Selective mobilization of fatty acids from white fat cells: evidence for a relationship to the polarity of triacylglycerols

Thierry RACLOT

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

Fatty acids are selectively released from white fat cells in accordance with well-defined rules relating their molecular structure and their mobilization rate, emphasizing the possible role of their physicochemical properties. Lipolysis is widely reported to work for conditions where only small amounts of substrate are available. We hypothesize that the preferential hydrolysis of a substrate fraction enriched in the most polar triacylglycerols (TAGs) reflects the pattern of selective fatty acid mobilization. Rat adipose tissue was first manipulated by dietary means to obtain a wide spectrum of fatty acids. Fat cell TAGs were separated into eight fractions according to polarity by liquid–liquid partition chromatography and their fatty acid proportions and compositions were determined by GLC. In the most polar TAG fractions, the relative enrichment of fatty acids (percentage in a TAG fraction divided by percentage in total TAGs) increased

with the number of double bonds for a given chain length, whereas it decreased with increasing chain length for a given degree of unsaturation. The relative enrichment of highly mobilized fatty acids (16–20 carbon atoms and four or five double bonds) was very high (more than 2.5) in the most polar TAG fractions, whereas that of weakly mobilized fatty acids (20–24 carbon atoms and no or one double bond) was very low (less than 0.5). The relative enrichment of moderately mobilized fatty acids (comprising all the others) was close to unity. Our study shows that the relative enrichment of fatty acids in the most polar adipose tissue TAGs is consistent with their relative mobilization rates. This supports our hypothesis and raises the possibility that the molecular species of TAGs might be one of the regulating factors.

INTRODUCTION

Lipolysis involves two cellular lipases that yield free fatty acids (FFAs) and glycerol from adipose tissue triacylglycerol (TAG) hydrolysis [1–3]. However, the mechanism of FFA release is still poorly understood [4–6]. In addition to phosphorylation of hormone-sensitive lipase (HSL) [7], other mechanisms exist that could influence lipolysis, such as the access of the enzyme to its lipid substrate [8–12]. The release of fatty acids has been shown to be selective [13–16]. This selectivity is a general metabolic feature of adipose tissue whatever the dietary manipulations [13,14]. Three categories of fatty acids have been defined by taking into account their mobilization rate, chain length and unsaturation. Highly mobilizable fatty acids have 16–20 carbon atoms and four or five double bonds, whereas weakly mobilizable fatty acids have 20–24 carbon atoms and no or one double bond. Moderately mobilizable fatty acids comprise all the others [14].

The mechanism through which the molecular structure of fatty acids affects their mobilization is at present unclear. During lipolysis the breakdown of stored TAGs by HSL is the rate-limiting step [17,18]. HSL selectively releases some polyunsaturated fatty acids [19] and preferentially cleaves the outer positions of TAGs [17,20]. However, the selective mobilization of fat cell fatty acids is not based on their positional distribution in TAGs [21]. Lipolysis has also been described as a process working at the lipid/water interface, i.e. for conditions where only small amounts of substrate are directly available to the enzymes [22,23]. Moreover the close relationships between the molecular structure of fatty acids and their mobilization rate have been shown to reflect their relative retention on a non-polar

GLC column [13]. From these results, a preferential location and thus accessibility to HSL of TAGs with the highest polarity at the lipid/water interface where the reaction occurs has been previously hypothesized. If valid, such a hypothesis predicts that the fatty acid composition of the most polar TAGs might be expected to be representative of the fatty acids hydrolysed, i.e. the released FFAs.

The aim of this study was therefore to test whether the fatty acid enrichment in the most polar adipose tissue TAGs is consistent with their mobilization rate. Rat adipose tissue was first manipulated by the use of fish-oil diets. This allows us (1) to overcome the difficulty of the determination of individual fatty acids found in very low concentrations in adipose tissue TAGs from animals in their normal dietary state, (2) to obtain a wide spectrum of fatty acids including those known to be highly and weakly mobilizable [14], (3) to relate the fatty acid molecular structure confidently to their metabolic fate, and (4) to obtain a wide range of TAG molecular species varying in their respective proportions [24].

METHODS

Chemicals

Analytical-grade solvents, precoated TLC plates (silica gel 60, 0.25 mm × 200 mm × 200 mm), precoated reverse-phase TLC plates (RP-18F₂₅₄S, 0.25 mm × 200 mm × 200 mm) and butylated hydroxytoluene were supplied by Merck (Darmstadt, Germany). All other reagents were purchased from Sigma (St. Louis, MO, U.S.A.).

Abbreviations used: FFA, free fatty acid; HSL, hormone-sensitive lipase; IPN, integral partition number; TAG, triacylglycerol; MEN and HER groups and adipose tissues, animals fed on a diet high in very-long-chain polyunsaturated fatty acids and low in very-long-chain monounsaturated fatty acids, and the reverse, respectively, and adipose tissues from these respective animals.

Animals and diets

Male Wistar rats (IFFA CREDO, l'Arbresle, France) weighing 240–260 g were individually housed in a room having a 12 h light/dark cycle at 25 °C. They were fed *ad libitum* for 4 weeks with semi-synthetic diets containing 19 % by weight of menhaden (MEN group, 6 rats) or herring (HER group, 6 rats) oil. Other components of the diets and their fatty acid compositions were as previously reported [14,25]. We have previously shown that retroperitoneal adipose tissue TAGs reached a new stable fatty acid composition after a similar dietary manipulation [26].

