11	2.24	2.60	3.17 ± 0.17	2.84	3.31
5:0	0.02 ± 0.01	0.01	0.04	0.02 ± 0.01	0.01	0.04
6:0	2.36 ± 0.12	2.22	2.43	2.53 ± 0.18	2.26	2.75
7:0	0.03 ± 0.01	0.02	0.03	0.03 ± 0.01	0.03	0.05
8:0	2.62 ± 0.15	2.46	2.88	2.49 ± 0.23	2.07	2.72
9:0	0.05 ± 0.01	0.04	0.06	0.06 ± 0.01	0.05	0.09
10:0	9.00 ± 0.44	8.25	9.56	7.81 ± 0.91	6.11	8.93
10:1	0.20 ± 0.04	0.18	0.30	0.23 ± 0.02	0.20	0.26
11:0	0.09 ± 0.01	0.07	0.11	0.09 ± 0.02	0.08	0.13
12:0	4.18 ± 0.56	3.31	5.23	4.50 ± 0.47	3.72	5.08
13:0	0.10 ± 0.04	0.06	0.18	0.10 ± 0.02	0.08	0.13
iso 14:0	0.13 ± 0.03	0.10	0.17	0.17 ± 0.04	0.14	0.23
14:0	9.88 ± 1.18	8.36	12.08	10.75 ± 0.37	10.22	11.27
iso 15:0	0.25 ± 0.02	0.20	0.34	0.32 ± 0.05	0.23	0.40
a-*iso*15:0[b]	0.37 ± 0.07	0.26	0.46	0.51 ± 0.03	0.46	0.55
14:1	0.15 ± 0.04	0.11	0.23	0.18 ± 0.02	0.16	0.20
15:0	0.99 ± 0.15	0.80	1.26	1.10 ± 0.06	1.01	1.17
iso 16:0	0.32 ± 0.05	0.25	0.45	0.29 ± 0.04	0.25	0.37
16:0	26.02 ± 1.73	23.51	28.08	23.78 ± 1.62	21.44	26.26
trans-16:1	0.18 ± 0.06	0.11	0.23	0.19 ± 0.06	0.13	0.30
cis-7 16:1	0.31 ± 0.06	0.25	0.37	0.22 ± 0.09	0.13	0.37
iso 17:0	0.42 ± 0.08	0.37	0.53	0.48 ± 0.08	0.39	0.61
cis-9 16:1	0.56 ± 0.05	0.50	0.60	0.74 ± 0.08	0.66	0.90
a-*iso* 17:0	0.52 ± 0.07	0.43	0.62	0.50 ± 0.06	0.42	0.59
cis-11 16:1	0.23 ± 0.07	0.15	0.33	0.17 ± 0.05	0.13	0.27
17:0	0.76 ± 0.11	0.64	0.94	0.78 ± 0.09	0.64	0.90
cis-9 17:1	0.42 ± 0.09	0.29	0.67	0.38 ± 0.06	0.34	0.40
18:0	10.12 ± 2.51	6.39	13.93	9.81 ± 1.19	8.62	11.71
trans + *cis* 18:1[c]	21.31 ± 1.52	19.23	22.92	21.21 ± 2.19	17.94	24.14
isom. 18:2[d]	0.95 ± 0.43	0.40	1.66	1.44 ± 0.42	0.80	1.95
18:2n-6[e]	1.97 ± 0.22	1.68	2.27	1.80 ± 0.39	1.15	2.37
20:0	0.29 ± 0.12	0.15	0.50	0.32 ± 0.06	0.25	0.41
18:3n-3	0.64 ± 0.15	0.45	0.88	0.93 ± 0.28	0.60	1.18
conj. 18:2[f]	0.45 ± 0.15	0.27	0.69	0.82 ± 0.19	0.77	1.04
others	1.74 ± 0.58	0.48	2.31	2.08 ± 0.28	1.41	2.20

[a]Number of samples.
[b]Values for a-*iso* 15:0 and 14:1 acids, not separated during gas–liquid chromatography analysis of total fatty acid isopropyl esters (FAIPE), are obtained by combining results of analyses of total FAIPE and of analyses of saturated plus *trans*-monoenoic FAIPE, with 15:0 acid as internal standard.
[c]Sum of all peaks corresponding to *cis* or *trans* 18:1 acids, or to mixtures of these.
[d]Group of several peaks that do not correspond to either saturated, *cis*- or *trans*-monoenoic acids and that are supposed to be *cis* and/or *trans* isomers of methylene- and/or nonmethylene-interrupted octadecadienoic acids. May contain trace amounts of 19:0 acid.
[e]18:2n-6 and 18:3n-3 acids are the all-*cis* isomers.
[f]Conjugated 18:2 acid(s).

same as those found in cow milk fat. However, the content of 10:0 acid is significantly higher in both goat and ewe milk fats, an observation that was previously made by several authors (13–19). To ascertain the genuineness of our samples (no adulteration with cow milk), we calculated the ratios 12:0/10:0. These ratios are 0.58 ± 0.02 for ewe cheeses and 0.46 ± 0.05 for goat cheeses instead of 1.14 for cow milk fat (calculated from data in Ref. 1). They are identical to those established by Iverson and Sheppard (19) for ewe, goat, and cow cheeses purchased in the United States (0.58, 0.46, and 1.16, respectively). This demonstrates that cheeses analyzed in the present study were made with either pure ewe or pure goat milk. *Trans*-octadecenoic acids average 4.53 ± 1.11% (minimum value: 3.02%; maximum value: 6.17%) of total fatty acids in ewe cheese fat, and 2.68 ± 0.88% (minimum value: 1.75%; maximum value: 4.50%) in goat cheese fat (Table 2). These acids were previously characterized in ewe and goat milk, but only partial quantitative data were reported (20,21). On the other hand, older studies (22,23) mention that the perirenal fat of both species may contain up to 11% *trans*-unsaturated fatty acids as determined by IR absorption at 970 cm^{-1}. In cheeses of both species, the level of *trans*-18:1 acids may double between the minimum and maximum values. This means that the *trans*-18:1 acid content in ewe and goat milk fats is variable. However, the parameters that control this content are unknown. It is probable that the feed may be of major importance, as for cows, but the period of lactation also seems to affect this content. In ewe milk, the *trans*-11 18:1

TABLE 2
***Trans*-Octadecenoic Acid Contents (as weight percentages relative to total fatty acids) in Various Fats Analyzed in this Study**

Origin of fat	*Trans*-18:1 acid content[a]		
	Mean ± SD	Minimum value	Maximum value
Goat cheeses (8)[b]	2.68 ± 0.88	1.75	4.50
Ewe cheeses (7)	4.53 ± 1.11	3.02	6.17
Beef meat TL[c] (10)	1.95 ± 0.94	0.75	3.54
Beef meat TG (10)	2.53 ± 0.94	1.14	4.10
Beef meat PL (10)	0.76 ± 0.34	0.49	1.69
Beef tallow (2)	4.6	–	–
Human milk (10)	1.99 ± 0.57	1.20	3.17

