Human tissue lipids: occurrence of fatty acid isomers from dietary hydrogenated oils

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Abstract Hydrogenation of vegetable oils produces fatty acids with unusual structures having *trans* double bonds and double bonds in new positions of the acyl chain. This study was designed to determine which of these fatty acid isomers are incorporated or accumulated in humans during long-term dietary consumption of hydrogenated fats. The double bond position and configuration of the octadecenoate fraction of total lipids extracted from human heart, brain, liver, aorta, and adipose tissue were determined. The level of *trans* octadecenoate in the tissues as determined by both direct gas–liquid chromatography (GLC) and by GLC after silver nitrate thin-layer chromatography ranged between 0.4 and 5.0%, with an average of 2.7%. Tissues were found to contain *trans*-octadecenoic isomers having double bonds between the 6 and 15 positions, whereas *cis* double bonds were found to occur between the 6 and 14 positions. The distribution of double bonds in adipose tissue correlated very closely with the composition of dietary hydrogenated fat. Thus, essentially all of the unusual octadecenoic fatty acid isomers that are produced during vegetable oil hydrogenation are incorporated into human tissue. However, in contrast to results of short-term (1–6 months) feeding studies of animals, our results suggest that long-term (20–60 years) consumption of hydrogenated fats by humans does not lead to substantial preferential accumulation of positional isomers in human tissue total lipids.—**Ohlrogge, J. B., E. A. Emken, and R. M. Gulley.** Human tissue lipids: occurrence of fatty acid isomers from dietary hydrogenated oils. *J. Lipid Res.* 1981. **22:** 955–960.

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Consumption of vegetable oils in western diets has increased steadily over the past 60 years so that now vegetable fat contributes approximately 40% of the fat and 10–15% of total calories in western diets (1).

After refining, about 70% of dietary vegetable oils are hydrogenated to increase flavor stability or to achieve desired physical properties (2). Dairy products and fats from ruminant animals present an additional dietary source of hydrogenated fats as a result of microbial biohydrogenations occurring in the stomachs of ruminants (3).

Industrial hydrogenation and biohydrogenations lead to several structural modifications of the constituent fatty acids. In addition to elimination of double bonds through the addition of hydrogen, some double bonds rearrange from the *cis* to the *trans* configuration and some double bonds migrate to new positions in the fatty acid chain.

In human lipids, the 18-carbon monoenoic fatty acids have *cis* double bonds predominantly in the 9 and 11 positions. In contrast, hydrogenated vegetable oils contain a much larger number of 18-carbon monoenoic isomers. Double bonds occur in all positions between 6 and 16 and 5–50% of these double bonds may be in the *trans* configuration (2, 4). Ruminant fats also contain *trans* unsaturation but at a level of 2–8% and with the *trans* double bond occurring predominantly in the 11 position (5). Thus the rise in dietary consumption of hydrogenated vegetable oils presents to human metabolism increasing quantities of both *cis* and *trans* positional isomers having structures previously encountered only in trace amounts in human diets. For example, the average per capita consumption of *trans*-10-octadecenoic acid can be estimated to be 1–2 g per day (1, 2, 4).

If rates of metabolism or turnover of these unusual fatty acids are low relative to intake rates, long-term consumption might lead to their accumulation in tissue lipids. In animals fed hydrogenated fat for 1–6 months, accumulation in the tissue of certain positional isomers above levels present in the diet has been reported by three laboratories (6–8). Earlier analyses of human tissues have revealed the presence of *trans* isomers in various organ lipids (9, 10), but no complete description of both double bond position and configuration has appeared. Thus it is

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

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of particular interest from the perspective of both nutritionists and lipid biochemists to examine the capacity of human metabolic reactions to incorporate or process dietary *cis* and *trans* positional fatty acid isomers. In light of the large and growing consumption of hydrogenated fats, basic information on the incorporation, distribution, and metabolism of these fatty acid isomers in humans is needed to evaluate the nutritional suitability and safety of this major dietary constituent. The analysis of human tissue described in this study provides an opportunity to answer the question: how have these unusual fatty acid isomers been processed or metabolized in vivo by humans who have ingested them over a 20–60-year time span?

