Net release of individual fatty acids from white adipose tissue during lipolysis *in vitro*: evidence for selective fatty acid re-uptake

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During lipolysis, adipose tissue triacylglycerols (TAG) undergo concurrent breakdown and synthesis because some of the newly hydrolysed and released non-esterified ('free') fatty acids (NEFA) can subsequently be taken up and re-esterified. The present study examines whether and how the release of individual fatty acids is affected by the re-uptake of some of the newly hydrolysed fatty acids *in vitro* during lipolysis. To alter fatty acid release and re-uptake, adipose tissue fragments and isolated adipocytes from rats were incubated under various conditions, i.e. several cell concentrations or adipose fragment quantities, with or without glucose. In the various conditions tested, the NEFA/glycerol molar ratio ranged from 1.5 to 2.9. Whatever the incubation conditions, including those resulting in very low, medium or high fatty acid re-uptake (as assessed by the NEFA/glycerol ratio), the percentage weight of fatty acids in NEFA was significantly different from that in TAG for 20–24 of the 35 fatty acids that were considered. Thus the greater the fatty acid re-uptake, the higher the proportion of polyunsaturated fatty acids and the lower the proportion of long-chain saturated and monounsaturated fatty acids in NEFA. Moreover, the relative mobilization (%NEFA/%TAG) of the least readily mobilized fatty acid ($C_{22:1,n-11}$) was 6.2-fold lower than that of the most readily mobilized fatty acid ($C_{20:5,n-3}$) under conditions of very low fatty acid re-uptake, and 14.8-fold lower under conditions of high fatty acid re-uptake, indicating a widening of the range of relative mobilizations. We conclude that the composition of the NEFA pool is affected by the rate of fatty acid re-uptake. This provides strong evidence for the selective re-uptake of adipose tissue fatty acids during lipolysis.

Key words: fat cells, fatty acid mobilization/re-esterification, fatty acid molecular structure, triacylglycerols.

INTRODUCTION

Adipose tissue represents the primary site of fuel storage, usually found in the form of triacylglycerols (TAG) [1]. Its main metabolic functions are lipid accumulation through lipid synthesis and lipid mobilization via TAG breakdown. The relative rates of lipid deposition and lipid removal can vary greatly in response to nutritional state and endocrine factors [1,2]. TAG fatty acids stored in adipose tissue are derived primarily from the action of lipoprotein lipase on circulating lipoproteins, but non-esterified ('free') fatty acids (NEFA) bound to albumin and derived from the circulation can also be taken up by adipose tissue independently of lipoprotein hydrolysis. However, in both cases, esterification or re-esterification of NEFA requires α-glycerophosphate, which originates mainly from the conversion of glucose, as glycerokinase activity is extremely low in adipose tissue [3,4]. The regulation of lipolysis is under fine control and can be considerably increased by a variety of hormones through increased adenylate cyclase activity [5]. In adipose tissue, the hydrolysis of the first ester bond of TAG by hormone-sensitive lipase (HSL) is the rate-controlling step for lipid mobilization. It prevents the accumulation of the intermediate di- and monoacylglycerols and precludes the NEFA/glycerol ratio from reaching a value greater than 3, which could be due to the partial hydrolysis of TAG. Therefore glycerol production largely reflects TAG degradation, and fatty acid re-uptake and subsequent re-esterification can be validly assessed *in vitro* by the NEFA/glycerol ratio [6].

The release of NEFA from adipose tissue is the result of the lipolytic and re-esterification pathways. During lipolysis, the main metabolic fate of fatty acids is net output from adipose tissue. Until quite recently, it was believed that the turnover (including the mobilization) of fatty acids was unselective [7]. However, we and others have disproved this hypothesis by demonstrating that fatty acids are mobilized selectively from adipose tissue [8–13]. Thus the fatty acid composition of the NEFA pool has been shown to differ from that of TAG under conditions where the principal metabolic fate of NEFA was their release, indicating net selective mobilization. However, it is possible that lipolysis occurs in a completely or relatively non-selective manner, followed by selective fatty acid re-uptake and then re-esterification. The present study was undertaken to determine whether the composition of the NEFA pool originating from adipose tissue via lipolysis is affected by the NEFA/glycerol ratio, i.e. the re-uptake and subsequent re-esterification of some of the newly hydrolysed NEFA. If so, this would indicate that the re-uptake of fatty acids by the adipose tissue is selective during lipolysis and could influence not only the quantitative but also the qualitative supply of fatty acids to tissues. We examined the net release of individual fatty acids *in vitro* under various incubation conditions. Special emphasis was placed on the relationship between the molecular structure of fatty acids and their metabolic properties. Fatty acids usually found in adipose tissue have between 14 and 18 carbon atoms, with between zero and three double bonds, and their relative mobilization is roughly close to unity. This is the reason why rat adipose tissue was first

Abbreviations used: NEFA, non-esterified ('free') fatty acids; HSL, hormone-sensitive lipase; TAG, triacylglycerol(s); VLC-MUFA, very-long-chain monounsaturated fatty acids; VLC-PUFA, very-long-chain polyunsaturated fatty acids.

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manipulated by dietary means using fish-oil-based diets, although the composition of normal diets would never reach similar proportions of fatty acids. A wide spectrum of fatty acids with between 14 and 22 carbon atoms and between zero and six double bonds was accurately identified by detailed analysis. This included high levels of very-long-chain polyunsaturated fatty acids (VLC-PUFA) and very-long-chain monounsaturated fatty acids (VLC-MUFA), which are known to be very selectively mobilized [10,12]. This allowed us to obtain reliable data on the relationship between the molecular structure of fatty acids and their metabolic fate.

MATERIALS AND METHODS

Chemicals

Analytical-grade solvents were purchased from SDS (Peypin, France), and TLC plates coated with silica gel 60 were supplied by Merck (Darmstadt, Germany). All other reagents were from Sigma (St. Louis, MO, U.S.A.).

