Selective mobilization of adipose tissue fatty acids during energy depletion in the rat

Thierry Raclot and René Groscolas[1]

Centre d'Ecologie et Physiologie Energétiques, CNRS, associé a l'Université Louis Pasteur, 23 rue Becquerel, 67087 Strasbourg, France

Abstract This study extends our previous work (Raclot, T., and R. Groscolas. 1993. *J. Lipid Res.* **34:** 1515–1526) which demonstrated that in the fed state fatty acids are selectively released from white adipocytes in vitro. It aims at determining whether such selectivity operates in vivo during energy depletion and has physiological relevance. This question was examined in rats by simultaneously measuring, after 1, 7, or 10 days of fasting, the fatty acid content of retroperitoneal adipose tissue (RP), and the composition of fatty acids released by isolated RP adipocytes. A preliminary dietary manipulation (fish oil feeding) allowed us to study the mobilization of a wide spectrum of fatty acids. Fasting resulted in a relative depletion of adipose tissue in fatty acids such as α-linolenic, arachidonic, and eicosapentaenoic, and in a relative enrichment in all very long chain saturated and monounsaturated fatty acids. After a 56% depletion of total fatty acids, 20% (22:1n–11) to 90% (20:5n–3) of the initial mass of individual fatty acids was lost. The in vivo relative mobilization of fatty acids (% in lost fatty acids / % in RP triacylglycerols) ranged from 0.31 to 2.54. For a given chain length it increased with unsaturation whereas for a given degree of unsaturation it decreased with chain length. The in vitro relative mobilization of fatty acids (% in released fatty acids/% in RP triacylglycerols) was similarly dependent on their molecular structure and, to a significant extent, directly related to in vivo mobilization. It is concluded that during fasting-induced energy depletion, the net in vivo mobilization of fatty acids from adipose tissue is selective. The selectivity of mobilization *i)* is based on the molecular structure of fatty acids, *ii)* is fully accounted for by their selective release from adipocytes, *iii)* leads to a profound remodelling of the composition of adipose tissue fatty acids, and *iv)* does not seem directed towards a preferential retention or sparing of particular fatty acids.—**Raclot, T., and R. Groscolas.** Selective mobilization of adipose tissue fatty acids during energy depletion in the rat. *J. Lipid Res.* 1995. **36:** 2164–2173.

Supplementary key words fatty acid metabolism and structure • fasting • adipocytes • lipolysis

Fatty acids stored as triacylglycerols (TAG) in adipose tissues are massively mobilized as energy sources during periods of energy deficit. This includes prolonged exercise (1), fasting (2), cachexia associated with pathological conditions such as cancer (3), and dietary restriction in obese persons (e.g., very low calorie dieting) (4, 5). Mobilized fatty acids, including essential fatty acids, are also used for the turnover of cell membrane lipids and some of these acids also serve as precursors of lipid mediators (e.g., eicosanoids) (6). Whereas the overall process of fatty acid mobilization from adipose tissue (lipolysis) has received considerable attention, the mobilization of individual fatty acids has not been frequently studied. In particular, whether stored fatty acids are equivalently or selectively released and how the qualitative supply of free fatty acids (FFA) to the circulation by adipose tissues depends on their content of individual fatty acid is not well known.

Earlier in vivo and in vitro studies on individual fatty acid mobilization have considered a very limited number of fatty acids (five to seven fatty acids with 14–18 carbon atoms and 0–2 double bonds) and have yielded conflicting results. Fatty acids were found either randomly (7–10) or selectively (11–13) mobilized, and data on this selectivity are inconsistent. Thus, until recently, the view that fatty acid mobilization was a random process was generally accepted (e.g., 5). This view has been partly challenged by recent results. A preferential loss of α-linolenic acid from human fat stores has been observed during diet-induced weight loss (4, 5, 14). Rat adipose hormone-sensitive lipase (HSL) has been reported to preferentially release several polyunsaturated fatty acids from TAG (15). More thorough evidence was provided by the demonstration of a differential mobilization of fatty acids from adipocytes in vitro on grounds of molecular structure (16). From a comparison of the release of 52 fatty acids, we found that, as a rule, fatty acids are more readily mobilized when they are short, unsaturated, and have double bonds close to the terminal methyl group of the chain. However, whether such

Abbreviations: RP, retroperitoneal adipose tissue; TAG, triacylglycerols; FFA, free fatty acids; HSL, hormone-sensitive lipase.

[1]To whom correspondence should be addressed.

selectivity operates in vivo and has physiological relevance is unknown.

The purpose of the present study was *1)* to examine the in vivo mobilization of individual fatty acids from adipose tissue during a prolonged energy depletion, and *2)* to determine whether this mobilization is affected by the molecular structure of fatty acids, with dependence upon a selective release from adipocytes. The net in vivo loss and the net in vitro release as FFA of 39 fatty acids were studied from rat retroperitoneal adipose tissue (RP) after 7 and 10 days of fasting, i.e., at a medium and high level of energy depletion. This large number of fatty acids included very long chain mono- and n-3 polyunsaturated fatty acids. As the metabolic properties and the sensitivity to energy depletion of adipose tissue differ according to its location (17–19), the mobilization of fatty acids from subcutaneous, epididymal, and mesenteric adipose tissues was also examined by determining the fasting-induced changes in their fatty acid composition.

