Structure and amount of positional isomers of monounsaturated fatty acids in human depot fat

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ABSTRACT   Positional isomers of decenoic, dodecenoic, and tetradecenoic acids of human adipose tissue have been separated by gas–liquid chromatography and their amounts determined by oxidative cleavage.

The following acids have been shown to be present: 7-decenoic, 8-decenoic, 9-decenoic, 7-, 8-, and 9-dodecenoic, and 7-, 8-, 9-, and 11-tetradecenoic acids. Among all isomers of the even-numbered acids from C_{10} to C_{18} the cis-9-isomer predominates. With increasing chain length, however, the content of 9-isomer decreases and the number of isomers increases. No 3- or 5-enoic acids could be detected.

The origin and biosynthesis of all isomers are discussed.

SUPPLEMENTARY KEY WORDS   decenoic · dodecenoic · tetradecenoic · biosynthesis

IN A PREVIOUS PAPER we reported the occurrence of positional isomers of hexadecenoic and octadecenoic acids in human depot fat, which contains as much as 58% of these fatty acids (1). The multitude of positional isomers found in these two groups of fatty acids suggested to us a search for shorter-chain, monounsaturated fatty acids with the double bond in unusual positions.

We have now isolated the decenoic, dodecenoic, and tetradecenoic acids from human depot fat and have determined their content of positional isomers by studying the products of cleavage with permanganate and iodate.

These acids have been shown earlier to be constituents of natural products; for instance 9-decenoic acid was shown first by Grün and Wirth (2) to be a component of butter-fat, from which Bosworth and Brown (3) later isolated dodecenoic and tetradecenoic acids. Hilditch and co-workers (4, 5) demonstrated that the dodecenoic acid

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Abbreviation: GLC, gas–liquid chromatography.

consisted chiefly of the Δ^9-isomer. The results were confirmed repeatedly, e.g., by Smith, Freeman, and Jack (6).

MATERIAL

The investigations were carried out on three different human depot fats; these fats showed an almost identical fatty acid composition after methylation. The positional isomers of the monoenoic acids were investigated, however, only on one sample of these fats (woman aged 85 yr; cause of death, apoplexy). The fat was methanolyzed with 5% methanolic HCl. The methyl esters of the acids of equal chain length were prepared by repeated distillation in a Jantzen split-ring column (Destillationstechnik Stage K.G., Cologne, West-Germany; 50 cm in length, about 22 theoretical plates) under reduced pressure (10^{-3} mm of Hg). The first distillation gave eight fractions, each of which was redistilled. Finally 238 fractions were obtained; their composition was determined by GLC. Fractions with equal composition were combined. Saturated, mono-, and polyunsaturated methyl esters were separated as their mercuric acetate adducts. These methods and the total composition of human depot fat examined in the present investigation were described in our previous paper (1). The shorter-chain monounsaturated fatty acids occur only in small amounts in human depot fat (decenoic acid less than 0.01%, dodecenoic acid 0.1%, tetradecenoic acid 0.9%).

We could demonstrate by GLC that there was no migration of the double bonds during saponification of the methyl esters of the unsaturated fatty acids with 2 N methanolic KOH: the ratios of the isomers before and after saponification followed by reesterification were the same. Thus, 10 mg of the authentic methyl dodecenoate (96% cis-9-dodecenoate) were saponified with 5 ml of 2 N methanolic KOH for 2 hr at 100°C. The mixture was cooled to room temperature, diluted with 7 ml of water,

and extracted twice with 10 ml of cyclohexane. After acidification with concentrated HCl the free fatty acids were extracted with cyclohexane and reesterified with 5% methanolic HCl at 100°C for 45 min. After this procedure the composition of the mixture was unchanged; GLC on capillary columns and analysis by oxidative cleavage again gave 96% of cis-9-dodecenoic acid. Authentic decenoic and tetradecenoic methyl esters were treated in the same manner. No difference between the composition of the original and that of the reesterified methyl esters could be observed.

METHODS

GLC and Oxidative Cleavage

The conditions of GLC and the oxidative cleavage of fatty acids were described previously (1), but the latter has been applied now also to the methyl esters. The results of the two methods—cleavage of the acids and of the methyl esters—did not differ by more than 0.4%.