[a]Values established by combining results obtained by gas–liquid chromatography (GLC) coupled with argentation thin-layer chromatography, except for beef fat triglycerides and phospholipids that were by direct GLC and use of a correction factor (see text). Palmitic and 18:0 acids were used as internal standards.
[b]Number of samples in parentheses.
[c]TL, total lipids; TG, triglycerides; PL, phospholipids.

acid would increase from 2.5 to 4% between 0 and 100 d *post partum* (20). Perhaps the breed may also be of concern. The mean level of *trans*-18:1 acids is significantly higher ($P < 0.01$) in ewe milk fat than in goat milk fat. Combining results of the present study with those on cow milk fat (1), fats can be classified in the following decreasing order with regard to their mean *trans*-18:1 acid content: ewe > cow > goat. For those people who wish to limit their *trans* intake, goat cheeses should thus be preferred to cow or ewe cheeses.

The distribution profiles of *trans*-18:1 isomers in ewe and goat milk fats are illustrated in Figure 1. The patterns for the two species are qualitatively identical and close to that presented by *trans*-18:1 isomers in cow milk fat (1). Identification of individual isomers was realized by co-injecting the *trans*-18:1 acid fraction, isolated by Ag–TLC together, with each individual synthetic *trans*-18:1 isomer with a double bond between positions 5 and 15. Isomers with double bonds between positions 6 and 9 are unresolved, but it would appear that the apex of this group of peaks corresponds to *trans*-9 18:1 acid (Fig. 1). Although the *trans*-10 and *trans*-11 18:1 isomers are incompletely separated, it is clear that the main component is *trans*-11 18:1 (vaccenic) acid, as in cow milk fat (see also Fig. 2). This was previously observed in goat (21) and ewe (20) milks. Following the main vaccenic acid peak are three close-eluting peaks that correspond to *trans*-12, *trans*-13 plus *trans*-14, and *trans*-15 18:1 acids. This last component was unambiguously located, and our observation is thus at variance with a recent GLC study that concluded, on a theoretical basis, to the absence of the *trans*-15 18:1 isomer in chromatograms obtained from butterfat fatty acid butyl esters (24). On the other hand, this acid was reported by several authors in different ruminant fats (21,25–29), using either products obtained by ozonolysis or direct GLC of the in-

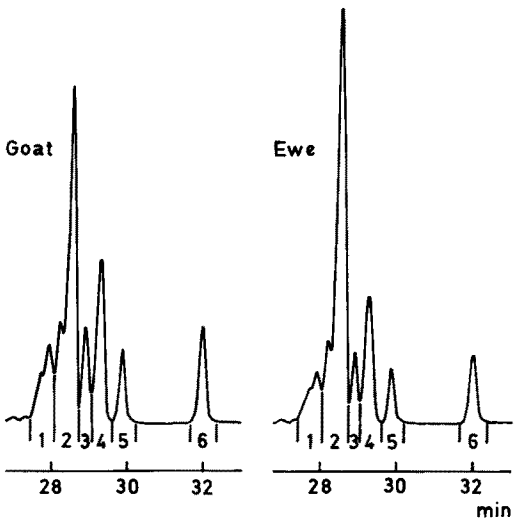

FIG. 1. Characteristic profiles of *trans*-18:1 acid isopropyl esters isolated by argentation thin-layer chromatography (Ag–TLC) from isopropyl esters prepared with goat and ewe cheese fat, and analyzed on a CP Sil 88 capillary column (50 m × 0.25 mm i.d., 0.20 μm film; Chrompack, Middelburg, The Netherlands). Temperature, 160°C; helium pressure, 100 kPa. Left chromatogram: *trans*-18:1 acids from goat cheese fat (composite sample made up with the *trans*-monoenoic acid fractions isolated by Ag–TLC from the eight samples used in this study). Right chromatogram: *trans*-18:1 acids from ewe cheese fat (mixture of the *trans*-monoenoic acid fractions isolated Ag–TLC from the seven samples used in this study). Injections at approximately the same load. Peak numbers refer to Table 3.

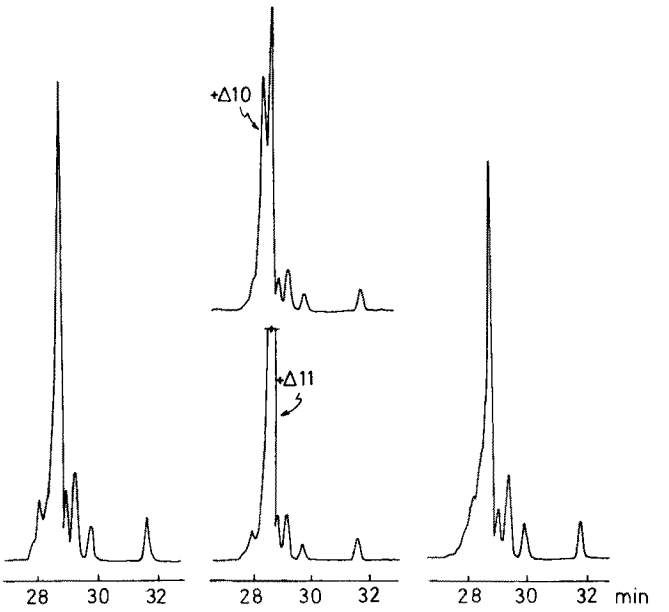

FIG. 2. Characteristic profiles of *trans*-18:1 acid isopropyl esters isolated by argentation thin-layer chromatography (Ag–TLC) from isopropyl esters prepared with beef meat fat and tallow. Left chromatogram: *trans*-18:1 acids from meat fat (mixture of *trans*-monoenoic acid fractions isolated by Ag–TLC from the ten samples used in this study). Middle chromatograms: same as left chromatogram, but spiked with either *trans*-10 or *trans*-11 18:1 acids. Right chromatogram: *trans*-18:1 acids from tallow (mixture of the *trans*-monoenoic acid fractions isolated by Ag–TLC from the two samples used in this study). Company source of the column, characteristics, and chromatographic conditions as in Figure 1.

tact *trans*-18:1 acids. The last-emerging peak is the *trans*-16 18:1 isomer (29). The detailed compositions of partially resolved individual *trans* isomers are given in Table 3. Apparently, there would be a slightly higher level of vaccenic acid (plus *trans*-10 18:1 acid) in ewe milk fat than in goat milk fat. The distribution of *trans*-18:1 isomers from ewe milk fat is close to that of milk fat from cows in spring (Table 3). This would indicate that the profile of *trans*-18:1 acids is related to the feeding conditions of the animals, where cows in spring and ewes all throughout the year mainly eat grass. On the other hand, the *trans*-18:1 acid distribution in goat milk fat resembles that of cow milk fat in autumn (Table 3). We can thus hypothesize that the proportion of the *trans*-11 18:1 isomer is probably related to the feed, with a trend toward the highest values under grazing conditions. Based on the similarity of the *trans*-18:1 acid profiles in milk fats from the three ruminant species, it can be deduced that the biohydrogenation mechanisms that take place in rumen bacteria are also similar and that they do not depend on the species. This conclusion is also supported by analyses of *trans*-18:1 acids in rumen digesta of cow and goat, which show great similarities (21,30).