METHODS

Animals and diets

Twenty four 2-month-old male Wistar rats (200 g, IFFA CREDO, L'Arbresle, France) were housed individually in plastic cages with a wire-mesh bottom, at 25 ± 1°C and under a 12-h light/dark cycle. The animals were maintained for 1 week on the same standard laboratory diet (AO4, Usine d'Alimentation Rationnelle, Villemoisson, France) as that fed since weaning. The fatty acid composition of the diet has been described previously (20). Then, 6 rats (control group) were killed and used to determine the fatty acid composition of adipose tissues in animals fed the standard diet. The 18 remaining rats were fed for 3 weeks on the powdered standard diet supplemented with a mixture of MaxEPA oil (RP Scherer, Beinheim, France) and herring oil (Norwegian Herring oil and Meal Industry Research Institute, Bergen, Norway). This dietary manipulation aimed at obtaining adipose tissues with a wide spectrum of individual fatty acids, including very long chain mono- and n-3 polyunsaturated fatty acids. Based on a previous study (21), we estimated that this duration of fish oil feeding was sufficient for the achievement of a new and steady fatty acid composition of adipose tissue. The proportions in the fish oil diet were 100 g standard diet/34 g MaxEPA oil/4 g herring oil. The gross composition of the fish oil diet, by weight, was 30% lipid, 15% protein, and 48% carbohydrate, the remaining being cellulose, minerals, and vitamins. Seven and 32%, by weight, of its fatty acids were very long chain mono- and n-3 polyunsaturated fatty acids, respectively. The fish oil diet was prepared weekly and stored in daily rations at -20°C, with α-tocopherol (40 mg/100 g) as an antioxidant. It was provided ad libitum and replaced daily.

At the end of the dietary treatment, the 18 rats fed the fish oil diet were randomly matched as groups of 3 and within each group an individual rat was randomly and totally fasted for 1 (overnight), 7, or 10 days (6 animals at each time). Rats fasted overnight (day 1 of fasting) were used as fish oil-fed rats. Overnight fasting standardizes individual rats with respect to the metabolic state of their adipose tissue. Because such a short fast causes insignificant depletion of adipose tissue (22), it probably does not affect its fatty acid composition (see Discussion). The other periods of fasting were chosen from Belkhou et al. (23), who reported that rats of the same strain and of similar body mass can tolerate 13-day fasting and a 40% body mass loss without detrimental effects, as demonstrated by the capacity of the animals to be successfully refed. In this case, a safety margin was provided by limiting the duration of fasting to 10 days. Tap water was given ad libitum and body mass was measured daily. This protocol was approved by the local Ethical Committee, and followed the CNRSs (Centre National de la Recherche Scientifique) guide for the care and use of laboratory animals.

Tissue sampling and lipid extraction

Rats were killed by cervical dislocation. RP of fasted rats was rapidly and totally dissected and weighed to the nearest milligram. It was minced with scissors and a representative aliquot was extracted according to Folch, Lees, and Sloane Stanley (24). The lipid content of RP was determined gravimetrically after solvent evaporation under vacuum. Fifty- to 100-mg samples of epididymal, mesenteric, and subcutaneous (inguinal) adipose tissue from fasted rats, and of RP from rats maintained on the standard diet, were also taken and extracted. Lipid extracts were dissolved in chloroform–methanol 2:1 (v/v) and kept at -20°C under nitrogen until analysis. Analytical grade solvents supplemented with butylated-hydroxytoluene as an antioxidant were used throughout the experiment.

In vitro mobilization of fatty acids

This experiment was designed to determine at the three stages of fasting (days 1, 7, and 10) the fatty acid composition of FFA released by isolated RP adipocytes in comparison to that of TAG from which they originated through lipolysis. The procedure was as previously reported (16). Briefly, a 100- to 500-mg sample of fresh RP was rapidly digested with collagenase (type II, Sigma, L'Isle d'Abeau Chesnes, France), filtered through a nylon sieve, decanted, and rinsed to obtain

isolated adipocytes. A 30- to 50-mg sample of adipocytes was incubated for 90 min in 4 ml of Krebs-Ringer bicarbonate buffer (pH 7.4, 37°C) containing 40 mg/ml bovine serum albumin (Sigma, A 6003, 0.005% fatty acid) and glucose (5.5 mmol/l). The gas phase was O_2/CO_2 (95/5) and lipolysis was stimulated by 10^{-6} M norepinephrine. At the end of the incubation, the medium containing released FFA and fat cells containing TAG were separated by filtration on Whatman glass microfiber filters. FFA in the incubation medium and TAG in adipocytes were extracted according to Dole and Meinertz (25). Under these conditions, about 1 mg FFA derived mainly from TAG hydrolysis is released, and the molar ratio of FFA/glycerol in the medium is close to 3/1 (16).

Lipid analysis

RP TAG were isolated by thin-layer chromatography (plates coated with Kieselgel 60, Merck, Darmstadt, Germany) after mixing with a known amount of triheptadecanoin (Sigma) as an internal standard. The developing solvent system was hexane–diethyl ether–acetic acid 70:30:1 (v/v/v). FFA from the incubation medium were similarly purified. Fatty acids in TAG and FFA and in lipid extracts of subcutaneous, epididymal, and mesenteric adipose tissues were converted to methyl esters using 14% boron trifluoride in methanol (Sigma), according to Morrison and Smith (26). Fatty acid methyl esters were separated and quantified by gas–liquid chromatography using a Chrompack CP 9000 gas chromatograph (Chrompack, Les Ulis, France) equipped with a flame ionization detector and a Spectra-Physics SP 4290 integrator (Spectra-Physics, Les Ulis, France). Chromatography was performed using an AT-WAX fused silica capillary column (60 m × 0.25 mm I.D.; 0.25 μm thickness, Alltech, Templeuve, France). Fatty acid peaks were identified by comparison of their retention times with those of authentic standards and by criteria described previously (16). Only *cis* positional isomers were considered.