Animals and diets

Male Wistar rats, weighing 200–220 g, were obtained from IFFA CREDO (l'Arbresle, France). They were individually caged under a 12 h light/dark cycle at 25 °C and fed on a standard laboratory diet [A04; Usine d'Alimentation Rationnelle (UAR), Villemoisson, France] for 1 week. They were then fed for 4 weeks on a powdered standard diet supplemented with a mixture of menhaden oil and herring oil (4:1; 20% of diet by weight) to obtain a wide spectrum of adipose tissue fatty acids, including VLC-MUFA and VLC-PUFA. Such a duration is long enough to reach a new steady-state fatty acid composition in retroperitoneal adipose tissue [14]. The content of the diet with regard to saturated, monounsaturated and polyunsaturated fatty acids was 25, 31 and 44% respectively. Its content of VLC-PUFA was high (sum 31.0%; including 13.5% $C_{20:5,n-3}$ and 12.9% $C_{22:6,n-3}$), as was its content of VLC-MUFA, although to a lesser extent (sum 9.4%; including 5.4% $C_{22:1,n-11}$ and 3.4% $C_{20:1,n-9}$).

Isolation and incubation of fat cells and adipose tissue fragments

All animals (350–390 g) were killed by cervical dislocation in the post-absorptive state. Retroperitoneal adipose tissue was dissected, weighed and then minced with scissors in warm Krebs–Ringer bicarbonate buffer without glucose, pH 7.4, containing 4% (w/v) BSA (essentially fatty acid-free), at 37 °C. Adipose tissue was then divided into two parts, one half being used for incubation of adipose fragments and the other half for preparation of a fat cell suspension. Each adipose tissue sample was cut into small pieces (about 2–10 mg) which were incubated straight away, after rinsing three times with buffer. Fat cells were prepared according to Rodbell [15] with minor modifications, using glucose-free buffer. Thus approx. 2 g of adipose tissue was digested in 6 ml of buffer containing 6 mg of collagenase for 1 h with agitation at 120 strokes/min under O_2/CO_2 (95:5). The fat cell suspension was filtered, decanted and rinsed three times with warm buffer.

Isolated fat cells and adipose fragments were incubated for 90 min in 20 ml polypropylene flasks containing 4 ml of buffer under various conditions, i.e. several cell concentrations or quantities of adipose fragments (low, medium, high and very high, representing approx. 12.5, 25, 50 and 100 mg respectively of fat cells or adipose fragments per ml of incubation buffer, determined gravimetrically) with glucose (5.5 mmol/l) or without glucose (see Table 1). In order to obtain a large efflux of NEFA, lipolysis was stimulated by the addition of noradrenaline at a final concentration of 1 μmol/l. Under such conditions, NEFA present in the medium at the end of the incubation represented the net efflux of NEFA, i.e. production from TAG hydrolysis minus re-uptake. Incubations were stopped by removal of isolated fat cells or adipose fragments (containing the TAG) by filtration, and the incubation medium (containing the NEFA) was collected.

Glycerol measurement, and lipid extraction and separation

The lipids from 3 ml of incubation medium, with a known quantity of heptadecanoic acid added as an internal standard, and from adipose fragments or cells were similarly extracted by the method of Dole and Meinertz [16]. The concentration of glycerol was determined enzymically as reported previously [17]. The lipid content of incubated adipose fragments or isolated adipocytes was determined gravimetrically after extraction to validate the measurements of various cell concentrations or adipose fragment quantities. All lipid extracts were purified by TLC, using plates washed beforehand with chloroform/methanol (1:1, v/v). The developing solvent system was hexane/diethyl ether/acetic acid (70:30:1, by vol.). The TLC plates were then dried under nitrogen and sprayed with primulin [0.05 mg/ml in acetone/water (4:1, v/v)]. The NEFA (incubation medium) or TAG (adipose fragments or cells) bands were collected and fatty acid derivatives were prepared by boron trifluoride-catalysed transmethylation. Butylated hydroxytoluene was added to all solvent mixtures (0.05%) as an antioxidant. Fatty acid methyl esters were analysed by GLC using a CP 9000 chromatograph (Chrompack, Les Ulis, France) equipped with an AT-WAX capillary column [0.25 mm (internal diam.) × 60 m; 0.25 mm thickness; Alltech, Templeuve, France). They were quantified using an SP 4290 integrator (Spectra-Physics, Les Ulis, France). Analyses were run at least in duplicate.

Calculations and statistics

The relative mobilization of each fatty acid was calculated as the ratio between its percentage weight in released NEFA to that in fat cell TAG. A relative mobilization greater than, equal to or lower than unity means that the fatty acid is mobilized respectively more, equally or less compared with the fatty acids as a whole. Statistical analysis of the data among groups was performed using the Peritz *F*-test for multiple comparisons [18]. The criterion of significance was $P < 0.05$.

RESULTS

Effects of incubation conditions on rates of lipolysis and re-uptake

The various incubation conditions used enabled us to show that: (1) rates of lipolysis were higher in isolated adipocytes than in adipose fragments when corrected for fat cell concentration or quantity of adipose fragments in the incubation medium; (2) rates of re-uptake of NEFA arising from lipolysis were higher for adipose fragments than for isolated adipocytes; (3) rates of re-uptake of NEFA were extremely low in isolated adipocytes when no glucose was present in the incubation medium; and (4) increasing concentrations of adipose tissue increased the rate of re-uptake of NEFA (Table 1). The concentration of glycerol in the medium and the mass of NEFA released were clearly affected by the incubation conditions, ranging from 200 to 1285 μM and from 638 to 3551 μg respectively. When expressed per 100 mg of incubated cells or adipose fragments, the degree of lipolysis, as assessed by glycerol release, was no longer different between

Table 1 Effects of incubation conditions on lipolysis and the NEFA/glycerol ratio in isolated adipocytes and tissue fragments from rats

To alter rates of fatty acid release and re-uptake, adipose fragments and isolated adipocytes were incubated for 90 min in 20 ml polypropylene flasks containing 4 ml of buffer under various conditions, i.e., several cell concentrations (low, medium, high and very high, representing approx. 12.5, 25, 50 and 100 mg of fat cells per ml of incubation buffer respectively, determined gravimetrically), in the absence or presence of glucose (5.5 mmol/l). Under such conditions, NEFA present in the medium at the end of the incubation represent the net efflux of NEFA, i.e. production from TAG hydrolysis minus re-uptake. Values are means ± S.E.M. ($n = 6$–8). Within a row, values that do not share the same superscript letter are significantly different ($P < 0.05$).