To evaluate the daily intake of *trans*-18:1 acids by populations who usually consume foods manufactured with goat and ewe milk, it is necessary to know the fat content in these milks. From literature data, it would appear that the fat content in goat milk lies in the range 3–6%. Based on about 75 values collected in the literature (13,17,31–34), we could estimate a mean fat content of 4.4 ± 1.0%. However, it should be emphasized that the amount of fat in goat milk depends on several factors. Among these are the breed (genetic factors), the physiological state (e.g., number of days after parturition), and the nutritional status (feed). Consequently, some variations may occur from one country or one region to another, or even from one flock to another. The fat content in ewe milk varies from *ca.* 6 to 8.5% with a mean value close to 7.0% (16,32,35,36). The fat content is thus significantly higher than in goat milk. This probably explains why we observe a higher fat content in ewe cheeses than in goat cheeses.

In the EEC, countries that produce goat and ewe milk rarely export their production. As a result, the production data roughly correspond to the consumption data. Recent data (1992 and 1993) on ewe and goat milk production by southern countries of the EEC (France, Italy, Greece, Spain, and Portugal) (32–34,37–39) are displayed in Tables 4 and 5. These figures, together with the fat content of ewe and goat milks and their *trans*-18:1 acid content determined in the present study, allow calculation of the mean daily per-capita intake of *trans* fatty acids from these sources (Tables 4 and 5). In most countries, the contribution of goat milk fat to the *trans* intake is almost negligible, except in Greece where it accounts for *ca.* 0.14 g (Table 4). The contribution to the *trans* intake from ewe milk fat is higher than that from goat milk fat (Table 5). Here too, this contribution is low for most countries, Greece being an exception (*ca.* 0.5 g/person/day). In previous estimates (1), it was established that the contribution of cow milk fat to the *trans* intake was 0.71 g/person/day for Greek people. The *trans* intake from ewe and goat milks totals 0.63 g/person/day, which represents 47% of the total *trans* intake from all milk fats (1.34 g/person/day). Moreover, Greek people appeared to have a low consumption of *trans* acids as compared to other EEC countries when only cow milk was considered (0.71 g vs. 1.16 g for the EEC) (1). The *trans* intake was comparable to that of two other southern countries, Spain and Portugal (1). When goat and ewe milks are taken into account, the individual *trans* intake by people in Greece almost doubles and becomes comparable to that of people from northern countries such as Germany, Belgium-Luxembourg, or Ireland (1.37, 1.35, and 1.34 g, respectively) (1). On the other hand, the *trans* intake from all milks by Spanish and Portuguese populations remains low (0.70 and 0.55 g, respectively).

Trans-18:1 acids in beef meat fat and tallow. The fatty compositions of total lipids from raw beef meat are summarized in Table 6. These values agree well with those published

TABLE 3
Proportions of the Different Parts of the Gas–Liquid Chromatographic Profiles of *trans*-18:1 Acids Isolated by Argentation Thin-Layer Chromatography (Ag–TLC) from Fatty Acid Isopropyl Esters Prepared with Goat and Ewe Cheese Fat and Comparison with Cow Milk Fat

Peak number[b]	Identification[c]	Proportions[a] Goat (n = 8)[d]	Ewe (n = 7)	Cow (spring)[e] (n = 12)	Cow (autumn) (n = 12)
1	*trans*-6 to *trans*-9	12.6 ± 2.9	8.3 ± 2.6	7.2 ± 0.8	9.6 ± 1.3
2	*trans*-10 + *trans*-11	44.9 ± 4.0	56.7 ± 5.3	58.2 ± 3.8	50.4 ± 3.9
3	*trans*-12	8.7 ± 0.2	6.7 ± 1.0	6.2 ± 0.7	7.2 ± 0.7
4	*trans*-13 + *trans*-14	18.2 ± 2.5	15.6 ± 1.5	15.4 ± 1.7	17.2 ± 1.6
5	*trans*-15	6.8 ± 1.4	5.2 ± 0.5	5.7 ± 0.6	6.6 ± 0.6
6	*trans*-16	8.8 ± 2.4	7.5 ± 1.8	7.3 ± 0.9	9.0 ± 1.2

[a]As percentages of total *trans*-18:1 acids (means ± SD).
[b]Peak numbers refer to Figure 1.
[c]By coinjection of pooled *trans*-18:1 acids from goat cheese fat (isolated by Ag–TLC) with each of individual standards with double bonds between positions 5 and 15. For other samples, by comparison with *trans*-18:1 acids from goat cheese fat.
[d]Number of samples analyzed.
[e]Data for cow milk fats are from Reference 1.

TABLE 4
Mean Daily per Capita Consumption of Goat Milk Fat and of *trans*-Octadecenoic Acids Present in Goat Milk Fat by People from Southern Countries of the European Economic Community

		Individual consumption		
	Production[a]	Milk/year	Fat/day[b]	*trans*-18:1/day[c]
Country	(x10^3 t)	(kg)	(g)	(mg)
France	480	8.35	1.01	26
Greece	470	44.76	5.40	139
Spain	437	11.35	1.37	35
Italy	124	2.16	0.26	7
Portugal	42	4.00	0.48	12

[a]Averaged annual values calculated from References 32–34.
[b]Mean content of fat in goat milk used for calculation: 4.4%.
[c]Values obtained by multiplying the daily consumption of milk fat by 0.95 (proportion of fatty acids in triglycerides) and by 0.027 (mean content of *trans*-18:1 acids in total fatty acids of goat milk fat).

TABLE 5
Mean Daily per Capita Consumption of Ewe Milk Fat and of *trans*-Octadecenoic Acids Present in Ewe Milk Fat by People from Southern Countries of the European Economic Community

		Individual consumption		
	Production[a]	Milk/year	Fat/day[b]	*trans*-18:1/day[c]
Country	(x 10^3 t)	(kg)	(g)	(mg)
France	230	4.17	0.80	34
Greece	604	59.80	11.47	490
Spain	271	7.04	1.35	58
Italy	604	10.52	2.02	86
Portugal	83	8.06	1.55	66

[a]Annual values estimated from graphical data in Reference 37.
[b]Mean content of fat in ewe milk used for calculation: 7%.
[c]Values obtained by multiplying the daily consumption of milk fat by 0.95 (proportion of fatty acids in triglycerides) and by 0.045 (mean content of *trans*-18:1 acids in total fatty acids of ewe milk fat).