METHODS

Human tissue, obtained during routine autopsies performed within 4 hr of death, was frozen until extraction procedures were initiated. The following specimens were obtained from undamaged tissue from subjects who, in most cases, died of traumatic injuries: the apex of the left ventricle of the heart, the entire thoracic and abdominal aorta, slices of normal-appearing liver, adipose tissue either from omentum or anterior abdominal wall, and slices of normal-appearing frontal lobes of the brain. Representative microscopic sections of these tissues were reviewed to assure that they were normal.

The age, sex, and cause of death of the eight subjects were as follows: 26 year-old female, head injury; 29 year-old male, head injury; 21 year-old male, head injury; 27 year-old male, head injury; 69 year-old female, myocardial infarction; 80 year-old female, pneumonia; 57 year-old male, myocardial infarction; 31 year-old male, chest wound. Brain and liver double bond analyses were performed on tissue from four of these subjects.

Dietary histories of some of the subjects were obtained from relatives, and this information confirmed the presence of hydrogenated vegetable oils in their diets.

Lipids were extracted from the tissue by the procedure of Hara and Radin (11). Five to 20 g of tissue was homogenized using a Brinkman Polytron homogenizer in 20 volumes of hexane–isopropanol 3:2. The extract was partitioned against one-half volume of aqueous sodium sulfate and the hexane-rich upper layer was concentrated under vacuum. One hundred to 200 mg of the lipid extract was transesterified by adding 3 ml each of benzene and 10% anhydrous HCl in methanol and heating for 3 hr at 65°C in a sealed tube. After adding 5 ml of water, the methyl esters were extracted with petroleum ether and the extract was washed with one-half volume of 0.1 M $NaHCO_3$.

The total fatty acid composition was determined by gas–liquid chromatography on 0.125 in × 6 ft 3%-EGSS-X columns or on 0.125 in × 20 ft 15%-OV-275 columns using methylpentadecanoate or methyl heptadecenoate as internal standards.

Silver nitrate thin-layer chromatography

Trace quantities of methyl[1-^{14}C]oleate and methyl [9,10-^{3}H]elaidate were added as internal standards to the methyl esters. Twenty to 40 mg of the methyl ester mixture was separated by silver nitrate thin-layer chromatography on 2-mm preparative silica gel plates dipped in 15% $AgNO_3$ solution and developed in benzene. Standards of *cis* and *trans* monoenes were visualized under UV light after spraying the plates with 0.1% 1-anilinonaphtalene-8-sulphonate. Regions containing the *cis* and *trans* monoenoic fractions were scraped from the TLC plate and the methyl esters were eluted from the silica gel using water-saturated ethyl ether. Using the ^{14}C and ^{3}H double label internal standards, the completeness of separation and the recovery of the *cis* and *trans* fractions were checked by scintillation counting of aliquots of the eluted fractions. Elution efficiencies from the TLC were better than 90%. Less than 1% of the [^{14}C]oleate was recovered in the *trans* fraction while less than 10% of the [^{3}H]elaidate was recovered in the *cis*-monoene fraction.

Unsaturated dimethyl acetals arising from the HCl-MeOH treatment of plasmalogens were observed to have R_f values on silver nitrate TLC below the *cis*-monoene methyl esters. Therefore, these structures were not included in the subsequent analyses.

Preparative GLC

Prior to ozonolysis, the *cis* and *trans* monoenoic fractions that had been isolated by $AgNO_3$-TLC were further purified by preparative GLC on either 20% DEGS or 20% OV17 on 0.25 in × 3 ft stainless steel columns. This procedure removed 16:1, 20:1, or other chain length monoenes from the samples. The 18-carbon chain length monoene isomers were collected from the GLC effluent after injections of 50–200 μg of each *cis* or *trans* monoenoic fraction. The GLC effluent was split approximately 10:1 and the larger portion was collected (as it passed from the oven) in a 30-cm section of 24 gauge Teflon tubing. Using radioactive methyl oleate as a standard, the collection efficiencies using this method were determined to be

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at least 80% if the amount collected was less than 200 μg.

The collected 18-carbon monoene fractions were rinsed from the Teflon tubing using pentane (nanograde AR) and their specific activity was determined by scintillation counting of the radioactivity and by quantitative gas–liquid chromatography using methyl heptadecanoate as internal standard.

The specific activity of the *trans*-18:1 fraction together with the specific activity of total fatty acids were used to calculate the percentage of *trans* 18:1 in the total fatty acids (Table 1) according to the equation: % *trans* 18:1 = 100 × [(mg *trans* 18:1/dpm [^{3}H]elaidate)/(mg total fatty acid/dpm [^{3}H]elaidate)].

