Differential mobilization of white adipose tissue fatty acids according to chain length, unsaturation, and positional isomerism

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Abstract The present study aims at determining whether, and how, the molecular structure of fatty acids influences their mobilization from fat cells. The in vitro mobilization of 52 fatty acids ranging in chain length from 12 to 24 carbon atoms, in unsaturation from 0 to 6 double bonds, and including 23 pairs of positional isomers was examined. Fat cells were isolated from adipose tissue of rats fed a fish-oil diet and treated with norepinephrine to stimulate lipolysis. Fatty acid composition of free fatty acids (FFA) released from these cells was compared to that of the triacylglycerols (TAG) from which they originated. The percentage weight of most fatty acids, and especially that of very long-chain fatty acids, was significantly different between FFA and TAG. The percentage of 20:5n-3 (eicosapentaenoic acid) and of 20:4n-6 (arachidonic acid) was 2.7 and 1.7 times higher in FFA than in TAG, respectively, whereas that of 20:1(n-11, 9 or 7), 22:1(n-11, 9 or 7) and 24:1n-9 was 1.7-2, 2.4, and 3.5 times lower, respectively. The relative mobilization (% in FFA / % in TAG) of the least readily mobilized fatty acid (24:1n-9) was 15-fold lower than that of the most readily mobilized (18:5n-3). For a given chain length, the relative mobilization increased exponentially with unsaturation, e.g., increasing from 0.45 to 2.7 in C 20 fatty acids when the number of double bonds increased from 0 to 5. Amongst the fatty acids with 18 to 22 carbon atoms, the shorter the chain was, the more steeply relative mobilization increased with unsaturation. On the other hand, for a given unsaturation the relative mobilization decreased with increasing chain length, e.g., decreasing from 1.15 to 0.3 in monoenes when the chain length increased from C 14 to C 24. A two-carbon-atom shortening of the chain length was on average equivalent to inserting one double bond for increasing the relative mobilization, i.e., by about 40%. The relative mobilization was also affected by the position of the double bond(s); increasing on average by 10% when there was a two-carbon-atom displacement towards the methyl end of the chain. These results demonstrate that under conditions of stimulated lipolysis individual fatty acids are more readily mobilized from fat cells when they are short and unsaturated, and when their double bonds are closer to the methyl end of the chain. The relationships between the molecular structure of fatty acids and their relative mobilization are strongly analogous to those between their structure and their retention time on nonpolar GLC columns. This suggests that the differential mobilization is related to a differential solubility of fatty acids, perhaps in water at the lipid-water interface where hormone-sensitive lipase hydrolyses TAG. As a result, TAG-fatty acids would be differentially accessible to the enzyme and hydrolyzed. The differential mobilization of fatty acids could markedly influence the storage of individual fatty acids in adipose tissue, and their utilization and supply to tissues and organs when lipolysis is enhanced, e.g., during fasting.—**Raclot, T., and R. Groscolas.** Differential mobilization of white adipose tissue fatty acids according to chain length, unsaturation, and positional isomerism. *J. Lipid Res.* 1993. **34:** 1515–1526.

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The first step in the utilization of fatty acids stored in adipose tissue is their release through lipolysis. Whereas a considerable number of studies have dealt with the overall process of total fatty acid release, the mobilization of individual fatty acids has rarely been considered. Depending mainly on the fatty acid composition of the diet (1), adipose tissue triacylglycerols contain up to 60 individual fatty acids varying in chain length, degree of unsaturation, and position of double bonds (2). Are these fatty acids equivalently released during lipolysis, i.e., in direct proportion to their content in the triacylglycerols, or is this release differential and dependent on their molecular structure? This is a very important question because a differential mobilization could greatly influence *1)* the storage and the subsequent utilization of individual fatty acids, and *2)* the type of fatty acids supplied by adipose tissue to other tissues and organs, notably in situations of negative energy balance. The latter possibility is of considerable interest because released fatty acids are not only used as fuels, but also as components of cell

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Abbreviations: GLC, gas-liquid chromatography; PUFA, polyunsaturated fatty acids; FFA, free fatty acids; TAG, triacylglycerols; KRBA, Krebs-Ringer buffer with albumin; TLC, thin-layer chromatography.

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membranes and, for some of them, as precursors of eicosanoids (see Discussion).

The idea that the release of free fatty acids from adipose tissue is not just a random process has been presented previously, from both in vivo and in vitro studies (3, 4). However, the supporting evidence was not strong, with the exception of that from the work of Hollenberg and Angel (5) and of Gavino and Gavino (6). The few other studies on the mobilization of individual fatty acids have provided no evidence for a differential mobilization (7-10). In all these previous studies, only the release of 4 to 8 different fatty acids was considered. Moreover, apart from the recent study of the mobilization of two long-chain polyunsaturated fatty acids (6), only fatty acids with chain length and unsaturation ranging from 14 to 18 carbon atoms and from 0 to 3 double bonds, respectively, have been examined. No study has considered in detail the relative mobilization of very long-chain (20 to 24 carbon atoms) mono- and poly-unsaturated (PUFA, 2 to 6 double bonds) fatty acids, or that of positional isomers. This is a major gap, notably for n-3 PUFAs such as eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) which are readily incorporated into adipose tissue, together with very long-chain monoenes, when ingested as fish oils (11). The consumption of these oils is recommended in human nutrition for their various beneficial health effects (12), but the metabolism of their characteristic n-3 PUFAs in fat cells is poorly understood.

Therefore, the relative mobilization of 52 different fatty acids was studied in vitro by comparing the fatty acid composition of free fatty acids (FFA), released by isolated fat cells, to that of the triacylglycerols (TAG) from which they originated through lipolysis. Rats fed a fish-oil diet were used to obtain adipose tissue with a wide spectrum of individual fatty acids, including very long-chain monoenes and PUFAs. To look for a potential confounding effect of this dietary treatment upon fatty acid mobilization, the same study was done using rats maintained on a laboratory chow. It was found that under both dietary conditions the fatty acids of white adipose tissue are differentially released according to chain length, unsaturation, and positional isomerism: the shorter and more unsaturated fatty acids, with double bond(s) closest to the methyl end of the chain, being more readily mobilized.