TABLE 6
Fatty Acid Compositions as Weight Percentages of Total Fatty Acids of Beef Meat Fat and of Beef Tallow from Animals Slaughtered in Winter

	Beef meat fat			Beef tallow
	Mean ± SD	Minimum	Maximum	Mean
Fatty acid	(n = 10)[a]	values	values	(n = 2)
14:0	3.00 ± 1.18	1.66	5.68	3.14
iso 15:0	0.14 ± 0.06	0.08	0.27	0.23
a-*iso* 15:0	0.18 ± 0.09	0.08	0.27	0.26
14:1	0.87 ± 0.39	0.41	1.53	0.42
15:0	0.40 ± 0.13	0.24	0.70	0.55
iso 16:0	0.17 ± 0.04	0.10	0.24	0.22
16:0	25.82 ± 2.55	20.89	30.00	24.49
16:1 + *iso* 17:0	0.68 ± 0.09	0.53	0.86	0.73
cis-9 16:1	5.73 ± 1.74	4.62	9.87	2.13
a-*iso* 17:0	0.19 ± 0.07	0.09	0.33	0.75
17:0	0.84 ± 0.18	0.49	1.06	1.42
cis-17:1	1.06 ± 0.15	0.88	1.28	0.85
18:0	10.99 ± 1.17	8.95	12.29	22.72
trans + *cis* 18:1[b]	40.84 ± 3.90	34.43	44.85	38.11
isom. 18:2[c]	0.63 ± 0.21	0.37	0.96	0.79
18:2n-6[d]	2.99 ± 1.32	1.71	5.22	1.38
20:0	0.08 ± 0.01	0.07	0.10	0.14
18:3n-3	0.65 ± 0.19	0.39	1.01	0.49
20:1	0.06 ± 0.01	0.05	0.09	0.04
conj. 18:2[e]	0.53 ± 0.20	0.30	0.85	0.52
20:3n-6	0.41 ± 0.30	0.18	1.02	–
20:4n-6	1.14 ± 1.06	0.37	3.50	–
22:5n-3	0.36 ± 0.46	0.09	1.52	–
others	2.24 ± 0.74	3.05	1.15	0.61

[a]Number of samples.
[b]Sum of all peaks corresponding to *cis*- or *trans*-18:1 acids, or to mixtures of these.
[c]Group of several peaks that do not correspond to either saturated, *cis*- or *trans*-monoenoic acids and that are supposed to be *cis* and/or *trans* isomers of methylene- and/or nonmethylene-interrupted octadecadienoic acids. May contain trace amounts of 19:0 acid.
[d]18:2n-6 and 18:3n-3 are the all-*cis* isomers.
[e]Conjugated 18:2 acids.

by other authors, saturated fatty acids accounting for 40–43% of total fatty acids (5,40,41). However, this composition differs from that of beef tallow mainly by the content of stearic acid (11% instead of *ca.* 23%) (Table 6). The fatty acid composition we have established for tallow of animals slaughtered in winter is identical to the mean fatty acid composition determined for 47 samples of tallow and published by the French Technical Institute for Research on Fats and Oils (ITERG) (42). This suggests that the composition of tallow should not depend as much on the season as that of triglycerides from milk fat (1).

The mean content of *trans*-18:1 acids in the fat from trimmed beef meat is 1.95 ± 0.94% of total fatty acids (Table 2). The corresponding value for tallow is 4.6% (Table 2). The mean *trans* content we determined in beef meat is lower than those reported by Lanza *et al.* (40) for one sample of beef meat, and by Lanza and Slover (43) for the separable lean of one sample of beef rib roast, 4.5–4.6%. Wood (5) found 2.4–5.7% of *trans*-18:1 acids in triglycerides and 1.1–1.2% in phospholipids from three samples of beef *Sterno mandibularis* muscles. Lin *et al.* (44) analyzed the *trans*-18:1 acid content of the fat from six samples of beef *longissimus* muscles and found a mean value of 1.7%. No precision was given on the season the animals were killed. However, it is known that the *trans*-18:1 acid content of cow milk fat varies with the season, with higher values in spring than in winter (1). If a similar phenomenon occurs in beef meat lipids, the value we established should correspond to the lowest level in the year. Considering that values of 4.4–4.6% (40,43) are the highest levels that can be reached in beef meat total lipids, a mean annual value of 3.1–3.3% can be estimated. These figures are quite close to the mean value reported by Slover *et al.* (41) who analyzed 269 samples of the lean portion of fourteen beef retail cuts and reported 3.20%. Considering the wealth of samples analyzed in this study, one may speculate that this value takes into account all possible seasonal variations. Accordingly, we will adopt this number for our calculations. This value is slightly less than that reported for milk fat (3.8%) (1). However, one should keep in mind that milk fat is almost exclusively made up with triglycerides, whereas meat lipids contain both triglycerides and phospholipids in variable proportions. As observed by Wood (5) and by us

(Table 2), phospholipids contain a lower level of *trans*-18:1 acids than do triglycerides (two to five times less). Our determination of *trans*-18:1 acids in phospholipids and triglycerides was not by GLC coupled with Ag–TLC, but by direct GLC. During GLC, *trans*-18:1 isomers with double bonds between position 6 and 11 (or 12) emerge as a single asymmetrical peak just before the major *cis*-9 18:1 acid (results not shown). Other isomers are masked by the main *cis*-9 18:1 acid peak or elute after it. By combining results of direct GLC of total fatty acids and GLC coupled with Ag–TLC, we could experimentally establish that the visible *trans*-18:1 acid peak corresponds to 80 ± 4% of total *trans*-18:1 isomers. This proportion is identical to the value obtained by summing up the relative percentages of isomers with double bonds between positions 6 and 12 from either beef meat fat or tallow (Table 7). So, the true amount of *trans*-18:1 acids in triglycerides and phospholipids is obtained by multiplying the proportion of the visible *trans*-18:1 acid peak by 1.25. Sampugna *et al.* (45) also observed from single chromatographic runs on an SP-2340 capillary column that correction factors ranging from 1.15 to 1.33 were needed to calculate the true amount of *trans*-18:1 acids in margarines. The amount of *trans*-18:1 acids in triglycerides is *ca.* 25% higher than that in total lipids (Table 2). Taking into account this factor, the mean value established by Slover *et al.* (41) for total lipids should give a *trans*-18:1 acid content in triglycerides of 4.0%, which is quite close to the *trans*-18:1 acid content in milk fat triglycerides (3.8%) (1). Data in Table 2 also allow calculation of the relative contributions of phospholipids and triglycerides to the intake of *trans*-18:1 acids from meat fat, which should be around one and two thirds, respectively.