Ozonolysis

The double bond distribution in the 18-carbon *cis* and *trans* monoenoic fractions was determined by the ozonolysis procedure described by Wood, Falch, and Wiegand (12) adapted as follows: 10–100 μg of the octadecenoate fractions was dissolved in 20–50 μl CS_2 at −70°C and was mixed with 0.2–0.4 ml ozone saturated CS_2 at −70°C. Excess ozone was removed immediately by bubbling with N_2 and the sample was concentrated to 50–100 μl with a stream of N_2. Triphenylphosphine (100 mg/ml CS_2) was added at the level of 10 μg/μg sample and the sample was capped until injection into the gas chromatograph. Samples were chromatographed on 2 mm × 6 ft glass columns packed with a mixed phase of 2% OV-17 and 1% OV 210 on 100/120 mesh Supelcoport. Temperature was programmed from 50–260°C at 10°/min (1 min initial, 5 min final). These chromatographic conditions gave resolution of all the aldehyde and aldehyde ester fragments derived from octadecenoates with double bonds between the 5 and 16 positions. Peak areas were integrated by digital computer.

RESULTS AND DISCUSSION

The determination of low (<5%) levels of mixtures of *trans* fatty acid isomers by GLC is subject to a number of errors (13, 14). The heterogeneity in double bond position found in monoenes derived from hydrogenated oils results in incomplete resolution of the *trans* and *cis* positional isomers when chromatographed on either packed or capillary columns (14). From comparison of % *trans* determinations before and after silver nitrate TLC, Heckers, Melcher, and Dittmar (14) have concluded that this overlap of *trans* and *cis* isomers can lead to at least a 60% underestimation of the *trans* content in vegetable oils containing approximately 5% *trans* isomers.

The samples from this study were, therefore, analyzed by two procedures. The % *trans* 18:1 was estimated first by GLC on OV-275. In the second method, the level of *trans* 18:1 in human lipids was determined after $AgNO_3$-TLC separation of the geometric isomers. [^{3}H]Elaidate was used as an internal standard to correct for any losses occurring during TLC separation and extraction from silica gel. **Table 1** presents the weight percentage of *cis* and *trans* 18:1 observed in the total fatty acids from tissues analyzed in this study.

For the 18 tissue samples analyzed, we observed the average *trans* 18:1 content to be 2.6% when determined directly by OV-275 and 2.7% when analyzed after silver-nitrate TLC by the internal standard method. Thus, we did not observe any major consistant underestimation of *trans* 18:1 content when OV-275 was used to determine the level of *trans* isomers in human tissue samples.

Fig. 1 presents the distribution of double bond positions observed in the *cis* and *trans* octadecenoate fractions from several human tissues. In addition, published data on the average double bond distribution of hydrogenated vegetable oil and butter samples are presented for comparison. We were able to detect *trans* isomers ranging in double bond position from 6–15 and *cis* isomers from 6–14. Since, presumably only the *cis*-9 and *cis*-11 isomers are synthesized by humans, the presence of all other isomers is most likely derived from dietary sources.

The data in **Fig. 2** were selected to demonstrate the degree of variability in *trans* double bond distribution, which we observed from analysis of adipose tissue from three different subjects. The percentage of *trans* 18:1 in the total fatty acids is also represented

TABLE 1. Weight percentage of *cis* and *trans*-octadecenoate in human tissue total fatty acids

Tissue Source	Average Weight Percent			
	cis 18:1	*trans* 18:1		
		Direct[a]	Indirect[b]	Range
Adipose	43	3.4	4.3	2.0–5.8
Liver	24	1.6	1.7	1.1–2.5
Heart	44	2.8	2.1	1.2–4.1
Aorta	34	3.1	3.1	1.4–3.9
Brain	14	0.4	0.4	0.23–0.88

[a] Direct method: *trans*-18:1 was determined by GLC of total fatty acid methyl esters on a 15% OV-275 column.

[b] Indirect method: *trans*-18:1 was determined after silver nitrate TLC isolation of *trans*-monoene fraction using radioactive elaidate as internal standard.

