The selective mobilization of fatty acids is not based on their positional distribution in white-fat-cell triacylglycerols

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Fatty acids have been shown to be selectively mobilized from rat white fat-cells, whatever the dietary manipulations. For convenience, fatty acids have been classified as being highly, weakly and moderately mobilizable. The aim of this study was to examine whether the selective mobilization of fatty acids can be explained, even partly, by their positional distribution in adipose-tissue triacylglycerols (TAG) via the known specificity of hormone-sensitive lipase for the *sn*-1 and *sn*-3 positions. Adipose tissue was dietarily manipulated in order to obtain a wide spectrum of fatty acids, including large amounts of either very-long-chain polyunsaturated fatty acids (VLC-PUFA) or very-long-chain monounsaturated fatty acids (VLC-MUFA). The determination of fatty acid distribution in adipose tissue TAG was based on random formation of 1,2-diacyl-*rac*-glycerols by Grignard degradation, followed by synthesis of phosphatidic acids and hydrolysis in the *sn*-2 position by phospholipase A_2. Regardless of the fatty acid composition and location of fat depots, highly (e.g. 18:4*n*−3 and some of the VLC-PUFA) and weakly (e.g. VLC-MUFA) mobilizable fatty acids were located mainly in the outer (*sn*-1 and *sn*-3) positions of the glycerol moiety (79.5% and 92.5% on average, respectively). Other fatty acids, which are rather moderately mobilizable, were more randomly distributed. We conclude that the selective mobilization of white-fat-cell fatty acids is not based on their positional distribution in TAG.

INTRODUCTION

The rate of mobilization of fatty acids from white adipose tissue has been reported to be differential and to depend on the fatty acid chain length and unsaturation, that is, on molecular structure [1]. Among fatty acids with chain length ranging from 12 to 24 carbon atoms and with unsaturation of 0–6 double bonds, a fatty acid is all the more readily mobilized as its carbon chain is shorter and more unsaturated. From these results, and for convenience, fatty acids have been classified into three categories: highly mobilizable fatty acids, including those with 16–20 carbon atoms and 4–5 double bonds, and weakly mobilizable fatty acids, including those with 20–24 carbon atoms and 0–1 double bond. Other fatty acids have been defined as being moderately mobilizable [1,2].

The mechanism of the selective mobilization of fatty acids from fat-cells is at present unknown. We have shown that this selective metabolic process is a general feature of adipose tissue, being unrelated to the tissue location and independent of the relative proportions of stored fatty acids. This latter result does not lend support for a competition process between fatty acids as an explanation of their selective mobilization. Hormone-sensitive lipase (HSL), the main enzyme involved in lipolysis, has been reported to release preferentially some polyunsaturated fatty acids from triacylglycerols (TAG) of rat adipose tissue [3]. Among the mechanisms that could explain the selective mobilization of fatty acids, a differential hydrolysis of adipose-tissue TAG could therefore be proposed. Because HSL is known to hydrolyse preferentially the *sn*-1 and *sn*-3 positions in TAG molecules [4], the selective hydrolysis of fatty acids might result from 'kinetic advantages' via a positioning of the more readily released fatty acids on these outer positions of the glycerol backbone, as previously proposed [3]. This would be in line with previous studies [5–8] describing the positional distribution of fatty acids among the three positions in TAG of animal fat depots as a non-random process being influenced by the fatty acid chain length and unsaturation [8,9].

The aim of this study was to determine whether a selective positional distribution of fatty acids in TAG accounts for their selective mobilization from fat cells. According to such a putative mechanism, highly and weakly mobilizable fatty acids should be preferentially located, respectively, in the outer (*sn*-1 and *sn*-3) and inner (*sn*-2) positions of the glycerol moiety, whereas moderately mobilizable fatty acids should be more randomly distributed among the three positions of the glycerol backbone.

