Fatty acid specificity of hormone-sensitive lipase: implication in the selective hydrolysis of triacylglycerols

Thierry Raclot,[1,*,†] Cecilia Holm,[§] and Dominique Langin*

INSERM Unité 317,[*] Institut Louis Bugnard, Faculté de Médecine, Université Paul Sabatier, Hôpital Rangueil, 31403 Toulouse Cedex 4, France; Centre d'Ecologie et Physiologie Energétiques,[†] CNRS UPR 9010, associé à l'Université Louis Pasteur, 23 rue Becquerel, 67087 Strasbourg Cedex 2, France; and Section for Molecular Signaling,[§] Department of Cell and Molecular Biology, Lund University, BMC, C11, SE-221 84 Lund, Sweden

Abstract **The selective mobilization of fatty acids from white fat cells depends on their molecular structure, in particular the degree of unsaturation. The present study was designed to examine if the release of fatty acids by hormone-sensitive lipase (HSL) in vitro *i)* is influenced by the amount of unsaturation, *ii)* depends on the temperature, and *iii)* could explain the selective pattern of fatty acid mobilization and notably the preferential mobilization of certain highly unsaturated fatty acids. Recombinant rat and human HSL were incubated with a lipid emulsion. The hydrolysis of 35 individual fatty acids, ranging in chain length from 12 to 24 carbon atoms and in unsaturation from 0 to 6 double bonds was measured. Fatty acid composition of in vitro released NEFA was compared with that of fat cell triacylglycerols (TAG), the ratio % NEFA/% TAG being defined as the relative hydrolysis. The relative hydrolysis of individual fatty acids differed widely, ranging from 0.44 (24:1n–9) to 1.49 (18:1n–7) with rat HSL, and from 0.38 (24:1n–9) to 1.67 (18:1n–7) with human HSL. No major difference was observed between rat and human HSL. The relative release was dependent on the number of double bonds according to chain length. The amount of fatty acid released by recombinant rat HSL was decreased but remained robust at 4°C compared with 37°C, and the relative hydrolysis of some individual fatty acids was affected. The relative hydrolysis of fatty acids moderately, weakly, and highly mobilized by adipose tissue in vivo was similar and close to unity in vitro. We conclude that *i)* the release of fatty acids by HSL is only slightly affected by their degree of unsaturation, *ii)* the ability of HSL to efficiently and selectively release fatty acids at low temperature could reflect a cold adaptability for poikilotherms or hibernators when endogenous lipids are needed, and *iii)* the selectivity of fatty acid hydrolysis by HSL does not fully account for the selective pattern of fatty acid mobilization, but could contribute to explain the preferential mobilization of some highly unsaturated fatty acids compared with others.**—Raclot, T., C. Holm, and D. Langin. **Fatty acid specificity of hormone-sensitive lipase: implication in the selective hydrolysis of triacylglycerols.** ***J. Lipid Res.*** **2001.** 42: **2049–2057.**

Supplementary key words lipolysis • fatty acid molecular structure • lipid emulsion • adipose tissue

If various fatty acids are stored as triacylglycerols (TAG) in adipose depots that represent the lipid storage of mammals and birds (1), the relative rate of their deposition and mobilization can vary greatly in response to nutritional states and endocrine factors (2). The fine regulation of lipolysis can be considerably increased by a variety of hormones through increased adenylate cyclase activity (3). The process involves the hydrolysis of stored TAG by fat cell lipases into NEFA and glycerol, followed by their release.

The mobilization of fatty acids from white fat cells is selective and depends on the fatty acid chain length and unsaturation (4). Among fatty acids usually found in adipose tissue TAG (12 to 24 carbon atoms and 0 to 6 double bonds), a fatty acid is more readily mobilized as its carbon chain is shorter and more unsaturated. From these results, fatty acids have been grouped into three categories by taking into account their relative mobilization rate, chain length, and unsaturation (5). Highly mobilized fatty acids include 16–20 carbon atom fatty acids with 4–5 double bonds, whereas weakly mobilized fatty acids include 20–24 carbon atom fatty acids with 0–1 double bond.

The precise mechanism of the selective mobilization of fat cell fatty acids remains to be elucidated. Lipolysis is mainly achieved by hormone-sensitive lipase (HSL), which has a critical role in the control of energy homeostasis and catalyzes the rate-limiting step in the breakdown of adipocyte TAG (3). Among the possible mechanisms that could explain the selective mobilization of fatty acids, differential hydrolysis of adipose tissue TAG by HSL is one. Hence, the selective hydrolysis of fatty acids might be a consequence of the enzymological properties of HSL.

Abbreviations: HSL, hormone-sensitive lipase; TAG, triacylglycerols; VLC-MUFA, very long-chain monounsaturated fatty acids.

[1] To whom correspondence should be addressed at Centre d'Ecologie et Physiologie Energétiques, CNRS UPR 9010, 23 rue Becquerel, 67087 Strasbourg Cedex 2, France.
e-mail: Thierry.Raclot@c-strasbourg.fr

This would be in line with a previous study in our laboratories showing that fatty acids are selectively hydrolyzed from TAG by HSL according to carbon atom chain length (6).

The first objective of this study was therefore to determine *i)* whether HSL shows selectivity toward highly unsaturated fatty acids, and *ii)* whether the hydrolysis of fatty acids by HSL is affected by incubation conditions. Interestingly, at low temperatures, HSL shows a 3- to 5-fold higher relative catalytic activity compared with lipoprotein lipase (7). Moreover, it was proposed that the homology between HSL and lipase 2 from the antarctic bacterium Moraxella TA144 could reflect a common cold adaptability. This question was examined by comparing the in vitro hydrolysis of individual fatty acids by HSL from lipid emulsion at various temperatures.

Another aim of this study was to test whether the selective release of fatty acids from lipid emulsion by HSL is consistent with their mobilization rate from adipocytes. It was of particular interest to determine *i)* whether the relationships between the molecular structure of fatty acids and their relative mobilization rates were found in vitro for the relative hydrolysis of fatty acids by HSL, and *ii)* whether the most readily mobilized fatty acids were readily released by HSL, and conversely, whether the least readily mobilized fatty acids were resistant to hydrolysis.