6.3 mg of methyl decenoate was dissolved in 7.3 ml of t-butanol–water 9:1, and 3 ml of 2% $NaIO_4$, 1.4 ml of 1% K_2CO_3, and 0.02 ml of 0.5% $KMnO_4$ were added. After 4 hr the mixture was decolorized with Na_2SO_3, made alkaline by addition of 10% KOH, and finally evaporated under reduced pressure. The residue was acidified with 10% H_2SO_4 and the aqueous layer extracted twice with ether. After evaporation under reduced pressure at 15°C the residue was methylated with 5% methanolic HCl.

In order to prove the accuracy of the method of oxidative cleavage in cases where the double bond is located in ω-position, we applied the method to a specimen of synthetic 9-decenoic acid, which was prepared by hydrogenation of highly purified 9-decynoic acid (7) with hydrazine hydrate. We could demonstrate that under the conditions of the Rudloff cleavage the amount of further oxidation products was less than 0.4%. We shall report details in another paper.

The hydrogenation of the investigated decenoic, dodecenoic, and tetradecenoic acids with Pd/H_2 in methanol resulted in each case only in one product: decanoic, dodecanoic, and tetradecanoic acid, respectively. The purity of the saturated fatty acids was greater than 99% (GLC).

RESULTS

The composition of the isomers of the even-numbered, monounsaturated fatty acids with chain length 10–18 are shown in Table 1. The values in parentheses are uncertain because the accuracy of the method is not better than about 0.9%.

TABLE 1 ABUNDANCE OF POSITIONAL ISOMERS OF EVEN-NUMBERED, MONOUNSATURATED FATTY ACIDS WITH CHAIN LENGTHS OF 10–18 ISOLATED FROM HUMAN DEPOT FAT

	Position of the Double Bond							
	5	6	7	8	9	10	11	12
	*moles %**							
C_{10}	—	—	3.9	9.4	86.8	—	—	—
C_{12}	—	(0.5)	1.4	1.9	96.2	—	—	—
C_{14}	(0.1)	(0.3)	3.0	1.7	91.2	(0.5)	2.9	(0.1)
C_{16}†	—	1.2	8.4	5.8	82.3	2.5	—	—
C_{18}†	—	—	(0.4)	1.9	73.0	2.5	19.0	3.3

Values in parentheses are uncertain (accuracy of the method is not better than about 0.9%).
* Deduced from the nature and amount of dicarboxylic acids that result from the oxidative cleavage of the monoenoic acids.
† From published data (1).

Table 1 shows that the 9-isomer predominates in all fractions; the content of the 9-isomer decreases from C_{12} to C_{18} with increasing chain length; the number of isomers tends to increase with the chain length; there are no isomers with double bonds in the 3- or 5-positions; and the decenoic acid shows a remarkably high content of the 8-isomer.

The percentage contents of saturated and 9-monoenoic acids for each chain length are shown in Fig. 1. Fig. 2 expresses the results from several different depot fats by showing the ratio of saturated to *total* monounsaturated acids corresponding to each chain length.
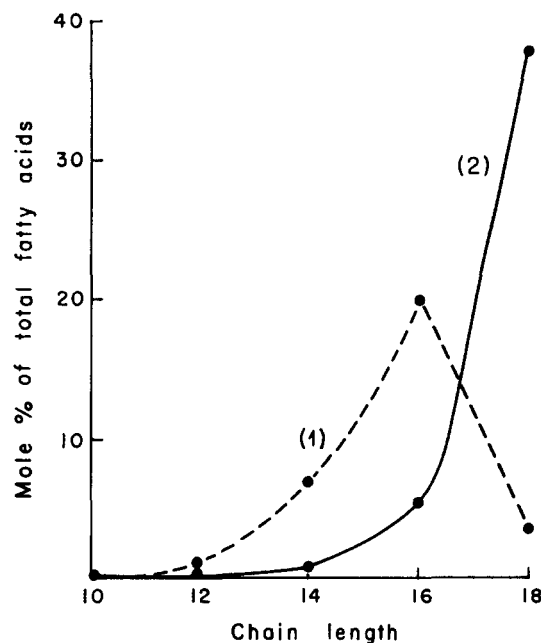