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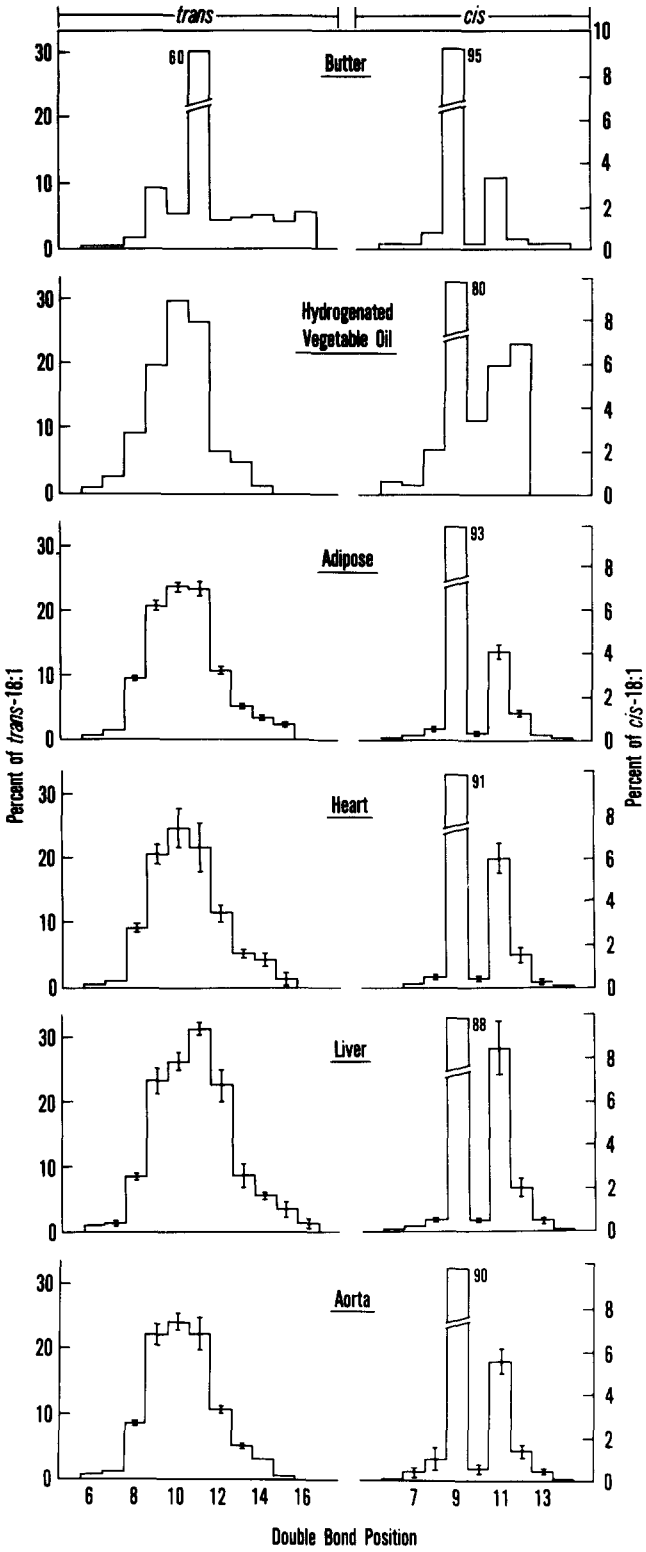

Fig. 1. Average double bond distribution in *cis* and *trans* octadecenoate fraction of total fatty acids from human tissues, from hydrogenated vegetable oils, and from butter. Error bars represent the standard error of the mean. The pattern shown for hydrogenated oils is calculated by averaging data published for 20 commercial margarines and cooking oils (2, 4). Data for butter is from reference 5.

for each analysis. Although the level of total *trans* 18:1 in the tissue varied more than twofold in these subjects, the pattern of positional isomers was remarkably similar. Thus, there appear to be no major differences between subjects in their ability to incorporate and metabolize these isomers.

The fatty acid profile of adipose tissue has been suggested to partially reflect the fatty acid composition of the diet (15–17). As seen from Fig. 1, the distribution of *trans* double bond positions in adipose tissue is remarkably similar to the pattern observed in dietary hydrogenated vegetable oils (2, 4), but rather different from the pattern reported for butter and ruminant fat (5). Although complete dietary histories were unavailable for this study, this close correlation between the distribution of double bonds in hydrogenated vegetable oils and adipose tissue lipids (Fig. 1) suggests that, for the subjects analyzed in this study, the major source of *trans* isomers is hydrogenated vegetable oils, whereas contributions from dairy and ruminant fat are relatively minor.