As in our previous studies on fatty acid mobilization [1,2], and on account of the strong relationships between dietary and adipose-tissue fatty acids [10], rats were first fed on two high-fat diets in order to enrich their adipose-tissue TAG with a wide spectrum of fatty acids in amounts high enough to allow accurate determination of their positional distribution. These fatty acids include very-long-chain polyunsaturated fatty acids (VLC-PUFA), some of which are among the highly mobilizable fatty acids, and very-long-chain monounsaturated fatty acids (VLC-MUFA), which are among those weakly mobilizable.

MATERIALS AND METHODS

Chemicals

Analytical-grade solvents, TLC plates coated with silica gel 60 and butylated hydroxytoluene were purchased from Merck (Darmstadt, Germany). Other chemical reagents were supplied by Sigma (St. Louis, MO, U.S.A.).

Abbreviations used: HSL, hormone-sensitive lipase; TAG, triacylglycerols; VLC-MUFA, very-long-chain monounsaturated fatty acids; VLC-PUFA, very-long-chain polyunsaturated fatty acids; MEN (menhaden) and HER (herring) diet and group, diet high in VLC-PUFA and low in VLC-MUFA, and the reverse, respectively, and animals fed on these respective diets.

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Animals and diets

Eight male Wistar rats, weighing 240–260 g, were housed in plastic cages at 25 °C with a 12 h light/dark cycle. They were divided into two groups (four rats per group) and fed *ad libitum* for 4 weeks on semi-synthetic diets containing 19 % by weight of menhaden (MEN group) or herring (HER group) oil, and 1 % of sunflower oil to meet the daily requirement in essential $n-6$ fatty acids. Other components of the diets were (in g/100 g): 45 sucrose, 25 casein, 4.5 agar-agar, 4.5 mineral mix [205 B, Usine d'Alimentation Rationnelle (UAR), Villemoisson, France], and 1 vitamin mix (200, UAR). The fatty acid compositions of the diets have been described in detail in a previous study [11]. The fatty acid composition of menhaden oil was characterized by a very high content of $n-3$ VLC-PUFA (sum, 39 % by weight; 20:5$n-3$, 17.5 %; 22:6$n-3$, 14.2 %), whereas that of herring oil was typified by a very high proportion of VLC-MUFA (sum, 36 %; 22:1$n-11$, 21.3 %; 20:1$n-9$, 12.5 %). Diets were prepared weekly and added with α-tocopherol (300 mg/kg) as an antioxidant. They were kept in rations at −20 °C and renewed daily. The animals had free access to water.

Lipid extraction and separation

Rats (360–400 g) were killed by cervical dislocation in the post-absorptive state. Samples of retroperitoneal (the two experimental groups) and epididymal and mesenteric adipose tissues (HER group) were rapidly excised and extracted as described by Folch et al. [12]. About 10 mg of TAG was isolated from total lipid extracts by chloroform elution on silicic acid columns and then purified by TLC using hexane/diethyl ether/acetic acid (70:30:1, by vol.) as the developing solvent. TLC plates were then dried under nitrogen and sprayed with primulin. The TAG band was scraped off and extracted three times with hexane/diethyl ether (1:1, v/v). Butylated hydroxytoluene at a final concentration of 0.05 % was added to all the solvent mixtures as an antioxidant.