Total lipid extraction and purification of TAGs

Rats were killed by cervical dislocation in the post-absorptive state. Adipose tissue samples from retroperitoneal fat depots were rapidly excised and their total lipids were extracted [27]. Because the methodology used was very time-consuming (see the next paragraph), aliquots of total lipids (approx. 2.5 mg) were collected from each individual ($n = 6$) and were randomly pooled in three sample sets of two individuals each within each experimental group. TAGs (approx. 5 mg) were isolated by silicic acid column chromatography with chloroform for elution, and were then purified by TLC with hexane/diethyl ether/acetic acid (70:30:1, by vol.) as the developing solvent [26]. TAGs were then scraped into vials, extracted three times with 5 ml of hexane/diethyl ether (1:1, v/v) and stored at -20 °C. Butylated hydroxytoluene at a final concentration of 0.05 % was added to all solvent mixtures as an antioxidant.

Separation of adipose tissue TAGs into fractions according to polarity by liquid–liquid partition chromatography

This method separates molecules on the basis of their differential solubilities in two immiscible phases [28]. TAGs are fractionated according to their partition number, which is a predictable function of both molecular mass and unsaturation. The integral partition number (IPN) can be calculated for any TAG from the formula: $\text{IPN} = \text{carbon number} - 2 \times (\text{number of double bonds})$. TAGs of the same IPN will travel together in the same band [28]. Lipid samples enriched in a wide spectrum of fatty acids are too complex to produce an adequate separation of TAGs into single components. A total separation of TAGs into distinct molecular species was not needed here, but a broad TAG spreading was required.

The procedure used precoated reverse-phase TLC plates (RP-18F₂₅₄S). The plates were prewashed by development with chloroform/acetonitrile (4:1, v/v.) to remove impurities and increase the sensitivity of the detection reagents [29], and were then activated for 1 h at 110 °C. After cooling, approx. 0.5 mg of adipose tissue TAGs from each of the MEN and HER groups was deposited under nitrogen on either side of the same plate (deposit over 7 cm) while 100 µg of the standard TAGs mixture was deposited between adipose tissue samples (deposit over 1 cm). The standard TAGs have to be unsaturated to be detected by phosphomolybdic acid [29] and were prepared from linseed oil, which contained TAGs with an IPN ranging from 36 to 48 [28], enriched with triolein (IPN = 48), 1,2-dioleoyl-3-stearoyl-*rac*-glycerol (IPN = 50), 1,2-distearoyl-3-oleoyl-*rac*-glycerol (IPN = 52), and tri-11-icosenoin (IPN = 54). The developing solvent was a polar binary phase of acetone/acetonitrile (70:30, v/v) and was run three times consecutively to optimize TAG spreading. After the third run the plate was cut into three pieces on both sides of the part containing the standard TAGs, which was then sprayed with phosphomolybdic acid at 5 % in ethanol, heated for 10 min at 110 °C to reveal the standard TAG spots

[29] and then replaced to its initial location. The standard TAG spots corresponding to given IPNs were used as markers to define the boundaries between fractions within the adipose tissue TAG samples from the MEN and HER groups. The number of TAG fractions has to be large enough to show clear changes in their respective fatty acid compositions. However, the amount of TAG in each fraction has to be sufficient to allow an accurate analysis of their fatty acid compositions by GLC. The TAG fractions were therefore arbitrarily delimited by taking into account the adipose tissue TAG spreading. The number of TAG fractions was equal to eight in each of the two experimental groups: fraction 1, $\text{IPN} < 40$; fraction 2, $40 \leq \text{IPN} < 42$; fraction 3, $42 \leq \text{IPN} < 44$; fraction 4, $44 \leq \text{IPN} < 46$; fraction 5, $46 \leq \text{IPN} < 48$; fraction 6, $48 \leq \text{IPN} < 50$; fraction 7, $50 \leq \text{IPN} < 52$; fraction 8, $\text{IPN} \geq 52$. Each TAG fraction was scraped into a vial and 20 µg of trionadecanoin, used as an internal standard, was added before extraction. Each TAG fraction was then extracted three times with 2 ml of hexane/diethyl ether (1:1, v/v). The TAG fractions were purified by TLC as reported above and stored at -20 °C with butylated hydroxytoluene for further fatty acid analysis.

Fatty acid analysis

Fatty acid methyl esters were prepared by using 14 % (v/v) boron trifluoride in methanol after saponification [30]. They were then purified by TLC with hexane/diethyl ether (90:10, v/v) as the developing solvent. Analysis of fatty acid derivatives by GLC was performed with a Chrompack CP 9000 chromatograph (Chrompack, Les Ulis, France) equipped with a flame ionization detector, an AT-WAX capillary column (0.25 mm internal diam. $\times$ 60 m; Alltech, Templeuve, France) and a Spectra-Physics SP 4290 integrator (Spectra-Physics, Les Ulis, France). Identification of fatty acids was performed by comparison with standard fatty acids and as described earlier [13]. Chromatographic analyses were run at least in duplicate.

Calculations and statistics

Within each experimental group, results are expressed as means $\pm$ S.E.M. (Tables 1 and 2) for three data sets of two individuals each. As a consequence of the methodology used (preadsorbent C₁₈ chemically bonded reverse-phase plates), stearic acid was omitted from the final calculations. The relative enrichment of each fatty acid was calculated as the ratio of their percentage by mass in a given TAG fraction to that in total adipose tissue TAGs. This ratio represents the relative degree of enrichment of the fatty acid in the considered fractions. A ratio greater than, equal to, or less than unity shows that the fatty acid proportion is, respectively, more, equally, or less represented than in the total adipose tissue fatty acids. The best-fit curves relating the relative enrichment to fatty acid unsaturation at a given chain length were determined by using a non-linear regression data analysis program [31] (Enzfitter, Elsevier-Biosoft, U.K.). The statistical significance of differences between means was assessed with the Peritz *F*-test for multiple comparisons [32]. The criterion of significance was $P < 0.05$.

