Selective release of human adipocyte fatty acids according to molecular structure

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The objective of the present study was to investigate the mobilization of individual fatty acids from human white fat cells. Mammary adipose tissue from eight healthy non-obese women in their normal dietary state was collected, and isolated adipocytes were incubated with lipolytic agents. The mobilization of 34 individual fatty acids was measured by comparing the composition of non-esterified fatty acids (NEFA) with that of the triacylglycerols (TAG) from which they originated through lipolysis. Compared with TAG, NEFA were enriched in some polyunsaturated fatty acids with 18–20 carbon atoms. Conversely, the percentage of very-long-chain (20–22 carbon atoms) saturated and monounsaturated fatty acids was approx. 2 times lower in NEFA than in TAG. The relative mobilization (% in NEFA/% in TAG) of the most readily mobilized fatty acid ($C_{20:5,n-3}$; 2.25) was more than 6-fold higher than that of the least readily mobilized ($C_{22:1,n-11}$; 0.37). Relationships were found between the molecular structure of fatty acids and their mobilization rate. For a given chain length, the relative mobilization rate increased with increasing unsaturation, whereas for a given unsaturation, it decreased with increasing chain length. The relative mobilization rate for essential fatty acids decreased in the following order: $C_{20:5,n-3} > C_{20:4,n-6} > C_{18:3,n-3} > C_{18:2,n-6} > C_{22:6,n-3}$. Interestingly, $C_{20:5,n-3}$ and $C_{20:4,n-6}$, which are respectively precursors of the 3- and 2-series of prostaglandins, were preferentially mobilized. It is concluded that fatty acids are selectively mobilized from human fat cells according to molecular structure, in full agreement with animal studies. By modulating the qualitative fatty acid supply to organs and by remodelling the fatty acid composition of adipose tissue, this selectivity would be relevant for consideration in physiology, health and epidemiology.

INTRODUCTION

In humans, the most important lipid store of the body is represented by triacylglycerols (TAG), found mainly in adipose tissue, which is the major site of lipolysis [1]. Adipose tissue TAG contain a complex mixture of fatty acids. The fatty acid composition of TAG largely reflects the dietary intake of fatty acids that are not synthesized *de novo* via lipogenesis [2]. The adipose tissue content of fatty acids depends also on their post-intake metabolism, including uptake in and release from adipose tissue. At the present time, it is widely believed that in humans the release of fatty acids is proportional to their content in adipose tissue [3]. However, it has recently been suggested that the rate of release of some fatty acids *in vivo* is higher than that of the uptake [4], and that the systemic plasma concentration of individual non-esterified fatty acids (NEFA) is not strictly related to their content in adipose tissue [5]. Therefore the question of whether some fatty acids are preferentially released from human adipose tissue is now a subject of debate.

In animal studies, a selective mobilization of adipose tissue fatty acids has been demonstrated, both *in vitro* [6,7] and *in vivo* [8,9]. A consistent picture is emerging, that the mobilization rate of fatty acids depends upon their molecular structure, increasing with degree of unsaturation at a given chain length and decreasing with chain length at a given unsaturation [6–9]. If such a selective metabolic process exists in humans, it would undoubtedly have important implications for epidemiology, physiology and health [10]. Concerning epidemiology, the fatty acid pattern of adipose tissue is widely used in humans as an indicator of dietary fatty acids in studies aimed at relating health disorders such as obesity or cancer to environmental factors, namely diet [11,12]. A selective release of fatty acids from adipose tissue could affect the relationships between the intake of fatty acids and their content in adipose tissue, e.g. through remodelling the fatty acid composition of adipose tissue in situations of intense lipolysis, such as during food restriction, prolonged exercise, or fasting [8]. Implications of a selective release of fatty acids in physiology and health could exist in the modulation of the supply of biologically active fatty acids to specific tissues [10,13,14]. Indeed, released fatty acids are not only used in various tissues as energy substrates but also are involved in non-oxidative pathways such as cell membrane synthesis [15,16] and, for some dietarily essential polyunsaturated fatty acids (PUFAs) of the n−6 and n−3 series, as precursors of eicosanoids [10,13,14].