METHODS

Animals and diets

Fifteen 7-week-old male Wistar rats (200-220 g, IFFA CREDO, L'Arbresle, France) were housed individually in stainless-steel cages. Six of them (chow group) were maintained for 5 weeks on the same laboratory chow (A04, UAR, Villemoisson, France) as that used from the time of weaning (3 weeks). The total duration of chow-diet feeding was therefore 9 weeks. The nine other rats (fish-oil group) were fed the laboratory chow for 1 week. They were then fed, for 4 weeks, a synthetic diet containing 19% by weight of fish oil (MaxEPA, R.P. Scherer France, Beinheim, France), and 1% of sunflower oil to meet the daily requirement in essential *n*-6 fatty acids. From the results of a previous study (13), this feeding regime was considered adequate to obtain a steady fatty acid composition of retroperitoneal adipose tissue. Other components of the diet were (in % weight) sucrose (45%), casein (25%), agar-agar (4.5%), mineral mixture (4.5%, UAR 205B), and vitamin mixture (1%, UAR 200). The n-3 PUFA content of this diet was 18.4% 20:5n-3, 14.4% 22:6n-3, 2.3% 22:5n-3, and 3.5% 18:4n-3. The content of long-chain monoenes was about 3%. The diet was prepared weekly and stored in daily rations at -20°C under vacuum, with α-tocopherol (40 mg/100 g) as an antioxidant. It was provided ad libitum and renewed daily. There were no significant changes in the fatty acid composition of the diets during 1 week at -20°C or during the 1-day exposure at 25°C in the animal facilities.

Fat cell preparation and incubation

Isolated fat cells were obtained from retroperitoneal adipose tissue. Rats in the post-absorptive state (320-350 g) were killed by cervical dislocation. Samples of adipose tissue were rapidly excised and placed in warm (37°C) Krebs-Ringer bicarbonate buffer, pH 7.4, containing 4% (w/v) of dialyzed bovine serum albumin (Sigma, A 6003, essentially fatty acid-free) and 5 mmol/l of glucose. This buffer, hereafter called KRBA, was used throughout all the remaining experiments. Each adipose tissue sample was cut into small pieces that were used for fat cell isolation according to Rodbell (14) with some minor modifications. About 1 g of adipose tissue was digested at 37°C, for 1 h, in 3 ml KRBA containing 3 mg collagenase (Sigma, type II) under agitation (120 strokes/min) and with O_2/CO_2 95%/5% as the gas phase. The fat cell suspension was filtered through a nylon sieve (250-μm mesh), decanted, and rinsed three times with 10 ml warm KRBA.

About 50 mg fat cells (equivalent to 1.2×10^5 cells with an average 0.4 μg mass) was incubated in 20-ml polypropylene flasks containing 4 ml KRBA and glucose (5 mmol/l). Incubations were done in duplicate for 2 h at 37°C, under O_2/CO_2 95%/5% and with a 120-stroke/min agitation rate. To obtain large amounts of FFA for further analysis, their release was maximally stimulated by adding norepinephrine (the main lipolytic hormone in mammals) at a final concentration of 10^{-6} mol/l. Under these conditions, lipolysis was stimulated by about tenfold and the molar ratio FFA/glycerol in the medium was close to 3/1. This indicates that the major fate of FFA after TAG hydrolysis was their transfer out of the cells. FFA present in the medium at the end of incubation represented the net efflux of FFA. The incubation was stopped by filtering the content of the flask on Whatman glass microfiber filters. Fat cells retained on the filter were rinsed with KRBA to eliminate adhering medium.

Lipid extraction and purification

The lipids from 2.5 ml of incubation medium (i.e., FFA) and from non-incubated fat cells (i.e., TAG), were extracted according to Dole and Meinertz (15) with analytic grade solvents (Merck). Butylated hydroxytoluene (Merck) was added to the extraction solvents at the final concentration of 0.05% to prevent the oxidation of PUFAs. Under the conditions used, FFA production averaged 1 mg per incubation, i.e., equivalent to 2% of TAG-fatty acids from incubated cells. FFA extracts were purified by thin-layer chromatography (TLC). TLC plates coated with Kieselgel 60 (Merck) were used after washing with chloroform–methanol 1:1 (v/v). Five hundred to 1000 μg of FFA extract, corresponding to three-fourths of the total extract, was mixed with 100–200 μg of heptadecanoic acid (Sigma) as an internal standard and applied under nitrogen. The developing solvent system was hexane–diethyl ether–acetic acid 70:30:1 (v/v/v). Though the lipid extract obtained from fat cells was $>$ 99% TAG, it was similarly treated by TLC. After development, TLC plates were quickly dried under nitrogen and sprayed with primulin (Sigma, 0.05 mg/ml in acetone–water 4:1 v/v). The FFA and TAG bands were scraped into capped glass tubes for preparation of fatty acid methyl esters.

Fatty acid analysis

Fatty acids in FFA and TAG were converted to methyl esters using 14% boron trifluoride in methanol (Sigma). TAG were saponified 15 min at 80°C with 0.5 ml of 0.5 mol/l NaOH in methanol under nitrogen. FFA were mixed with 1 ml boron trifluoride solution and heated at 80°C for 15 min under nitrogen. After cooling, 1 ml water and 1 ml pentane were added, followed by agitation and centrifugation at 3500 rpm for 10 min. The upper phase, containing fatty acid methyl esters, was run through a small column containing dried Na_2SO_4 to remove any trace of water. After drying under nitrogen, the sample was dissolved in hexane. Fatty acid methyl esters were separated and quantified by gas-liquid chromatography using a Chrompack CP 9000 gas chromatograph equipped with a flame ionization detector and a Spectra-Physics SP 4290 integrator. Chromatography was performed using an AT-WAX fused silica capillary column (60 m × 0.25 mm I.D.; 0.25 μm thickness, Alltech France). Helium was used as the carrier gas at a flow rate of 2.1 ml/min and an inlet pressure of 230 kPa. The inlet splitter was set at 25:1. The oven temperature was maintained at 210°C and chromatography was completed in 75 min. The injection port temperature was 250°C and that of the detector was 260°C. Fatty acid peaks were identified by comparing their retention times with authentic standards (Nu-Chek Prep, Elysian, MN) and by calculating and plotting Equivalent Chain Length. Moreover, TLC on $AgNO_3$-impregnated silica gel plates and hydrogenation with platinum as catalyst were used to help in determining unsaturation of some fatty acids. Comparison with literature data from studies using the same GLC procedure was also performed. Appropriate corrections were made for the differential response of the flame ionization detector after using reference fatty acid mixture (Nu-Chek Prep). Analyses were run at least in duplicate. To look for a possible contamination of FFA during the overall procedure, a blank extraction was made on five 2.5-ml aliquots of KRBA mixed with 3 μg heptadecanoic acid. The blank lipid extracts were treated exactly as FFA extracts and found to contain, on average, 5 μg of compounds with a retention time identical to that of a fatty acid. These compounds originated mostly from albumin, but TLC and methylation of fatty acids directly on Kieselgel added no further contaminants. The contaminants represented only 0.5% of the mass of total FFA, and corresponded exclusively to a few fatty acids with 16 to 18 carbon atoms and with 0 to 2 double bonds. Though representing less than 5% of the mass of these individual fatty acids, the blank value was deducted before calculating the percentage weight. No contamination was found for the minor fatty acids (percentage weight less than 0.3%) considered in this study. All fatty acids confidently identified and with a percentage higher than 0.01% were considered for analysis.