RESULTS

Fatty acid composition of total and fractional TAGs from retroperitoneal adipose tissue

The fatty acid compositions, the amounts and the proportions of the eight TAG fractions from rat retroperitoneal adipose tissue of the MEN and HER groups are summarized in Tables 1 and

Table 1 Fatty acid compositions (wt.%), amounts and proportions of TAG fractions from rat adipose tissue of the MEN group

Fat cell TAGs were separated into eight fractions according to polarity by liquid–liquid partition chromatography and their fatty acid proportions and compositions were determined by GLC. Values are means $\pm$ S.E.M. for three data sets of two individuals each. Recombinant total is calculated from fatty acid compositions and proportions of the TAG fractions. Minor fatty acids (less than 0.02 %) were not considered. Within a line, values that do not share the same superscript letter are significantly different ($P < 0.05$). Abbreviation: n.d., not determinable.

Fatty acids	Total	Fraction ... 1	2	3	4	5	6	7	8	Recombinant total
Saturated sum (wt. %)	38.5 $\pm$ 1.7 ^a	15.0 $\pm$ 1.6 ^b	25.6 $\pm$ 1.5 ^c	40.1 $\pm$ 1.3 ^a	40.6 $\pm$ 1.3 ^a	41.4 $\pm$ 1.5 ^a	56.3 $\pm$ 2.9 ^d	68.6 $\pm$ 4.2 ^d	68.2 $\pm$ 5.2 ^d	41.9 $\pm$ 2.3 ^a
Mono-unsaturated sum (wt. %)	37.6 $\pm$ 1.1 ^{ac}	19.2 $\pm$ 0.9 ^b	33.5 $\pm$ 0.4 ^{ab}	35.4 $\pm$ 0.3 ^a	35.1 $\pm$ 1.1 ^a	42.2 $\pm$ 1.1 ^c	40.2 $\pm$ 2.5 ^{ac}	29.7 $\pm$ 3.9 ^{ab}	29.6 $\pm$ 4.8 ^{ab}	36.0 $\pm$ 1.2 ^{ac}
Di-unsaturated sum (wt. %)	11.6 $\pm$ 0.3 ^a	8.3 $\pm$ 0.2 ^b	13.8 $\pm$ 0.3 ^c	12.2 $\pm$ 0.3 ^{ac}	15.8 $\pm$ 0.2 ^d	14.1 $\pm$ 0.3 ^c	3.4 $\pm$ 0.3 ^e	1.7 $\pm$ 0.3 ^f	2.2 $\pm$ 0.4 ^g	10.9 $\pm$ 0.3 ^a
Tri-unsaturated sum (wt. %)	1.3 $\pm$ 0.1 ^a	2.6 $\pm$ 0.2 ^b	2.7 $\pm$ 0.2 ^b	1.7 $\pm$ 0.2 ^a	1.6 $\pm$ 0.1 ^a	0.6 $\pm$ 0.1 ^c	0.1 $\pm$ 0.0 ^d	n.d.	n.d.	1.1 $\pm$ 0.1 ^a
Tetra-unsaturated sum (wt. %)	2.6 $\pm$ 0.1 ^a	10.8 $\pm$ 0.2 ^b	7.1 $\pm$ 0.2 ^c	3.0 $\pm$ 0.2 ^a	1.6 $\pm$ 0.1 ^d	0.4 $\pm$ 0.1 ^e	0.1 $\pm$ 0.0 ^f	n.d.	n.d.	2.3 $\pm$ 0.1 ^a
Penta-unsaturated sum (wt. %)	4.1 $\pm$ 0.2 ^a	21.0 $\pm$ 0.9 ^b	9.7 $\pm$ 0.2 ^c	4.0 $\pm$ 0.1 ^a	2.5 $\pm$ 0.1 ^d	0.6 $\pm$ 0.1 ^e	n.d.	n.d.	n.d.	3.8 $\pm$ 0.3 ^a
Hexa-unsaturated sum (wt. %)	4.2 $\pm$ 0.1 ^a	23.1 $\pm$ 1.3 ^b	7.7 $\pm$ 0.2 ^c	3.8 $\pm$ 0.2 ^a	2.8 $\pm$ 0.1 ^d	0.6 $\pm$ 0.1 ^e	n.d.	n.d.	n.d.	4.0 $\pm$ 0.3 ^a
Amount (μ g)	419.0 $\pm$ 6.6 ^a	40.4 $\pm$ 2.1 ^b	21.5 $\pm$ 0.2 ^c	50.9 $\pm$ 0.6 ^d	75.7 $\pm$ 2.8 ^e	114.5 $\pm$ 1.8 ^f	70.0 $\pm$ 2.2 ^e	21.1 $\pm$ 2.4 ^c	4.8 $\pm$ 1.1 ^g	398.8 $\pm$ 7.7 ^a
Proportion (%)	100.0	10.2 $\pm$ 0.6 ^a	5.4 $\pm$ 0.1 ^b	12.8 $\pm$ 0.3 ^c	19.0 $\pm$ 0.4 ^d	28.7 $\pm$ 0.1 ^e	17.6 $\pm$ 0.2 ^d	5.3 $\pm$ 0.5 ^b	1.2 $\pm$ 0.2 ^f	95.2 $\pm$ 0.4 ^g

Table 2 Fatty acid compositions (wt.%), amounts and proportions of TAG fractions from rat adipose tissue of the HER group

Fat cell TAGs were separated into eight fractions according to polarity by liquid–liquid partition chromatography and their fatty acid proportions and compositions were determined by GLC. Values are means $\pm$ S.E.M. for three data sets of two individuals each. Recombinant total is calculated from fatty acid compositions and proportions of the TAG fractions. Minor fatty acids (less than 0.02 %) were not considered. Within a line, values that do not share the same superscript letter are significantly different ($P < 0.05$). Abbreviation: n.d., not determinable.