The aim of the present study was therefore to determine whether fatty acids are selectively released from adipose tissue in humans and whether the same rules as those described in animal studies relate the mobilization rate of fatty acids to their molecular structure. The release from human fat cells of 34 individual fatty acids was studied *in vitro*. The composition of NEFA released by isolated fat cells under conditions of stimulated lipolysis was compared with that of the TAG from which they originated. In contrast with previous studies using laboratory animals fed on various different fish oil diets to enrich adipose tissue with particular fatty acids [6–9], the present study was performed on fat cells from humans in their normal dietary state.

MATERIALS AND METHODS

Chemicals

Analytical-grade solvents, TLC plates and butylated hydroxytoluene were supplied by Merck (Darmstadt, Germany).

Abbreviations used: NEFA, non-esterified fatty acids; TAG, triacylglycerols; HSL, hormone-sensitive lipase; PUFAs, polyunsaturated fatty acids.

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Table 1 Fatty acid composition of fat cell TAG in human, and of NEFA released by these cells *in vitro*, and relative mobilization (% NEFA/% TAG) of fatty acids

Values are means ± S.E.M. ($n = 8$). The fatty acids column gives the number of carbon atoms:number of double bonds and the position of the first double bond from the methyl end of the molecule. Asterisks indicate a significant difference in the percentage of the fatty acid between NEFA and TAG: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Fatty acid	TAG (weight %)	NEFA (weight %)	Relative mobilization
$C_{12:0}$	0.50 ± 0.07	0.45 ± 0.06	0.88 ± 0.02
$C_{14:0}$	3.08 ± 0.13	2.94 ± 0.15	0.94 ± 0.01
$C_{14:1,n-7}$	0.03 ± 0.00	0.03 ± 0.00	1.07 ± 0.14
$C_{14:1,n-5}$	0.20 ± 0.01	0.19 ± 0.02	0.96 ± 0.03
$C_{15:0}$	0.33 ± 0.02	0.35 ± 0.02	1.05 ± 0.02
$C_{16:0}$	22.79 ± 0.56	23.51 ± 0.74	1.02 ± 0.01
$C_{16:1,n-9}$	0.54 ± 0.01	0.42 ± 0.02***	0.77 ± 0.01
$C_{16:1,n-7}$	2.77 ± 0.21	3.69 ± 0.34*	1.31 ± 0.02
$C_{17:1,n-8}$	0.29 ± 0.02	0.36 ± 0.02*	1.21 ± 0.03
$C_{18:0}$	6.67 ± 0.35	6.41 ± 1.39	0.95 ± 0.06
$C_{18:1,n-9}$	40.79 ± 0.52	39.77 ± 0.57	0.96 ± 0.01
$C_{18:1,n-7}$	1.90 ± 0.05	2.12 ± 0.10	1.10 ± 0.03
$C_{18:1,n-5}$	0.27 ± 0.01	0.31 ± 0.03	1.12 ± 0.04
$C_{18:2,n-6}$	16.23 ± 0.86	16.21 ± 0.62	0.99 ± 0.01
$C_{18:3,n-6}$	0.04 ± 0.00	0.05 ± 0.01	1.27 ± 0.07
$C_{18:3,n-3}$	0.51 ± 0.02	0.75 ± 0.03***	1.43 ± 0.03
$C_{20:0}$	0.21 ± 0.02	0.10 ± 0.01***	0.47 ± 0.04
$C_{20:1,n-11}$	0.17 ± 0.01	0.11 ± 0.01***	0.66 ± 0.03
$C_{20:1,n-9}$	0.84 ± 0.02	0.53 ± 0.02***	0.62 ± 0.01
$C_{20:1,n-7}$	0.03 ± 0.00	0.02 ± 0.00*	0.67 ± 0.03
$C_{20:2,n-9}$	0.04 ± 0.00	0.02 ± 0.00**	0.63 ± 0.06
$C_{20:2,n-6}$	0.31 ± 0.02	0.26 ± 0.01*	0.82 ± 0.04
$C_{20:3,n-6}$	0.26 ± 0.03	0.24 ± 0.03	0.90 ± 0.05
$C_{20:3,n-3}$	0.03 ± 0.00	0.03 ± 0.00	0.90 ± 0.06
$C_{20:4,n-6}$	0.35 ± 0.03	0.57 ± 0.04***	1.60 ± 0.04
$C_{20:4,n-3}$	0.03 ± 0.01	0.04 ± 0.01	1.13 ± 0.16
$C_{20:5,n-3}$	0.04 ± 0.01	0.10 ± 0.01***	2.25 ± 0.08
$C_{22:0}$	0.04 ± 0.01	0.02 ± 0.01*	0.42 ± 0.05
$C_{22:1,n-11}$	0.03 ± 0.01	0.01 ± 0.00*	0.37 ± 0.02
$C_{22:1,n-9}$	0.07 ± 0.01	0.03 ± 0.00**	0.45 ± 0.03
$C_{22:4,n-6}$	0.17 ± 0.02	0.10 ± 0.01**	0.58 ± 0.03
$C_{22:5,n-6}$	0.02 ± 0.01	0.01 ± 0.00	0.59 ± 0.05
$C_{22:5,n-3}$	0.20 ± 0.03	0.11 ± 0.01**	0.55 ± 0.02
$C_{22:6,n-3}$	0.21 ± 0.04	0.14 ± 0.02*	0.65 ± 0.04