Statistics

To allow a satisfactory statistical analysis of data on minor fatty acids, all percentage values were calculated to the nearest 1/1000. This does not mean that such an accuracy was warranted. However, note the very low values of SEM in Table 1. This indicates that our analyses were highly reproducible and that there were very low inter-individual differences. Thus, data on very minor fatty acids might be confidently used.

The percentage weight of each fatty acid in FFA and TAG was compared using the two-tailed *t*-test for paired data after transforming the percentage values into arcsin (16). The comparison of relative mobilization between positional isomers was done using the Student's *t*-test. When comparing pairs of positional isomers within a series of three isomers, a conservative Bonferroni approach was used (17) and the criterion of significance was set at 0.017 (i.e., 0.05/3). Otherwise, the criterion of significance was $P < 0.05$. The equation of the curves relating the relative mobilization of fatty acids to their unsaturation at a given chain length was determined for each animal by using a computerized nonlinear regression data analysis program (ENZFITTER, Elsevier-BIOSOFT, U. K.). The means ± SEM of the parameters of individual curves were calculated and compared using the Peritz' *F*-test (18).

RESULTS

Comparison of TAG and FFA composition in fish-oil-fed rats

Only a few fatty acids with chain length lower than 18 C could not be confidently identified, and thus were not considered further. As expected, fish-oil feeding resulted in a wide spectrum of fatty acids and in a marked enrichment in long-chain mono- and n−3 polyunsaturated fatty acids in fat cell TAG (**Table 1**). Compared to chow-fed rats (see Table 3), the percentage weight of tetra-unsaturated fatty acids with 16 and 18 carbon atoms, and of one saturated fatty acid (14:0), was also markedly increased. This was mainly at the expense of 18:2n−6. The percentage weight of long-chain saturated fatty acids (20:0, 22:0) and of 20:4n−6 was unaffected by fish-oil feeding. In total, the mobilization of 52 fatty acids was studied in fish-oil-fed rats. Their chain length ranged from 12 to 24 carbon atoms and their unsaturation ranged from 0 to 6 double bonds. Moreover, 23 pairs of positional isomers, including 6 triplets, were identified among unsaturated fatty acids, as well as 4 saturated fatty acids with the iso configuration and 1 with the anteiso configuration.

Table 1 shows the fatty acid composition of fat cell TAG and of FFA released by these cells. For 36 of the 52 fatty acids that were considered, the percentage weight in FFA was significantly different from that in TAG. Compared to TAG, FFA were enriched in polyunsaturated fatty acids with 18–20 carbon atoms and 4–5 double bonds. For example, the percentage weight of 20:5n−3 and of 20:4n−6 was, respectively, 2.7 and 1.7 times higher in FFA than in TAG. In contrast, the percentage of saturated and monounsaturated fatty acids with 20–24 carbon atoms was 2–3.5 times lower in FFA than in TAG. Fatty acids with 18 carbon atoms or less, and with less than 3 double bonds, differed less in percentage weight in FFA and TAG.

To compare the mobilization of all fatty acids, the ratio between their percentage in FFA to that in TAG was calculated; it was called "relative mobilization." A ratio greater, equal, and lower than one means that the fatty acid is, respectively, more, equally, and less intensively mobilized than the total fatty acids. As shown in Table 1, the relative mobilization of the most intensively mobilized fatty acid (18:5n−3) was 15-fold higher ($P < 0.001$) than that of the least (24:1n−9). Considering only major fatty acids (percentage weight > 1), the relative mobilization of 20:5n−3 was 5-fold higher than that of 20:1n−9 ($P < 0.001$).

Influence of chain length and unsaturation on relative mobilization

Data obtained from fish-oil-fed rats were used to examine in detail the possible influence of the molecular structure of fatty acids on their mobilization. Relative mobilization was plotted versus chain length, at a given unsaturation, and against unsaturation, at a given chain length. In a first step, the position of the double bond(s) was not considered and when there were several positional isomers their average relative mobilization was calculated. This allowed the consideration of a greater number of individual fatty acids in the study of relationships. **Fig. 1** shows that, for a given number of double bonds, the relative mobilization decreased as the number of carbon atoms increased. This decrease was more marked for chain lengths longer than 18 carbon atoms. In addition, relative mobilization fell more steeply (as the chain length was increased) for highly unsaturated compounds compared to those with few double bonds. For example, increasing the chain length from 20 to 22 carbon atoms significantly decreased the relative mobilization by 1.3-, 2.1-, 2.3-, and 3.2-fold in mono-, tri-, tetra-, and penta-unsaturated fatty acids, respectively.

Fig. 2 shows that for a given chain length the relative mobilization increased with the number of double bonds. The increase was more marked for compounds with more than 3 double bonds and with 18 to 20 carbon atoms. Amongst the fatty acids with 18 to 22 carbon atoms, the increase in relative mobilization with increasing unsaturation was best described by an exponential. Each of the three parameters of the equation of the line of best fit differed significantly according to chain length (**Table 2**). Notably, the exponent increased significantly with decreasing chain length. This demonstrates that chain length and unsaturation significantly influence relative mobilization, but in an opposite way.