Calculations and statistics

The total mass of RP fatty acids was given by 0.91 × total mass of RP TAG, the fatty acid moiety contributing 91% of the molecular weight of RP TAG. The total mass of a given RP fatty acid was given by weight % in RP TAG × total mass of RP fatty acids/100. During each fasting period (days 1–7 and days 7–10), three parameters were calculated to evaluate and compare the loss (in vivo) or release (in vitro) of fatty acids. The in vivo fractional mobilization was a quantitative measure of the loss of each fatty acid, and corresponded to the fraction of its initial mass that was lost from RP. It was calculated as: (initial - final mass)/initial mass, and expressed in %. The ratio of the weight % of each fatty acid in lost or released fatty acids to that in RP TAG from which it originated was calculated and called relative mobilization. The value of this ratio represents the relative degree of enrichment of the fatty acid in lost or released fatty acids. A ratio greater, equal, and lower than unity indicates that the fatty acid is mobilized, respectively, more, equally, or less readily than total RP fatty acids. To calculate in vivo relative mobilization (% in lost fatty acids/% in RP TAG) during a fasting period, the weight % of a fatty acid in lost fatty acids was determined as: (initial - final mass of the fatty acid)/(initial - final mass of total RP fatty acids). Weight % in RP TAG was the mean of the initial and final weight %. During a fasting period, in vitro relative mobilization (% in released FFA/% in RP TAG) was the mean of initial and final values. Initial and final values were taken from paired animals (see above).

Statistical significance of differences between means was assessed using the Peritz' *F*-test for multiple comparison, or the Student's *t*-test for unpaired or paired values, as appropriate, after transforming the percentage values into arcsin. The equation of the curves relating the relative mobilization of fatty acids to their unsaturation at a given chain length was determined as described previously (16). Linear regression analyses with the *F*-test were performed for statistical analysis of correlations (SIGMASTAT, Jandel Corporation, Erkrath, Germany). In all cases the criterion of significance was $P < 0.05$.

RESULTS

Total body and fatty acid mass loss

Fasting for 7 or 10 days induced a significant 18 and 28% decrease in body mass, respectively (**Table 1**). The corresponding decrease in the mass of total RP fatty acids was 56 and 93%, respectively.

TABLE 1. Body mass and mass of fatty acids in retroperitoneal adipose tissue (RP) of fasted rats

Days of Fasting	Body Mass			RP Fatty Acids	
	Initial	Final	Loss	Mass	Loss
	g	*g*	%	*g*	%
1	323.2[a] ± 5.1	314.9[a] ± 4.4	2.6[a] ± 0.4	3.28[a] ± 0.16	
7	328.7[a] ± 3.8	270.0[b] ± 2.3	17.8[b] ± 0.7	1.45[b] ± 0.12	55.7[a] ± 3.1
10	324.0[a] ± 5.5	234.2[c] ± 1.9	27.7[c] ± 1.0	0.24[c] ± 0.09	92.7[b] ± 4.3

Values are means ± SEM (n = 6). Within a column values that do not share the same superscript letter are significantly different, $P < 0.0001$.

TABLE 2. Changes in the fatty acid composition of retroperitoneal adipose tissue TAG during fish oil feeding (day 1 vs. control) followed by fasting

		Days of Fasting		
	Control	Day 1	Day 7	Day 10
Fatty Acids*	Weight %	Weight %	Weight %	Weight %
Saturated				
12:0	0.11 ± 0.02[a]	0.22 ± 0.02[b]	0.24 ± 0.03[b]	0.17 ± 0.01[b]
14:0	1.76 ± 0.07[a]	3.51 ± 0.22[b]	3.20 ± 0.14[b]	2.66 ± 0.08[c]
15:0	0.38 ± 0.03[a]	0.41 ± 0.02[a]	0.37 ± 0.02[a]	0.26 ± 0.02[b]
16:0	28.32 ± 0.78[a]	24.73 ± 0.51[b]	21.43 ± 0.44[c]	16.11 ± 0.59[d]
18:0	3.28 ± 0.12[a]	3.32 ± 0.07[a]	4.04 ± 0.16[b]	5.20 ± 0.21[c]
20:0	0.07 ± 0.01[a]	0.10 ± 0.01[a]	0.18 ± 0.02[b]	0.43 ± 0.04[c]
22:0	tr	tr	0.05 ± 0.01[a]	0.15 ± 0.03[b]
24:0	tr	0.04 ± 0.01[a]	0.08 ± 0.01[b]	0.19 ± 0.02[c]
Mono-unsaturated				
16:1n-9	0.18 ± 0.06[a]	0.54 ± 0.03[b]	0.58 ± 0.01[b]	0.63 ± 0.01[c]
16:1n-7	7.20 ± 0.56[a]	6.07 ± 0.16[a]	3.52 ± 0.31[b]	1.57 ± 0.18[c]
16:1n-5	tr	0.11 ± 0.01[a]	0.08 ± 0.01[a]	0.04 ± 0.00[a]
17:1n-8	0.29 ± 0.04[a]	0.52 ± 0.03[b]	0.41 ± 0.04[b]	0.24 ± 0.03[a]
18:1n-9	23.89 ± 0.56[a]	20.21 ± 0.67[b]	23.30 ± 0.53[a]	25.26 ± 0.34[c]
18:1n-7	4.75 ± 0.25[a]	3.33 ± 0.12[b]	3.88 ± 0.12[c]	3.58 ± 0.07[b,c]
18:1n-5	0.08 ± 0.02[a]	0.12 ± 0.01[a]	0.11 ± 0.01[a]	0.10 ± 0.01[a]
20:1n-11	0.12 ± 0.02[a]	0.50 ± 0.04[b]	0.93 ± 0.08[c]	2.81 ± 0.22[d]
20:1n-9	0.45 ± 0.03[a]	1.36 ± 0.08[b]	2.08 ± 0.11[c]	3.67 ± 0.26[d]
20:1n-7	0.51 ± 0.05[a,c]	0.26 ± 0.01[b]	0.39 ± 0.03[a]	0.61 ± 0.06[c]
22:1n-11	0.06 ± 0.01[a]	0.56 ± 0.06[b]	1.14 ± 0.10[c]	2.78 ± 0.23[d]
22:1n-9	tr	0.11 ± 0.01[a]	0.22 ± 0.02[b]	0.49 ± 0.08[c]
22:1n-7	tr	tr	0.06 ± 0.01[a]	0.14 ± 0.03[b]
Di-unsaturated				
18:2n-6	25.08 ± 1.58[a]	18.38 ± 0.97[b]	22.12 ± 0.74[a]	21.72 ± 0.70[a]
18:2n-4	tr	0.15 ± 0.02[a]	0.11 ± 0.02[a]	0.05 ± 0.01[b]
20:2n-9	tr	0.14 ± 0.02[a]	0.21 ± 0.01[b]	0.35 ± 0.05[c]
20:2n-6	0.21 ± 0.02[a]	0.24 ± 0.02[a]	0.27 ± 0.02[a]	0.36 ± 0.03[b]
Tri-unsaturated				
18:3n-6	0.08 ± 0.01[a]	0.12 ± 0.00[b]	0.10 ± 0.01[a,b]	0.07 ± 0.01[a]
18:3n-4	tr	0.18 ± 0.02[a]	0.18 ± 0.01[a]	0.16 ± 0.01[a]
18:3n-3	1.52 ± 0.11[a]	1.06 ± 0.07[b]	0.80 ± 0.05[c]	0.42 ± 0.04[d]
20:3n-6	0.10 ± 0.01[a]	0.19 ± 0.02[b]	0.19 ± 0.01[b]	0.32 ± 0.03[c]
Tetra-unsaturated				
16:4n-1	tr	0.33 ± 0.06[a]	0.16 ± 0.03[b]	0.06 ± 0.01[c]
18:4n-3	tr	0.98 ± 0.08[a]	0.62 ± 0.06[b]	0.28 ± 0.02[c]
18:4n-1	tr	0.25 ± 0.04[a]	0.12 ± 0.02[b]	0.03 ± 0.00[c]
20:4n-6	0.52 ± 0.04[a]	0.67 ± 0.02[b]	0.44 ± 0.03[a]	0.35 ± 0.03[c]
20:4n-3	tr	0.44 ± 0.05[a]	0.29 ± 0.04[b]	0.16 ± 0.02[c]
Penta-unsaturated				
20:5n-3	0.14 ± 0.03[a]	3.57 ± 0.28[b]	0.90 ± 0.12[c]	0.20 ± 0.03[a]
21:5n-3	tr	0.36 ± 0.05[a]	0.24 ± 0.02[b]	0.18 ± 0.01[c]
22:5n-6	tr	0.34 ± 0.07[a]	0.36 ± 0.06[a]	0.94 ± 0.10[b]
22:5n-3	0.26 ± 0.03[a]	1.21 ± 0.08[b]	1.36 ± 0.08[b]	1.90 ± 0.11[c]
Hexa-unsaturated				
22:6n-3	0.59 ± 0.06[a]	5.37 ± 0.33[b]	5.26 ± 0.37[b]	5.36 ± 0.27[b]