Fatty acid distribution in adipose-tissue TAG

The stereospecific analysis of TAG was performed as previously reported [13]. Representative *sn*-1,2- and *sn*-2,3-acylglycerols were obtained from about 10 mg of TAG by the Grignard reaction [14]. Phosphatidic acids were then chemically synthesized by a diacylglycerol derivative-formation procedure [14] with slight modifications. Briefly, diacylglycerols (about 1.5–2 mg) in ice-cold chloroform were mixed slowly with 2 ml of a chilled $POCl_3$ solution (chloroform/pyridine/$POCl_3$, 9.5:9.5:1, by vol.) with stirring. After 1 h, 4 ml of chloroform, 2 ml of $NaHCO_3$ (0.5 M), and 0.4 ml of EDTA (0.5 M) were added to the mixture, which was vortex-mixed (2 h) and then centrifuged. The lower phase was collected, evaporated to dryness under nitrogen, dissolved with 1 volume of chloroform/methanol (1:1, v/v) and partitioned with 1 vol. of water. After evaporation of the lower phase under nitrogen, the phosphatidic acid was then purified by TLC [15]. The hydrolysis of *sn*-1,2-phosphatidic acid was carried out with snake venom phospholipase A_2, as previously reported [16], with minor modifications. Briefly, phosphatidic acid (about 1 mg) was vortex-mixed (2 h) with 2 ml of diethyl ether and 1 ml of Tris buffer (50 mM Tris/HCl, 4 mM $CaCl_2$, pH 7.5) containing 5 units of phospholipase A_2. The hydrolysis products, i.e. non-esterified fatty acids released from the *sn*-2 position and lyso-phosphatidic acid (*sn*-1 position), were purified by TLC [15]. The fatty acid composition in the *sn*-3 position was determined by the following calculation: $3 \times (TAG) - (sn\text{-}1 + sn\text{-}2$ positions). The procedure was tested for accuracy on synthetic 1,3-dioleoyl-2-palmitoyl-*rac*-glycerol and showed very low contamination from one position to another. On the other hand, despite reproducible fatty acid compositions in the *sn*-1 and *sn*-2 positions in both experimental groups, minor fatty acids (< 0.3 % by wt.) caused large discrepancies in the calculation of the *sn*-3 position. They were omitted in the final calculations.

Fatty acid analysis

The preparation of fatty acid methyl esters was performed with 14 % BF_3 in methanol, as described by Morrison and Smith [17]. Fatty acid methyl esters were separated and quantified by GLC using a Chrompack CP 9000 chromatograph (Chrompack, Les Ulis, France) equipped with an AT-WAX capillary column (0.25 mm internal diam. × 60 m, 0.25 μm thickness; Alltech, Templeuve, France), a flame ionization detector and a Spectra-Physics SP 4290 integrator (Spectra-Physics, Les Ulis, France), as previously described [1]. Identification of fatty acids was performed by comparison with standard fatty acids (Nu-Check Prep, Elysian, MN, U.S.A.).

Statistics

Results are expressed as means ± S.E.M. of four determinations, except for the positional distribution of fatty acids from different adipose-tissue TAG of the HER group, where values were obtained from one animal. The comparison of fatty acid percentages was made with the Peritz *F*-test for multiple comparisons, after arcsine transformation [18]. The criterion of significance was $P < 0.05$.

RESULTS

Composition and positional distribution of fatty acids in TAG of retroperitoneal fat-cells

The percentage weight of fatty acids and the proportions of each fatty acid in the three positions of retroperitoneal adipose-tissue TAG are shown in Tables 1 (MEN group) and 2 (HER group). The fatty acid composition of the high-fat diet influenced largely that of the adipose tissue. Retroperitoneal adipose tissue TAG of the MEN group were notably rich in VLC-PUFA (Table 1, left column) whereas those of the HER group were rich principally in VLC-MUFA (Table 2, left column).

Although the amounts of most fatty acids in adipose-tissue TAG were markedly different between the two experimental groups, there were only slight differences in their positional distribution on the glycerol backbone. About half of the 16:0 and 18:1$n-7$ was found in the *sn*-1 position, whereas 18:1$n-9$ was located predominantly in the *sn*-2 and *sn*-3 positions. Half or more of the 18:2$n-6$ and 18:3$n-3$ was located in the *sn*-2 position. There was a more even distribution of 14:0 and 16:1$n-7$ among the three positions. About 50 % of 18:4n-3 was located in the *sn*-3 position, whereas 20:4n-3 was found mostly in the *sn*-1 and *sn*-3 positions in the MEN group, and mainly in the *sn*-1 position in the HER group. The 20:5$n-3$ and 20:1$n-9$ were mainly distributed in the *sn*-3 position (40–60 %) and also in the *sn*-1 position (about 30 % or more). The 22:5$n-3$ was mainly found in the *sn*-3 position in the MEN group, but was more evenly distributed in the HER group. The 22:6$n-3$ was located mostly in the *sn*-3 position, and to a lesser extent in the *sn*-2 position. The 20:1$n-11$, 20:1$n-7$ and 22:1n-11, present in significant amounts only in the HER group, were found nearly exclusively in the *sn*-1 and *sn*-3 positions.