These questions were examined by comparing the in vitro hydrolysis by HSL of individual fatty acids from a lipid emulsion containing a wide spectrum of fatty acids, especially PUFA and very long chain-monounsaturated fatty acids (VLC-MUFA). According to previous results (4, 5), PUFA include some of the most readily mobilized fatty acids, whereas VLC-MUFA are among the least readily mobilized.

MATERIALS AND METHODS

Materials

Reagent grade chemical solvents were supplied by SDS (Peypin, France). TLC plates coated with silica gel 60 and butylated hydroxytoluene were purchased from Merck (Darmstadt, Germany). All other reagents were supplied by Sigma (St. Louis, MO, USA). The recombinant rat and human HSL proteins were overexpressed and purified to >99% homogeneity using a baculovirus/insect-cell system (8, 9). Omegavenous lipid emulsion (10%) was kindly provided by Fresenius France (Sèvres, France).

Enzyme activity

Recombinant HSL was assayed as previously reported with slight modifications (6). Briefly, a lipid emulsion (Omegavenous, 10% TAG from fish oil) was incubated with HSL in a phosphate buffer, 37°C, pH 7.0, containing 8% (w/v) of bovine serum albumin (essentially fatty acid-free) as fatty acid acceptor in a shaking bath at 200 cycles/min. Different incubation times (0, 2, 4, 8, 12 h) and various enzyme concentrations were tested in order to obtain a large efflux of NEFA from TAG hydrolysis and to allow an accurate analysis of their fatty acid composition. Finally, recombinant rat HSL (1.5 μl of a solution at 1 mg/ml; 200 U/ml) and recombinant human HSL (25 μl of a solution at 0.12 mg/ml; 12 U/ml) were incubated in 5-ml polypropylene flasks in 200 μl of buffer containing 10 μl of lipid emulsion for 6 h. To check whether temperature affects the release of individual fatty acids by HSL, various incubation temperatures were tested. Incubations were carried out at 37, 20, and 4°C in the shaking bath. To verify that no NEFA arose from chemical degradation of the lipid emulsion, incubations were also performed without HSL as controls.

Lipid extraction and separation

At the end of the experiment, the incubation media (containing TAG and NEFA) were extracted according to the Folch et al. procedure (10). Total lipids from lipid emulsion samples were mixed with 100 mg of heptadecanoic acid and 250 mg of tripentadecanoin as internal standards for NEFA and TAG, respectively. The exact composition of the lipid emulsion (including cholesterol esters, monoacylglycerols, diacylglycerols, and phospholipids) was determined using a known amount of internal standard (heptadecanoic acid) added to each lipid fraction. The initial fatty acid composition of NEFA and TAG was determined on incubation medium containing buffer and lipid emulsion without HSL. Lipid extracts were purified by TLC by using plates previously washed with chloroform–methanol 1:1 (v/v). TLC plates coated with silica gel G were impregnated with boric acid in ethanol (2.3%, w/v) and activated at 110°C for 10 min (11). Then lipid extracts were separated by TLC using hexane–diethyl ether–acetic acid (70:30:1, v/v/v) as the developing solvent. The TLC plates were dried under nitrogen and sprayed with primulin. The NEFA or TAG bands were then scraped into vials. Butylated hydroxytoluene at a final concentration of 0.05% was added to all solvent mixtures as an antioxidant.

Fatty acid analysis

Fatty acid methyl esters were prepared using 14% boron trifluoride in methanol after saponification (12). They were then purified by TLC using plates coated with silica gel 60 and hexane–diethyl ether–acetic acid 70:30:1 (v/v/v) as the developing solvent. The plates were dried under nitrogen and sprayed with primulin (4). Gas–liquid chromatography analysis of fatty acid methyl esters was performed with a Chrompack CP 9001 chromatograph (Chrompack, Les Ulis, France) equipped with a flame ionization detector, an AT-WAX capillary column (30 m × 0.25 mm I.D., 0.25 mm thickness, 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 (Nu Check Prep, Elysian, MN, USA) as described before (4). Chromatographic analyses were run at least in duplicate.

Calculations and statistics

Values are expressed as means ± SEM of 4–6 determinations. The weight percentage of each fatty acid in NEFA and TAG was compared using the Peritz F-test for multiple comparisons (13). The relative hydrolysis rate of individual fatty acids was calculated as the ratio of their percentage weight in released NEFA to that in substrate TAG. A ratio greater than, equal to, or lower than unity shows that the fatty acid is released, respectively, more, equally, or less readily than the total TAG-fatty acids. Comparisons between two groups and multiple comparisons were performed by using the Student *t*-test or the Peritz F-test, respectively. The criterion of significance was $P < 0.05$.

RESULTS

Lipid and fatty acid composition of lipid emulsion TAG

In the original lipid emulsion, TAG represented 92–95% of total lipids. Cholesterol esters and diacylglycerols (*sn*-1,2-(2,3-) and (*sn*-1,3-) were found in very low amounts

(less than 0.5% of total lipids), whereas monoacylglycerols were found even lower. Their fatty acid composition was roughly similar to that of TAG. Phospholipids represented 5–7% of the total lipids. At the onset of the experiment, NEFA found in the incubation medium (present in the lipid emulsion and bound to albumin) represented about 1% of the total lipids. They were deducted before final calculations of released NEFA. Incubating the lipid emulsion with recombinant HSL resulted in significant changes in the relative proportion of NEFA and TAG, which were markedly increased and decreased, respectively (results not shown).

Among the 35 fatty acids that were considered, their weight percentages ranged from 0.1 (12:0) to 24.3% (20:5n–3) (**Table 1**). The content of TAG in total saturated, monounsaturated, and polyunsaturated fatty acids was 16.1, 23.3, and 60.6%, respectively. The chain length and unsaturation of fatty acids ranged from 12 to 24 carbon atoms and from 0 to 6 double bonds, respectively. VLC-MUFA (20:1n–9, 22:1n–11, and 24:1n–9) were present in low amounts, while the content of TAG in the total PUFA and above all in tetra-, penta-, and hexa-unsaturated fatty acids, was high.