Fatty acids	Total	Fraction ... 1	2	3	4	5	6	7	8	Recombinant total
Saturated sum (wt. %)	30.3 $\pm$ 0.7 ^a	23.4 $\pm$ 0.6 ^b	19.3 $\pm$ 0.3 ^c	24.8 $\pm$ 1.5 ^b	26.8 $\pm$ 0.2 ^b	32.2 $\pm$ 0.2 ^a	36.3 $\pm$ 0.2 ^d	31.1 $\pm$ 0.4 ^a	24.9 $\pm$ 0.3 ^b	30.9 $\pm$ 0.7 ^a
Mono-unsaturated sum (wt. %)	53.1 $\pm$ 0.1 ^a	31.4 $\pm$ 0.7 ^b	33.0 $\pm$ 0.1 ^{bc}	36.9 $\pm$ 1.6 ^{cd}	41.0 $\pm$ 0.2 ^d	49.0 $\pm$ 0.4 ^e	55.6 $\pm$ 0.4 ^f	64.4 $\pm$ 0.6 ^g	73.4 $\pm$ 0.5 ^h	52.8 $\pm$ 0.2 ^a
Di-unsaturated sum (wt. %)	10.8 $\pm$ 0.3 ^a	11.0 $\pm$ 0.4 ^a	14.9 $\pm$ 0.1 ^b	17.8 $\pm$ 0.4 ^c	21.0 $\pm$ 0.1 ^d	14.6 $\pm$ 0.1 ^b	6.4 $\pm$ 0.1 ^e	3.8 $\pm$ 0.2 ^f	1.6 $\pm$ 0.2 ^g	10.7 $\pm$ 0.3 ^a
Tri-unsaturated sum (wt. %)	1.6 $\pm$ 0.1 ^a	3.9 $\pm$ 0.0 ^b	6.9 $\pm$ 0.2 ^c	5.1 $\pm$ 0.4 ^c	3.5 $\pm$ 0.1 ^b	1.3 $\pm$ 0.1 ^a	0.7 $\pm$ 0.1 ^d	0.4 $\pm$ 0.1 ^d	0.1 $\pm$ 0.0 ^e	1.6 $\pm$ 0.1 ^a
Tetra-unsaturated sum (wt. %)	1.6 $\pm$ 0.1 ^a	11.0 $\pm$ 0.7 ^b	11.3 $\pm$ 0.1 ^b	6.2 $\pm$ 0.8 ^c	2.8 $\pm$ 0.0 ^d	1.2 $\pm$ 0.2 ^a	0.4 $\pm$ 0.0 ^e	0.2 $\pm$ 0.0 ^e	0.1 $\pm$ 0.0 ^f	1.6 $\pm$ 0.1 ^a
Penta-unsaturated sum (wt. %)	1.2 $\pm$ 0.1 ^a	9.7 $\pm$ 0.1 ^b	8.1 $\pm$ 0.2 ^c	4.7 $\pm$ 0.7 ^d	2.3 $\pm$ 0.0 ^e	0.8 $\pm$ 0.0 ^f	0.3 $\pm$ 0.0 ^g	0.1 $\pm$ 0.0 ^h	n.d.	1.2 $\pm$ 0.1 ^a
Hexa-unsaturated sum (wt. %)	1.3 $\pm$ 0.2 ^a	9.6 $\pm$ 0.1 ^b	6.6 $\pm$ 0.2 ^c	4.4 $\pm$ 0.6 ^d	2.6 $\pm$ 0.1 ^e	0.9 $\pm$ 0.0 ^a	0.3 $\pm$ 0.0 ^f	n.d.	n.d.	1.2 $\pm$ 0.2 ^a
Amount (μ g)	445.7 $\pm$ 3.8 ^a	6.8 $\pm$ 0.4 ^b	10.7 $\pm$ 0.1 ^c	27.3 $\pm$ 0.7 ^d	67.6 $\pm$ 1.4 ^e	116.2 $\pm$ 2.2 ^f	123.4 $\pm$ 1.6 ^f	82.3 $\pm$ 0.9 ^g	35.4 $\pm$ 0.2 ^h	469.6 $\pm$ 2.7 ^f
Proportion (%)	100.0	1.5 $\pm$ 0.1 ^a	2.3 $\pm$ 0.0 ^b	5.8 $\pm$ 0.1 ^c	14.4 $\pm$ 0.2 ^d	24.7 $\pm$ 0.3 ^e	26.3 $\pm$ 0.4 ^e	17.5 $\pm$ 0.2 ^f	7.5 $\pm$ 0.1 ^g	105.4 $\pm$ 0.5 ^h

2 respectively. For each experimental group, the eight TAG fractions, which ranged from the most polar (fraction 1) to the least polar (fraction 8), showed clear changes in their fatty acid compositions from fraction 1 to fraction 8. The most polar TAG fractions showed a trend for enrichment in polyunsaturated fatty acids, reaching 65.5 % in fraction 1 of the MEN group and 45.5 % in that of the HER group, i.e. approx. three times more than in total TAGs. This tendency was even more pronounced for tetra-, penta- and hexa-unsaturated fatty acids. In contrast, these polar TAG fractions showed a trend to be depleted in saturated and mono-unsaturated fatty acids, the latter being 19 % in fraction 1 of the MEN group and 31 % in that of the HER group, i.e. less than in total TAGs by 1/1.7 to 1/2. This tendency towards depletion was even more clearly shown for very-long-chain saturated and mono-unsaturated fatty acids (see Table 3). Compared with the most polar TAGs, the least polar fractions showed a reverse trend in their fatty acid compositions. They were depleted in polyunsaturated fatty acids and enriched in very-long-chain saturated and mono-unsaturated fatty acids (results not shown).