Table 1 Composition and positional distribution of fatty acids in TAG of retroperitoneal fat-cells from rats of the MEN group

The determination of fatty acid distribution in adipose-tissue TAG was based on random formation of 1,2-diacyl-*rac*-glycerols by Grignard degradation, followed by synthesis of phosphatidic acids and hydrolysis in the *sn*-2 position by phospholipase A_2. Values are means ± S.E.M. of four determinations. * Expressed as percentage weight of total fatty acids. Minor fatty acids (< 0.3%, by wt.) were not considered. †For a given fatty acid, the sum of the three positions equals 100. Within a line, values that do not share the same superscript letter are significantly different ($P < 0.05$). The relative mobilization rate of fatty acids is based on results from a previous study [2]: H, high; M, moderate; W, weak.

Fatty acids	All*	Glycerol position†			Relative mobilization rate
		sn-1	*sn*-2	*sn*-3	
Saturated					
14:0	5.06 ± 0.03	37.3 ± 2.2^a	34.4 ± 0.3^a	28.3 ± 2.5^b	M
16:0	28.58 ± 0.42	51.0 ± 2.1^a	23.6 ± 2.9^b	25.3 ± 5.0^b	M
18:0	4.38 ± 0.08	39.5 ± 3.5^a	18.0 ± 2.4^b	42.6 ± 5.9^a	M
Mono-unsaturated					
16:1*n*−7	8.48 ± 0.23	28.9 ± 1.2^a	38.6 ± 0.3^b	32.5 ± 1.5^a	M
18:1*n*−9	20.02 ± 0.31	20.1 ± 2.1^a	42.0 ± 0.6^b	37.9 ± 2.7^b	M
18:1*n*−7	4.90 ± 0.07	49.8 ± 1.2^a	22.0 ± 2.6^b	28.2 ± 1.4^b	M
20:1*n*−9	1.15 ± 0.03	33.3 ± 6.5^a	5.1 ± 2.7^b	61.6 ± 1.5^c	W
Di-unsaturated					
18:2*n*−6	11.71 ± 0.47	15.3 ± 0.8^a	64.7 ± 3.7^b	20.0 ± 4.5^a	M
Tri-unsaturated					
18:3*n*−3	0.81 ± 0.03	22.2 ± 0.4^a	50.1 ± 0.5^b	27.7 ± 0.2^c	M
Tetra-unsaturated					
18:4*n*−3	1.37 ± 0.05	31.8 ± 1.9^a	18.5 ± 0.4^b	49.7 ± 1.6^c	H
20:4*n*−6	0.67 ± 0.03	17.8 ± 1.9^a	36.9 ± 2.4^b	45.3 ± 4.3^b	H
20:4*n*−3	0.66 ± 0.01	47.2 ± 4.8^a	5.7 ± 2.4^b	47.1 ± 0.9^a	H
Penta-unsaturated					
20:5*n*−3	4.10 ± 0.22	30.7 ± 3.6^a	13.7 ± 1.0^b	55.5 ± 2.6^c	H
22:5*n*−3	1.68 ± 0.02	12.4 ± 1.0^a	14.6 ± 0.6^a	73.0 ± 0.4^b	M
Hexa-unsaturated					
22:6*n*−3	6.43 ± 0.11	13.6 ± 0.1^a	38.4 ± 1.5^b	48.0 ± 1.6^c	M

Table 2 Composition and positional distribution of fatty acids in TAG of retroperitoneal fat-cells from rats of the HER group

The determination of fatty acid distribution in adipose-tissue TAG was based on random formation of 1,2-diacyl-*rac*-glycerols by Grignard degradation, followed by synthesis of phosphatidic acids and hydrolysis in the *sn*-2 position by phospholipase A_2. Values are means ± S.E.M. of four determinations. * Expressed as percentage weight of total fatty acids. Minor fatty acids (< 0.3%, by wt.) were not considered. † For a given fatty acid, the sum of the three positions equals 100. Within a line, values that do not share the same superscript letter are significantly different ($P < 0.05$). The relative mobilization rate of fatty acids is based on results from a previous study [2]: H, high; M, moderate; W, weak.