TABLE 1. Fatty acid composition (wt. %) of TAG and relative hydrolysis determined after enzymatic hydrolysis of the omegavenous lipid emulsion

		Relative Hydrolysis (% NEFA/% TAG)	
Fatty Acids	TAG	Rat HSL	Human HSL
Saturated			
12:0	0.105 ± 0.005	1.06 ± 0.07	1.08 ± 0.08
14:0	5.880 ± 0.321	0.96 ± 0.05	0.95 ± 0.06
16:0	8.827 ± 0.060	1.27 ± 0.05[a]	1.24 ± 0.08[a]
18:0	1.286 ± 0.114	1.15 ± 0.13	1.14 ± 0.14
Mono-unsaturated			
16:1n–7	9.407 ± 0.557	0.91 ± 0.03	0.94 ± 0.12
16:1n–5	0.207 ± 0.005	1.10 ± 0.06	1.03 ± 0.08
17:1n–8	1.595 ± 0.065	0.74 ± 0.03[a]	0.77 ± 0.11
18:1n–9	7.048 ± 0.292	1.35 ± 0.16[a]	1.20 ± 0.09[a]
18:1n–7	2.635 ± 0.088	1.49 ± 0.04[a]	1.67 ± 0.20[a]
18:1n–5	0.107 ± 0.012	1.16 ± 0.06[a]	1.19 ± 0.05[a]
20:1n–11	0.146 ± 0.018	1.07 ± 0.07	0.94 ± 0.08
20:1n–9	0.924 ± 0.076	1.18 ± 0.03[a]	1.27 ± 0.19
20:1n–7	0.186 ± 0.016	0.93 ± 0.06	0.98 ± 0.09
22:1n–11	0.645 ± 0.062	0.78 ± 0.03[a]	0.95 ± 0.11
22:1n–9	0.156 ± 0.007	0.99 ± 0.06	0.87 ± 0.09
24:1n–9	0.251 ± 0.032	0.44 ± 0.12[a]	0.38 ± 0.08[a]
Poly-unsaturated			
16:4n–3	2.814 ± 0.134	0.78 ± 0.03[a]	0.56 ± 0.09[a]
18:2n–6	1.744 ± 0.023	1.18 ± 0.13	0.92 ± 0.11
18:2n–4	0.344 ± 0.002	1.32 ± 0.08[a]	1.30 ± 0.19
18:3n–6	0.312 ± 0.005	0.75 ± 0.11	0.67 ± 0.08[a]
18:3n–3	1.252 ± 0.030	1.01 ± 0.02	0.91 ± 0.06
18:4n–3	3.886 ± 0.140	0.96 ± 0.06	0.75 ± 0.13
18:4n–1	0.349 ± 0.007	0.84 ± 0.12	0.82 ± 0.17
20:2n–6	0.176 ± 0.009	1.19 ± 0.09[a]	1.14 ± 0.08
20:3n–6	0.162 ± 0.014	1.06 ± 0.06	1.11 ± 0.06
20:4n–6	1.368 ± 0.020	1.03 ± 0.03	1.31 ± 0.11[a]
20:3n–3	0.117 ± 0.056	1.06 ± 0.05	1.27 ± 0.07[a]
20:4n–3	1.043 ± 0.014	1.18 ± 0.07[a]	1.30 ± 0.10[a]
20:5n–3	24.362 ± 0.234	1.06 ± 0.05	1.26 ± 0.09[a]
21:5n–3	0.782 ± 0.011	1.05 ± 0.06	1.04 ± 0.12
22:4n–6	0.127 ± 0.014	0.56 ± 0.03[a]	0.53 ± 0.06[a]
22:5n–6	0.393 ± 0.103	0.70 ± 0.04[a]	0.56 ± 0.11[a]
22:5n–3	2.885 ± 0.093	0.57 ± 0.02[a]	0.60 ± 0.08[a]
22:6n–3	18.480 ± 0.445	0.61 ± 0.02[a]	0.64 ± 0.10[a]

Values are means ± SEM (n = 5–7). Minor fatty acids (less than 0.1% in emulsion TAG) are not shown.

[a] Within a column, values that share a superscript a indicate for these fatty acids that their percentages in NEFA are significantly different from those in TAG ($P < 0.05$).

Relative release of individual unsaturated fatty acids by recombinant rat and human HSL

The extent of hydrolysis of the lipid emulsion by recombinant rat and human HSL was dependent on the incubation conditions. The NEFA content ranged from 8–12 μg (native emulsion; baseline) to 230–270 μg (recombinant rat HSL for 6 h). The quantity of NEFA recovered after hydrolysis of the lipid emulsion by recombinant human HSL for 6 h was lower, reaching only about 130–160 μg on the average.

The relative release (% NEFA/% TAG) of 35 fatty acids from the lipid emulsion by recombinant rat and human HSL was calculated (Table 1). The relative release ranged from 0.44 to 1.49 with rat HSL, and from 0.38 to 1.67 with human HSL. In both groups, the least and most readily released fatty acids were 24:1n–9 and 18:1n–7, respectively. For most fatty acids, the relative release was not significantly different between rat and human HSL. Among major fatty acids, the relative release of the least and the most readily released fatty acid was, respectively, 0.61 (22:6n–3) and 1.35 (18:1n–9) with rat HSL, and 0.64 (22:6n–3) and 1.26 (20:5n–3) with human HSL.

Among monounsaturated fatty acids, all isomers of 18:1 were preferentially released, whereas 24:1n–9 was 2–3 times less readily released than total fatty acids (Table 1). Among the PUFA, 16:4n–3, 22:4n–6, 22:5n–3, and 22:6n–3 were 1.5 to 2 times less readily released than total fatty acids. Interestingly, 20:5n–3 was about 2 times more readily released than 22:5n–3 and 22:6n–3. Data were similar for human and rat HSL. Differences were observed for a few fatty acids, such as 20:5n–3, which was preferentially hydrolyzed only by human HSL.