Even though the influence of chain length was dependent on degree of unsaturation, and vice versa, the average influence on relative mobilization of a two-carbon shortening of the chain or of a one double bond increase in unsaturation was calculated. On average, and without taking account of the degree of unsaturation, subtracting two carbon atoms increases relative mobilization by 44 ± 12% (n = 20 pairs of fatty acids differing in chain length by two carbons). On average, and without taking account of the chain length, adding one double bond increases relative mobilization by 38 ± 8% (n = 19 pairs of fatty acids differing in unsaturation by one double bond). Thus, among the fatty acids studied, the influence of adding one double bond is roughly equivalent to subtracting two carbon atoms. This result only allows the comparison of the average effect of chain length and unsaturation. It cannot be used to predict the relative mobilization of a

TABLE 1. Fatty acid composition of fat cell triacylglycerols (TAG) in fish-oil-fed rats and of free fatty acids (FFA) released by these cells in vitro, and relative mobilization (%FFA / %TAG) of fatty acids

Fatty Acids[a]	Triacylglycerols		Free Fatty Acids	Relative Mobilization
	weight %		*weight %*	
Saturated				
12:0	0.250 ± 0.004		0.256 ± 0.043	1.04 ± 0.10
13:0	0.021 ± 0.001		0.023 ± 0.002	1.10 ± 0.10
14:0	4.571 ± 0.154	*	4.003 ± 0.133	0.88 ± 0.04
15:0	0.569 ± 0.020	*	0.518 ± 0.010	0.92 ± 0.03
16:0	24.347 ± 0.404	**	21.981 ± 0.643	0.90 ± 0.02
18:0	3.486 ± 0.130	***	2.628 ± 0.057	0.76 ± 0.03
20:0	0.116 ± 0.011	***	0.051 ± 0.003	0.46 ± 0.03
22:0	0.041 ± 0.004	***	0.013 ± 0.002	0.32 ± 0.02
Saturated, iso-				
14:0	0.024 ± 0.001		0.022 ± 0.001	0.93 ± 0.06
16:0	0.160 ± 0.009		0.154 ± 0.007	0.97 ± 0.03
17:0	0.189 ± 0.008		0.177 ± 0.016	0.94 ± 0.08
18:0	0.238 ± 0.023	**	0.167 ± 0.004	0.73 ± 0.04
Saturated, anteiso-				
17:0	0.176 ± 0.008		0.190 ± 0.020	1.09 ± 0.10
Mono-unsaturated				
14:1 n-9	0.031 ± 0.003		0.033 ± 0.004	1.10 ± 0.14
14:1 n-7	0.044 ± 0.002	***	0.051 ± 0.003	1.16 ± 0.03
14:1 n-5	0.095 ± 0.007		0.106 ± 0.008	1.12 ± 0.06
16:1 n-9	0.619 ± 0.019		0.571 ± 0.023	0.93 ± 0.04
16:1 n-7	7.864 ± 0.231	***	9.888 ± 0.305	1.26 ± 0.02
16:1 n-5	0.121 ± 0.006		0.137 ± 0.009	1.15 ± 0.08
17:1 n-8	0.636 ± 0.017		0.670 ± 0.027	1.06 ± 0.05
18:1 n-9	18.446 ± 0.390	***	14.792 ± 0.501	0.80 ± 0.02
18:1 n-7	4.114 ± 0.137	***	3.569 ± 0.167	0.87 ± 0.03
18:1 n-5	0.112 ± 0.003		0.118 ± 0.007	1.05 ± 0.05
20:1 n-11	0.250 ± 0.034	***	0.122 ± 0.017	0.49 ± 0.01
20:1 n-9	1.055 ± 0.073	***	0.592 ± 0.042	0.56 ± 0.01
20:1 n-7	0.399 ± 0.039	***	0.233 ± 0.025	0.58 ± 0.01
22:1 n-11	1.131 ± 0.083	***	0.465 ± 0.025	0.41 ± 0.03
22:1 n-9	0.162 ± 0.008	***	0.068 ± 0.003	0.43 ± 0.03
22:1 n-7	0.061 ± 0.004	***	0.027 ± 0.002	0.44 ± 0.04
24:1 n-9	0.092 ± 0.008	***	0.026 ± 0.004	0.28 ± 0.03
Di-unsaturated				
18:2 n-6	12.600 ± 0.636	*	10.440 ± 0.608	0.84 ± 0.06
18:2 n-4	0.302 ± 0.011	**	0.361 ± 0.013	1.20 ± 0.05
20:2 n-9	0.231 ± 0.013	***	0.149 ± 0.009	0.65 ± 0.02
20:2 n-6	0.171 ± 0.004	***	0.134 ± 0.006	0.79 ± 0.04
22:2 n-6	0.168 ± 0.007	***	0.087 ± 0.012	0.51 ± 0.07
Tri-unsaturated				
18:3 n-6	0.160 ± 0.004	*	0.178 ± 0.009	1.11 ± 0.04
18:3 n-4	0.232 ± 0.005		0.264 ± 0.015	1.14 ± 0.07
18:3 n-3	0.809 ± 0.032	***	1.025 ± 0.032	1.27 ± 0.08
20:3 n-3	0.049 ± 0.002		0.050 ± 0.002	1.00 ± 0.02
22:3 n-6	0.060 ± 0.010	**	0.032 ± 0.071	0.54 ± 0.10
Tetra-unsaturated				
16:4 n-3	0.093 ± 0.003	***	0.184 ± 0.019	1.95 ± 0.14
18:4 n-3	1.145 ± 0.044	***	1.697 ± 0.130	1.49 ± 0.11
18:4 n-1	0.294 ± 0.007	***	0.577 ± 0.031	1.97 ± 0.10
20:4 n-6	0.681 ± 0.052	***	1.150 ± 0.091	1.69 ± 0.04
20:4 n-3	0.612 ± 0.020	***	0.900 ± 0.034	1.47 ± 0.05
22:4 n-6	0.177 ± 0.009	***	0.122 ± 0.018	0.69 ± 0.01
Penta-unsaturated				
18:5 n-3	0.022 ± 0.002	***	0.093 ± 0.009	4.25 ± 0.33
20:5 n-3	4.275 ± 0.226	***	11.681 ± 0.946	2.70 ± 0.15
21:5 n-3	0.306 ± 0.007	**	0.418 ± 0.017	1.37 ± 0.05
22:5 n-6	0.207 ± 0.011	***	0.146 ± 0.004	0.72 ± 0.03
22:5 n-3	1.527 ± 0.065		1.355 ± 0.054	0.89 ± 0.02
Hexa-unsaturated				
22:6 n-3	6.089 ± 0.280		6.224 ± 0.266	1.03 ± 0.03

Values are means ± SEM (n = 9). The third decimal place is given *1)* to help in understanding the results of the statistical analysis of data on very minor fatty acids (see Statistics), and *2)* to allow an accurate calculation of their relative mobilization.