Fatty acids	All*	Glycerol position†			Relative mobilization rate
		sn-1	*sn*-2	*sn*-3	
Saturated					
14:0	5.32 ± 0.50	27.0 ± 1.1^a	28.8 ± 1.6^a	44.2 ± 2.2^b	M
16:0	24.71 ± 0.93	52.8 ± 2.9^a	29.4 ± 0.4^b	17.7 ± 3.2^c	M
18:0	2.15 ± 0.15	56.6 ± 3.1^a	27.9 ± 3.6^b	15.5 ± 6.6^b	M
Mono-unsaturated					
16:1*n*−7	5.98 ± 0.45	20.3 ± 0.3^a	38.4 ± 2.0^b	41.4 ± 2.0^b	M
18:1*n*−9	23.20 ± 0.75	23.7 ± 0.3^a	41.5 ± 1.2^b	34.9 ± 0.9^c	M
18:1*n*−7	2.44 ± 0.04	48.8 ± 1.0^a	21.0 ± 1.6^b	30.2 ± 2.3^c	M
20:1*n*−11	3.31 ± 0.16	24.8 ± 0.1^a	17.5 ± 0.4^b	57.7 ± 0.4^c	W
20:1*n*−9	8.42 ± 0.29	48.8 ± 1.0^a	10.8 ± 1.0^b	40.4 ± 1.7^c	W
20:1*n*−7	0.37 ± 0.01	47.7 ± 2.9^a	5.9 ± 3.3^b	46.4 ± 2.8^a	W
22:1*n*−11	6.94 ± 0.26	33.5 ± 1.1^a	3.8 ± 0.5^b	62.7 ± 1.6^c	W
Di-unsaturated					
18:2*n*−6	10.70 ± 0.20	13.1 ± 0.1^a	64.2 ± 2.3^b	22.7 ± 2.3^c	M
Tri-unsaturated					
18:3*n*−3	1.60 ± 0.02	18.3 ± 0.4^a	51.2 ± 1.8^b	30.5 ± 1.8^c	M
Tetra-unsaturated					
18:4*n*−3	1.21 ± 0.07	21.8 ± 0.8^a	25.4 ± 0.8^b	52.8 ± 0.7^c	H
20:4*n*−3	0.42 ± 0.01	61.7 ± 6.0^a	26.2 ± 3.4^b	12.1 ± 7.4^b	H
Penta-unsaturated					
20:5*n*−3	0.98 ± 0.05	25.7 ± 1.3^a	15.4 ± 1.5^b	58.9 ± 2.8^c	H
22:5*n*−3	0.54 ± 0.04	31.5 ± 9.2^a	29.6 ± 9.7^a	38.9 ± 1.5^a	M
Hexa-unsaturated					
22:6*n*−3	1.71 ± 0.12	19.9 ± 3.1^a	36.9 ± 5.5^a	43.2 ± 8.2^a	M

Table 3 Positional distribution of weakly, moderately and highly mobilizable fatty acids in the three positions of TAG from retroperitoneal adipose tissue for the two experimental groups

The determination of fatty acid distribution in adipose-tissue TAG was based on random formation of 1,2-diacyl-*rac*-glycerols by Grignard degradation, followed by synthesis of phosphatidic acids and hydrolysis in the *sn*-2 position by phospholipase A_2. Highly mobilizable fatty acids have 16–20 carbon atoms and 4–5 double bonds; weakly mobilizable fatty acids have 20–24 carbon atoms and 0–1 double bond; moderately mobilizable fatty acids include all the others [2]. Values are means ± S.E.M. of four determinations and are computed from Tables 1 and 2. For a given category of fatty acids, the sum of the three positions equals 100. Within each experimental group and for a given position, values that do not share the same superscript letter are significantly different ($P < 0.05$).