Relative enrichment of individual fatty acids in the most polar TAGs

The fatty acid compositions of the most polar TAG fractions (alone or summed) were compared with those of total TAGs by

calculating the percentage in polar TAG fractions divided by the percentage in total TAGs (Table 3). In the MEN and HER groups this ratio showed a tendency to be either higher or lower than unity for most fatty acids in all fractions considered, indicating a selective enrichment. On average, this ratio was greater than unity for tetra-, penta- and hexa-unsaturated fatty acids, being close to 3–6.4, 3.5–8.2 and 4.5–5.5 respectively, but lower than unity for very-long-chain saturated and mono-unsaturated fatty acids (0–0.45) in the two most polar TAG fractions (fraction 1, and fractions 1 plus 2) of the MEN group. Similarly, in the HER group, this ratio was much higher than unity for tetra-, penta- and hexa-unsaturated fatty acids, reaching 3–14.7, 4–15.5 and 4.5–7.6 respectively, but also much lower than unity for very-long-chain saturated and mono-unsaturated fatty acids (0–0.35) in the three most polar TAG fractions (fractions 1, 1 plus 2, and 1 plus 2 plus 3). It should be noted that each fraction did not contain the same quantity of TAGs, so that comparisons between fractions should be taken cautiously. One can see that the most polar TAG fraction (fraction 1) of the MEN group represented approx. 10 % of total TAGs (Table 1), whereas the three most polar TAG fractions (fraction 1, 1.5 %; fraction 2, 2.3 %; and fraction 3, 5.8 %) in the HER group have to be summed to reach a similar proportion (Table 2). As a practical outcome, these comparably defined fractions will be used when showing the fatty acid enrichments in the most polar TAGs between the MEN and HER groups (Figure 1).

Table 3 Fatty acid compositions (wt.%) of total TAGs and relative enrichment of fatty acids (percentage in a given fraction divided by percentage in total TAGs) in the most polar TAG fractions from rat adipose tissue of the MEN and HER groups

Fat cell TAGs were separated into eight fractions according to polarity by liquid–liquid partition chromatography and their fatty acid proportions and compositions were determined by GLC. Values are means for three data sets of two individuals each. Fractions 1–2 and 1–3 represent the combinations 1 + 2 and 1 + 2 + 3 respectively. Minor fatty acids (less than 0.02%) were not considered; 0.0 indicates that the value is more than 0.02 and less than 0.05. Abbreviation: n.d., not determinable.

Fatty acids	MEN group			HER group			
	Total	Relative enrichment		Total	Relative enrichment		
		Fraction 1	Fractions 1–2		Fraction 1	Fractions 1–2	Fractions 1–3
Saturated							
C _{14:0}	4.8	0.8	0.9	5.1	1.2	1.2	1.5
C _{15:0}	0.6	0.5	0.7	0.6	1.3	1.0	1.2
C _{16:0}	32.7	0.3	0.4	24.6	0.7	0.6	0.6
C _{20:0}	0.2	n.d.	0.2	0.1	n.d.	0.4	0.1
C _{22:0}	0.0	n.d.	n.d.	0.0	n.d.	n.d.	n.d.
Mono-unsaturated							
C _{14:1} , <i>n</i> –5	0.2	1.5	1.8	0.1	2.1	2.5	1.7
C _{16:1} , <i>n</i> –9	0.6	1.2	1.4	0.2	1.8	1.9	1.8
C _{16:1} , <i>n</i> –7	8.3	0.9	1.1	5.4	1.2	1.5	1.8
C _{17:1} , <i>n</i> –8	0.7	1.5	1.4	0.6	1.4	1.2	1.7
C _{18:1} , <i>n</i> –9	20.1	0.4	0.5	22.9	0.7	0.7	0.7
C _{18:1} , <i>n</i> –7	5.7	0.3	0.5	3.4	1.0	0.9	0.8
C _{20:1} , <i>n</i> –11	0.2	0.4	0.5	2.5	0.2	0.2	0.3
C _{20:1} , <i>n</i> –9	1.2	0.3	0.4	10.1	0.3	0.3	0.3
C _{20:1} , <i>n</i> –7	0.5	0.1	0.3	0.6	n.d.	0.2	0.3
C _{22:1} , <i>n</i> –11	0.2	0.1	0.3	7.3	0.2	0.1	0.2
Di-unsaturated							
C _{18:2} , <i>n</i> –6	11.1	0.7	0.9	10.5	1.1	1.3	1.5
C _{18:2} , <i>n</i> –4	0.3	0.9	1.0	0.1	n.d.	3.6	2.4
C _{20:2} , <i>n</i> –6	0.2	0.6	0.9	0.2	n.d.	0.7	0.9
Tri-unsaturated							
C _{18:3} , <i>n</i> –6	0.2	2.0	2.2	0.1	n.d.	3.0	2.2
C _{18:3} , <i>n</i> –4	0.3	1.8	1.8	0.1	n.d.	2.8	1.9
C _{18:3} , <i>n</i> –3	0.7	2.2	2.3	1.4	2.9	3.8	3.6
C _{20:3} , <i>n</i> –6	0.2	2.1	2.2	0.1	n.d.	2.1	1.9
Tetra-unsaturated							
C _{16:4} , <i>n</i> –1	0.3	6.4	4.9	0.1	14.7	11.6	7.5
C _{18:4} , <i>n</i> –3	1.0	4.9	4.2	0.9	7.3	7.7	5.7
C _{18:4} , <i>n</i> –1	0.3	5.0	4.6	0.1	12.0	10.9	7.2
C _{20:4} , <i>n</i> –6	0.5	2.6	2.8	0.2	2.9	3.3	2.8
C _{20:4} , <i>n</i> –3	0.5	3.7	3.4	0.3	5.2	5.2	3.9
Penta-unsaturated							
C _{18:5} , <i>n</i> –3	0.0	8.2	6.6	0.0	15.6	12.9	7.6
C _{20:5} , <i>n</i> –3	3.2	5.8	4.6	0.8	9.4	7.8	5.7
C _{22:5} , <i>n</i> –3	1.0	3.8	3.5	0.4	4.6	5.4	4.0
Hexa-unsaturated							
C _{22:6} , <i>n</i> –3	4.2	5.5	4.5	1.3	7.6	6.2	4.6