[a] Number of carbon atoms:number of double bonds, position of the first double bond from the methyl end of the molecule. Asterisks between triacylglycerol and free fatty acid values indicate a significant difference in the percentage of the fatty acid between the two classes of lipids: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

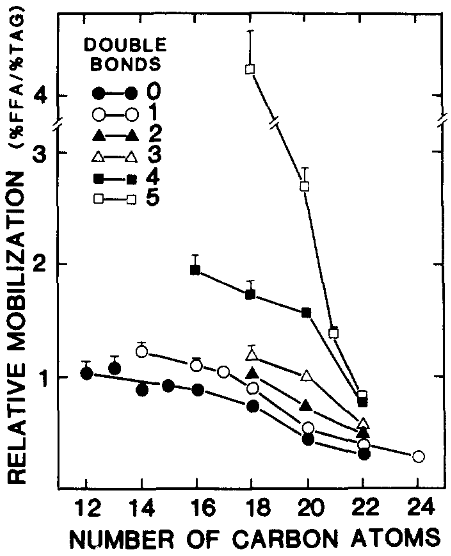

Fig. 1. Relationships between chain length and relative mobilization from fat cells of fatty acids at given unsaturations. The position of the double bond(s) was not considered and when there was more than one positional isomer the average value of relative mobilization was calculated. T-bars show SEM (n = 9); when not shown, SEM was smaller than the size of the symbol.

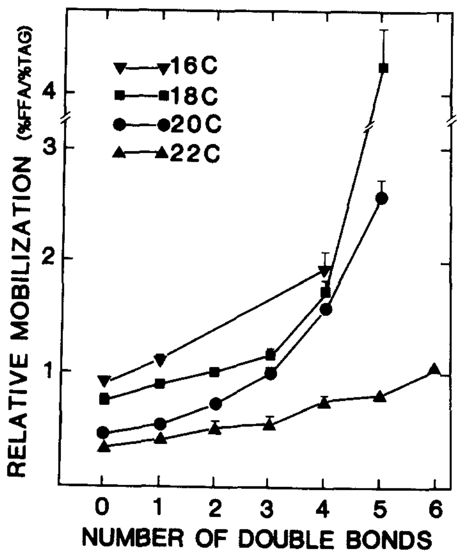

Fig. 2. Relationships between unsaturation and relative mobilization from fat cells of fatty acids at given chain lengths. T-bars show SEM (n = 9). Legend as in Fig. 1.

given fatty acid from its molecular structure. The equations relating relative mobilization to unsaturation at given chain length (Table 2) should be used for this purpose.

Influence of positional isomerism and of iso and anteiso configuration

A detailed analysis of the relationships between positional isomerism and relative mobilization was not possible in this study. Indeed, for given chain lengths and unsaturations no more than three positional isomers were available. Thus, only average calculations were done by pooling data from all positional isomers, regardless of absolute chain length and unsaturation. The influence of the position of the double bond(s) on relative mobilization was examined by pairwise comparison of positional isomers. Twenty-three pairs of positional isomers were compared; a triplet of isomers yielding 3 pairs. Within each pair, the positional isomer with the double bond(s) closest to the methyl end of the chain had a relative mobilization significantly higher for 9 pairs, nonsignificantly higher for 11 pairs, nonsignificantly lower for 2 pairs, and significantly lower for 1 pair. Thus, there was a general trend for the displacement of the double bond(s) towards the methyl end to result in an increase in relative mobilization (see Fig. 3). The average increase in relative mobilization (following, for example, a 2- or 3-carbon-atom displacement towards the methyl end) did not depend significantly upon the absolute position of the displacement within the carbon chain (e.g., from carbon 9 to carbon 7 or from carbon 3 to carbon 1). Among the 23 pairs of positional isomers, the position of the double bond(s) differed by 2.5 ± 0.3 carbon atoms. On average, a 2.5-carbon-atom displacement of the double bond(s) towards the methyl end of the chain resulted in a 13 ± 4% increase of the relative mobilization. This roughly corresponds to a 10% increase for a 2-carbon-atom displacement. Therefore, positional isomerism influences fatty acid mobilization, although far less than chain length and unsaturation. On average, an 8-carbon displacement of the double bond(s) towards the methyl end of the chain is necessary to yield an increase in relative mobilization equivalent to that resulting from a 2-carbon shortening of the chain or a 1-double bond increase in unsaturation.

TABLE 2. Parameters of the equations of the best-fit curves relating relative mobilization of fatty acids to their unsaturation at given chain lengths

	Parameters of the Equations		
Chain Length	b	a	k
18 C	0.79 ± 0.02	0.02 ± 0.01	1.08 ± 0.08
20 C	0.46 ± 0.02	0.13 ± 0.05	0.64 ± 0.05
22 C	0.34 ± 0.02	1.11 ± 0.42	0.23 ± 0.06

The relationship of relative mobilization (Y) to number of double bonds (n) was best described by the equation: $Y = b + a(\exp^{k \cdot n} - 1)$, in which the Y-intercept b is the relative mobilization of the saturated fatty acid. Data obtained from fish-oil-fed rats were used in calculations. Values are means ± SEM (n = 9). The three values of b and k were significantly different, $P < 0.0001$; the three values of a were significantly different, $P < 0.05$ or less. Calculations were not made for 16 C fatty acids because of the limited number of double bonds. The corresponding curves are shown in Fig. 2.

This conclusion is based on the calculation of the average influence of positional isomerism. Of course, this does not preclude the possibility that the influence of positional isomerism on relative mobilization depends on unsaturation and/or chain length. This is suggested by the calculation that a 2-carbon-atom displacement of the double bond(s) towards the methyl end of the chain resulted in a lower increase in relative mobilization in monoenes (8.4 ± 4.0%, n = 10) than in polyunsaturated fatty acids (21.4 ± 5.8%, n = 6; P = 0.08).

No significant influence of the iso or anteiso configurations of saturated fatty acids was found. However, the relative mobilization tended to be higher in the iso than in the normal configuration and higher in the anteiso than in the iso one. It must be stressed that only 5 fatty acids with chain length equal or less than 18 carbon atoms could be compared and that the relative mobilization of these types of fatty acids was not markedly different.