	Isolated adipocytes			Adipose fragments	
Quantity of incubated cells or fragments …	Medium	Low	High	Medium	Very high
Glucose …	−	+	+	+	+
Fatty acid re-uptake …	Very low	Low	Low	Medium	High
Glycerol (μM)	200.4 ± 35.6[a]	307.0 ± 36.7[a,c]	1284.8 ± 94.7[b]	399.5 ± 27.3[c]	1116.1 ± 62.1[b]
Quantity of NEFA (μg)	637.7 ± 97.7[a]	920.7 ± 113.3[a]	3550.7 ± 205.9[b]	761.6 ± 43.8[a]	1908.4 ± 106.2[c]
Glycerol (μM)/100 mg of incubated cells or adipose fragments	200.4 ± 35.6[a]	614.0 ± 73.4[b]	642.4 ± 47.4[b]	399.5 ± 27.3[c]	279.0 ± 15.5[a]
Quantity of NEFA (μg)/100 mg of incubated cells or adipose fragments	637.7 ± 97.7[a,c]	1841.4 ± 226.6[b]	1775.3 ± 103.0[b]	761.6 ± 43.8[a]	477.1 ± 26.6[c]
NEFA*/glycerol	2.86 ± 0.07[a]	2.69 ± 0.03[b]	2.48 ± 0.07[c]	1.71 ± 0.03[d]	1.53 ± 0.02[e]

* NEFA values were converted into units of μM before calculations.

Table 2 Fatty acid composition of adipose tissue TAG and of NEFA released by isolated adipocytes and tissue fragments *in vitro*

To alter rates of fatty acid release and re-uptake, adipose fragments and isolated adipocytes were incubated for 90 min in 20 ml polypropylene flasks containing 4 ml of buffer under various conditions, i.e. several cell concentrations (medium and very high, representing approx. 25 and 100 mg of fat cells/ml of incubation buffer respectively, determined gravimetrically), in the absence or presence of glucose (5.5 mmol/l). Under such conditions, NEFA present in the medium at the end of the incubation represent the net efflux of NEFA, i.e. production from TAG hydrolysis minus re-uptake. Values are means ± S.E.M. ($n = 6$–8). Within a row, values that do not share the same superscript letter are significantly different ($P < 0.05$).

	Composition (wt.%)		
		Isolated adipocytes	Adipose fragments
Quantity of incubated cells or fragments …		Medium	Very high
Glucose …		−	+
	TAG	Fatty acid re-uptake … Very low	High
$C_{14:0}$	3.91 ± 0.09[a]	3.74 ± 0.07[a,b]	3.43 ± 0.08[b]
$C_{15:0}$	0.43 ± 0.01[a,b]	0.47 ± 0.01[a]	0.39 ± 0.02[b]
$C_{16:0}$	26.90 ± 0.43[a,b]	27.63 ± 0.46[a]	25.14 ± 0.50[b]
$C_{18:0}$	3.49 ± 0.08[a]	3.33 ± 0.15[a]	2.60 ± 0.09[b]
$C_{20:0}$	0.09 ± 0.01[a]	0.05 ± 0.00[b]	0.03 ± 0.00[a,b]
$C_{14:1,n-5}$	0.12 ± 0.01[a]	0.15 ± 0.01[a]	0.13 ± 0.01[a]
$C_{16:1,n-7}$	7.16 ± 0.27[a]	9.86 ± 0.29[b]	9.47 ± 0.32[b]
$C_{16:1,n-5}$	0.21 ± 0.01[a]	0.11 ± 0.00[b]	0.10 ± 0.00[b]
$C_{17:1,n-8}$	0.58 ± 0.01[a]	0.69 ± 0.02[b]	0.74 ± 0.02[b]
$C_{18:1,n-9}$	19.50 ± 0.43[a]	16.97 ± 0.55[b]	14.52 ± 0.58[c]
$C_{18:1,n-7}$	3.16 ± 0.10[a,b]	3.25 ± 0.09[a]	2.70 ± 0.13[b]
$C_{18:1,n-5}$	0.11 ± 0.00[a]	0.10 ± 0.01[a]	0.10 ± 0.00[a]
$C_{20:1,n-11}$	0.67 ± 0.03[a]	0.36 ± 0.03[b]	0.23 ± 0.02[c]
$C_{20:1,n-9}$	1.79 ± 0.07[a]	1.15 ± 0.05[b]	0.82 ± 0.04[c]
$C_{20:1,n-7}$	0.30 ± 0.01[a]	0.17 ± 0.01[b]	0.14 ± 0.01[b]
$C_{22:1,n-11}$	1.10 ± 0.04[a]	0.48 ± 0.05[b]	0.27 ± 0.01[c]
$C_{18:2,n-6}$	15.82 ± 0.67[a]	11.94 ± 0.43[b]	11.87 ± 0.63[b]
$C_{18:2,n-4}$	0.16 ± 0.00[a]	0.19 ± 0.01[b]	0.21 ± 0.01[b]
$C_{20:2,n-9}$	0.06 ± 0.00[a]	0.04 ± 0.00[b]	0.03 ± 0.00[b]
$C_{20:2,n-6}$	0.15 ± 0.01[a]	0.10 ± 0.00[b]	0.10 ± 0.00[b]
$C_{18:3,n-6}$	0.14 ± 0.01[a]	0.16 ± 0.00[a]	0.18 ± 0.00[b]
$C_{18:3,n-4}$	0.23 ± 0.01[a]	0.24 ± 0.01[a]	0.24 ± 0.01[a]
$C_{18:3,n-3}$	1.40 ± 0.03[a]	1.57 ± 0.07[a]	1.93 ± 0.05[b]
$C_{20:3,n-6}$	0.10 ± 0.01[a]	0.10 ± 0.00[a]	0.10 ± 0.00[a]
$C_{20:3,n-3}$	0.05 ± 0.00[a]	0.05 ± 0.00[a]	0.06 ± 0.00[a]
$C_{16:4,n-3}$	0.04 ± 0.00[a]	0.06 ± 0.00[b]	0.10 ± 0.01[c]
$C_{16:4,n-1}$	0.29 ± 0.01[a]	0.32 ± 0.03[a]	0.51 ± 0.04[c]
$C_{18:4,n-3}$	0.85 ± 0.03[a]	1.03 ± 0.02[b]	1.56 ± 0.05[c]
$C_{18:4,n-1}$	0.24 ± 0.01[a]	0.38 ± 0.01[b]	0.58 ± 0.02[c]
$C_{20:4,n-6}$	0.62 ± 0.04[a]	0.93 ± 0.07[b]	1.15 ± 0.06[b]
$C_{20:4,n-3}$	0.39 ± 0.02[a]	0.52 ± 0.02[b]	0.67 ± 0.03[c]
$C_{20:5,n-3}$	3.45 ± 0.31[a]	8.31 ± 0.62[b]	12.70 ± 1.10[c]
$C_{21:5,n-3}$	0.25 ± 0.01[a]	0.27 ± 0.01[a]	0.35 ± 0.01[c]
$C_{22:5,n-3}$	1.24 ± 0.09[a]	0.93 ± 0.07[b]	1.07 ± 0.05[a,b]
$C_{22:6,n-3}$	4.98 ± 0.36[a,b]	4.37 ± 0.38[a]	5.76 ± 0.34[b]