FIG. 1. Mole percentages of saturated fatty acids (curve 1, —) and of 9-monounsaturated fatty acids (curve 2, —) in the total fatty acids of human depot fat.
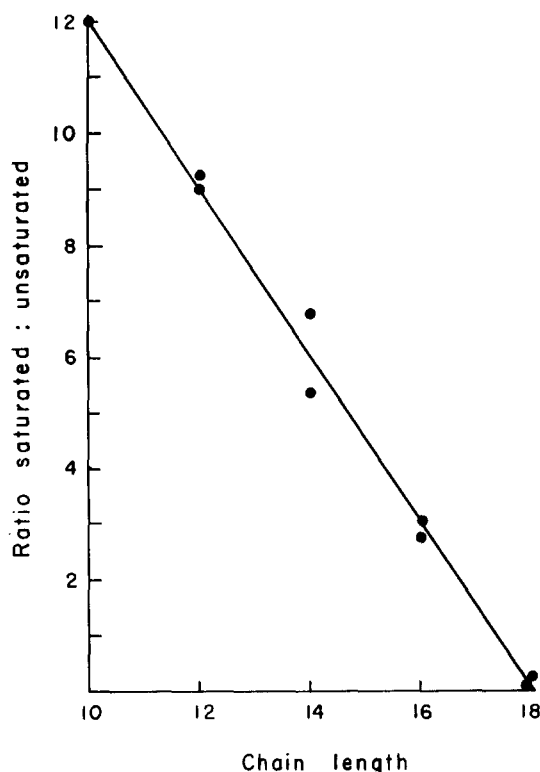
Fig. 2. Plot of the ratio of saturated to total monounsaturated fatty acids against chain length (mole percentages, several different human depot fats).

DISCUSSION

Curve *1* of Fig. 1 seems to rise exponentially only up to C_{16}, while curve *2* is exponential over the whole interval shown. The value for C_{18} in curve *1* is significantly lower than the value for C_{16} or C_{14}. Curve *1* can be interpreted easily if one supposes either that palmitate synthetase produces exclusively palmitic acid and that the acids of shorter chain length (C_{10}–C_{14}) are products of β-oxidation, or that the enzyme system is not entirely specific and forms not only palmitic acid but shorter-chain fatty acids also. The low level of stearic acid suggests a different biogenesis for this acid; as has been discussed repeatedly (8, 9) this acid arises mainly by two-carbon elongation of palmitic acid.

Curve *2* has no discontinuity, which we interpret to mean that the biogenesis of all 9-monounsaturated fatty acids proceeds by the same mechanism: the 9-dehydrogenation of saturated fatty acids (10, 11). This reaction seems to proceed more readily as the chain length of the acids increases; it reaches a high rate at C_{18}, which may be the optimum chain-length for the enzyme. The linearity of Fig. 2 supports these conclusions.

The origin of all the 7-isomers can be explained by β-oxidation of the higher Δ^9-homologues, and of all the 11-isomers by chain elongation of the lower Δ^9-homologues. Stoffel (8) reported, for example, that oleic acid is

converted into 11-eicosenoic acid to the extent of 10%.

Another biosynthesis of 11-octadecenoic acid has been shown to occur in microorganisms (12, 13): 9-hexadecenoic and 11-octadecenoic acids are formed from 3-decenoic acid. 3-Dodecenoic acid can be also converted by microorganisms into 7-hexadecenoic and 9-octadecenoic acids. As all these acids occur in the human depot fat it is worth considering whether these biosynthetic pathways also occur in man, although the total absence of 3-enoic acids does not argue for the possibility.

The origin of the 8-, 10-, and 12-isomers is not clear. Possibly, they originate from dietary fats; this could be true for all minor components. The formation of 12-octadecenoic acid during the hydrogenation of linoleic acid under technical conditions, as in the production of margarine, is well known (14). β-Oxidation of that acid should yield 8-tetradecenoic and 10-hexadecenoic acids, both found in human depot fat. Migration of double bonds during the hydrogenation of unsaturated fatty acids has also been reported (15, 16) and could explain the presence of 8- and 10-isomers in dietary and hence in human depot fat. Finally, 6- and 8-hexadecenoic acids could result from the β-oxidation of 8- and 10-octadecenoic acids.

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