The influence of the fatty acid molecular structure on enrichment in the most polar TAG fractions was investigated by plotting relative enrichment against unsaturation at a given chain length, and against chain length at a given unsaturation (Figure 1). The positions of double bonds were not taken into consideration and an average relative enrichment was calculated when there were positional isomers. In both groups, for a given chain length, the relative enrichment increased with the number of double bonds (left panels). These relationships are best described by exponential formulae identical with that relating mobilization of fatty acids to their unsaturation at a given chain length, i.e. $y = b + a(e^{kn} - 1)$, in which b is the y intercept and n the number of double bonds. This is further illustrated in Figure 1 by the best-fit curves from this exponential formula. In contrast, for a given number of double bonds, the relative enrichment decreased in both groups when the fatty acid chain length increased (right panels). This was observed mainly among tetra-

and penta-unsaturated fatty acids. In all of the most polar TAG fractions reported in Table 3, similar relationships to those described above and illustrated in Figure 1 were found between relative enrichment and fatty acid chain length or unsaturation (results not shown).

Relative enrichment of fatty acids in TAG fractions according to mobilization rates

Fatty acids have already been classified roughly into three categories according to their mobilization rates (see the Introduction section). Highly mobilized fatty acids were mainly represented in the most polar TAG fractions. The relative enrichment of this class of fatty acids was 4.9 and 7.7 in fraction 1 of the MEN and HER groups respectively, and then progressively decreased to a value close to zero in fraction 8 in both experimental groups (Figure 2). Weakly mobilized fatty acids

Figure 1 Relationships between relative enrichment of fatty acids in the most polar TAG fractions of adipose tissue and their unsaturation at given chain lengths (left panels) or their chain length at given unsaturations (right panels)

Upper panels, MEN group (fraction 1); lower panels, HER group (fractions 1–3). Values are means for three data sets of two individuals each. When there were positional isomers, an average value was calculated. Abbreviations: C, carbon atoms; DB, double bond(s).

were found mainly in the least polar TAG fractions. The relative enrichment of this class of fatty acids corresponded to approx. 0.2 in fraction 1 of both experimental groups, and then tended to increase progressively in all fractions to reach 6.2 and 2.8 in fraction 8 of the MEN and HER groups respectively. Moderately mobilized fatty acids were represented in significant proportions in all fractions. The relative enrichment of this class of fatty acids was close to unity in most fractions considered in both experimental groups (Figure 2).

DISCUSSION

The selective steps in the mobilization of fatty acids from fat cells and the underlying mechanism remain unresolved questions. Mature adipocytes contain a large unilocular lipid droplet [12] that presents a small surface-to-volume ratio and thus a limited substrate availability [23]. The neutral lipids are water-insoluble and lipases are water-soluble [22,23]. The catabolic process of apolar lipids seems closely related to the lipid/water interface, where most lipases act [18,22,23]. This is also true for HSL, for which a translocation of the enzyme to the lipid storage droplet is involved in the mechanism of hormone-stimulated lipolysis within adipocytes [11,12]. This strengthens the notion that substrate accessibility is a key step during lipolysis [22,23]. The

Figure 2 Relative enrichment of three categories of fatty acids according to their mobilization rates in the TAG fractions of adipose tissue

TAG fractions are arranged from left to right in decreasing order of polarity. Upper panel, MEN group; lower panel, HER group. Values are means for three data sets of two individuals each. Highly mobilized fatty acids have 16–20 carbon atoms and four or five double bonds; weakly mobilized fatty acids have 20–24 carbon atoms and no or one double bond; moderately mobilized fatty acids have 14–20 carbon atoms and zero to three double bonds, and 22 carbon atoms and four to six double bonds [14].

physicochemical state of the substrate that could condition its availability has not been extensively studied during investigations into HSL substrate specificity. On the basis of their interactions with water, biologically active lipids have been classified according to physical properties and partition coefficients between several phases differing by their polarity [33,34]. It has been reported for lipoproteins that apolar lipids, mainly TAGs, enriched in polyunsaturated fatty acids have a higher partition coefficient between an interfacial phase and an apolar phase than TAGs enriched in saturated fatty acids [34]. As a result, lipolytic enzymes would be more efficient towards TAGs enriched in polyunsaturated fatty acids. This could explain the preferential release of some polyunsaturated fatty acids from TAGs by HSL [19], be consistent with our results [13–16], and meet previous suggestions [35,36]. Moreover it has been reported that a low molecular mass of a TAG molecule in a heterogeneous TAG mixture facilitates hydrolysis by the lipase despite similar hydrolysis rates for both long and short fatty acids, and a higher hydrophilicity or diffusional mobility of the lower-molecular-mass TAGs has been proposed [37]. The accumulation of these molecules in the surface of the oil droplets, which might partly account for a more rapid hydrolysis of lower-molecular-mass TAGs, has also been suggested [37]. Therefore a selective fatty acid mobilization by the preferential hydrolysis of a TAG fraction might be possible.