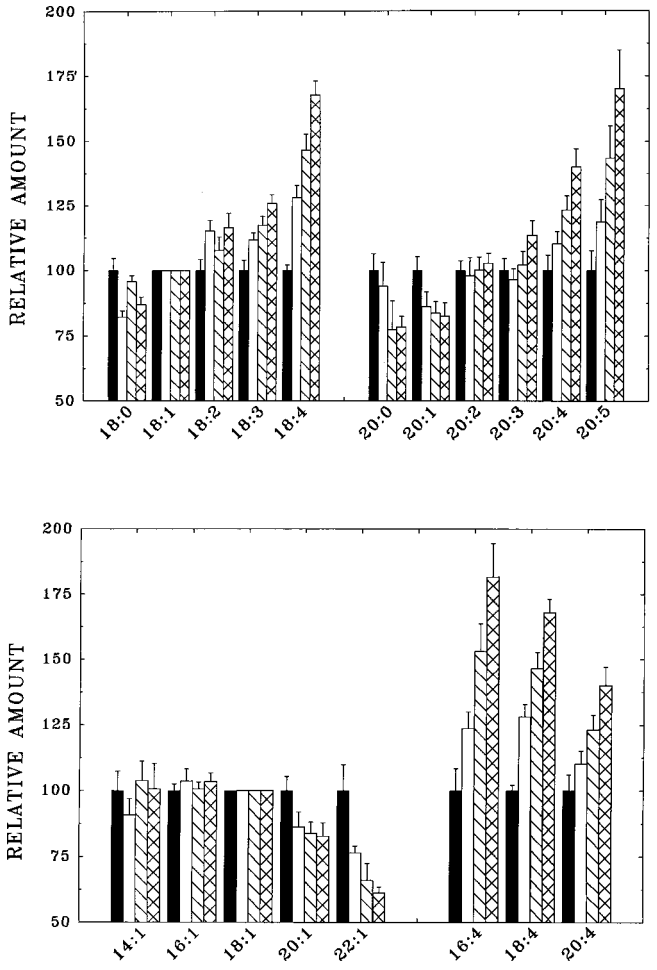

Figure 1 Relationships between adipose tissue and NEFA contents of individual fatty acids stored in and released from rat retroperitoneal adipose tissue

Solid bars represent fatty acid content in adipose tissue. Open bars represent NEFA content of fatty acids released from adipose tissue under conditions of very low fatty acid re-uptake (isolated adipocytes in the absence of glucose). Hatched and double-hatched bars represent NEFA contents of fatty acids released from adipose tissue under conditions of medium and high fatty acid re-uptake (adipose fragments in the presence of glucose). Upper panel, influence of unsaturation at given chain lengths; lower panel, influence of chain length at given degrees of unsaturation. NEFA present in the medium at the end of the incubation represented the net efflux of NEFA, i.e. production from TAG hydrolysis minus re-uptake. Values (means ± S.E.M., $n = 7$) are expressed as a percentage of the value for oleic acid ($C_{18:1,n-9}$) arbitrarily set to 100 in each fraction. Data are partly from Table 2.