Relative mobilization in rats maintained on a laboratory chow

Only 27 fatty acids were identified in adipose tissue of rats maintained on a laboratory chow diet from weaning to 12 weeks of age. The percentage weight of long-chain mono- and polyunsaturated fatty acids was very low, usually not exceeding 0.5%. Nevertheless, a differential mobilization of fatty acids very similar to that found in fish-oil-fed rats was observed (**Table 3**). The percentage

TABLE 3. Fatty acid composition of fat cell triacylglycerols (TAG) in rats maintained on a laboratory chow and of free fatty acids (FFA) released by these cells in vitro, and relative mobilization (%FFA / %TAG) of fatty acids

Fatty Acids[a]	Triacylglycerols		Free Fatty Acids	Relative Mobilization
	weight %		*weight %*	
Saturated				
14:0	1.521 ± 0.039		1.668 ± 0.079	1.10 ± 0.06
15:0	0.392 ± 0.020		0.433 ± 0.018	1.11 ± 0.02
16:0	25.644 ± 0.772		27.340 ± 0.623	1.07 ± 0.02
18:0	3.005 ± 0.152	**	2.364 ± 0.123	0.79 ± 0.03
20:0	0.069 ± 0.003	***	0.028 ± 0.001	0.40 ± 0.03
22:0	0.032 ± 0.001	**	0.012 ± 0.001	0.38 ± 0.01
Mono-unsaturated				
16:1 n-7	4.834 ± 0.473	**	7.435 ± 0.585	1.54 ± 0.06
17:1 n-8	0.326 ± 0.028	*	0.420 ± 0.027	1.29 ± 0.02
18:1 n-9	22.596 ± 0.573	*	20.282 ± 0.691	0.90 ± 0.02
18:1 n-7	3.308 ± 0.212		3.446 ± 0.088	1.06 ± 0.08
18:1 n-5	0.089 ± 0.001		0.096 ± 0.002	1.09 ± 0.06
20:1 n-11	0.110 ± 0.001	***	0.059 ± 0.002	0.53 ± 0.06
20:1 n-9	0.435 ± 0.076	***	0.247 ± 0.044	0.58 ± 0.02
20:1 n-7	0.468 ± 0.064	**	0.273 ± 0.025	0.58 ± 0.02
22:1 n-11	0.093 ± 0.083	***	0.044 ± 0.008	0.48 ± 0.05
Di-unsaturated				
18:2 n-6	31.987 ± 1.114		28.974 ± 1.047	0.91 ± 0.01
20:2 n-6	0.291 ± 0.018	*	0.234 ± 0.023	0.78 ± 0.02
Tri-unsaturated				
18:3 n-6	0.089 ± 0.008		0.095 ± 0.009	1.08 ± 0.05
18:3 n-3	1.784 ± 0.034	***	2.535 ± 0.102	1.42 ± 0.04
20:3 n-3	0.028 ± 0.004		0.033 ± 0.002	1.19 ± 0.08
22:3 n-6	0.062 ± 0.010	*	0.033 ± 0.081	0.53 ± 0.09
Tetra-unsaturated				
20:4 n-6	0.693 ± 0.072	*	1.220 ± 0.241	1.76 ± 0.21
20:4 n-3	0.019 ± 0.006		0.033 ± 0.004	1.74 ± 0.36
22:4 n-6	0.183 ± 0.013		0.147 ± 0.030	0.79 ± 0.01
Penta-unsaturated				
20:5 n-3	0.122 ± 0.008	***	0.335 ± 0.028	2.76 ± 0.15
22:5 n-3	0.294 ± 0.025		0.275 ± 0.034	0.93 ± 0.09
Hexa-unsaturated				
22:6 n-3	0.574 ± 0.039		0.665 ± 0.091	1.15 ± 0.12

Values are means ± SEM (n = 6).

[a] Number of carbon atoms:number of double bonds, position of the first double bond from the methyl end of the molecule. Asterisks between triacylglycerol and free fatty acid values indicate a significant difference in the percentage of the fatty acid between the two classes of lipids: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

weight of 15 fatty acids was significantly different between FFA and TAG. The values of relative mobilization in chow-fed rats were similar or even the same as those measured in fish-oil-fed rats. Notably, no significant difference was found between the two groups for the 8 long-chain mono- and n-3 polyunsaturated fatty acids, the percentage weight of which was the most affected by fish-oil feeding. This includes the more (20:5n-3) and the less (20:1 and 22:1) readily mobilized fatty acids. Despite an almost 3-fold lower percentage weight, the relative mobilization of 18:2n-6 in chow-fed rats was also the same as in fish-oil-fed rats. Lastly, relationships very similar to that calculated in fish-oil-fed rats were found between relative mobilization and chain length or unsaturation (not shown). Thus, a similar differential mobilization of fatty acids was found under two markedly different dietary situations.

DISCUSSION

This study is the first to compare the mobilization from fat cells of almost all the fatty acids that can be found in adipose tissue of mammals. This includes long- and very long-chain mono- and polyunsaturated fatty acids. It is also the first to detail the relationships between the mobilization of fatty acids and their chain length, unsaturation, and positional isomerism. Our results clearly demonstrate that under conditions of stimulated lipolysis in vitro fatty acids are not mobilized from fat cells in direct proportion to their content in TAG, but differentially according concurrently to chain length, unsaturation, and positional isomerism. As a rule, fatty acids are more readily mobilized when they are short and unsaturated, and when their double bonds are closer to the methyl end of the chain. This differential mobilization is not related to the fatty acid content or to the interval administration of the diet.

Methodological considerations

Why has a differential mobilization of fatty acids not been clearly demonstrated and thoroughly characterized previously? In this study, the range of relative mobilization was very wide (0.32-4.25) for fatty acids with chain length longer than 18 carbon atoms and with unsaturation higher than 3 double bonds. On the other hand, among fatty acids with 14 to 18 carbon atoms and 0 to 3 double bonds, there were not such large differences, the relative mobilization ranging from 0.76 to 1.26 (i.e., only a 66% difference). Only the latter types of fatty acids have been considered in earlier studies on mobilization of individual fatty acids. This probably explains the previous failures to demonstrate clear and consistent relationships between fatty acid molecular structure and mobilization. However, Hollenberg and Angel (5) obtained results comparable to ours. They found that after incubation of rat adipose tissue with corticotrophin, the ratio of the amount of each free acid in the medium to that in the tissue increased with decreasing chain length and increasing unsaturation. Unfortunately, this was based on the comparison of only a few fatty acids and was not supported by statistical analysis of the data. Moreover, in this study as in others, the positional isomerism was not considered. This is mostly because positional isomers were not separated in pioneer studies because of the lower resolution of GLC columns. Very recently, a preferential release of two polyunsaturated fatty acids (18:3n-3 and 20:4n-6) over three other fatty acids (16:0, 18:1n-9 and 18:2n-6) has been reported (6). These limited data fit the general pattern of differential mobilization demonstrated here. However, the conclusion of the authors that "hormone-sensitive lipase preferentially releases polyunsaturated fatty acids from triglycerides" seems an over-generalization as in the present study polyunsaturated fatty acids with 22 carbon atoms were not preferentially released.