The fact that the proportion of *trans*-18:1 acids is higher in tallow than in meat triglycerides from animals slaughtered at the same period of the year is intriguing. Does this situation occur all through the year? One explanation would be that the *trans* content in muscle lipids depends on the feed, as in milk triglycerides, but not that of adipose tissue. Alternately stated, the turnover rate of *trans*-18:1 acids in muscle lipids should be higher than in the fat from adipose tissue. Another possibility would be that the *trans* content of adipose tissue varies with the season, being systematically higher than in muscle lipids. Slover *et al.* (41) have observed that fatty tissues surrounding the lean of beef cuts have a significantly higher content of *trans*-18:1 acids than the separable lean: 6.5% instead of 3.2%. This would support our second hypothesis. However, nothing is known with certainty thus far about these possibilities, and we are currently investigating the *trans* content of tallow as a function of the month the animals are slaughtered. For the present study, we will adopt a median value of 5.5%, a figure that is probably not too far from the true mean annual value.

As shown in Figure 2, the distribution patterns of *trans*-18:1 acids in meat fat and tallow are closely related to those displayed by ruminant milk fats, vaccenic acid being the prominent isomer. The distribution profile of *trans*-18:1 isomers in beef meat fat is in good agreement with profiles established by different authors (25,26) for beef adipose tissue and by us for beef tallow (Table 7), but not with Wood's data (5) for beef meat triglycerides. This author has observed that the main isomer was the *trans*-10 18:1 acid, and he speculated that the animals from which the cuts were obtained might have eaten some partially hydrogenated fat at one time (5). Because he also found the same profile in the fat from processed foods that contained beef as the sole source of *trans*-18:1 acids, this would imply that most beef animals in the United States are fed hydrogenated fats. This seems un-

TABLE 7
Distribution of *trans*-18:1 Isomers in Beef Meat Fat and Tallow and Comparison with Literature Data

Double bond position	Origin of fat					
	Perirenal	Subcutaneous		Meat[b]		
	(25)[a]	(25)	(26)	(5)	Meat[c]	Tallow
6	—[d]	0.3	0.2	3.3–5.6	8.7 ± 2.4	11.6
7	1.0	0.5	0.3			
8	2.1	1.6	1.5			
9	5.0	8.9	13.6	4.5–9.8		
10	11.9	5.4	6.4	40.8–56.2	66.9 ± 7.2	63.8
11	46.9	68.6	64.4	23.9–36.9		
12	6.0	2.7	2.4	2.1–5.0	5.9 ± 2.2	4.7
13	6.6	2.6	2.3	3.3–8.3	9.5 ± 2.4	10.5
14	7.4	3.6	3.6	1.0–2.4		
15	5.5	2.6	2.3	–	4.1 ± 1.2	4.6
16	7.6	3.2	3.0	–	4.9 ± 1.5	4.7

[a]References in parentheses.
[b]Triglycerides. Minimum and maximum values for analyses of three cuts.
[c]Mean ± SD of analyses of total lipids from the lean part of ten cuts.
[d]Not reported.

likely. Spiking the *trans*-18:1 acid fraction, isolated by Ag–TLC, with either *trans*-10 or *trans*-11 18:1 acid shows that these two isomers cannot be confused under our analytical conditions (Fig. 2).

The fat content of lean beef ranged from a low of 1.6% to a high of 17.5% (mean: 6.1 ± 4.3%), which is comparable to data of Slover *et al.* (41) (which varied from 3.2 to 14.6% with a mean of 7.4 ± 2.9% for 269 samples). These means are close to the value of 8% established and recommended for calculation by the French Center of Information on Meats (CIV) (46). However, the estimation of the true fat amount that is actually ingested is complicated by the cooking operations. Generally, meat cuts are trimmed in the dish, after cooking. In some instances, the fat in a cut as a whole decreases because of drip loss. On the other hand, fat increases in the separable edible lean because of the infiltration of fat from surrounding fatty tissues (41). Experimental values from the present study and from Slover *et al.* (41) may thus be slightly underestimated, and we will use the value of 8% for our estimates of *trans*-18:1 acid intake from beef meat.

Consumption of beef carcasses (including veal) is given in Table 8. These values have been calculated for the year 1993 by the National Interprofessional Office of Meat from Cattle and Poultry (OFIVAL) (47). However, these figures should be corrected for both professional and household wastage. For the first correction, we will apply a factor of 0.7 (calculated by the CIV, personal communication) that leads to the true marketable portion of a carcass, which is the part of the animal that is effectively sold for edible purposes. A second correction factor, estimated to be 0.9 and corresponding to household wastage (trimming of bones, connective and fatty tissues), is also applied. The resulting values should correspond to the meat that is actually ingested. As indicated above, the fat and the *trans*-18:1 acid contents of the lean are 8 and 3.2%, respectively. From these calculations, it appears that the individual intake of *trans*-18:1 acids in most EEC countries is around 0.1 g/day (Table 8), which is less than one-tenth the consumption of *trans*-18:1 acids from dairy products (1).

Apparently, there are no official statistics about beef tallow consumption in the EEC. However, the French Syndicate for Animal Proteins and Fats could provide us some estimates that were given by similar national organizations for half of the EEC countries (Table 9). A mean value of 0.06 ± 0.02 g/person/day can be calculated from these data. Wastage of tallow should be low, because most food industries use tallow as an ingredient in processed foods, and not for cooking or deep frying. Consequently, no corrections will be made for wastage.

There is another source of *trans*-18:1 acids for which literature data are unfortunately lacking: sheep and lamb meat. This source is not negligible in countries such as Greece, the United Kingdom, France, and Spain (Table 10). Here too, rough consumption data published by OFIVAL (47) should be corrected. We will use 0.8 for professional wastage (value calculated by the CIV) and 0.8 for household wastage. We can at best make gross estimates for this source of *trans*-18:1 acids, but this may be of use due to the unavailability of published data. It is a common observation that sheep meat contains more fat than beef meat. Moreover, from our observations on cow and ewe milk fats, one can deduce that the fat from sheep meat contains more *trans*-18:1 acids than beef meat. Consequently, we will use the following figures for our calculations: fat content, 12%; *trans*-18:1 acid content, 5%. We are aware of the fact that these values are somewhat arbitrary, but this should not considerably affect our final calcula-

TABLE 8
Mean Daily per Capita Consumption of Beef Meat and of *trans*-Octadecenoic Acids from Meat Fat by People from the Twelve Countries of the European Economic Community

Country	Total consumption[a] ($x10^3$ t/year)	Individual consumption: Carcass (g/d)	Lean meat[b] (g/d)	Meat fat[c] (g/d)	*trans*-18:1 acid[d] (mg/d)
France	1681	80.1	50.5	4.04	129
Germany	1525	53.8	33.9	2.71	87
Italy	1395	66.5	41.9	3.35	107
The Netherlands	297	56.1	35.3	2.83	90
Belgium-Luxembourg	208	55.3	34.0	2.79	89
United Kingdom	1068	51.9	32.7	2.62	84
Ireland	61	32.1	20.2	1.62	52
Denmark	105	56.4	35.5	2.84	91
Greece	220	59.7	37.6	3.01	96
Spain	495	35.2	22.2	1.77	57
Portugal	165	43.9	27.7	2.21	71

[a]Quantities of carcasses from slaughterhouses for edible purposes. Values are from the National Interprofessional Office of Meat from Cattle and Poultry (Ref. 47).
[b]Values obtained by multiplying the daily consumption of carcasses by 0.7 (professional wastage) and by 0.9 (household wastage).
[c]Values obtained by multiplying lean meat values by 0.08 (fat content).
[d]Values obtained by multiplying meat fat value by 0.032 (*trans*-18:1 acid content; datum from Ref. 41).