The influence of fatty acid molecular structure on hydrolysis by HSL was investigated by plotting relative release versus unsaturation at a given chain length and versus chain length at a given unsaturation (**Fig. 1**). The position of double bonds was not considered and an average relative enrichment value was calculated when there were several positional isomers. In both groups, for a given chain length, the relative release slightly increased with the number of double bonds for fatty acids with 20 carbon atoms, whereas it decreased with the number of double bonds for fatty acids with 16, 18, and 22 carbon atoms (Fig. 1A). On the other hand, for a given number of double bonds, the relative release increased and then decreased as the chain length increased in both groups (Fig. 1B). This is particularly seen for mono-, tetra-, and penta-unsaturated fatty acids.

Relative release of fatty acids by HSL at different temperatures

The extent of hydrolysis of the lipid emulsion by both recombinant rat and human HSL was dependent on the

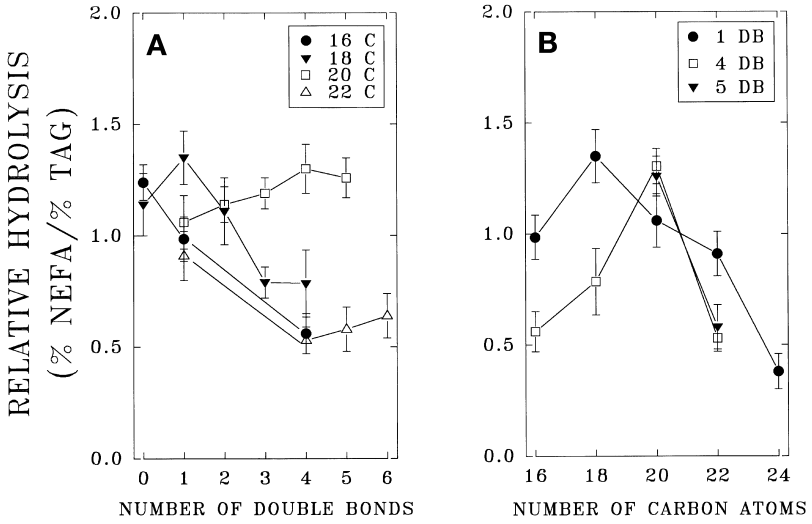

Fig. 1. Relationships between in vitro relative hydrolysis of individual fatty acids from the lipid emulsion by recombinant human HSL and their unsaturation at given chain lengths (A) and of their chain length at given unsaturations (B). The relative hydrolysis of each fatty acid was calculated as the ratio between its weight percentage in released NEFA to that in lipid emulsion TAG. Data are from Table 1. Values are expressed as means ± SEM (n = 5–7). C, carbon atoms; DB, double bonds.

temperature. At 4°C, the NEFA production ranged from 50–80 μg (recombinant human HSL for 6 h) to 80–120 μg (recombinant rat HSL for 6 h). The hydrolysis rate at 4°C represented 35–45% that of the 37°C rate. At 20°C, the NEFA quantity recovered after hydrolysis was similar to that obtained at 37°C.

The relative release of 18 major fatty acids from lipid emulsion by recombinant rat HSL was measured at 37, 20, and 4°C (**Table 2**). The relative release of all fatty acids but one (14:0) was not significantly different between incubations performed at 37°C and 20°C. Conversely, the relative release by HSL of 7 fatty acids was significantly different between incubations performed at 37°C and 4°C. Among saturated and monounsaturated fatty acids, a significant difference in the relative release was found for 14:0, 16:1n–7, and 17:1n–8. Concerning PUFA, the relative release was significantly decreased for 16:4n–3 and 22:6n–3, and significantly increased for 20:4n–6 and 20:5n–3. Similar trends were obtained for the relative release of the same fatty acids by recombinant human HSL measured at 37, 20, and 4°C (not shown).

TABLE 2. Effects of temperature on in vitro relative hydrolysis of some representative fatty acids from the omegavenous lipid emulsion by recombinant rat HSL

	Relative Hydrolysis (% NEFA/% TAG)		
Fatty Acids	37°C	20°C	4°C
14:0	0.85 ± 0.06^a	0.58 ± 0.03^b	0.35 ± 0.03^c
16:0	1.32 ± 0.04	1.22 ± 0.04	1.31 ± 0.14
16:1n–7	0.90 ± 0.05^a	0.85 ± 0.05^a	0.64 ± 0.05^b
17:1n–8	0.75 ± 0.04^a	0.68 ± 0.02^a	0.47 ± 0.03^b
16:4n–3	0.66 ± 0.03^a	0.59 ± 0.02^a	0.35 ± 0.05^b
18:0	1.21 ± 0.10	1.17 ± 0.12	1.22 ± 0.11
18:1n–9	1.46 ± 0.13	1.40 ± 0.08	1.71 ± 0.13
18:1n–7	1.45 ± 0.07	1.52 ± 0.06	1.50 ± 0.07
18:2n–6	1.27 ± 0.07	1.27 ± 0.07	1.49 ± 0.13
18:3n–3	0.97 ± 0.06	0.98 ± 0.03	0.90 ± 0.06
18:4n–3	1.02 ± 0.08	1.06 ± 0.05	1.02 ± 0.14
20:1n–9	1.24 ± 0.12	1.18 ± 0.10	1.08 ± 0.13
20:4n–6	1.07 ± 0.03^a	1.02 ± 0.07^a	1.30 ± 0.05^b
20:4n–3	1.15 ± 0.08	1.16 ± 0.09	1.26 ± 0.09
20:5n–3	0.97 ± 0.04^a	1.11 ± 0.03^a	1.29 ± 0.08^b
21:5n–3	1.00 ± 0.06	1.02 ± 0.14	1.08 ± 0.13
22:5n–3	0.59 ± 0.05	0.61 ± 0.02	0.52 ± 0.02
22:6n–3	0.64 ± 0.07^a	0.69 ± 0.03^a	0.44 ± 0.02^b

Values are means ± SEM (n = 5–7).
^{a,b,c} Within a row, values that do not share the same superscript letter are significantly different ($P < 0.05$).