Values are means ± SEM (n = 6); tr, trace. Within a line values that do not share the same superscript letter are significantly different, $P < 0.05$ or less. Control: rats fed the standard laboratory diet; day 1 of fasting: fish oil-fed rats fasted overnight. *Number of carbon atoms: number of double bonds, position of the first double bond from the methyl end of the molecule.

Changes in the fatty acid composition of adipose tissues

The fatty acid composition of RP TAG in rats maintained on the standard diet (control group), fed the fish oil diet (day 1 of fasting), and then fasted for 7 or 10 days is shown in **Table 2.** As expected, compared to the control group, RP TAG of fish oil-fed rats contained higher amounts of some very long chain (20–22 carbon atoms) monounsaturated fatty acids and of n–3 polyunsaturated fatty acids with 4–6 double bonds. During fasting, the weight % of all but five fatty acids changed significantly. The weight % of highly unsaturated (4–5 double bonds) fatty acids with 16–20 carbon atoms, and of 18:3n–3 and 16:1n–7 decreased by 2- to 18-fold ($P <$ 0.001 or less), the weight % of 20:5n–3 showing the most dramatic decrease. In contrast, the weight % of very long chain (20–24 carbon atoms) saturated and monounsaturated fatty acids increased by 3- to 5-fold ($P < 0.001$ or less). Very similar changes were observed in subcutaneous, epididymal, and mesenteric adipose tissues (data not shown).

Comparison of the changes in the fatty acid composition of RP TAG induced by fish oil feeding (day 1 vs. control) and fasting (day 7 or 10 vs. day 1) shows that these changes were unrelated. For example, whereas the percentage weight of most very long chain mono- and of n-3 polyunsaturated fatty acids markedly increased during fish oil feeding, it either decreased (18:4n-3, 20:5n-3), increased (20:1, 22:1, 22:5n-3), or remained unchanged (22:6n-3) during fasting. At the end of the fast (day 10) the weight % of all but five fatty acids was significantly different from that in control rats fed the standard diet, indicating that for almost all fatty acids the fasting-induced changes were different from those induced by fish oil feeding.

Quantitative mobilization of individual RP fatty acids

From day 1 to 7, the fractional mobilization (the fraction of the initial mass that was lost) of 16 of the 37 fatty acids was significantly different ($P < 0.05$ or less) from 56%, that is, from the fraction of total fatty acids that was lost during this period (see Table 1). This is illustrated for quantitatively major fatty acids (weight % ≥ 0.5) in **Fig. 1.** Among these fatty acids, the fractional mobilization ranged from 20% (22:1n-11) to 89% (20:5n-3), i.e., a 4.5-fold difference ($P < 0.001$). For the total fasting period (days 1-10) during which 93% of total fatty acids were lost, the fractional mobilization of major fatty acids ranged from 79 ± 5% (22:1n-11) to 99.5 ± 0.4% (20:5n-3), i.e., a 1.4-fold difference ($P < 0.0001$).