Our demonstration of a differential mobilization of fatty acids was largely based on data obtained from rats changed from a laboratory chow diet to a fish-oil diet. It could be argued that this may have some confounding effects. This is because it is generally accepted that recent dietary fatty acids may be mobilized more readily (19, 20). Actually, the finding of a similar differential mobilization by using rats maintained on a chow diet from weaning allows this possibility to be ruled out. This conclusion is reinforced by the observation that in fish-oil-fed rats recent dietary fatty acids were either preferentially (20:5n-3, 18:4n-3), equivalently (22:5n-3, 22:6n-3) or less (20:1, 22:1) released in comparison to total fatty acids. A third argument is that a similar differential mobilization of fatty acids has been observed without any dietary treatment in a wild seabird, the emperor penguin, that feeds on fish throughout all its life (21). All these observations argue against the "last-in first-out" hypothesis as an explanation for the differential mobilization of fatty acids demonstrated here. Perhaps, in short-term experiments a preferential release by adipose tissue of recently uptaken fatty acids has been observed because they do not mix immediately with the bulk of lipids (19). In contrast, during long-term dietary treatments such as that used in this study, a thorough mixing within the various pools occurs. This is reflected by the achievement of a steady fatty acid composition of adipose tissue TAG.

Molecular structure of fatty acids also influences other aspects of their metabolism and in vivo mobilization

Other aspects of fatty acid metabolism have been reported to depend on their chain length and unsaturation. This includes their total rate of oxidation in vivo, that of saturated fatty acids decreasing with increasing chain length (22), and their absorption and intestinal catabolism, the shorter and more unsaturated fatty acids being more oxidized (23). The capacity of fatty acids to bind to proteins, e.g., albumin (24), and to modify the binding of various hormones to their transport proteins (25) or their receptors (26) also depends on their chain length and unsaturation. Only a limited number of fatty acids were compared in the latter studies, similarly to previous studies on fatty acid mobilization. Thus, to our knowledge, our study is the first to give a detailed analysis of the relationships between the molecular structure of fatty acids and one aspect of their metabolism.

On the other hand, the present data are in full agreement with the recent demonstration, both in vitro and in vivo, of a similar differential mobilization of fatty acids from subcutaneous adipose tissue in a long-term fasting wild bird, the emperor penguin (21). They are also consistent with the finding that the adipose tissue of long-term fasting rats becomes proportionately enriched and depleted in fatty acids with low and high relative mobilization, respectively (T. Raclot, R. Groscolas and E. Mioskowsky, unpublished data). Thus, the structure-related differential mobilization of fatty acids does not seem to be a unique property of retroperitoneal adipose tissue and to be restricted to mammals or to in vitro conditions. Interestingly, it is operating in vivo, at least in situations of enhanced fat store mobilization.

Basis of differential mobilization: a differential aqueous solubility of fatty acids?

The relationships presently found between fatty acid molecular structure and relative mobilization strongly resemble their pattern of retention times on nonpolar GLC columns. On this type of column, for a given unsaturation and positional isomerism, the retention time of fatty acids increases exponentially with increasing chain length (2). On the other hand, for a given chain length and positional isomerism, retention time decreases with increasing unsaturation. The retention time of unsaturated fatty acids is also governed, though to a lesser extent, by the position of the double bond(s). The analogy between the relative mobilization of fatty acids and their retention on nonpolar GLC columns is illustrated in **Fig. 3**; the "retention" of individual fatty acids within fat cells during lipolysis (the reciprocal of their relative mobilization, that is % in TAG/% in FFA) is plotted in a manner similar to the retention time on GLC chromatograms. As for GLC on nonpolar columns, at a given unsaturation, the "retention" of fatty acids increases with chain length and at a given chain length it decreases with unsaturation. The differential retention of fatty acids on a nonpolar GLC column is due to their different partition coefficient, or solubility, between a nonpolar stationary liquid phase