The influence of the fatty acid molecular structure on the relative release was investigated by plotting relative release versus chain length at a given unsaturation and versus unsaturation at a given chain length in the three incubation conditions (**Fig. 2**). Whatever the incubation conditions, relationships very similar to those described in the preceding section were found between relative release by HSL and fatty acid chain length or unsaturation. At 4°C compared with incubations carried out at 20°C and 37°C, the relative release of most fatty acids shorter than 18:0 (except 16:0) was significantly decreased independently of their degree of unsaturation, indicating an effect of the chain length (Fig. 2A). For a given chain length, the relative release was not significantly affected by the number of double bonds for fatty acids with 18 carbon atoms (Fig. 2A), whereas it was slightly increased for fatty acids with 20 carbon atoms when incubations were carried out at 4°C compared with other conditions of temperature (Table 2).

Relative release of fatty acids by HSL according to their mobilization rates

Fatty acids have previously been roughly classified into three categories depending on their mobilization rates (see Discussion). The relative release of these three classes of fatty acids by HSL is shown in **Fig. 3** for the two experimental groups. The relative release of moderately mobilized fatty acids, defined as those with 14–18 carbon atoms and 0–3 double bonds or with 22 carbon atoms and 4–6 double bonds, was close to unity in the two experimental groups, in conformity with their mobilization rate. The relative release of highly mobilized fatty acids, defined as those with 16–20 carbon atoms and 4–5 double bonds, and of weakly mobilized fatty acids, those with 20–

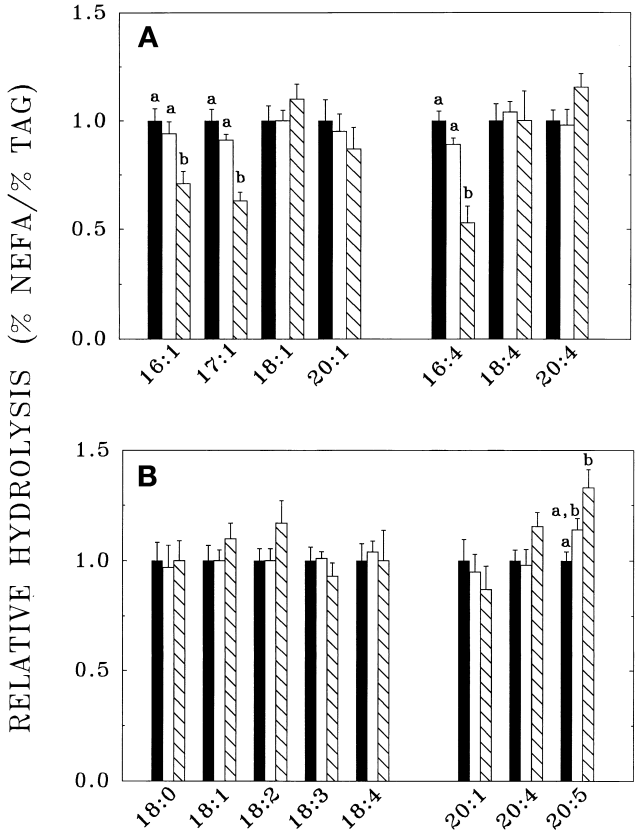

Fig. 2. Relative hydrolysis of individual fatty acids from the lipid emulsion by recombinant rat HSL at various temperatures. Solid, open, and hatched bars represent the relative hydrolysis of some representative fatty acids from the lipid emulsion by recombinant rat HSL at 37, 20, and 4°C, respectively. A: Influence of chain length at given unsaturations; B: Influence of unsaturation at given chain lengths. For each fatty acid, values (means ± SEM, n = 5–7) are expressed as proportions of the value measured at 37°C arbitrarily set to 1. Data are from Table 2. For a given fatty acid, bars that do not share the same letter show significant differences ($P < 0.05$).

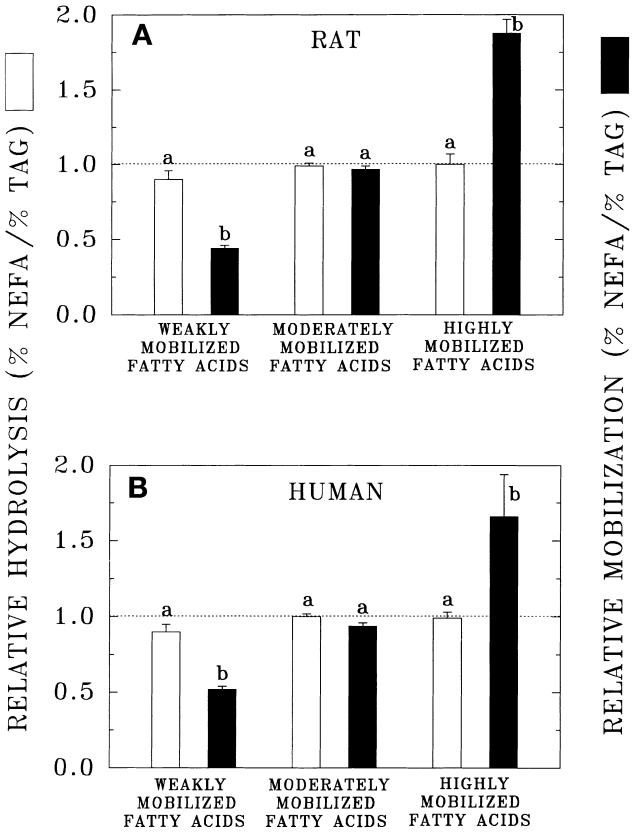

Fig. 3. Relative hydrolysis of weakly, moderately, and highly mobilized fatty acids from the lipid emulsion by recombinant rat (A) and human (B) HSL and relative mobilization from fat cells of the same three categories of fatty acids. Highly mobilized fatty acids have 16–20 carbon atoms and 4–5 double bonds; weakly mobilized fatty acids have 20–24 carbon atoms and 0–1 double bond; moderately mobilized fatty acids are all the others. Relative mobilization rates of the three categories of fat cell fatty acids are computed from the results of previous studies in the rat (4) and humans (35). Values are means ± SEM of 5–7 determinations. For a given category of fatty acids, bars that do not share the same letter show significant differences ($P < 0.05$). Data are from Table 1.

24 carbon atoms and 0-1 double bond, was close to unity in both groups, in contrast to their mobilization rate.