Collagenase and adenosine deaminase were purchased from Boehringer (Mannheim, Germany). All other reagents were obtained from Sigma (St. Louis, MO, U.S.A.).

Subjects and sampling

The subjects comprised eight young, healthy, drug-free non-obese women undergoing plastic surgery. Age and body mass index were 31 ± 4 years and 24 ± 2 kg/m² respectively. Mammary adipose tissue was collected at the beginning of the operation performed in the morning. The study conformed to INSERM guidelines and to those of the Toulouse University Hospital Ethics Committee.

Fat cell preparation and incubation

Adipose tissue was dissected out and adipocytes were isolated using the method described by Rodbell [17] with minor modifications [18]. Briefly, a Krebs–Ringer bicarbonate buffer containing 1 mg/ml type III collagenase, 3.5 % (w/v) BSA and 6 mM glucose adjusted to pH 7.4 (incubation medium) was used. Digestion and cell washing procedures were carried out at 37 °C. Isolated adipocytes were washed three times and the packed cells were used for lipolysis experiments. Approx. 100 mg of fat cells were incubated in duplicate in 4 ml of incubation medium with gentle shaking under an air phase at 37 °C. After 90 min, the incubation tubes were placed in an ice bath. Adipocytes and 3 ml of the infranatant were collected for further lipid analysis. Maximal stimulation of lipolysis was obtained in the presence of isoprenaline (10^{-5} M), a non-selective β-adrenergic agonist [18], and adenosine deaminase (10 μg/ml) that hydrolyses endogenous adenosine and thereby relieves its *in vitro* antilipolytic effect [19]. The intensity of stimulation [on average 5.7(± 1.1)-fold] was calculated by dividing the maximal lipolysis rate by the basal lipolysis rate determined in the absence of the drugs.