		MEN group			HER group		
Fatty acids	Glycerol position...	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3
Weakly mobilizable		33.3 ± 6.5	5.1 ± 2.7[a]	61.6 ± 1.5[a]	38.7 ± 3.1	9.5 ± 1.8[a]	51.8 ± 2.8[a]
Moderately mobilizable		29.0 ± 3.2	34.6 ± 3.4[b]	36.3 ± 3.4[b]	30.2 ± 3.1	38.8 ± 2.9[b]	31.0 ± 2.2[b]
Highly mobilizable		31.9 ± 4.1	18.7 ± 4.5[c]	49.4 ± 1.8[c]	36.4 ± 6.6	22.3 ± 2.0[c]	41.3 ± 7.7[a,b]

Table 4 Positional distribution of three representative fatty acids in the three positions of TAG from different adipose tissues of the HER group

The determination of fatty acid distribution in adipose-tissue TAG was based on random formation of 1,2-diacyl-*rac*-glycerols by Grignard degradation, followed by synthesis of phosphatidic acids and hydrolysis in the *sn*-2 position by phospholipase A_2. 20:1*n*-9, 16:0 and 20:5*n*-3 are representative of weakly, moderately and highly mobilizable fatty acids, respectively, as described in detail in the text. For a given fatty acid, the sum of the three positions equals 100. Values were obtained from one rat. Abbreviations: RP, retroperitoneal; EPI, epididymal; MES, mesenteric.

		RP adipose tissue			EPI adipose tissue			MES adipose tissue		
Fatty acids	Glycerol position...	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3	*sn*-1	*sn*-2	*sn*-3
20:1*n*−9		48.4	10.8	40.8	49.7	12.9	37.4	49.2	9.6	41.2
16:0		52.5	29.4	18.2	48.5	28.6	22.9	58.0	26.9	15.1
20:5*n*−3		25.5	15.4	59.1	27.7	17.4	55.0	23.1	12.5	64.4

Positional distribution of fatty acids in the outer positions of TAG

In both experimental groups, the relative distribution of each fatty acid on the *sn*-1 plus *sn*-3 positions has been calculated from Tables 1 and 2. For a given fatty acid, a sum higher than 66.7% indicates its preferential positional distribution on the TAG outer positions. Among the 15 fatty acids considered in the MEN group, this sum showed a trend to be higher than 66.7% for 8 fatty acids. They include mainly 18:4*n*−3 and some VLC-PUFA (e.g. 20:5*n*−3, 20:4*n*−3 and 22:5*n*−3), but also a VLC-MUFA (20:1*n*−9, 90% being found in the outer positions). Among the 17 fatty acids considered in the HER group, the sum of the proportions in the outer positions show a tendency to be higher than 66.7% for 12 fatty acids. They include mainly 18:4*n*−3 and some VLC-PUFA (e.g. 20:4*n*−3 and 20:5*n*−3), but also all VLC-MUFA (e.g. 20:1*n*−11, 20:1*n*−9, 20:1*n*−7 and 22:1*n*−11, 96% of the latter being found in the outer positions). On the contrary, in both experimental groups, the sum of the proportions of 18:2*n*−6 and 18:3*n*−3 on the outer positions showed a tendency to be lower than 66.7%, indicating a preferential location in the inner position. All VLC-MUFA (20:1 and 22:1) were nearly exclusively found in the *sn*-1 and *sn*-3 positions (from 82.5 to 96.2%). The *n*-3 tetra-unsaturated fatty acids and 20:5*n*−3 were also mainly distributed in the *sn*-1 and *sn*-3 positions.