DISCUSSION

Methodological considerations

The use of a stable lipid emulsion and recombinant HSL has allowed us to investigate the enzymological properties of HSL and its selectivity in the fatty acid hydrolysis of lipid substrate TAG. Because HSL was the only lipolytic enzyme present in the assay, TAG hydrolysis could be ascribed solely to this lipase. Indeed, crude or semi-purified enzyme mixtures contain monoglyceride lipase that could contribute to the reaction. Moreover, a very limited amount of HSL can be obtained from adipose tissue because of its low tissue abundance and the multistep purification scheme needed to purify the protein (3, 9). A stable lipid emulsion was chosen to overcome the variability in hydrolysis rates observed with laboratory-prepared emulsions. Therefore, the combination of pure HSL and a stable lipid emulsion allowed reproducible incubation conditions. Also, substrate TAG containing a large spectrum of fatty acids, including those known to be highly, weakly, and moderately mobilized were all present in the same stable lipid emulsion.

HSL shows relative, although not absolute, positional specificity for the *sn*-1 and *sn*-3 positions (14). Our data are in agreement with hydrolysis by HSL at all three positions in TAG, although the rate may differ among positions. The experimental conditions tested in our study were different from those used to evaluate the positional specificity mentioned above. Moreover, several incubation times and various HSL to TAG ratios were tested (data not shown), and in no case did we find any accumulation of 2-monoglycerides. The lack of accumulation of monoglyceride may be due to the relatively high ratios of HSL versus substrate compared with more physiological conditions. The monoglyceride lipase activity of HSL may be sufficient under our assay conditions to hydrolyze 2-monoglycerides. Furthermore, the necessity of mono-

glyceride lipase may be related to a compartmentalization of lipids and lipases, i.e., conditions that are difficult to reproduce in in vitro assays.

In the current study, recombinant HSL is non-phosphorylated, i.e., non-activated. It is unlikely that a conformational change due to phosphorylation by cyclic adenosine monophosphate-dependent protein kinase would change the fatty acid selectivity of HSL. In fat cells, an important step in lipolysis activation seems to be the translocation of HSL from a cytosolic compartment to the surface of the lipid droplet (15). In unstimulated cells, HSL is diffusively distributed throughout the cytosol. Upon stimulation with a β-adrenergic agonist, the enzyme translocates concomitantly with the onset of lipolysis. Hence, the in vitro incubation conditions used here allow the enzyme to hydrolyze the substrate as after translocation of HSL to the surface of the lipid droplet, i.e., during stimulated lipolysis.

Release of individual fatty acids by HSL from lipid emulsion

Our data show that the in vitro release of individual fatty acids by HSL from lipid emulsion is selective. Considering all fatty acids, the effect of carbon chain length on HSL selectivity was more marked than that of degree of unsaturation [present study and (6)]. Selectivity in fatty acid release has also been investigated for several other mammalian lipases, including lipoprotein lipase, hepatic lipase, and pancreatic lipase (16–19). The data for these lipases tend to support an effect of unsaturation degree rather than of chain length. Concerning the fatty acid selectivity of microbial lipases, TAG-containing medium-chain saturated fatty acids are good substrates for chromobacterium lipase B, while substrates containing longer chain fatty acids are poorly hydrolyzed, indicating a selective effect of the fatty acid chain length (20). Similarly, the stereoselective hydrolysis of TAG by some microbial lipases depends on the fatty acid chain length of the substrate (21). This observation is in line with the fact that HSL has no homology with other mammalian lipases but shares an amino acid sequence homology with a few microbial lipases (8). The present study clearly shows that the release of fatty acids by HSL is not a random process. In contrast with a previous study (22), we do not find that PUFA are readily released compared with total fatty acids (Table 1, Fig. 1). Conversely, most of them were resistant to the action of the lipase.

Considering the substrate specificity of HSL, its preference for certain fatty acids remains a possible selective step. For instance, it is known that pancreatic lipase selectively hydrolyzes TAG-fatty acids according to chain length, unsaturation (19), and positional isomerism (23). In the study of Bottino et al. (19), among eicosapentaenoic, docosahexaenoic, and docosapentaenoic acids (all mainly present in *sn*-1 and *sn*-3 positions of the TAG), only the latter fatty acid was not lipase-resistant. As proposed by these authors (19), there might be a limited accessibility of the lipase toward the ester bonds of the substrate due to the presence either of a double bond near the carboxyl group or of the terminal methyl group close to the carboxyl end (in the case of highly unsaturated fatty acids). Therefore, the consequence of the resulting steric hindrance would be a lowering of the hydrolysis rate of some fatty acids (e.g., fatty acids with the double bond closest to the carboxyl group of the chain) by the lipase. Such an explanation does not seem to apply to TAG-fatty acid hydrolysis by HSL, because in the present context and considering the same three fatty acids, eicosapentaenoic acid is about two to three times more readily released than the two others (Table 1, Fig. 1).

The catabolic process of apolar lipids by most lipases (including HSL) is closely related to the lipid-water interface (24, 25). Hydrolysis of apolar lipid molecules, i.e., TAG, generates products that move from the apolar phase to the interfacial phase and thereafter to the polar aqueous phase. According to their physical properties, substrates, products, and enzymes will partition themselves among these phases, and this distribution changes as lipolysis proceeds (26). It has already been suggested that in the lipolytic cascade, the substrate availability could be an activated process concomitant with the lipase activation (27, 28). HSL is active at a lipid-water interface and its action could be modulated by the availability and/or the reactivity of the substrate and also by the distribution of the reaction products that can act as surface-active compounds. In this regard, it is likely that the molecular structure of fatty acids is a determinant in the physical properties of substrate and lipase activation.

The activity of HSL might be influenced by the physical state of the substrate because the phase behavior of TAG molecular species could subsequently affect their metabolic fate (29–31). It is known that TAG containing a wide spectrum of fatty acids (different chain lengths and unsaturation degrees) have a complicated phase behavior. It is often reported that TAG are organized in a liquid or crystalline (partial or total) form according to temperature and fatty acid composition. On chylomicrons isolated at low temperatures, lipoprotein lipase seems to preferentially hydrolyze unsaturated TAG compared with saturated TAG, and this selective hydrolysis could result in a selective crystallization of the most saturated TAG in the remnant core, which would therefore be inaccessible to the lipase (30, 31). Thus, the changes in the physical state of TAG species may influence their metabolic fate (hydrolysis). Such an explanation does not seem to apply to the release of fatty acids by HSL because of similar relative hydrolysis rates among 18 C- and 20 C-fatty acids ranging in unsaturation from 0 to 4 (Table 2, Fig. 2). Furthermore, the relative release by HSL of 20:5n−3 but not of 22:6n−3 was higher at low temperature (4°C) compared with that at 37°C.