TABLE 9
Mean Daily per Capita Consumption of Beef Tallow and of *trans*-Octadecenoic Acids from Tallow of People from Some Countries of the European Economic Community

Country	Total consumption[a] (x10^3 t/year)	Individual consumption: g tallow/day[b]	Individual consumption: mg *trans*-18:1 acid/day[c]
France	20	0.95	43
Germany	25	1.12	51
Italy	40	1.91	86
The Netherlands	6.1	1.15	52
United Kingdom	35	1.70	76
Spain	20	1.42	64

[a]Values are personal communications from the French Syndicate for Animal Proteins and Fats. Values for all countries were not available.
[b]Assuming no wastage of tallow.
[c]Values obtained by multiplying the daily consumption of tallow by 0.055 (estimated *trans*-18:1 acid content).

tions, due to the generally low intake of sheep and lamb meat. Our data show that the per capita daily intake of *trans*-18:1 acids from this source is less than 0.025 g for people from most EEC countries, Greece being an exception (Table 10).

Total individual consumption of trans-18:1 acids in EEC countries. The *trans*-18:1 acid content of ruminant meat and milk fats is thus highly variable. It depends on the season (feed), the tissue (milk, meat or adipose), and perhaps on the animal species. We observed a low of 0.8% in a sample of meat from a beef slaughtered in winter and a high of 6.2% in a sample of ewe cheese (Table 2). However, higher values have been observed by Slover *et al.* (41) in beef fatty tissues, and the upper limit should be around 7–8%. This wide range (0.8–8%) was established with data obtained by GLC coupled with Ag–TLC (1, this study) or by GLC alone (41). This rules out variations linked to differences in analytical techniques, such as those that occur when one compares GLC determination and IR absorption measurements (1).

We have recapitulated all *trans*-18:1 acid intakes from ruminant sources (Table 11), which shows that the total *trans*-18:1 acid consumption varies from 1.3 to 1.8 g/person/day in most member states of the EEC, Spain and Portugal being exceptions (*ca.* 0.8 g/person/day). The mean value for all EEC countries is 1.4 g/person/day. Surprisingly enough, Greece, which is a Mediterranean country, is ranked third for *trans*-18:1 acid intake in the EEC.

It is interesting to try to determine the respective contributions of ruminant fats and margarines to the total *trans*-18:1 acid intake in EEC states. Consumption data for margarines are available from the Interprofessional National Center for Dairy Economics (CNIEL) (3), but the greatest difficulty is to estimate the mean *trans*-18:1 acid content in European margarines. From a recent survey (1), it would seem that this mean content is in the range 7–20%. We determined a mean content of 13% in French margarines (1), a value that is in agreement with that found in Swedish margarines (48). If we adopt this figure, which is known at best with a precision of about 6%, we obtain data that are illustrated in Figure 3. This figure clearly shows that the consumption of *trans*-18:1 acids increases from the southwest to the northeast of the EEC. One can draw a virtual line grossly spanning from Ireland to Greece that divides the EEC into two groups of countries, those who consume less than 3 g/person/day (beneath the line) and those who consume more than 3 g/person/day (northeast of the line). For those people whose intake is lower than 3 g/person/day, the prominent source of *trans*-18:1 acids is ruminant fat (except in Portugal). On the other side of the line, margarines are of major importance. So, the differences

TABLE 10
Mean Daily per Capita Consumption of Sheep Meat and of *trans*-Octadecenoic Acids from Meat Fat by People from the Twelve Countries of the European Economic Community

Country	Total consumption[a] (x10^3 t/year)	Individual consumption: Carcass (g/day)	Lean meat[b] (g/day)	Meat fat[c] (g/day)	*trans*-18:1 acid[d] (mg/day)
France	317	5.5	3.5	0.4	21
Germany	84	1.1	0.7	0.1	4
Italy	115	2.0	1.3	0.2	8
The Netherlands	18	1.2	0.8	0.1	5
Belgium-Luxembourg	22	2.1	1.4	0.2	8
United Kingdom	328	5.8	3.7	0.4	22
Ireland	33	6.4	4.1	0.5	26
Denmark	7	1.4	0.9	0.1	5
Greece	151	15.0	9.6	1.2	57
Spain	263	6.8	4.4	0.5	26
Portugal	43	4.2	2.7	0.3	16

[a]Quantities of carcasses from slaughterhouses for edible purposes. Values are from the National Interprofessional Office of Meat from Cattle and Poultry (Ref. 47).
[b]Values obtained by multiplying the daily consumption of carcasses by 0.8 (professional wastage) and by 0.8 (household wastage).
[c]Values obtained by multiplying lean meat values by 0.12 (estimated fat content).
[d]Values obtained by multiplying meat fat by 0.05 (estimated *trans*-18:1 acid content).

TABLE 11
Recapitulation of the Individual Daily *trans*-18:1 Acid Intake from Ruminant Milk, Meat, and Fat by People from the Twelve Countries of the European Economic Community

Country	Cow milk[a] (mg)	Goat milk (mg)	Ewe milk (mg)	Beef meat (mg)	Tallow (mg)	Sheep meat (mg)	Total (g)
France	1460	26	34	129	43	21	1.71
Germany	1370	_b	–	87	51	4	1.51
Italy	1080	7	86	107	86	8	1.37
The Netherlands	1140	–	–	90	56	5	1.29
Belgium-Luxembourg	1350	–	–	89	(60)[c]	8	1.51
United Kingdom	1130	–	–	84	76	22	1.31
Ireland	1340	–	–	52	(60)	24	1.48
Denmark	1660	–	–	91	(60)	5	1.82
Greece	710	139	490	96	(60)	57	1.55
Spain	610	35	58	57	64	26	0.85
Portugal	570	12	66	71	(60)	16	0.80

[a]Data from Reference 1.
[b]Lower than the lowest value in the column.
[c]Values in parentheses correspond to the mean value of countries for which data were available.

in *trans*-18:1 acid intake between countries mainly come from differences in margarine consumption. One may also note that the 3-g line extends across France and divides the country into two parts. This indeed is the reflection of the heterogeneity in fat consumption in this country: People from northernmost regions purchase almost four times more margarine and twice as much butter as people from the southwest and southeast of France (49,50).

