Occurrence of positional isomers of octadecenoic and hexadecenoic acids in human depot fat

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ABSTRACT Positional isomers of hexadecenoic aud octadecenoic acids of human adipose tissue have been separated by gas–liquid chromatography and their amounts determined by oxidative cleavage (MnO_4 and IO_4). The following isomeric octadecenoic acids were present: 7-octadecenoic acid (0.4%), 8- (1.9%), 9- (73.0%), 10- (2.5%), 11- (19.0%) and 12- (3.2%). The hexadecenoic acids have also been shown to be a mixture of positional isomers, in which the *cis*-9-isomer predominates. 10-Hexadecenoic and 12-octadecenoic acids could conceivably be precursors of linoleic acid.

The following branched fatty acids have also been determined in human depot fat: 13-methyltetradecanoic, 12-methyltetradecanoic, 14-methylpentadecanoic, 14-methylhexadecanoic, and 16-methylheptadecanoic acid. They were present in percentages of 0.02–0.6% and their identification rests solely on comparison of their gas–liquid chromatographic retention times with those of synthetic compounds.

KEY WORDS man · adipose tissue · positional isomers · hexadecenoic and octadecenoic acids · oxidative cleavage · branched fatty acids

Investigations of Klenk (1) have shown that 5,8,11-eicosatrienoic acid-10-^{14}C is formed by dehydrogenation of 9-octadecenoic acid-8-^{14}C by rats fed a fat-free diet, whereas the reverse process, hydrogenation of unsaturated fatty acids such as palmitoleic acid, occurs to a very limited extent (2). Polyenoic acids are also formed from di- or triunsaturated fatty acids [arachidonic from linoleic (3), and 20:5 and 22:6 from linolenic (4)].

Dehydrogenation seems to occur only at positions between an existent double bond and the carboxylic group; no formation of 9,12,15-octadecatrienoic acid from 9,12-octadecadienoic acid, for example, has been observed (4). Given these observations, it is theoretically possible that 10-hexadecenoic acid could be a precursor of linoleic acid, via dehydrogenation to 7,10-hexadecadienoic acid and chain elongation by two carbon atoms. 12-Octadecenoic acid could also be a precursor, for although no direct dehydrogenation to linoleic acid occurs, at least in the rat (5), conversion to 10-hexadecenoic acid by β-oxidation could be a first step.

From these general considerations it seemed to be of interest to investigate the isomeric monounsaturated acids in human depot fat a little further than has been done (6–9) till now.

The existence of isomeric octadecenoic and hexadecenoic acids in other biological materials has been described by many investigators (10). Weitkamp (11), for instance, described an 8-octadecenoic acid in the fat of human hair. This finding was later confirmed (12) and in addition 6-octadecenoic acid was found. Various isomers of octadecenoic acids (double bonds in the 13-, 12-, 11-, 10-, and 8-positions) have been detected in bovine body fat and butter fat (13) and in the sphingosines of pig brain (14). Isomeric hexa- and octadecenoic acids have been found in the milk and depot fat of rats of various ages (15), and Panos (16) found isomers of this type in *Streptococcus pyogenes*.

The gas chromatographic separation of positional isomeric unsaturated fatty acids has been described by Panos (16) and by Kuemmel and Chapman (17); before the time of these publications positional isomers were investigated by reductive or oxidative ozonolysis or by periodate–permanganate oxidation (18–28). The ozonolysis (19) of monoenoic acids is complicated by the formation of two isomeric ozonides (20) and possible degradation of the products after these ozonides have been split. We decided to use oxidative cleavage because (*a*) a calibration curve of the highly volatile aldehydes resulting from ozonolysis is more difficult to establish

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Abbreviation: GLC, gas–liquid chromatography. Fatty acids are designated by number of carbon atoms: number of double bonds.

than one of the methyl esters obtained from the non-volatile sodium salts of short-chain fatty acids, and (*b*) the primary cleavage products of periodate–permanganate oxidation are degraded only to the extent of 1% or less [(28), confirmed by ourselves].

METHODS

The investigations were carried out on two samples of human depot fat (women aged 85 and 84 yr; cause of death, apoplexy). After saponification with KOH in methanol and methylation, the compositions of the two samples, determined by GLC, were very similar.

Preparation of Unsaturated C_{15} and C_{18} Fractions

Some of the saturated fatty acids were eliminated by crystallization of the free acids from acetone. The methyl esters of the more unsaturated acids were then fractionated by distillation in a Jantzen split-ring column (Destillationstechnik Stage K.G., Cologne, West Germany; 50 cm in length, about 22 theoretical plates). The fractions containing mainly C_{16} and C_{18} esters were redistilled in the same apparatus and separated into saturated, monoenoic, and polyenoic esters by chromatography of their mercuric acetate adducts on silica gel. Details are as follows.