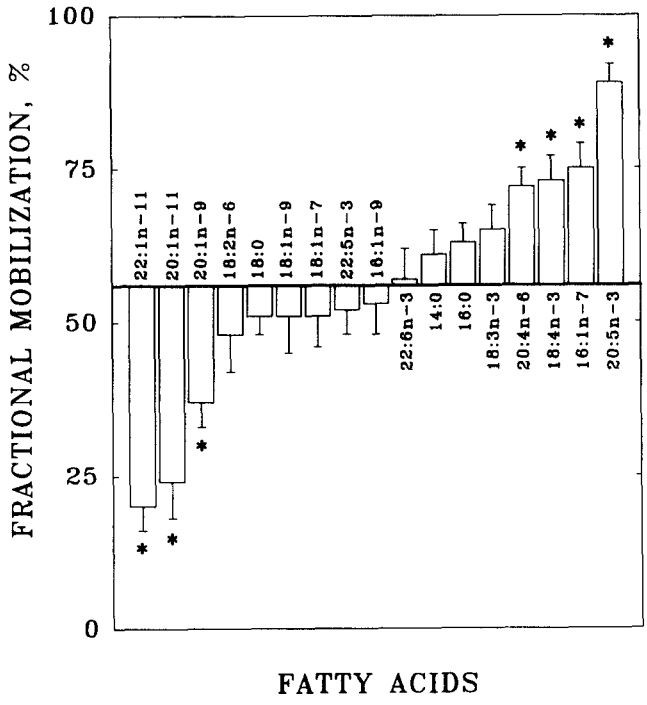

Fig. 1. In vivo fractional mobilization of major fatty acids between days 1 and 7 of the fast. Values are means ± SE (n = 6). Fractional mobilization is the fraction of the initial mass of the fatty acid that was lost from RP during the fast. Only fatty acids whose initial percentage weight was ≥ 0.5% are considered. The horizontal line at 56% shows the fractional mobilization of total fatty acids (see Table 1). *Significantly different from total fatty acids.

TABLE 3. In vivo relative mobilization of fatty acids from retroperitoneal adipose tissue between days 1 and 7 or 7 and 10 of fasting

Fatty Acids	Days 1-7	Days 7-10
Saturated		
12:0	0.91 ± 0.06	1.18 ± 0.09[a]
14:0	1.13 ± 0.06	1.10 ± 0.05
15:0	1.08 ± 0.04	1.23 ± 0.03[a]
16:0	1.16 ± 0.03	1.18 ± 0.03
18:0	0.82 ± 0.05	0.84 ± 0.03
20:0	0.41 ± 0.05	0.52 ± 0.04
22:0	nd	0.51 ± 0.05
24:0	0.31 ± 0.08	0.48 ± 0.05
Mono-unsaturated		
16:1n-9	0.85 ± 0.06	0.94 ± 0.01
16:1n-7	1.65 ± 0.03	1.50 ± 0.06[a]
16:1n-5	1.37 ± 0.06	1.43 ± 0.08
17:1n-8	1.31 ± 0.07	1.31 ± 0.04
18:1n-9	0.80 ± 0.05	0.94 ± 0.02[a]
18:1n-7	0.79 ± 0.05	1.04 ± 0.02[a]
18:1n-5	1.08 ± 0.06	1.10 ± 0.03
20:1n-11	0.35 ± 0.05	0.46 ± 0.05
20:1n-9	0.56 ± 0.04	0.67 ± 0.02[a]
20:1n-7	0.62 ± 0.06	0.73 ± 0.04
22:1n-11	0.34 ± 0.05	0.50 ± 0.03[a]
22:1n-9	0.44 ± 0.08	0.50 ± 0.03
22:1n-7	nd	0.47 ± 0.06
Di-unsaturated		
18:2n-6	0.68 ± 0.09	1.00 ± 0.04[a]
18:2n-4	1.45 ± 0.12	1.34 ± 0.12
20:2n-9	0.64 ± 0.06	0.70 ± 0.02
20:2n-6	0.84 ± 0.05	0.81 ± 0.03
Tri-unsaturated		
18:3n-6	1.26 ± 0.06	1.26 ± 0.07
18:3n-4	1.15 ± 0.08	1.09 ± 0.05
18:3n-3	1.25 ± 0.07	1.42 ± 0.06
20:3n-6	1.03 ± 0.06	0.70 ± 0.04[a]
Tetra-unsaturated		
16:4n-1	2.08 ± 0.12	1.58 ± 0.12[a]
18:4n-3	1.59 ± 0.08	1.46 ± 0.09
18:4n-1	1.90 ± 0.08	1.51 ± 0.07[a]
20:4n-6	1.50 ± 0.02	1.28 ± 0.06[a]
20:4n-3	1.50 ± 0.05	1.34 ± 0.05[a]
Penta-unsaturated		
20:5n-3	2.54 ± 0.08	1.83 ± 0.08[a]
21:5n-3	1.48 ± 0.10	1.20 ± 0.06[a]
22:5n-6	0.91 ± 0.11	0.53 ± 0.11[a]
22:5n-3	0.90 ± 0.06	0.79 ± 0.03
Hexa-unsaturated		
22:6n-3	1.06 ± 0.08	0.99 ± 0.06

Values (% in lost fatty acids/ % in RP TAG) are means ± SEM (n = 6).

[a]Significantly different from days 1-7, $P < 0.05$ or less; nd, not determinable.

In vivo relative mobilization of fatty acids: relationships with their molecular structure

The weight % of most fatty acids in the total fatty acids that were lost during the fast was significantly different from that in RP TAG. As shown in **Table 3,** the in vivo relative mobilization (the ratio of the weight % in lost