Published data for the fat intake from dairy products in the United States are rather homogenous: 23.4–25 g/person/day (4), which is in the range of values for the EEC. However, the *trans*-18:1 acid consumption deduced from the preceding figures varies from one author to another from a low of 0.44 to a high of 0.77 g/person/day (4). These discrepancies are even larger when *trans*-18:1 acid intakes from meat and dairy fats are summed up: 0.56 to 2.21 g/person/day (4). In fact, there are disagreements on the intake of meat fat and on the *trans* content of fats. Depending on the authors, beef fat consumption varies from 18.1 to 34.2 g/person/day (4), which appears to be high when compared to our estimates for EEC countries, and the *trans* content is arbitrarily taken between 0.5 and 5.8%. With such ranges of values, it is evident that no conclusions can be drawn. The *trans* contents described in the present study (3.8% in dairy products and 3.2% in beef meat fat) and the averaged consumption data (24 g of dairy fat and 25 g of beef meat fat per person per day) indicate that the mean *trans*-18:1 acid intake from ruminant fats by American people should not be too far from 1.7 g/person/day, which matches values estimated for most European populations (Table 11).

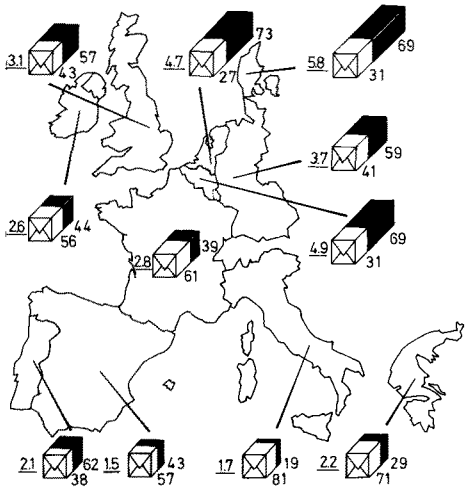

FIG. 3. Estimated consumptions of *trans*-18:1 acids by populations from the twelve member states of the European Economic Community. Black solids, sum of *trans*-18:1 acids from all ruminant sources. White solids, *trans*-18:1 acids from margarines. Underlined figures correspond to dietary intakes and are expressed as g/person/day. Other figures correspond to the relative contribution of the two sources (as percentage of the total).

Trans-18:1 acids in human milk. It has been clearly established that the fatty acid composition of milk from lactating women reflects to some extent the fatty acid composition of diets from the previous days (51). This is particularly true for *trans*-18:1 acids, which increase or decrease in milk lipids, depending on their amount in the diet (51–53). For women in the United States, *trans*-18:1 acids generally represent from 2 to *ca.* 6% of total milk fatty acids, although values as high as 11% can be reached (51,52,54). From data reported in two studies for fourteen apparently free-living subjects (analyses by GLC on packed columns) (51,54), a mean value of 3.37 ± 0.74% can be calculated for American women. Using similar analytical methods, Clark *et al.* (55) found 4.48 ± 1.33% of *trans*-18:1 acids in triacylglycerols for eleven samples of human milk. Craig-Schmidt *et al.* (52) found an average *trans*-18:1 acid content in milk collected from eight American women of 4.76 ± 2.06% (analyses by direct GLC on a capillary column without application of a correction factor). The corresponding figure for the milk of ten lactating French women established in the present study is 1.99 ± 0.57%

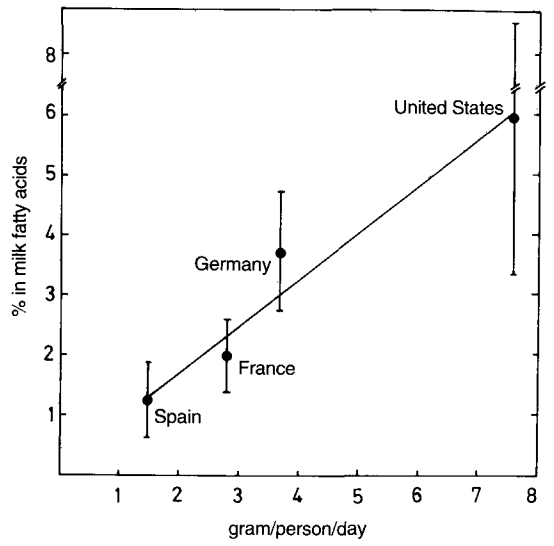

FIG. 4. Relationship between the estimated daily per capita *trans*-18:1 acid intake (g) and the *trans*-18:1 acid level (% of the total fatty acids) in human milk lipids as determined by gas–liquid chromatography on capillary columns. Original literature data for *trans*-18:1 acids in human milk lipids have been corrected (x1.25). Vertical bars correspond to standard deviations.

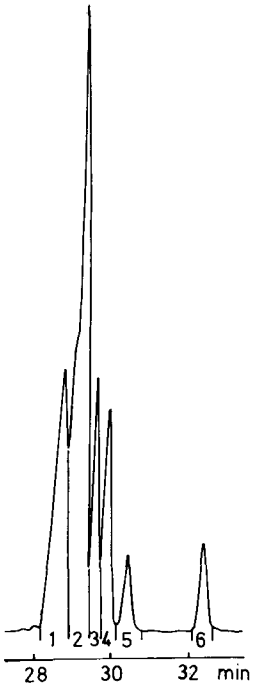

FIG. 5. Characteristic profile of *trans*-18:1 acid isopropyl esters isolated by argentation thin-layer chromatography (Ag–TLC) from isopropyl esters prepared with human milk lipids (composite sample made up with the *trans*-monoenoic acid fractions isolated by Ag–TLC from the ten samples used in this study). Company source of the column, characteristics, and chromatographic conditions as in Figure 1. Peak numbers refer to Table 12.

(Table 2), which is significantly lower than in the United States. This is explained by the fact that French people consume less *trans*-18:1 acids than do American people: 2.8 g/person/day (this study) instead of 7.6 or 13.3 g/person/day, depending on the calculation method (56,57). The value for French human milk is intermediate between those established in Spain (1.0 ± 0.5%, thirty-eight samples) (58) and in Germany (3.0 ± 0.7%, six samples) (59) (determinations by direct GLC on capillary columns, without application of correction factor). These data, after correction with the factor 1.25, our own values for France, corrected data from Craig-Schmidt *et al.* (52) and consumption data from Hunter and Applewhite (56) for the United States allow construction of the plot displayed in Figure 4. It is clear that there is a linear relationship between the mean daily per capita consumption of *trans*-18:1 acids and the percentage of these isomers in human milk fat ($y = 0.76x + 0.20$; correlation, 0.98). This supports the observation by Craig-Schmidt *et al.* (52) of a linear relation between the *trans* content in human milk lipids and that in the meals of the previous day.