We have previously shown that HSL retained more catalytic activity at low temperatures than carboxyester lipase and lipoprotein lipase (7). The molecular basis for cold adaptation of HSL has not been elucidated, although the role of candidate amino acid motifs was ruled out (32). In the previous studies, the psychrotolerant properties of HSL were studied through measurement of HSL esterase activity with the chromogenic substrate *p*-nitrophenylbu-

tyrate. Interestingly, we demonstrate here that the lipase activity of HSL is also cold-adapted with a marked enzymatic activity at 4°C. This psychrotolerant property probably has substantial survival value for obligatory hibernators whose body temperature can decrease to less than 5°C during hibernation. During arousal, these animals rely on mobilization of fatty acids from TAG stores in white and brown adipose tissues for the increase of body temperature.

Selective mobilization of fat cell fatty acids: implication of HSL

The second aim of this study was to test whether the hydrolysis of TAG-fatty acids by HSL is consistent with their mobilization rate. The selectivity of fatty acid mobilization from rat and human adipose tissue has been reported during fasting in vivo (33, 34) and during stimulated lipolysis on isolated fat cells (4, 35). The composition of NEFA, released by isolated fat cells or adipose tissue under conditions of stimulated lipolysis, was compared with that of the TAG from which they originated. For some fatty acids, their percentage in NEFA was different from that in TAG. The relative mobilization of individual fatty acids was calculated as the ratio between their percentage weight in released NEFA to that in fat cell TAG. As a rule, the relative mobilization decreases with increasing chain length for a given unsaturation degree and increases with increasing unsaturation for a given chain length. For instance, the relative mobilization increases from 0.75–0.80 to 1.75 in 18 C-fatty acids when unsaturation increases from 0 to 4 double bonds and from 0.5 to 2.7 in 20 C-fatty acids when unsaturation increases from 0 to 5 double bonds (4). The release of 18 C- and 20 C-fatty acids according to unsaturation by HSL (this study) does not vary as their mobilization from adipocytes (**Fig. 4A** and **B**). Conversely, it is interesting to note that the release of monounsaturated fatty acids according to chain length by HSL (this study) varies in the same way as their mobilization from adipocytes (Fig. 4C and D). This relationship was also found for saturated fatty acids in a previous work (6). Comparison of the relative hydrolysis of fatty acids by HSL and their mobilization rates from adipocytes supports the view that the low mobilization of some fatty acids could derive from a low release of fatty acids by HSL, whereas the high mobilization of other fatty acids seems unrelated to the enzymological properties of HSL. At a first glance, the results of the present study do not account for the broad trends of selective fatty acid mobilization (Fig. 3 and 4). However, our results support a role for HSL in the selective mobilization of some individual fatty acids, notably of 22:5n–3 and 22:6n–3 compared with 20:5n–3 (Fig. 4E and F).

HSL selectivity toward quantitatively important fatty acids might be seen as an adaptation of the lipase to the adipose tissue TAG composition (4, 35) as suggested for a seed lipase and vernolic acid (36). **Figure 5** supports this hypothesis because among monounsaturated fatty acids (Fig. 5A), the higher the amount of a fatty acid in adipose tissue TAG of individuals in their normal dietary state, the higher the relative hydrolysis of that fatty acid (Fig. 5A). Similarly, among fatty acids ranging from C16:1 to C22:6 for molecular structure and from about 43% (C18:1) to trace levels (C22:0 or C16:4), the higher the amount of the fatty acid in adipose tissue TAG of individuals in their normal dietary state, the higher the relative hydrolysis of the given fatty acid (Fig. 5B).

Because HSL and TAG accessibility can explain only part of fatty acid mobilization, other selective steps may be involved in the lipolytic process. Proper activation of lipolysis involves proteins that do not directly participate in the catalytic process. Hence, these proteins could play a critical role in fatty acid selectivity during lipolysis. Two pro-