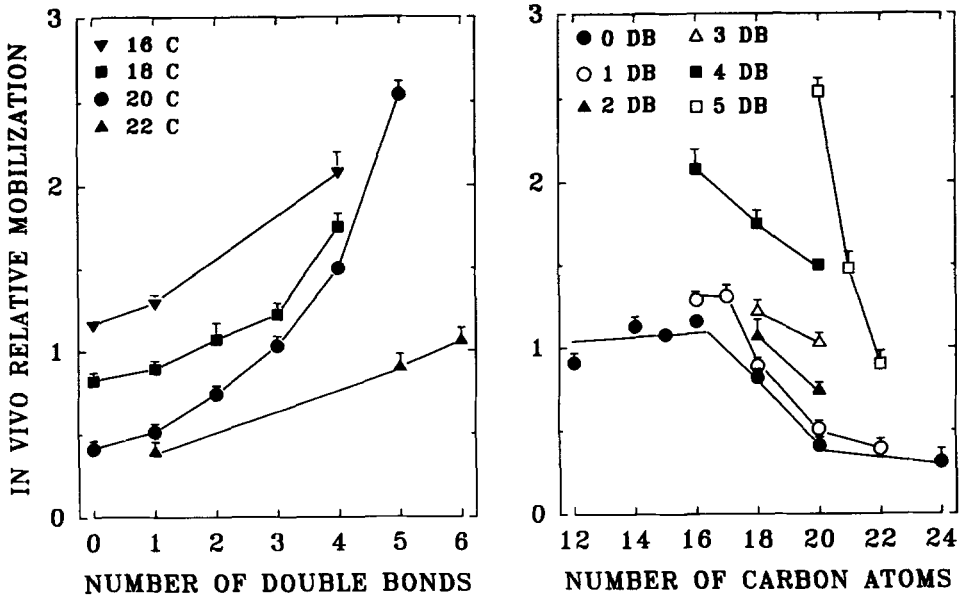

Fig. 2. Relationships between in vivo relative mobilization of fatty acids and their unsaturation at given chain lengths (left) or their chain length at given unsaturations (right). Values are from days 1–7 of fasting; the position of double bonds was not considered and when there were several positional isomers their average relative mobilization was calculated; T-bars show SE (n = 6). C, carbon atom; DB, double bond.

fatty acids to that in RP TAG) of most fatty acids was therefore different from 1. During the first period of fasting (days 1–7), the in vivo relative mobilization of the most readily lost fatty acid (20:5n–3) was 8-fold higher ($P < 0.0001$) than that of the least (24:0). During the second period (days 7–10), this difference was 4-fold ($P < 0.0001$).

The molecular structure of fatty acids influenced their in vivo mobilization. For a given chain length the in vivo relative mobilization increased with the number of double bonds (**Fig. 2,** left panel) whereas for a given number of double bonds it decreased as the chain length increased (Fig. 2, right panel). For fatty acids with 18 or 20 carbon atoms, the relative mobilization increased exponentially with unsaturation (**Table 4**), two of the three parameters of the equation of the line of best fit differing significantly. Notably, the exponent was higher for the shorter chain length, indicating that the shorter the fatty acids were, the greater was the effect of unsaturation. The relative mobilization of fatty acids with 16 or 22 carbon atoms also increased significantly with increasing unsaturation, but the limited number of double bonds precludes a detailed analysis of the relationships. A similar influence of unsaturation and chain length on relative mobilization was observed between days 7 and 10 (Table 3). However, owing to the narrowing of the range of relative mobilization (values tending to 1 as fatty acid stores approached exhaustion; see also Fig. 3) there was a flattening of the lines of best fit.

The pairwise comparison of the in vivo relative mobilization of *cis* positional isomers indicated that, regardless of chain length and unsaturation, the displacement of the double bond(s) towards the methyl end of the chain resulted in an increase in relative mobilization. This increase was significant for 10 of the 20 isomer pairs that were identified. On the average, a 2-carbon atom displacement of the double bond(s) towards the methyl end resulted in a 26 ± 8% (days 1–7) or 18 ± 5% (days 7–10) increase in relative mobilization.

TABLE 4. Parameters of the equations of the best-fit curves relating in vivo and in vitro relative mobilization of fatty acids between days 1 and 7 of fasting to their unsaturation at two given chain lengths

	Parameters of the Equations		
Chain Length	b	a	k
18 C			
In vivo	0.85 ± 0.06^a	0.10 ± 0.08^a	0.96 ± 0.13^a
In vitro	0.96 ± 0.07^a	0.05 ± 0.04^a	1.04 ± 0.12^a
20 C			
In vivo	0.40 ± 0.07^b	0.27 ± 0.11^a	0.54 ± 0.08^b
In vitro	0.48 ± 0.05^b	0.18 ± 0.09^a	0.62 ± 0.07^b

Values are means ± SEM (n = 6). 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. Within a column values that do not share the same superscript letter are significantly different, $P < 0.05$ or less. Average values for positional isomers were used in calculations. For in vivo relative mobilization, the corresponding curves are shown in Fig. 2.

In vitro relative mobilization

The weight % of 30 of the 39 fatty acids was significantly different ($P < 0.05$ or less) between FFA released in the medium from isolated adipocytes and RP TAG, indicating a selective release (not shown). The in vitro relative mobilization (the ratio of the percentage weight in FFA to that in TAG) of the most readily released fatty acid (20:5n-3) was 9.6- and 5-fold higher ($P < 0.0001$) than that of the least (24:0) during the first (days 1-7) and second (days 7-10) periods of fasting, respectively (**Fig. 3**).

In vitro relative mobilization increased with unsaturation, at a given chain length, and decreased with chain length, at a given unsaturation. As shown in Table 4, the parameters of the equations relating in vitro relative mobilization of fatty acids with 18 and 20 carbon atoms to their unsaturation were not significantly different from those relating in vivo relative mobilization of fatty acids to their unsaturation at the same chain lengths. Considering positional isomerism at given chain length and unsaturation, in vitro relative mobilization increased on the average by 16 ± 5% (days 1-7) or 12 ± 4% (days 7-10) when there was a 2-carbon atom displacement of the double bond(s) towards the methyl end of the chain.

In vivo versus in vitro relative mobilization

During the two periods of the fast, a significant direct relationship was found between in vivo and in vitro relative mobilization (Fig. 3). Further, significant correlations ($P < 0.0001$) were found between the weight % of fatty acids in total fatty acids lost from RP in vivo (Y) and in FFA released by adipocytes in vitro (X). The equations of the line of best fit were Y = 1.004 X - 0.015 (n = 37, r = 0.93, days 1-7) and Y = 1.006 X - 0.024 (n = 37, r = 0.90, days 7-10). In all cases, the slopes and the y-intercepts of the lines of best fit were not significantly different from 1 and zero, respectively ($P > 0.5$ or more). Thus, during a given period of the fast, the composition of total fatty acids lost from RP was not significantly different from that of FFA released by RP adipocytes.