A selective enrichment of substrate submitted to hydrolysis in

Figure 3 Relative enrichment of fatty acids in the most polar adipose tissue TAGs (upper panel) and relative mobilization rates of fatty acids from white fat cells (lower panel) plotted as a function of both fatty acid chain length and unsaturation

Values of relative enrichments of fatty acids (percentage in a TAG fraction divided by percentage in total TAGs) are from Table 3 (MEN group, fraction 1). Relative mobilization rates of fatty acids are computed from the results of a previous study [13]. The relative mobilization rate of a fatty acid was calculated as the ratio of its percentage weight in released FFAs to that in fat cell TAGs from which it originated through lipolysis *in vitro*. Abbreviations: sat, saturated; mono, mono-unsaturated; di, di-unsaturated; tri, tri-unsaturated; tetra, tetra-unsaturated; penta, penta-unsaturated fatty acids.

certain TAG species according to their physicochemical properties such as polarity can be considered. Figure 3 (upper panel) shows the relative enrichments of fatty acids in the most polar TAGs plotted as a function of both chain length and unsaturation. For convenience we choose the most polar TAG fractions, representing about 10 % of total TAGs. For a given chain length the relative enrichment of fatty acids in the most polar TAGs increases exponentially with unsaturation, whereas for a given unsaturation it decreases with increasing chain length (see also Figure 1). Taking into account our previous studies on mobilization of fatty acids from white fat cells [13,14,16], we plot in Figure 3 (lower panel) the relative mobilization rates of fatty acids as a function of both chain length and unsaturation. For a given chain length the relative mobilization rate of fatty acids increases exponentially with unsaturation, whereas for a given unsaturation it decreases with increasing chain length. The widening in the range of the relative enrichment of fatty acids in polar TAGs compared with their relative mobilization rate (Figure 3) can be explained by the stringent separation of TAGs according to polarity. The substrate subjected to hydrolysis would be enriched in polar TAGs only, but not necessarily constituted exclusively of polar TAGs, as already suggested

[34,37]. Therefore the relative enrichment of fatty acids in the most polar adipose tissue TAGs is consistent with their relative mobilization rates (i.e. the relative enrichment of fatty acids in released FFAs from fat cells). According to the dynamic concept of lipolysis, and taking into account our selective hydrolysis hypothesis, the TAG fraction subjected to hydrolysis would be permanently enriched in the most polar TAGs compared with total TAGs so that the released FFAs would reflect, at least partly, this substrate fatty acid enrichment. During fasting-induced energy depletion, adipose tissue would be relatively depleted in the most polar TAGs and, conversely, relatively enriched in the least polar TAGs on account of the continuous 'peeling off' of substrate enriched in the former TAG molecular species. The relative enrichment of fatty acids according to their mobilization rates in the TAG fractions of adipose tissue arranged in decreasing order of polarity (see Figure 2) is in full agreement with the fact that highly mobilized fatty acids will be preferentially lost during fat store depletion, whereas weakly mobilized fatty acids will be relatively spared [16]. Therefore this view is consistent with the reported selective patterns of fatty acid mobilization *in vitro* [13–15] and *in vivo* [16] in the rat.

It should be noticed that the method used in the present study allows an investigation of the physicochemical properties of the substrate independently of lipase specificities. The hydrolysis of TAGs by HSL would be preferential but not stringent, thus allowing a determination of substrate specificities of the lipase [19,21,39]. However, the specificity of HSL towards the substrate could reflect or be modulated by the accessibility and/or the availability of TAGs [23,40]. Melting points of TAG molecular species could also influence their metabolic fates [35,36,41]. Conceptually, such an explanation does not seem to apply for the mobilization of fatty acids because of similar selectivity, although there were marked differences in the fatty acid composition of TAGs [13,14], i.e. in the physical state of TAG molecules. Nevertheless it is known that TAG-containing fatty acyl chains with different degrees of unsaturation have complicated phase behaviours [42].

In conclusion, we propose that the selectivity of fatty acid mobilization from white fat cells lies in the hydrolysis of substrate enriched in the most polar TAGs. This mechanism would involve a heterogeneous distribution of TAGs according to their physicochemical properties, leading to a selective accessibility of substrate. TAG molecular species submitted to hydrolysis would be high in very-long-chain polyunsaturated fatty acids and low in very-long-chain saturated and mono-unsaturated fatty acids compared with those of the droplet core, so that they might have 'kinetic advantages'. This raises the possibility that the molecular species of TAGs might be one of the regulating factors. Although other selectivities could also intervene in the mobilization of white fat cell fatty acids, they would be subsequent to the proposed mechanism.

I thank Dr. A. C. Bach, Dr. R. Groscolas, Dr. J. Hauton and Dr. C. Leray for helpful discussions, and E. Mioskowski for her excellent technical assistance. Menhaden and herring oils were gifts from RP Scherer (Beinheim, France) and from the Norwegian Herring Oil and Meal Industry Research Institute (Bergen, Norway) respectively.