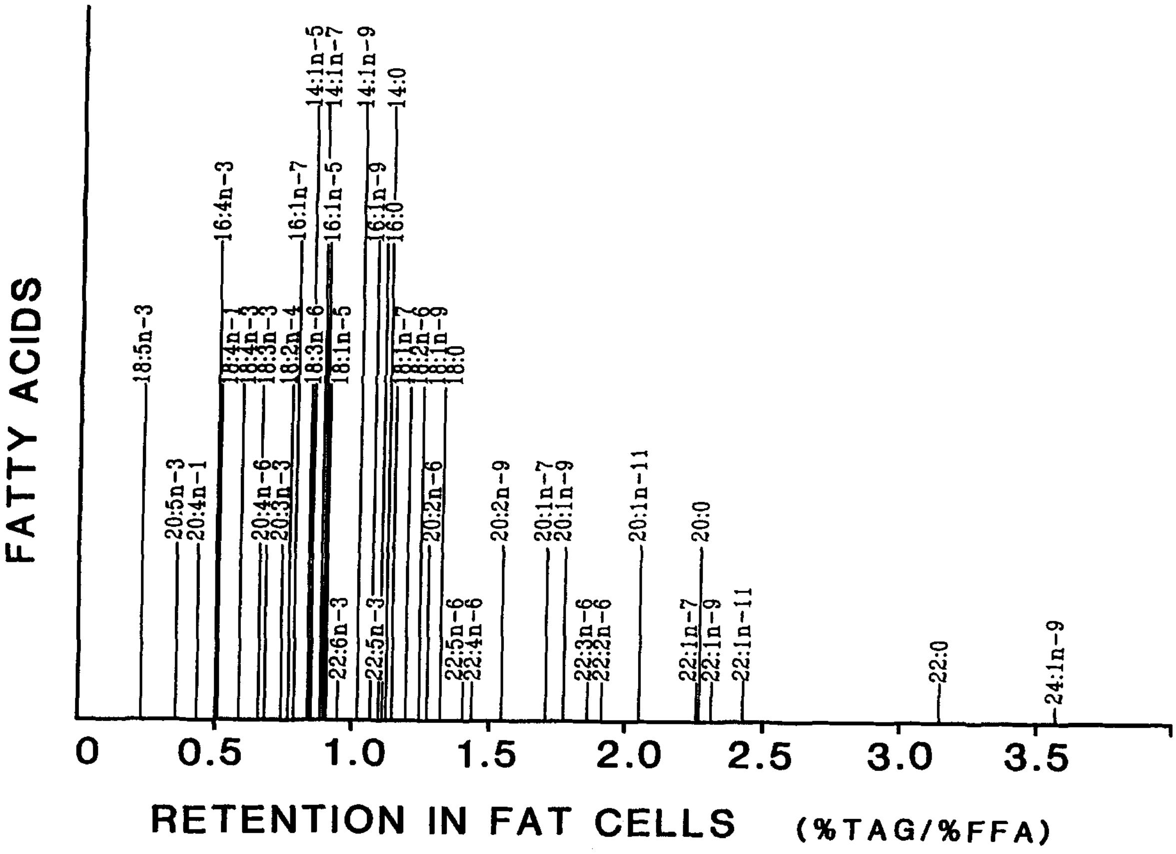

Fig. 3. GLC-like "retention" of fatty acids in fat cells during lipolysis.

and a mobile gaseous phase. The shorter and more unsaturated fatty acids are more polar (5) and thus are retained less in the nonpolar phase. By analogy, we suggest that the lower "retention" in fat cells (i.e., the higher mobilization) of the more polar fatty acids is due to a lower solubility in a nonpolar phase, or to a higher solubility in a polar phase.

The nonpolar and polar phases between which fatty acids can partition according to their polarity could be the lipid droplet containing TAG and the aqueous cytoplasm containing lipases, respectively. The hydrolysis of TAG is the rate-limiting step in fatty acid mobilization (27), is catalyzed by hormone-sensitive lipase (28), and occurs at the interface of these two phases (29, 30). We suggest that in the lipid droplet TAG are not distributed randomly but according to their polarity. The more polar TAG (the richer in polar fatty acids) would be preferentially located at the periphery and, as a consequence, polar TAG-fatty acids would be preferentially accessible to hormone-sensitive lipase and hydrolyzed. This meets the suggestion of Brockman (29) that the apparent specificity of lipolytic enzymes may primarily reflect the physical availability of substrate to the enzyme at the lipid–water interface. According to our hypothesis, the hydrolysis of the more polar fatty acids by hormone-sensitive lipase would be favored (preferential) but not stringent. Indeed, TAG molecules are highly heterogenous and less polar fatty acids (e.g., very long-chain saturated and monounsaturated) can be acylated on the same glycerol backbone as more polar ones, and thus made accessible to lipases. To our knowledge, this is the first suggestion that a differential release of fatty acids from fat cells can result from a distribution of TAG in the lipid droplet according to their polarity. On the other hand, a differential hydrolysis of TAG has been previously suggested. However, it has been related to some specificity of fatty acid distribution in the TAG molecule (6), notably in relation to the positional preference of lipases (31). The determination of the positional distribution of fatty acids in triacylglycerols from adipose tissue of rats fed a chow or a fish-oil diet (13) gave no supporting evidence for this hypothesis.

A differential aqueous solubility of fatty acids could also intervene at other sites in the fat cell and/or at other steps of mobilization. A major candidate is the transfer of free fatty acids to plasma membrane and then extracellular space by cytosolic and/or membrane fatty acid-binding proteins (FABP). These transport proteins have been characterized in various cells, including fat cells (32, 33), and the mechanism of transfer of FFA from cytosolic FABP to membranes has been recently suggested to be most likely through aqueous phase diffusion (34). In full agreement with the present findings, the shorter and more unsaturated fatty acids (i.e., the more water-soluble) were found to be more rapidly transferred. As a result of this higher transfer, the more water-soluble fatty acids would be less available for intra-cell re-esterification into TAG, and thus preferentially released from the cell. Whether the differential mobilization of fatty acids results from a differential hydrolysis of TAG or from a differential transfer of FFA cannot be presently stated. However, it is very likely that the basis of the differential mobilization of fatty acids is a differential aqueous solubility, that is a differential physicochemical property.

Methodological and physiological consequences

Several consequences result from the present demonstration of a differential mobilization of individual fatty acids. From a methodological point of view, this stresses the need for a careful choice of the tracer fatty acid when studying the metabolism of total fatty acids in adipose tissue, e.g., their rate of uptake or of release, by using only one individual fatty acid. Our results show that among fatty acids stored in adipose tissue of rats fed fish oils, docosahexaenoic acid (22:6 n−3) has a relative mobilization closest to 1, and thus is probably the more representative of total fatty acids with respect to their rate of mobilization. Palmitic (16:0) or oleic (18:1 n−9) acids are commonly used as tracers in studying fatty acid metabolism in adipose tissue, and our results indicate that they can be, respectively, 10 and 20% less easily mobilized than total fatty acids. More dramatically, their use as tracers could lead to a 2- to 3-fold under- and overestimation of the rate of release of specific fatty acids such as eicosapentaenoic acid and long-chain monoenes, respectively.

From a physiological point of view, a differential mobilization probably affects the storage of individual fatty acids. This could partly explain the observation that the partitioning of dietary fatty acids between storage and oxidation varies according to chain length and unsaturation (35, 36). For example, it has been shown that polyunsaturated fatty acids are oxidized as fuel sources more rapidly, and are less stored, than are saturated long-chain fatty acids (37). Similarly, the tendency of short-chain (38) or unsaturated (39) dietary fatty acids to reduce body mass gain or adipose tissue growth could result partly from a preferential mobilization, and thus from a lesser storage, of these types of fatty acids. The differential mobilization of fatty acids could greatly affect the qualitative supply of tissues and organs with fatty acids in the post-absorptive state and in situations of negative energy balance such as fasting. This could be of great metabolic significance if we consider that the two major precursors of eicosanoids, i.e., arachidonic and eicosapentaenoic acids, are very preferentially mobilized from adipose tissue. The fatty acid composition of cell membranes, and thus cell metabolism, could also be markedly influenced.

Because of these considerable potential methodological and physiological consequences, the mobilization of individual fatty acids should be further characterized. It is particularly important to determine whether the differential mobilization operates under conditions of basal lipolysis and not only when fatty acid release is enhanced. Hormone-sensitive lipase has recently been suggested to exhibit a preference for certain triacylglycerol species under stimulated conditions, but little preference under basal conditions (40). Is the relative mobilization of fatty acids dependent on the relative proportion of individual fatty acids in adipose tissue; is differential mobilization the same in the various fat depots; and what is the precise underlying mechanism, are also questions that deserve further study.

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