750 g of human depot fat (female, 85 yr) was refluxed with 3 liters of 2 N methanolic KOH for 2 hr. The mixture was cooled to room temperature, diluted with 3 liters of water, and acidified with HCl. The fatty acids were extracted twice with 6 liters of petroleum ether (bp 50–70°C).

The resulting 721 g of acids was crystallized from 2.5 liters of acetone at 0°C. The contents of the mother liquor, 583.5 g, were esterified with 2 liters of 5% methanolic HCl (2 hr under reflux). The methyl esters were extracted with petroleum ether after dilution of the solution with 2 liters of water.

206 g of methyl esters was distilled in a Jantzen column under reduced pressure (10^{-3} mm of Hg). The fraction distilling at 140–142°C (39.7 g) and the distillation residue (128.1 g) were collected, redistilled separately under the same conditions, and collected very carefully. The two fractions obtained contained only C_{16} (1.5 g) or C_{18} acids (7 g), respectively.

Separation of the mercuric acetate adducts was performed as follows (29–31). 1.497 g of the C_{16} methyl ester fraction was dissolved in 30 ml of 10% methanolic mercuric acetate solution. After 48 hr the methanol was evaporated at 40°C in 12 mm Hg; the residue was extracted three times with 60 ml of benzene. After removal of the benzene by evaporation, the adducts (2.8 g) were separated on a column of silica gel (30 g, containing 6% water); methyl palmitate (0.39 g) was eluted with 375 ml of benzene and the adduct of the hexadecenoates (2.4 g) with 150 ml of diethyl ether–acetic acid 95:5. The C_{18} fraction was treated in the same manner.

Gas–Liquid Chromatography

A fractograph F 7/F of the Perkin-Elmer & Co. (P-E), Bodenseewerk Uberlingen, Germany, with a flame ionization detector and an electronic integrator D 2, 159–008 was used.

Esters of Fatty Acids. (*a*) Capillary column, 50 m long, I.D. 0.25 mm, P-E type 1G54; stationary phase diethylene glycol adipate polyester; temperature 170°C; flow rate (N_2) 1.0 ml/min. (*b*) Capillary column, 25 m long, I.D. 0.25 mm, P-E type 2G51; stationary phase butanediol succinate; temperature 170°C; flow rate 1 ml/min. (*c*) Capillary column, 50 m long, I.D. 0.25 mm, P-E type 1G1; stationary phase Apiezon; temperature 190°C; flow rate 1 ml/min.

Esters of Dicarboxylic Acids Resulting from Oxidative Cleavage. (*a*) Capillary column, 25 m long, I.D. 0.25 mm, P-E type 2G51. (*b*) Packed column (steel), 2 m long, I.D. 3 mm; ethylene glycol succinate polyester (EGS), 20% on Celite, temperature 190°C; flow rate 30 ml/min.

Esters of Monocarboxylic Acids Resulting from Oxidative Cleavage. Packed column (steel), EGS, temperature 100°C, flow rate 32 ml/min (see above).

Oxidative Cleavage

4.5 mg of the acid to be cleaved was dissolved in 1.5 ml of a 1.5% solution of potassium carbonate. This usually takes 30 min. Then 3 ml of water, 3.5 ml of 1% $NaIO_4$ solution, and 0.05 ml of 0.1 M $KMnO_4$ solution were added and the mixture was allowed to stand for 15 hr at room temperature. After this time sufficient Na_2SO_3 was added to discolor the solution, which was acidified with about 1 ml of 10% H_2SO_4. The liberated iodine was reduced with just sufficient sodium sulfite.

The monocarboxylic acids were obtained by two extractions with 10 ml of petroleum ether (bp 50–70°C), the dicarboxylic acids with an equal volume of diethyl ether. The solution of monocarboxylic acids in petroleum ether was neutralized exactly with 0.1 N NaOH and the aqueous phase was evaporated in vacuo. The dry sodium salts were refluxed with 0.5 ml of 5% methanolic HCl for 45 min. The solution was diluted with twice the quantity of water and extracted with 1 ml of diethyl ether at 10–12°C. The ether phase was washed with 0.5 ml of water and analyzed by GLC without prior evaporation. The dicarboxylic acids were esterified in the usual manner.

Hydrogenation of the isolated hexadecenoic and octadecenoic esters with Pd/H_2 in methanol or acetic

acid in the usual way yielded only palmitic or stearic acid, respectively, as determined by GLC.

RESULTS

The composition of fatty acids in human depot fat obtained by GLC of the methyl esters is shown in Table 1. The results are taken from three runs on different columns; all compounds, especially the branched ones, were compared by GLC with reference samples (32). Nevertheless the branched-chain methyl esters must be investigated further and isolation of them is underway.