Lipid extraction and fatty acid analysis

NEFA from 3 ml of incubation medium and TAG from approx. 100 mg of fat cells were extracted according to Dole and Meinertz [20]. The lipid weight per incubation was 95.0 ± 13.3 mg as determined gravimetrically. Under conditions of maximal stimulation of lipolysis, NEFA production averaged 400.3 ± 58.3 μg per incubation, i.e. equivalent to 0.47 ± 0.07 % of the content of incubated fat cells in TAG fatty acids. NEFA extracts were mixed with 100 μg of heptadecanoic acid as an internal standard and purified by TLC using plates coated with Kieselgel 60. The developing solvent system was hexane/diethyl ether/acetic acid (70:30:1, by vol.). After development, the plates were dried under nitrogen and sprayed with primulin [0.05 mg/ml in acetone/water (4:1, v/v)]. TAG extracts were similarly purified. The NEFA and TAG bands were scraped into glass tubes for preparation of fatty acid methyl esters. To look for possible contamination of NEFA during the overall procedure, a blank extraction was also made on five 3-ml aliquots of incubation medium mixed with 10 μg of heptadecanoic acid. The blank extracts were treated exactly as the NEFA extracts. To prevent the oxidation of PUFAs, butylated hydroxytoluene was added to the extraction mixture at a final concentration of 0.05 % (w/v). Fatty acids in NEFA and TAG were converted into methyl esters using 14 % (w/v) BF_3 in methanol, as already reported [6]. Fatty acid derivatives were separated and quantified by GLC using a Chrompack CP 9001 gas chromatograph (Chrompack, Les Ulis, France) equipped with a flame ionization detector and a Spectra-Physics SP 4290 integrator (Spectra-Physics, Les Ulis, France). Chromatography was performed using an AT-WAX fused silica capillary column (30 m × 0.25 mm internal diameter, 0.25 μm thickness; Alltech, Templeuve, France). Fatty acid peaks were identified by comparison of their retention times with those of authentic standards and by criteria described previously [6]. Only *cis* positional isomers were considered. Analyses were done in duplicate.

Calculations and statistics

Values are expressed as means ± S.E.M. of eight determinations. The mass of each fatty acid in NEFA was calculated from that of the internal standard. Only data from stimulated fat cells were used in further calculations. Blank NEFA extracts were found to contain small amounts of compounds with retention times identical with those of several fatty acids with 16 or 18 carbon atoms and with 0 or one double bond [6]. For these fatty acids, the blank value was deducted before calculating the percentage by weight.

The weight percentage of each fatty acid in NEFA and TAG was compared using the two-tailed t test for paired data after transforming the percentages into arcsin, as previously reported [6]. The relative mobilization of individual fatty acids from fat

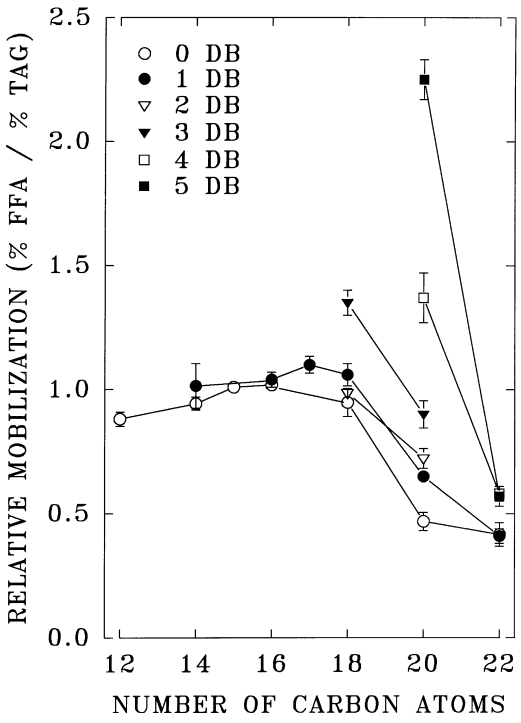

Figure 1 Relationship between chain length and relative mobilization from human fat cells of fatty acids at given degrees of unsaturation

The position of the double bond(s) is not taken into consideration. When there is more than one positional isomer the average value of relative mobilization was calculated. Bars show S.E.M. ($n = 8$). DB, double bond(s). FFA = NEFA.