As seen from Fig. 1, the distribution of *trans* double bonds in the heart, aorta, and liver tissues is similar to that of adipose tissue with the exception that in liver the 11 rather than 10 isomer was most prevalent. We have also observed similar double bond distributions in brain (data not shown), although the very low levels of *trans* 18:1 observed (Table 1) has made analysis of this tissue difficult. Since the data shown in Fig. 1 are derived from analysis of the methyl esters prepared from total lipid extracts, it is possible that analysis of specific neutral or phospholipid classes would reveal patterns different from those presented here.

The pattern of *cis* isomers in the tissue samples is more difficult to compare to the diet because of the de novo synthesis by humans of *cis*-9 and *cis*-11 18:1. However, comparison of the relative proportions of the 8, 10, and 12 isomers in the diet (2, 4) with their proportions in the tissue lipids suggests that the *cis*-10 isomer is selectively metabolized or excluded by human tissues. The apparent selective metabolism or low incorporation of the *cis* 10-octadecenoate isomers that we observe is similar to data obtained previously from animal feeding experiments (6–8).

The results from this study provide evidence for occurrence of a wider range of octadecenoate positional isomers in human tissues than had previously been recognized (18–21). Capillary GLC recently has been used to analyze the fatty acid composition of human heart (22) and red blood cells (23). The resolution of this technique was sufficient to allow identification of the 9, 11, and 12

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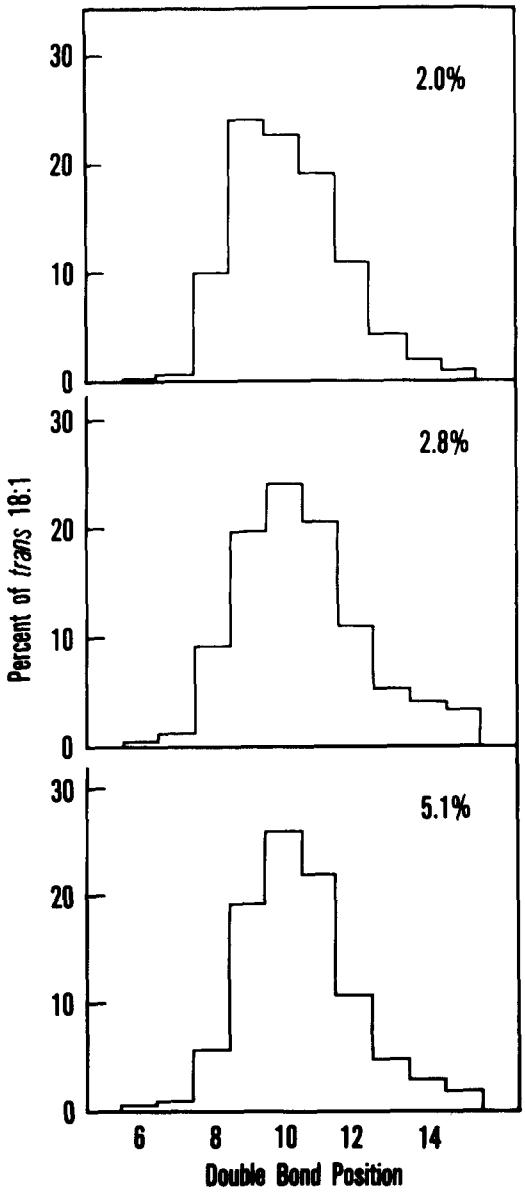

Fig. 2. *trans*-Octadecenoate double bond distribution in adipose tissue from three subjects. The percentage of *trans*-18:1 in total fatty acids is indicated above each profile.

cis-octadecenoate isomers and the 6, 9, and 11 *trans* isomers. However, the presence of the many other positional isomers observed in this study was not reported.

In conclusion, results from this study demonstrate that the wide range of positional isomers present in dietary hydrogenated fats is incorporated into human tissue lipids. In addition, although hydrogenated fats have been consumed by Americans for many years, the rates of turnover of the positional and geometric isomers by human metabolism are evidently sufficient to prevent major accumulation of these structures in total tissue lipids.

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