The C_{16} and C_{18} monounsaturated fractions were found by GLC and oxidative cleavage to be mixtures of isomers. By the use of capillary columns a complete separation of the 7-, 9-, and 10-hexadecenoic esters and of the 9-, 11-, and 12-octadecenoic esters can be achieved. The retention times of all isomers were compared with those of samples synthesized in our laboratory. The presence of *trans* isomers was excluded by GLC on Apiezon, on which the *trans* acids have measurably longer retention times, and by the absence of *trans* bands in the IR spectrum.

TABLE 1 FATTY ACID COMPOSITION* OF TWO SPECIMENS OF HUMAN DEPOT FAT

Acid	Specimen 1†	Specimen 2‡
	moles per cent	
Decanoic	0.2	0.1
Dodecanoic	1.4	1.2
Dodecenoic	0.1	0.2
Tetradecanoic	7.4	5.8
13-Methyltetradecanoic§	0.2	0.1
Tetradecenoic	0.9	1.1
12-Methyltetradecanoic§	0.3	0.3
Pentadecanoic	0.8	0.6
14-Methylpentadecanoic§	0.2	0.1
Pentadecenoic	0.4	0.2
Hexadecanoic	20.2	22.0
6-Hexadecenoic	0.1	9.2
7-Hexadecenoic	0.6	(all isomers)
8-Hexadecenoic	0.4	
9-Hexadecenoic	5.5	
10-Hexadecenoic	0.2	
15-Methylhexadecanoic§	0.2	0.8
14-Methylhexadecanoic§	0.4	0.6
Heptadecanoic	0.4	0.3
Heptadecenoic	0.7	0.6
16-Methylheptadecanoic§	trace	trace
Octadecanoic	3.5	2.9
7-Octadecenoic	0.2	49.1
8-Octadecenoic	1.0	(all isomers)
9-Octadecenoic	38.5	
10-Octadecenoic	1.3	
11-Octadecenoic	10.1	
12-Octadecenoic	1.7	
Octadecadienoic	3.9	5.3
Octadecatrienoic	0.1	0.1

* Determined by GLC on a capillary column coated with butanediol succinate.
† Woman aged 85 yr.
‡ Woman aged 84 yr.
§ Identified only by GLC.

TABLE 2 COMPOSITION OF THE HEXADECENOIC ACIDS, CALCULATED FROM PRODUCTS OF OXIDATIVE CLEAVAGE

Position of Double Bond	Percentage Calculated from: Dicarboxylic	Percentage Calculated from: Monocarboxylic
	moles per cent	
6	1.2	1.2
7	8.4	9.0
8	5.8	5.1
9	82.3	80.0
10	2.5	4.9

TABLE 3 COMPOSITION OF THE OCTADECENOIC ACIDS, CALCULATED FROM PRODUCTS OF OXIDATIVE CLEAVAGE

Position of Double Bond	Percentage Calculated from: Dicarboxylic	Percentage Calculated from: Monocarboxylic
	moles per cent	
7	0.4	0.3
8	1.9	1.4
9	73.0	73.0
10	2.5	2.9
11	19.0	19.7
12	3.2	3.1

The cleavage of both of the monounsaturated fractions by permanganate–iodate results in degradation products with reproducible composition if the conditions of reaction are well controlled. In the determination of the area of the GLC peaks with the aid of the electronic integrator, correction has been made for the different contributions made by the different proportions of carbon atoms in the various mono- and dicarboxylic acids. The correction was made with the aid of synthetic mixtures of mono- (C_4–C_{12}) and dicarboxylic (C_4–C_{13}) acids. Results of the oxidative cleavage of C_{16} and C_{18} mixtures are shown in Tables 2 and 3.

Model Test

In order to investigate further the possible oxidation of degradation products we attempted to prepare heptadecenoic acid for oxidative cleavage. We found, however, that several positional isomeric heptadecenoic acids result from the catalytical hydrogenation of 9-heptadecynoic acid, and that the amounts of these isomers very much depend upon the conditions of the reaction. Even by changing the catalyst we were unable to obtain pure *cis*-9-heptadecenoic acid (33), although the 9-heptadecynoic acid used was at least 99.1% pure. A migration of the double bond during catalytic hydrogenation has been described by various authors (34).

We decided, therefore, to subject 9-heptadecynoic acid itself to oxidative cleavage. The same amounts of de-

gradation products were registered by an integrator repeatedly at three different stages of sensitivity, and the reproducibility of the composition of the degradation products was excellent, i.e., further oxidation of the degradation products is less than 0.9%. Some pelargonic acid was found, which leads us to conclude that the 9-heptadecynoic acid contained traces of the 8-isomer.

DISCUSSION

The tables show that 10-hexadecenoic and 12-octadecenoic acid, as well as other positional isomers, are present in human adipose tissue. The two acids named have been singled out for attention since they could possibly be precursors of linoleic acid. Further investigations will show whether they are or not.

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