To our knowledge, no data on the distribution of individual *trans*-18:1 isomers in human milk are available in the literature. However, Ohlrogge *et al.* (60) have described the distribution profile of *trans*-18:1 isomers in triglycerides from human adipose tissue. Comparing this pattern with that of *trans*-18:1 acids in butter and margarines, they concluded that the distribution of double bonds in adipose tissue triglycerides correlated closely with the composition of dietary partially hydrogenated fats, the main isomers having their double bonds in positions 9, 10, and 11. From these data, they further deduced that this last source should account for 90–95% of the total *trans*-18:1 acid intake by American people (6), the remainder coming from ruminant fats. According to consumption data published by Hunter and Applewhite (56), the proportion of *trans*-18:1 acids from ruminant fats should be around 20% of the total intake. The profile determined for the milk fat of French women does not fit this model (Fig. 5 and Table 12). The main isomer is vaccenic acid, as in ruminant

TABLE 12
Proportions of the Different Parts of the Gas–Liquid Chromatographic Profiles of *trans*-18:1 Acids Isolated by Argentation Thin-Layer Chromatography from Fatty Acid Isopropyl Esters Prepared with Human Milk Lipids

Peak number[a]	Identification[b]	Proportions[c] (n = 10)[d]
1	*trans*-6 to *trans*-9	20.3 ± 7.8
2	*trans*-10 + *trans*-11	49.2 ± 7.3
3	*trans*-12	10.5 ± 6.9
4	*trans*-13 + *trans*-14	11.3 ± 2.1
5	*trans*-15	3.9 ± 1.0
6	*trans*-16	4.9 ± 1.7

[a]Peak numbers refer to Figure 5.
[b]By coinjection with authentic individual standards.
[c]As percentages of total *trans*-18:1 (means ± SD).
[d]Number of samples.

milk and meat fats. This confirms our conclusions based on consumption data: The main dietary sources of *trans*-18:1 acids in France are ruminant fats (mainly milk fat), margarines being of secondary importance. We tried to estimate the relative contributions of these two sources, starting with the *trans*-18:1 acid distribution in human milk. This estimation is based on the relative abundance of the *trans*-16 18:1 acid in ruminant fats, and more particularly in milk fat (*ca.* 8.1% of total *trans*-18:1 isomers) (1), and on its almost absence in partially hydrogenated vegetable oils. In these products, the relative proportion of the *trans*-16 18:1 isomer is comprised between 0 and 2% of total *trans*-18:1 acids, but it is generally absent (6,45,60–67). The mean value certainly lies around 0.5%. This value, together with the mean proportions of *trans*-18:1 acid in cow (8.1%) and human milk fat (4.9%) allows estimation of the respective contributions of cow milk fat and margarines to the *trans*-18:1 acid intake according to equations: $8.1 \times X + 0.5 \times Y = 4.9$ and $X + Y = 1$. The proportion of milk fat (X) is 58%, that of margarines (Y) is 42%. These figures correlate well with our estimations based on consumption data (*ca.* 60 and 40%).

Concluding remarks. It was recently established that the intake of *trans*-unsaturated acids (especially from margarines) was positively correlated with increased risks of coronary heart diseases (68). In this study, the median daily intake ranged from 2.4 g in the lowest quintile to 5.7 g in the highest. This is almost the same range as that presented by the twelve EEC countries (1.5–5.8 g/person/day). So, it was tempting to compare the daily intakes of *trans*-18:1 acids from ruminant fats and margarines established in this study with the age-standardized rates of ischaemic heart diseases (IHD) in EEC countries. These last values were obtained from the World Health Organization (Dr. I. Martin, World Health Organization, Geneva, personal communication). They correspond mainly to the year 1990 and apply to male subjects. As shown in Figure 6, a correlation similar to that observed by Willett *et al.* (68) is not evident at the level of the EEC. However, one can note that almost all countries beneath the 3-g line have the lower IHD risks, less than 140 deaths for 100,000 persons, Ireland being an exception. Other countries northeast of this line have IHD risks higher than 180 deaths for 100,000 persons, with the exception of Belgium-Luxembourg. Surprisingly, Ireland displays the highest risks, almost similar to those of the United Kingdom. However, in our calculations, we only took into account *trans*-18:1 acids, and not longer chains with *trans* double bonds that may occur in partially hydrogenated fish oils. In fact, it would appear that hydrogenated marine oils are a major source of *trans* acids in the United Kingdom (69). Moreover, we did not take into account partially hydrogenated oils for other use than margarines (shortenings, salad oils). Provided that Irish and English people consume such products, their total *trans* intake will probably be higher than those we calculated. According to a study by the British Nutrition Foundation (70), the total *trans* intake in the United Kingdom would be in the range of 6–7 g/person/day. If values for Ireland and the United Kingdom are omitted from our calculations, a pretty good correlation between *trans*-18:1 acid consumption and IHD risks is obtained ($y = 31.0x + 58.2$; correlation factor, 0.74; $P < 0.05$) (Fig. 6). Although there is a wealth of parameters that can influence IHD risks, our observations do not exclude the possibility of a potential effect of *trans*-18:1 acids on these risks. This would be in line with the conclusions of Willett *et al.* (68).

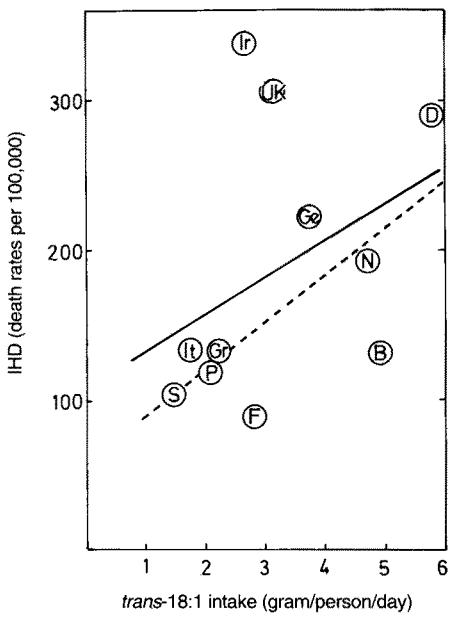

FIG. 6. Plots of age-standardized death rates (male subjects, all ages) for ischaemic heart disease (IHD) as a function of the daily *trans*-18:1 acid intake in populations from European Economic Community countries. Solid lines, all countries; broken line, Ireland and the United Kingdom excluded from calculations. S, Spain; F, France; P, Portugal; It, Italy; Gr, Greece; B, Belgium; N, The Netherlands; Ge, Germany; D, Denmark; Ir, Ireland; UK, the United Kingdom.

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