Mobilization rate of fatty acids and their positional distribution in adipose-tissue TAG

According to their mobilization rates, fatty acids have previously been roughly classified into three categories (see the Introduction). Their positional distribution among the three positions of TAG from retroperitoneal adipose tissue is shown in Table 3. Weakly mobilizable fatty acids, i.e. those with 20–24 carbon atoms and 0 or 1 double bond, were typically represented here by VLC-MUFA. In both groups, this class of fatty acids was almost exclusively located in the *sn*-1 plus *sn*-3 positions (95%, MEN group; 91%, HER group), less than 10% being found in the *sn*-2 position. On the other hand, moderately mobilizable fatty acids, i.e. those with 14–18 carbon atoms and 0–3 double bonds, or with 22 carbon atoms and 4–6 double bonds, were more randomly distributed among the three positions of the glycerol molecule in both groups. Finally, highly mobilizable fatty acids, i.e. those with 16–20 carbon atoms and 4 or 5 double bonds, were mainly represented in the present study by 18:4*n*−3, 20:4*n*−3, 20:4*n*−6 and 20:5*n*−3. In both groups, this class of fatty acids was mainly found in the *sn*-1 plus *sn*-3 positions (82%, MEN group; 78%, HER group).

Positional distribution of fatty acids in three different adipose tissues

In addition to the positional distribution of fatty acids in TAG of retroperitoneal adipose tissue, the proportions of 17 fatty acids in the three positions of adipose-tissue TAG were determined in epididymal and mesenteric adipose tissues from one rat of the HER group. The fatty acid composition of the three adipose tissues was similar, as was the positional distribution of all fatty acids (results not shown). As reported in Table 4, and similarly for the three adipose tissues considered, a weakly (20:1*n*−9) as well as a highly (20:5*n*−3) mobilizable fatty acid were preferentially located in the *sn*-1 and *sn*-3 positions, whereas a moderately mobilizable fatty acid (16:0) was found mainly in the *sn*-1 and, to a lesser degree, in the *sn*-2 position.

DISCUSSION

The aim of this study was to examine whether the previously reported selective mobilization of fat-cell fatty acids [1,2], which has already been shown to be unrelated to the tissue location [2], might be explained by their positional distribution in adipose-tissue TAG through the known positional specificity of HSL for the outer (*sn*-1 and *sn*-3) positions. For this purpose, the positional distribution of a large spectrum of fatty acids, including those known to be highly, weakly or moderately mobilizable [2], was determined after manipulating their proportions in adipose tissues. The present study shows that the positional distribution of fatty acids in adipose-tissue TAG (i) is not a random process, (ii) is similar, expressed on a relative basis, for two markedly different fatty acid compositions of adipose-tissue TAG, and (iii) is similar in three differently located white adipose tissues. These observations are in accordance with previous results that reported similar fatty acid distribution patterns among the three positions of the glycerol moiety [9,13,19–21], including those from different adipose-tissue sites [7,8]. The hypothesis on which the present work was based predicts that highly mobilizable fatty acids (20:5*n*−3, 20:4*n*−3, 18:4*n*−3, 20:4*n*−6) would be located mainly in the outer positions, whereas weakly mobilizable fatty acids (20:1*n*−11, 20:1*n*−9, 20:1*n*−7, 22:1*n*−11) would be found mostly in the inner position. Highly mobilizable fatty acids were mainly distributed in the *sn*-1 and *sn*-3 positions, which agrees with our hypothesis. The more even distribution of moderately mobilizable fatty acids (14:0, 16:0, 18:0, 16:1, 18:1, 18:2, 18:3, 22:5*n*−3, 22:6*n*−3) among the three positions of the glycerol molecule is also consistent with the hypothesis that the location of a substantial amount of this class of fatty acids in the *sn*-2 position of TAG might explain their moderate mobilization rate. However, and in marked contrast with the predictions of our hypothesis, weakly mobilizable fatty acids were almost exclusively distributed in the *sn*-1 and *sn*-3 positions. Nevertheless, it should be remembered that the hydrolysis of stored TAG from adipose tissue is a complex metabolic process that involves consecutively HSL and monoacylglycerol lipase, and that both enzymes are required for complete degradation of TAG to non-esterified fatty acids and glycerol [22]. Moreover, the HSL from rat adipose tissue has been reported to hydrolyse preferentially, but not exclusively, the outer positions, thus excluding an absolute specificity of HSL for primary ester bonds [4], whereas monoacylglycerol lipase does not exhibit positional specificity [23–25]. On the whole, our results demonstrate that the positional distribution of fatty acids in TAG cannot explain, even partly, the selective mobilization of fatty acids from fat-cells.