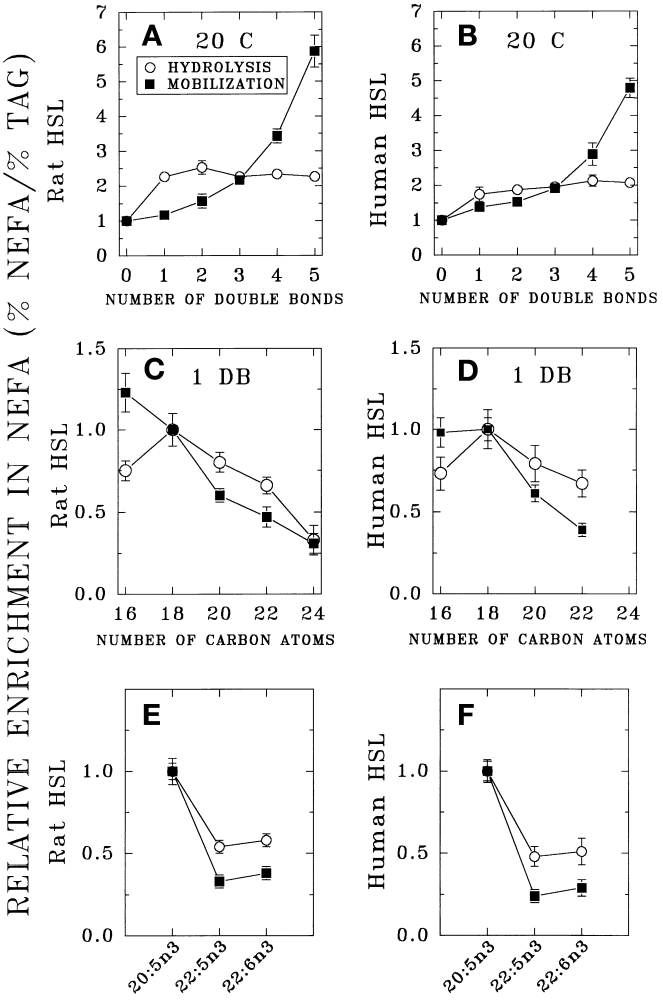

Fig. 4. Relationships between the molecular structure of fatty acids and their in vitro relative enrichment in NEFA after hydrolysis by HSL or mobilization from fat cells in the rat (A,C,E) and humans (B,D,F). Relationships between in vitro relative enrichment in NEFA of fatty acids and their unsaturation at a given chain length (A,B), between chain length at a given unsaturation (C,D), and at high unsaturation (E,F). The relative hydrolysis or mobilization of each fatty acid was calculated as the ratio between its weight percentage in released NEFA to that in TAG. Values of relative hydrolysis of TAG-fatty acids by HSL are from Table 1. Relative mobilization rates of fatty acids from adipocytes were computed from the results of previous studies in the rat (4) and humans (35). Values are means ± SEM (n = 5–7). C, carbon atoms; DB, double bonds.

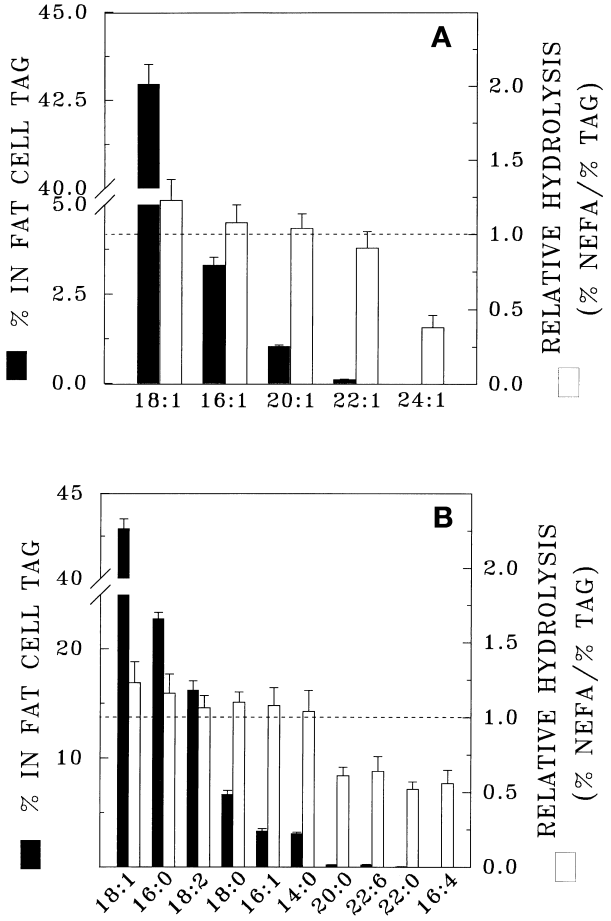

Fig. 5. Relationships between the relative hydrolysis of fatty acids from lipid emulsion by recombinant human HSL and their amount in fat cell TAG from humans in their normal dietary state. Relationships between the relative hydrolysis of fatty acids and their amount in fat cell TAG for monounsaturated fatty acids (A) and for some fatty acids differing by their molecular structure and their amount in fat cell TAG (B). From left to right, fatty acids are arranged in decreasing order of amount in adipose tissue TAG. Values of relative hydrolysis of TAG-fatty acids by HSL are from Table 1. Fatty acid composition in fat cell TAG was computed from the results of previous studies in humans (35). Values are means ± SEM (n = 5–7).

teins have recently been shown to interact with HSL: adipocyte lipid binding protein (ALBP) and lipotransin (37, 38). ALBP is an intracellular fatty acid-binding protein highly expressed in adipocytes. Its interaction with the N-terminal domain of HSL would facilitate rapid shuttling of the fatty acids out of the adipocyte. It would also prevent the deleterious effect of fatty acid accumulation during lipolysis. Consistent with such a role for ALBP is the observation that ALBP-null mice exhibit decreased lipolytic capacity (39, 40). Lipotransin is a member of the katanin family that may dock the protein to the surface of the lipid droplet. Knowledge of the exact contribution of lipotransin to catecholamine lipolytic and insulin antilipolytic effects awaits further studies. Access to the lipid droplet constitutes another potential mechanism for the control of lipolysis. Perilipins are proteins covering the large lipid droplets in adipocytes and a few other cell types (41). Perilipins shield stored triglycerides from cytosolic lipases (42). It has been hypothesized that upon phosphorylation, perilipins allow access to the lipid droplet and thereby allow HSL interaction with its substrates (43). In support of a critical role for perilipins, perilipin-deficient mice have a higher basal lipolysis rate and less adipose tissue than wild-type littermates (44).

In conclusion, our results clearly show that HSL selectively releases individual fatty acids from lipid emulsion TAG. A resistance of certain long-chain polyunsaturated fatty acids to HSL hydrolysis was demonstrated. Compared with the mobilization rates of fatty acids from adipocytes, our data support the view that the low mobilization of certain fatty acids could derive from a low release of fatty acids by HSL, whereas the high mobilization of other fatty acids seems unrelated to the enzymological properties of the lipase. The fatty acid specificities of HSL do not seem to be oriented toward a special demand by tissues or toward a preferential sparing of particular fatty acids. We obtained no evidence that dietary essential fatty acids of the n–6 and n–3 series are preferentially released or retained. Our study provides evidence that the fatty acid selectivity of HSL is related to the fatty acid composition of adipose tissue TAG, that is to say, HSL preferentially releases fatty acids usually stored in high amounts in adipose tissue of rats and humans in their normal dietary state.

We thank Dr. Jacqueline Rangaraj (Fresenius, France) for the Omegavenous lipid emulsion. T.R. was supported by Nestlé France (Nutrition Department) during the tenure of this study. We also thank C. Jeannerod and S. Diehl for expert technical assistance. Part of the work was supported by grants from the Groupe Danone and Produits Roche-France (to D.L.), the Swedish Research Council (11284 to C.H.), and the A. Påhlsson foundation (to C.H.).

Manuscript received 7 June 2001 and in revised form 31 August 2001.

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