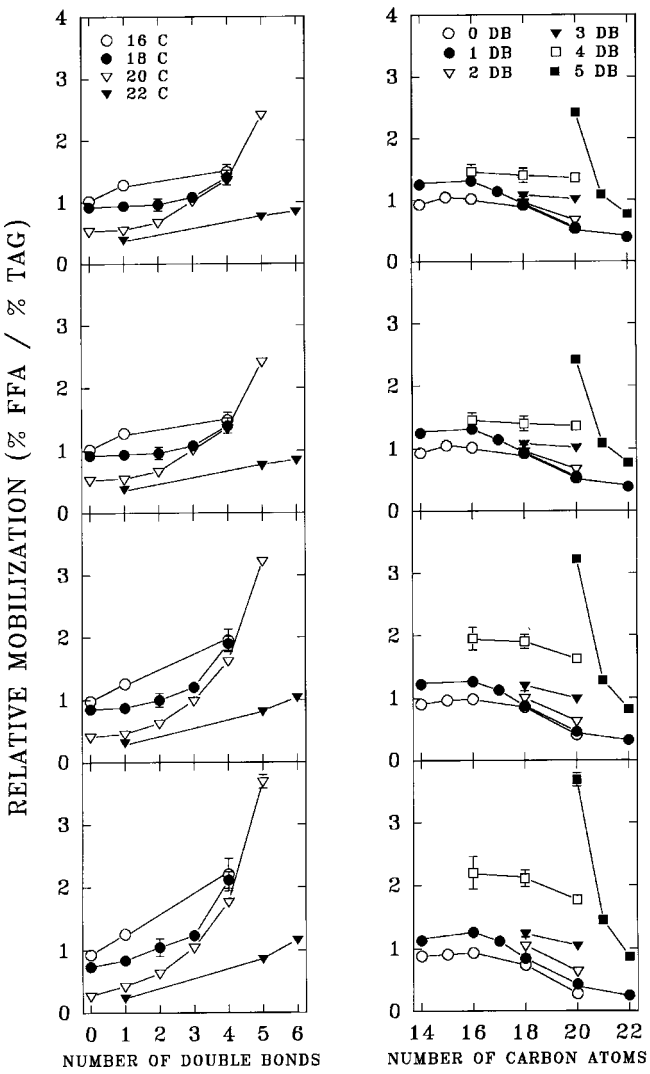

Figure 2 Relationships between individual fatty acids and their relative mobilization *in vitro* from rat retroperitoneal adipose tissue incubated under various conditions involving different rates of lipolysis and re-uptake

The influence of unsaturation at given chain lengths (left panels) and of chain length at given degrees of unsaturation (right panels) are shown. From top to bottom, plots are arranged in increasing order of fatty acid re-uptake, i.e. very low, low, medium and high re-uptake rates (isolated adipocytes without glucose, isolated adipocytes with glucose, medium and very high quantities of adipose fragments with glucose respectively). The relative mobilization of each fatty acid was calculated as the ratio between its percentage weight in released NEFA ('FFA') to that in fat cell TAG. NEFA present in the medium at the end of the incubation represented the net efflux of NEFA, i.e. production from TAG hydrolysis minus re-uptake. Values are means ± S.E.M. ($n = 7$). When there were several positional isomers, the average value for relative mobilization was calculated. C, carbon atoms; DB, double bonds.

incubations where NEFA were taken up to a very low extent (200 μM; isolated adipocytes at 25 mg/ml without glucose) and those where NEFA were taken up to a high extent (279 μM; adipose fragments at 100 mg/ml with glucose).

When incubations were carried out in the presence of glucose, the lipolytic rate (assessed by glycerol release) was not significantly affected by the adipocyte concentration (614 and 642 μM/100 mg of incubated cells at low and high concentrations of adipocytes respectively), whereas it was affected by the concentration of adipose fragments (399 and 279 μM/100 mg of incubated adipose fragments at low and high concentrations of fragments respectively). Similar trends were obtained when considering the quantity of NEFA released per 100 mg of incubated adipose fragments (Table 1). In incubations with glucose and when corrected for the quantity of incubated cells or fragments, the lower net output of NEFA by adipose fragments compared with isolated adipocytes is explained principally by a lower lipolytic rate and also by an approx. 1.6-fold higher re-uptake rate, as assessed by the NEFA/glycerol ratio (fragments, 1.53–1.71; adipocytes, 2.48–2.69). Among all the conditions tested, the molar NEFA/glycerol ratio ranged from 2.86 (isolated adipocytes incubated without glucose) to approx. 1.5 (high concentration of adipose fragments incubated with glucose). This indicates that, in the former case, the re-uptake of NEFA was extremely low, whereas in the latter case about half of the newly released fatty acids were taken up by adipose tissue after

Table 3 Effects of incubation conditions on relative mobilization *in vitro* of some representative fatty acids from isolated adipocytes and tissue fragments

To alter rates of fatty acid release and re-uptake, adipose fragments and isolated adipocytes were incubated for 90 min in 20 ml polypropylene flasks containing 4 ml of buffer under various conditions, i.e. several cell concentrations (medium, high and very high, representing approx. 25, 50 and 100 mg of fat cells/ml of incubation buffer respectively, determined gravimetrically), in the absence or presence of glucose (5.5 mmol/l). Under such conditions, NEFA present in the medium at the end of the incubation represent the net efflux of NEFA, i.e. production from TAG hydrolysis minus re-uptake. Values are means ± S.E.M. ($n = 6$–8). Within a row, values that do not share the same superscript letter are significantly different ($P < 0.05$).

	Relative mobilization (%NEFA/%TAG)		
	Isolated adipocytes		Adipose fragments
Quantity of incubated cells or fragments ...	Medium	High	Very high
Glucose ...	−	+	+
Fatty acid re-uptake ...	Very low	Low	High
Weakly mobilized			
$C_{20:0}$	0.52 ± 0.05^{a}	0.48 ± 0.09^{a}	0.28 ± 0.02^{b}
$C_{20:1,n-9}$	0.61 ± 0.01^{a}	0.52 ± 0.02^{b}	0.45 ± 0.01^{c}
Moderately mobilized			
$C_{16:0}$	1.02 ± 0.01^{a}	0.98 ± 0.02^{a}	0.93 ± 0.02^{b}
$C_{18:1,n-9}$	0.86 ± 0.02^{a}	0.84 ± 0.01^{a}	0.74 ± 0.02^{b}
$C_{18:2,n-6}$	0.76 ± 0.02^{a}	0.83 ± 0.01^{b}	0.75 ± 0.02^{a}
$C_{18:3,n-3}$	1.12 ± 0.04^{a}	1.32 ± 0.02^{b}	1.37 ± 0.02^{b}
$C_{22:5,n-3}$	0.75 ± 0.01^{a}	$0.76 \pm 0.03^{a,b}$	0.87 ± 0.03^{b}
$C_{22:6,n-3}$	0.85 ± 0.01^{a}	0.92 ± 0.02^{b}	1.17 ± 0.03^{c}
Highly mobilized			
$C_{18:4,n-3}$	1.19 ± 0.05^{a}	1.42 ± 0.03^{b}	1.83 ± 0.04^{c}
$C_{20:5,n-3}$	2.42 ± 0.09^{a}	2.75 ± 0.07^{b}	3.69 ± 0.11^{c}

TAG hydrolysis. The protocol used, including other incubation conditions (results not shown), thus allowed us to examine the release of fatty acids *in vitro* under conditions resulting in different lipolytic and/or re-uptake rates.