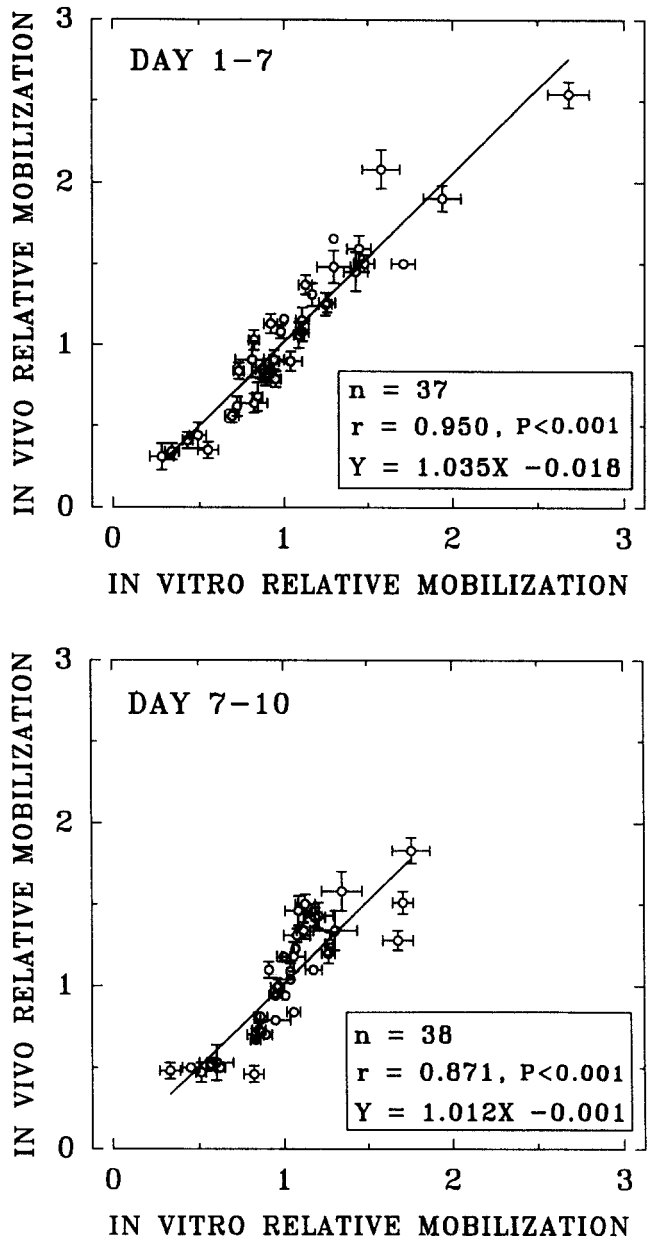

Fig. 3. Relationships between in vivo relative mobilization (Y) and in vitro relative mobilization (X) of fatty acids between days 1 and 7 (top) and 7 and 10 (bottom) of fasting. Each point corresponds to one fatty acid and bars show ± SE.

DISCUSSION

Experimental model

The present study demonstrates that during fasting fatty acids stored as TAG in adipose tissue are selectively mobilized. This demonstration was based on the measurement of the net loss of individual fatty acids from retroperitoneal adipose tissue (RP) after its fatty acid pattern had been modified by a dietary treatment and at various stages of fasting. It should, therefore, be considered whether and how our results depend on *1)* the pre-fasting dietary treatment, and *2)* the extent of adipose tissue depletion.

The pre-fasting dietary treatment (fish oil feeding) led to the enrichment of adipose tissue in specific fatty acids. As it is generally accepted that recently ingested dietary fatty acids may be mobilized more readily ("last in-first out" hypothesis, 27, 28), it could be argued that the selectivity we observed during fasting was actually related to the interruption of fish oil feeding. Our results (Table 2) do not support this hypothesis, showing no

relationship between feeding- and fasting-induced changes in RP fatty acid composition. Moreover, a very similar selectivity of fatty acid mobilization was observed in vitro (16) and in vivo during fasting (unpublished observations) in animals maintained on a standard diet. We therefore conclude that the present selective loss of RP fatty acids was unrelated to the pre-fasting fatty acid intake, and that it was due to adipose tissue depletion itself.

The same pattern of selective mobilization of fatty acids was observed at two markedly different extents of RP depletion (56 and 93%, Table 3 and Fig. 3), indicating that the selectivity of the mobilization of fatty acids under conditions of stimulated lipolysis is an intrinsic metabolic property of adipose tissue and is not based on the extent of fat store depletion. However, the range of relative mobilization between the most and the least readily mobilized fatty acids decreased with increasing depletion, the relative mobilization of each fatty acid tending towards 1 as depletion tended towards 100%. Thus, from a methodological point of view, the extent of adipose tissue depletion is a key factor in characterization of the selectivity of fatty acid mobilization in vivo. Based on present results and on those of a previous study showing no significant changes in the fatty acid composition of adipose tissue after a 1–2 day fast (22), i.e., roughly after a 10–20% depletion, we suggest that the attainment of a ca. 50% depletion is adequate for this characterization. Furthermore, the selectivity of fatty acid mobilization can be characterized no matter which fat depot is used. This conclusion is based on the present observation of similar effects of fasting on the fatty acid composition of adipose tissue at various sites, and on the finding that the selectivity of fatty acid mobilization in vitro is a general characteristic of adipose tissue (29).