REFERENCES

- Vaughan, M., Berger, J. E. and Steinberg, D. (1964) *J. Biol. Chem.* **239**, 401–409
- Fredrikson, G., Strålfors, P., Osten Nilsson, N. and Belfrage, P. (1981) *J. Biol. Chem.* **256**, 6311–6320
- Khoo, J. C., Jarrett, L., Mayer, S. E. and Steinberg, D. (1972) *J. Biol. Chem.* **247**, 4812–4818
- Zinder, O., Eisenberg, E. and Shapiro, B. (1973) *J. Biol. Chem.* **248**, 7673–7676
- Wise, L. S. and Jungas, R. L. (1978) *J. Biol. Chem.* **253**, 2624–2627
- Scow, R. O. and Blanchette-Mackie, E. J. (1991) *Brain Res. Bull.* **27**, 487–491

- 7 Strålfors, P. and Belfrage, P. (1983) *J. Biol. Chem.* **258**, 15146–15152
- 8 Blanchette-Mackie, E. J., Dwyer, N. K., Barber, T., Coxey, R. A., Takeda, T., Rondoni, C. M., Theodorakis, J. L., Greenberg, A. S. and Londos, C. (1995) *J. Lipid Res.* **36**, 1211–1226
- 9 Okuda, H., Morimoto, C. and Tsujita, T. (1994) *J. Lipid Res.* **35**, 36–44
- 10 Okuda, H., Morimoto, C. and Tsujita, T. (1994) *J. Lipid Res.* **35**, 1267–1273
- 11 Hirsch, A. H. and Rosen, O. M. (1984) *J. Lipid Res.* **25**, 665–677
- 12 Egan, J. J., Greenberg, A. S., Chang, M.-K., Wek, S. A., Moos, Jr., M. C. and Londos, C. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 8537–8541
- 13 Raclot, T. and Groscolas, R. (1993) *J. Lipid Res.* **34**, 1515–1526
- 14 Raclot, T., Mioskowski, E., Bach, A. C. and Groscolas, R. (1995) *Am. J. Physiol.* **269**, R1060–R1067
- 15 Raclot, T. and Groscolas, R. (1994) *Am. J. Clin. Nutr.* **60**, 72–78
- 16 Raclot, T. and Groscolas, R. (1995) *J. Lipid Res.* **36**, 2164–2173
- 17 Belfrage, P., Fredrikson, G., Strålfors, P. and Tornqvist, H. (1984) in *Lipases* (Borgström, B. and Brockman, H. L., eds.), pp. 365–416, Elsevier, Amsterdam
- 18 Soma, M. R., Mims, M. P., Chari, M. V., Rees, D. and Morrisett, D. (1992) *J. Biol. Chem.* **267**, 11168–11175
- 19 Gavino, V. C. and Gavino, G. R. (1992) *Lipids* **27**, 950–954
- 20 Fredrikson, G. and Belfrage, P. (1983) *J. Biol. Chem.* **258**, 14253–14256
- 21 Raclot, T., Leray, C., Bach, A. C. and Groscolas, R. (1995) *Biochem. J.* **311**, 911–916
- 22 Derewenda, Z. S. and Sharp, A. M. (1993) *Trends Biochem. Sci.* **18**, 20–25
- 23 Brockman, H. L. (1984) in *Lipases* (Borgström, B. and Brockman, H. L., eds.), pp. 3–46, Elsevier, Amsterdam
- 24 Huang, Y.-S., Lin, X., Smith, R. S., Redden, P. R., Jenkins, D. K. and Horrobin, D. F. (1992) *Lipids* **27**, 711–715
- 25 Belzung, F., Raclot, T. and Groscolas, R. (1993) *Am. J. Physiol.* **264**, R1111–R1118
- 26 Leray, C., Raclot, T. and Groscolas, R. (1993) *Lipids* **28**, 279–284
- 27 Folch, J., Lees, M. and Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- 28 Litchfield, C. (1972) in *Analysis of Triglycerides* (Litchfield, C., ed.), pp. 67–103, Academic Press, New York
- 29 Sherma, J. and Bennett, S. (1983) *J. Liquid Chromatogr.* **6**, 1193–1211
- 30 Morrison, W. R. and Smith, L. M. (1964) *J. Lipid Res.* **5**, 600–608
- 31 Motulsky, H. J. and Ransnas, L. A. (1987) *FASEB J.* **1**, 365–374
- 32 Harper, J. F. (1984) *Comput. Biol. Med.* **14**, 437–445
- 33 Small, D. M. (1970) *Fed. Proc.* **29**, 1320–1326
- 34 Hauton, J. C. and Lafont, H. (1987) *Biochimie* **69**, 177–204
- 35 Bennett Clark, S., Atkinson, D., Hamilton, J. A., Forte, T., Russell, B., Feldman, E. B. and Small, D. M. (1982) *J. Lipid Res.* **23**, 28–41
- 36 Florén, C. H. and Nilsson, Å. (1977) *Eur. J. Biochem.* **77**, 23–30
- 37 Brockerhoff, H. and Jensen, R. G. (1974) in *Lipolytic Enzymes* (Brockerhoff, H. and Jensen, R. G. eds.), pp. 25–34, Academic Press, New York
- 38 Reference deleted
- 39 Jensen, R. G., Dejong, F. A. and Clark, R. M. (1983) *Lipids* **18**, 239–252
- 40 Marangoni, A. G. (1994) *Biochem. Biophys. Res. Commun.* **200**, 1321–1328
- 41 Redgrave, T. G., Kodali, D. R. and Small, D. M. (1988) *J. Biol. Chem.* **263**, 5118–5123
- 42 Parks, J. S., Atkinson, D., Small, D. M. and Rudel, L. L. (1981) *J. Biol. Chem.* **256**, 12992–12999