Fatty acid composition of adipose tissue TAG and of NEFA released through lipolysis

Table 2 shows the fatty acid composition of adipose tissue TAG and of NEFA released through lipolysis, i.e. net mobilization. Feeding the rats on the fish oil diet for 4 weeks resulted in a wide spectrum of fatty acids being present in significant amounts in retroperitoneal adipose tissue TAG. The fatty acid chain length and degree of unsaturation ranged from 14 to 22 carbon atoms and from zero to six double bonds respectively. Among the 35 fatty acids that were considered, the content of saturated, monounsaturated and polyunsaturated fatty acids in adipose tissue TAG was 34.8, 34.7 and 30.5% respectively. The content in TAG of VLC-PUFA, and especially of tetra-, penta-, and hexa-unsaturated fatty acids, was high (sum 11%; including 3.5% $C_{20:5,n-3}$ and 5.0% $C_{22:6,n-3}$). VLC-MUFA were also found in significant amounts (sum 3.9%; including 1.1% $C_{22:1,n-11}$ and 1.8% $C_{20:1,n-9}$).

For 20–24 of the 35 fatty acids that were considered (depending on the various incubation conditions), the percentage weight in NEFA was significantly different from that in adipose tissue TAG. Compared with TAG, NEFA were enriched in polyunsaturated fatty acids with 16–20 carbon atoms and 4–5 double bonds, and depleted in very long-chain saturated and monounsaturated fatty acids with 20–22 carbon atoms and 0–1 double bond. Other fatty acids differed less between NEFA and TAG with regard to percentage weight. Figure 1 shows some representative fatty acid series of NEFA released under various incubation conditions (see above) and expressed relative to their content in NEFA and TAG (set at 100%), all normalized arbitrarily to oleic acid taken as reference. Fatty acids with 18 and 20 carbon atoms and varying in the degree of unsaturation are depicted in the upper panel. For the series $C_{18:0}$ to $C_{18:4}$ and $C_{20:0}$ to $C_{20:5}$ (i.e. for a given chain length), the relative amount of highly unsaturated fatty acids (more than three double bonds) tended to increase with increasing rate of fatty acid re-uptake. Similar trends are also apparent for the series $C_{16:0}$ to $C_{16:4}$ and $C_{22:1}$ to $C_{22:6}$. Fatty acids with one and four double bond(s) and varying in chain length are illustrated in the lower panel of Figure 1. For the series $C_{14:1}$ to $C_{22:1}$ (i.e. for a given degree of unsaturation), the relative amount of long-chain fatty acids (more than 18 carbon atoms) tended to decrease with increasing rate of fatty acid re-uptake. The effect of chain length is also valid for the series $C_{16:4}$ to $C_{20:4}$. Similar trends hold also for the series $C_{14:0}$ to $C_{20:0}$, $C_{18:2}$ to $C_{20:2}$, $C_{18:3}$ to $C_{20:3}$ and $C_{20:5}$ to $C_{22:5}$.

Relative mobilization of fatty acids from adipose tissue

The relative mobilization (%NEFA/% fat cell TAG) of 35 fatty acids was calculated under various incubation conditions, and results are shown in Figure 2 and Table 3. Relative mobilization ranged from 0.39 to 2.42 (i.e. a greater than 6-fold difference) when the re-uptake of fatty acids was very low (isolated adipocytes in the absence of glucose) and from 0.25 to 3.69 (i.e. almost a 15-fold change) when the re-uptake of fatty acids was very high (adipose fragments in the presence of glucose). However, the least and most readily mobilized fatty acids were $C_{22:1,n-11}$ and $C_{20:5,n-3}$ respectively in both cases. On the whole, the relative mobilization of 20 fatty acids differed significantly ($P < 0.05$) between these two extreme incubation conditions (results not shown). In the intermediate incubation conditions with respect to the re-uptake rate of fatty acids (see Table 1), the range of relative mobilizations was also intermediate, as was the number of fatty acids (between four and 12) with a relative mobilization different from that measured in conditions of very low re-uptake (results not shown). For all incubation conditions, fatty acids with 16, 18 or 20 carbon atoms and four or five double bonds showed the highest relative mobilization, whereas fatty acids

with 20 or 22 carbon atoms and zero or one double bond showed the lowest.

Relative mobilization was related to the molecular structure of the fatty acids. For a given chain length, mobilization increased with increasing unsaturation, whereas for a given unsaturation it decreased with increasing chain length (Figure 2). As a consequence of the effect of the re-uptake rate on relative mobilization described above, with a high re-uptake rate the relative mobilization of fatty acids was higher for highly polyunsaturated fatty acids, lower for VLC-MUFA and very-long-chain saturated fatty acids, and only slightly affected for other fatty acids compared with their respective relative mobilization obtained under conditions of very low fatty acid re-uptake.

DISCUSSION

The metabolic fate of fatty acids seems to be related to their molecular structure [8–13] to a greater extent than previously believed, increasing the interest in studying a wide spectrum of fatty acids that differ with regard to chain length and degree of unsaturation. We demonstrated previously that the selectivity of fatty acid mobilization is independent of recent dietary fatty acid intake, allowing us to reject the 'last in – first out' hypothesis [19] as an explanation. Likewise, it is unlikely that the selectivity of fatty acid movement (release/re-uptake) in adipose tissue is based on the fatty acid composition of the tissue. It has been proposed that NEFA re-esterification takes place by an extracellular route, whereby NEFA must leave the adipocyte before being taken up again [20]. Such a mechanism would imply that NEFA derived from lipolysis are not directly accessible to the enzymes involved in glycerolipid synthesis within adipocytes. On the other hand, the esterification pathway accounts for nearly 100 % of the influx of fatty acids, because their rate of oxidation is very low in adipose tissue [21]. Therefore, in our *in vitro* experiments, the metabolic origin of NEFA can be validly ascribed to the lipolytic/re-uptake pathway, so that NEFA present in the medium reflect the relative importance of these two processes.