Selectivity of in vivo mobilization depends on the molecular structure of fatty acids and is due to a selective release from adipocytes

Data in Tables 3 and 4 and in Fig. 2 clearly demonstrate that the selectivity of the mobilization of fatty acids depends simultaneously and according to complex relationships on chain length, unsaturation, and *cis* positional isomerism. The good fit of the value of their relative mobilization on the curves relating this parameter to chain length and unsaturation indicates that all of the fatty acids seem to be mobilized in vivo in accordance with what may be expected from their molecular structure, the only noticeable exception being 16:1n–7 (higher loss than expected from the structure, see Tables 2 and 3 and Fig. 1). On the other hand, and in contrast to previous suggestions in naturally (30) or experimentally (31) fasted animals, or in corpulent persons undergoing weight loss (4), our study provides no evidence that the selectivity of the mobilization of fatty acids is directed towards a special demand by tissues or towards a preferential retention or sparing of particular fatty acids. Notably, we obtained no evidence that dietary essential fatty acids of the n–6 and n–3 series were preferentially retained, 20:4n–6 as well as 18:3n–3 and 20:5n–3 actually being mobilized at a rate considerably higher than that of most long and very long chain saturated and monounsaturated fatty acids. Several physico–chemical properties (e.g., polarity, see below) depend on the molecular structure of fatty acids. On the basis of the present results on mobilization, we suggest that these physico–chemical properties may govern the metabolism of fatty acids to a much greater extent than has previously been recognized.

The present results on in vitro mobilization in fasted rats show, in close agreement with our previous findings in fed animals (16), that the release of fatty acids from adipocytes is selective and that the in vivo and in vitro selectivities of mobilization are similarly related to the molecular structure of fatty acids (Table 4). Moreover, the relative in vivo and in vitro mobilizations of fatty acids are significantly related (Fig. 3), and the compositions of in vivo lost and of in vitro released fatty acids are almost the same. This indicates that during the depletion of adipose tissue the selectivity of the release of fatty acids from adipocytes entirely accounts for the selectivity of their net loss. It is known that even in the fasted state a small proportion of mobilized fatty acids is not oxidized but recycled back to adipose tissue, including interconversions (32–34). The net loss of fatty acid, as measured here, is the result of the two processes. Our data suggest that the net recycling of released fatty acids is not selective or that, if selective, it is not extensive enough to significantly affect the selectivity of fatty acid release.

The same selectivity of fatty acid release from adipocytes in vitro and net loss from adipose tissue in vivo was demonstrated under conditions of intense lipolysis, i.e., when the net efflux and loss of fatty acids, respectively, massively reflects the hydrolysis of adipocyte TAG (35). This observation supports the suggestion that the selectivity of the release of fatty acids originates from a selective hydrolysis of TAG by HSL, as previously observed in vitro for several polyunsaturated fatty acids (15). Among putative mechanisms, a preferential location and thus accessibility to HSL of TAG with the highest polarity at the lipid–water interface (TAG droplet–cytoplasm) has been proposed (16). In consequence, during prolonged lipolysis, the least polar fatty acids would be released at a progressively increasing rate in proportion to the "peeling off" of the most polar ones by HSL. This would explain that the mobilization of fatty

acids is selective but not as stringent as it would be in the classical sense of enzyme specificity. The observation during fasting of a progressive decrease in the weight % (Table 2) and in the relative rate of mobilization (Table 3) of the most polar fatty acids (e.g., 18:4n–3, 20:4n–6, and 20:5n–3 in the present context) and of the reverse changes for those with the lowest polarity (e.g., very long chain saturated and monounsaturated fatty acids) is consistent with this hypothesis.

Implications for physiology and health

Our results suggest that the selectivity of the mobilization of fatty acids from adipose tissue may result in marked changes in their qualitative supply to the body during an energy deficit. For example, on the basis of the composition of FFA released in vitro at days 1 and 10, it can be estimated that during the fast the weight % of the fatty acid that is most lost (20:5n–3) in fatty acids released in the blood from RP decreased from 11.1 to 0.3%. Assuming that throughout the fast the output of fatty acids from RP remained steady, this would correspond to a 40-fold decrease in the supply of 20:5n–3. On the other hand, the weight % of the least lost fatty acid (22:1n–11) increased from 0.2 to 1.6%, corresponding to an 8-fold increase in its supply. It is known that changes in the qualitative supply of fatty acids induce alterations in the fatty acid composition of cell membranes, resulting in changes in the activity of membrane receptors (36) or in the production of eicosanoids from membrane-derived fatty acids (37). The synthesis and activity of various enzymes are known to be regulated or modulated by specific fatty acids (38) and may also be greatly affected. Consequently, the selectivity of fatty acid mobilization may have important implications for physiology and health. For example, the preferential mobilization of 18:3n–3 (Tables 2 and 3) may be a reason for its selective depletion during weight cycling in rats (39) and humans (5) and during loss of body mass induced by very low calorie dieting in humans (4, 14); this possibly contributes to the significant increase in cardiovascular risk associated with weight cycling (39). Present and previous (16, 29) results indicate that the selectivity of the mobilization of fatty acids is a metabolic property strictly inherent to their molecular structure, suggesting that it very likely operates in humans. As an energy deficit induces a profound remodelling of the fatty acid composition of adipose tissue (Table 2), the use in epidemiological studies of this composition as a marker of dietary fatty acid intake (40) in persons with fluctuating body mass and/or energy balance, e.g., "yo-yo" dieters, appears questionable.

In conclusion, this study demonstrates that, during a prolonged fasting-induced energy deficit, individual fatty acids are selectively mobilized from adipose tissue. This selectivity is based on the molecular structure of fatty acids, and this holds also for release of the fatty acids from isolated adipocytes. Thus, the selectivity of the release of fatty acids operates in vivo and it fully accounts for the net selective loss of fatty acids. Evidence is also provided that this selectivity is a general characteristic of adipose tissue and that it is not related to the pre-fasting fatty acid intake. As it may markedly modulate the supply of biologically active fatty acids to the whole body or to specific tissues, and as it leads to a profound remodelling of the fatty acid composition of adipose tissue, the selectivity of mobilization may have important implications for health, physiology, and epidemiology.

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