However, this conclusion does not preclude the hydrolysis of TAG as the selective step in the mobilization of fatty acids. Indeed, in addition to its positional specificity, HSL could exhibit other selectivities, including preference for some fatty acids and/or different rates of substrate hydrolysis [26,27]. Such a lipase specificity could also derive from a selective accessibility of the substrate through its limited availability at the lipid/water interface [26,28]. Concerning the preference of the lipase for fatty acids based on their molecular structure, it is known that pancreatic lipase selectively hydrolyses some fatty acids from TAG according to chain length and unsaturation [29], as well as according to their positional isomerism [30]. Therefore, in the present context, an influence of the fatty acid molecular structure by itself in this selective metabolic process cannot be ruled out [3]. As postulated above, different rates of substrate hydrolysis could result in the selective release of fatty acids from substrates [31]. It has been reported that the relative maximal activities of HSL towards tri-, di- and mono-oleoylglycerol were 1:10:4 respectively [24]. These hydrolysis rates were obtained from homogeneous substrates constituted of one single fatty acid, whereas natural TAG are heterogeneous molecules covering a wide spectrum of fatty acids differing by their molecular structure. Therefore, adipose-tissue lipases are confronted with many analogous molecular species of substrates that could exhibit different hydrolytic kinetics [26,31]. Belfrage et al. [24] reported higher relative activity of monoacylglycerol lipase towards mono-octanoylglycerol compared with mono-oleoylglycerol. On the basis of our previous results [1,2], and considered simply, the higher relative activity of HSL towards trieicosapentaenoylglycerol (20:5*n*−3 is a highly mobilizable fatty acid) compared with tridocosenoylglycerol (22:1*n*−11 is a weakly mobilizable fatty acid) could be expected. More generally, substrates enriched in some VLC-PUFA (that include some highly mobilizable fatty acids) would have kinetic advantages leading to their preferential hydrolysis by lipases, in contrast with substrates enriched in VLC-MUFA (which are among the weakly mobilizable fatty acids). On the other hand, a selective mobilization of fatty acids might also result from a limited accessibility of the substrate at the lipid/water interface, where HSL, like most acylglycerol hydrolases, acts [26,28]. One can hypothesize that the most polar TAG (those enriched notably in VLC-PUFA) were more abundant at the interface than in the droplet core. Therefore they would be preferentially hydrolysed by HSL, thus leading to the release of the corresponding fatty acids, the highly mobilizable ones.

In conclusion, our results clearly demonstrate that the selective mobilization of white-fat-cell fatty acids is not based on their positional distribution in TAG. Among the other hypotheses discussed above, and as reported for many lipases [32,33], HSL could have several hydrolytic selectivities for substrates that might explain the pattern of selective fatty acid mobilization previously reported [1,2]. In this context, it would be interesting to examine the influence of the chain length and unsaturation of substrate fatty acids on HSL specificities. It would also be of interest to investigate the fatty acid composition on the most polar TAG fraction susceptible to being preferentially hydrolysed at the lipid/water interface.

We are grateful to E. Mioskowski for her excellent technical assistance and Dr. A. Pape for his help in editing the manuscript. Menhaden and herring oils were generously given by RP Scherer (Beinheim, France) and the Norwegian Herring Oil and Meal Industry Research Institute (Bergen, Norway) respectively.

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Received 24 March 1995/ 27 June 1995; accepted 11 July 1995