In the present study we show that, regardless of the incubation conditions and amount of fatty acid re-uptake, the rules relating the relative mobilization of fatty acids to their molecular structure are the same (Figure 2). The higher the re-uptake rate, the higher the proportion of some polyunsaturated fatty acids in NEFA and the lower that of very-long-chain saturated and monounsaturated fatty acids. This shows that, whereas the rate of re-uptake of fatty acids by adipose tissue affects their individual relative mobilization values, it does not affect the overall selectivity of their mobilization. Therefore the selectivity of fatty acid mobilization is a general feature of adipose tissue which is still valid when some newly released fatty acids are taken up again. On average, the relative mobilization of all fatty acids was 1.14 times greater under conditions of high fatty acid re-uptake than when re-uptake was low. However, not all fatty acids were similarly affected. Therefore the relative mobilization of a given fatty acid should be determined under well-known experimental conditions and interpreted cautiously. This reinforces our previous dismissal of the hypothesis that the relative mobilization of a fatty acid is constant, and confirms the need to exercise caution in relating a plasma fatty acid profile to the fatty acid composition of adipose tissue TAG *in vivo*, since the lipolytic/re-uptake ratio is usually unknown [8,9,13].

In previous studies dealing with the incorporation [22,23], the esterification [24–26] and the rates of uptake [27] of NEFA by adipose tissue, no conclusive evidence has been obtained for selectivity of these metabolic processes. Some caution should be used in comparing and interpreting these data since, in some studies, either a mixture or only one type of NEFA was added to the medium. When fatty acids are employed as substrates, their degree of emulsification during the assay is of importance. The effects of chain length or degree of unsaturation of fatty acids do not necessarily reflect the selectivity of the metabolic pathways. Thus no clear picture could be drawn from the results, except for the pioneering study of Hollenberg and Angel [25], who considered the mobilization and re-uptake of only a few fatty acids. In accordance with these authors, we found a similar relationship between fatty acid molecular structure and mobilization rate. In contrast, we did not find that, for a given degree of unsaturation, shorter fatty acids are more readily taken up and thereby esterified, nor that, for a given chain length, the more unsaturated fatty acids are more readily taken up.

It is very unlikely that these differences could be explained by the incubation conditions, which were roughly similar except for the lipolytic agent. The discrepancies probably lie in the comparison of only a few fatty acids, with 14–18 carbon atoms and 0–3 double bonds, in the pioneering studies, mainly due to the low resolution of GLC columns. Indeed, among fatty acids with 14–18 carbon atoms and 0–3 double bonds in the present study, the relative mobilization ranged only from 0.9 to 1.37, i.e. a 0.65-fold difference. A detailed analysis allowed us to identify a wide spectrum of fatty acids (14–22 carbon atoms and 0–6 double bonds). Their range of relative mobilizations was very wide, from 0.28 ($C_{20:0}$) to 3.69 ($C_{20:5,n-3}$), i.e. a 13.2-fold difference. This is likely to contribute to the differences in the relationship between fatty acid molecular structure and metabolic fate. In other words, we report here that the more polar NEFA (shorter and more highly unsaturated) are more readily mobilized than the average of all the fatty acids, whereas they seem to be less readily taken up. Conversely, the less polar NEFA are less readily mobilized than the average of the fatty acids, and seem to be more readily taken up. Thus similar physicochemical properties of fatty acids cannot in itself be responsible for selective mobilization and selective re-uptake, because the two selectivities vary in opposite ways. This would be consistent with the fact that the overall esterification process is not the reverse of lipolysis, at least with regard to the enzymic processes involved.

Our results strongly suggest that the basis of the selectivity observed during mobilization differs from that of the selectivity observed during the re-uptake process. It is unlikely that the different rates of re-uptake of individual fatty acids are due to differences in fatty acid availability, since in the present study all fatty acids originated from lipolysis. It could be proposed that the composition of the NEFA pool depends on the relative quantities of substrate hydrolysed during lipolysis. This view is not supported by our data, since the composition of the NEFA pool (Figure 1) and the relative mobilization values (Figure 2) were similar whether the quantity of NEFA present in the incubation medium was close to 600 or 3500 μg (i.e. approx. 0.6 or 1.8 % of substrate fatty acids hydrolysed) (Table 1). That is, the selectivity of fatty acid release *in vitro* was similar in different situations of enhanced lipolysis and roughly similar re-uptake rates, giving no support to an explanation based on the selective hydrolysis of a very small pool of substrate fatty acids. The fact that the same selective mobilization pattern of fatty acids was observed with two different extents (56 and 93 %) of adipose tissue TAG depletion *in vivo* [13] also argues against such a hypothesis.

Previous data have raised the possibility that adipocytes contain several (at least two) lipid-metabolizing compartments: a small one associated with cytoplasmic particles and having very rapid turnover, and one associated with the lipid droplet and

exhibiting very slow turnover [23]. During lipolysis, part of the newly synthesized TAG belonging to the small pool with a high turnover rate could be more prone to lipolysis than the bulk of the lipid, whereas another part could be equilibrated with the lipid droplet [28]. This would lead the lipolytic products to reflect mainly the hydrolysis of a small fraction of the substrate, which could be enriched in certain TAG species, as we have suggested previously [12,29].

The mechanism through which the molecular structure of fatty acids influences their metabolic fate remains to be elucidated. The selectivity of fatty acid mobilization is not based on the proportions of stored fatty acids, giving no support to a competition process between fatty acids [10]. An explanation could lie in a selective partition of released NEFA within fat cells. This would lead to a progressive enrichment of an intracellular pool of NEFA in the least readily mobilized fatty acids, to counteract the fatty acid pattern of released NEFA. During lipolysis, NEFA do not accumulate, since they are known to inhibit HSL activity [30]. Thus, to sustain active lipolysis, active re-esterification should occur within the cell rather than via the more common extracellular pathway [20]. Moreover, this improbable intracellular re-esterification would need to be intensive and mainly involve the less readily mobilized fatty acids (e.g. VLC-MUFA) through acyltransferase and/or acyl-CoA lyase selectivities. It has also been shown that the rates of hydration of fatty acids are dependent on both fatty acid chain length and unsaturation, with a trend to increase this rate with a decrease in chain length or with an increase in unsaturation [31]. While these rates could account for intracellular transfer between different compartments, their influence remains uncertain, because of carrier proteins.

Therefore it seems reasonable to propose that the selectivity of fatty acid mobilization lies, at least partly, in the selective hydrolysis of TAG. Among known lipase specificities, HSL has been reported to cleave preferentially at the *sn*-1 and *sn*-3 positions of TAG [5]. However, the selective mobilization of fatty acids is not based on their positional distribution in TAG [32]. Nevertheless, a role for HSL can still be proposed through the selective hydrolysis of TAG due to a preference for certain fatty acids or different rates of substrate hydrolysis. Lipolysis occurs at a lipid/water interface, where only small amounts of substrate are available to the enzyme. It has been suggested that some TAG species are preferentially hydrolysed under stimulated lipolysis [33]. A selective enrichment in some TAG species due to their physicochemical properties, such as polarity at the lipid/water interface where HSL acts, could be proposed [12,29]. In support of this hypothesis, it has been reported for lipoproteins that apolar lipids, mainly TAG, enriched in polyunsaturated fatty acids display a higher partition coefficient between an interfacial phase and an apolar phase than TAG enriched in saturated fatty acids [34]. Hence, at the lipid/water interface, lipolytic enzymes would be more efficient towards polyunsaturated fatty acids. As a consequence, the relative amount of polyunsaturated fatty acids released will be greater than that of saturated fatty acids. This strengthens the hypothesis that the role of the physicochemical properties of fatty acids operates at the level of the substrate (TAG), and consequently of intermediate hydrolysis products. The accessibility of the ester group at the lipid/water interface should not be discarded as a preferential or poor-cleavage form [35]. Indeed, the orientation of the substrate at the interface may play an important role, e.g. whether a fatty acid part of the molecule is oriented towards the aqueous phase or away from it [36].

Among possible mechanisms underlying the selectivity of fatty acid re-uptake by adipose tissue, several hypotheses can be proposed, including competitive reactions between fatty acids at the transfer or transport level, or selectivity of fatty acid binding proteins. High enzyme selectivities could also promote re-uptake and subsequent re-esterification of certain fatty acids at several steps. The glycerophosphate and acylglycerophosphate acyltransferases might be expected to display substrate specificities [37]. Indeed, the rate of incorporation of a number of acyl-CoA homologues depends on the fatty acid molecular structure, with a preference for unsaturated fatty acids. The specificity of monoacylglycerol acyltransferase showed that the chain length of the substituting fatty acid in monoacylglycerols was important. A preference of the enzyme for long-chain acyl-CoA was also described [38,39]. The properties of diacylglycerol acyltransferase showed that the fatty acid composition of the diacylglycerols was important. On the other hand, taking a known diacylglycerol as substrate, different reaction rates were obtained according to the acyl-CoA tested [38,40]. It is well known that the formation of fatty acid thiol esters is necessary during the esterification process. An explanation for the low re-uptake of some fatty acids could be their low activation in adipose tissue [41]. Indeed, the activation of fatty acids is brought about by long-chain acyl-CoA synthetase, which exhibits substrate specificity [42]. Thus it is proposed that the selectivity of fatty acid re-uptake is the result of NEFA availability as well as of enzyme and/or fatty acid binding protein selectivities.

Our data show that adipose tissue does not preferentially spare essential fatty acids. Indeed, $C_{18:3,n-3}$ and $C_{20:4,n-6}$ were preferentially mobilized, and their proportions in released NEFA tended to increase as the rate of fatty acid re-uptake increased, whereas that of $C_{18:2,n-6}$ was not significantly affected (Table 3). The control of the storage of individual fatty acids, and notably essential fatty acids, in adipose tissue remains unclear. Fatty acid bioavailability and enzyme and/or binding-protein specificities probably affect the composition of adipose tissue TAG, in addition to rate and selectivity of fatty acid mobilization and uptake. Moreover, although TAG are the predominant products of esterification in adipose tissue, some of the fatty acids can also be transformed into cholesterol esters and phospholipids. Based on previous data showing the preferential esterification of myristate, palmitate and palmitoleate into TAG and the concentration of stearate into phospholipids in the adipocyte [28], one cannot exclude the possibility that the selective esterification of fatty acids in these various lipid fractions could partly explain their selective re-uptake during lipolysis. In this framework, the measurement of the composition of newly synthesized TAG and/or phospholipids would be of interest, in order to determine whether individual fatty acids are incorporated into these two lipid fractions to the same extent.

It is concluded that the individual proportions of each fatty acid in NEFA are affected when various conditions are present that result in different lipolytic and re-uptake rates. Enzymes or transfer proteins are capable of exhibiting hydrolytic or transport selectivities for substrates or reaction end-products that might explain the composition of released NEFA. It seems clear at present that the physicochemical properties of fatty acids play a key role, but more information is required in order to better understand the mechanisms by which the molecular structure of fatty acids affects their metabolic fate. Because of the physiological relevance of a selective fatty acid supply to tissues, the extent to which the re-uptake rate of fatty acids *in vivo* affects their release needs close examination. A better knowledge of the mechanisms by which individual fatty acids are mobilized from and taken up by adipose tissue is of major importance for understanding the selective storage of fat-depot fatty acids and, as a result, the regulation of fat cell hypertrophy by dietary fats.

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