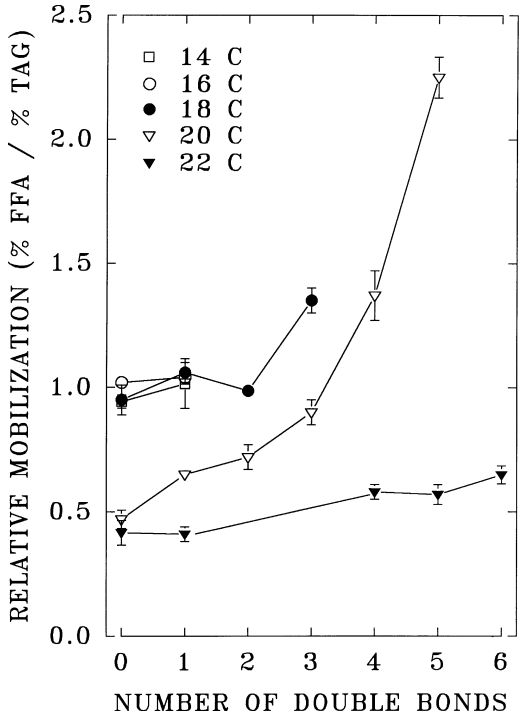

Figure 2 Relationship between degree of unsaturation and relative mobilization from human fat cells of fatty acids at given chain lengths

The position of the double bond(s) is not taken into consideration. When there is more than one positional isomer the average value of relative mobilization was calculated. Bars show S.E.M. ($n = 8$). C, carbon atoms. FFA = NEFA.

cells was calculated as the ratio between their weight percentage in released NEFA to that in fat cell TAG. A ratio greater or lower than unity indicates that the fatty acid is mobilized respectively more or less readily than total fatty acids. The relative mobilization of individual fatty acids was compared using the Peritz *F*-test for multiple comparisons [21]. Unless otherwise indicated, the criterion of significance was $P < 0.05$.

RESULTS

As shown in Table 1, 34 fatty acids with weight percentages ranging from 0.02 to 40.8 could be confidently identified and quantified in human adipose tissue. Chain length and degree of unsaturation ranged from 12 to 22 carbon atoms and from 0 to six double bonds respectively. For 18 of the 34 fatty acids, the weight percentage in released NEFA was significantly different from that in TAG. Compared with TAG, NEFA were enriched in some polyunsaturated fatty acids, i.e. $C_{18:3,n-3}$, $C_{20:4,n-6}$ and $C_{20:5,n-3}$. Their relative mobilization (% NEFA/% TAG) ranged from 1.4 to 2.3. In contrast, the percentages of very-long-chain (20–22 carbon atoms) saturated and monounsaturated fatty acids were approx. 2 times lower in NEFA than in TAG. The weight percentages of highly unsaturated (five to six double bonds) and very long (22 carbon atoms) fatty acids, such as $C_{22:5,n-3}$ and $C_{22:6,n-3}$, were also approx. 1.5 times lower in NEFA than in TAG. With the exceptions of $C_{16:1,n-9}$, $C_{16:1,n-7}$ and $C_{17:1,n-8}$, fatty acids with 12 to 18 carbon atoms and 0 to two double bonds had weight percentages not significantly different between NEFA and TAG.

The relative mobilization differed greatly between fatty acids (Table 1), being 6-fold higher ($P < 0.0001$) for the most ($C_{20:5,n-3}$; 2.25), than for the least ($C_{22:1,n-11}$; 0.37), readily mobilized fatty acid. There are relationships between the molecular structure of the fatty acids and their relative mobilization. As illustrated in Figure 1, for each class of unsaturation (0 to five double bonds) and for chain lengths longer than 16 carbon atoms, the relative mobilization decreased as the number of carbon atoms increased. For example, there was a 4-fold decline ($P < 0.0001$) in relative mobilization of penta-unsaturated fatty acids when chain length increased from 20 to 22 carbon atoms. On the other hand, for a given chain length the relative mobilization increased with the number of double bonds (Figure 2). For example, among the fatty acids with 20 carbon atoms, there was a 5-fold increase in relative mobilization ($P < 0.0001$) when the unsaturation

Table 2 Relative mobilization (% NEFA/% TAG) of n−6 and n−3 polyunsaturated fatty acids from human fat cells

Values are means ± S.E.M. ($n = 8$) and come from Table 1. Within a column, values not sharing the same superscript letter are significantly different ($P < 0.05$, Peritz *F*-test for multiple comparisons).

n−6 polyunsaturated fatty acids	Relative mobilization	n−3 polyunsaturated fatty acids	Relative mobilization
$C_{18:2,n-6}$	0.99 ± 0.01^{a}	$C_{18:3,n-3}$	1.43 ± 0.03^{a}
$C_{20:2,n-6}$	0.82 ± 0.04^{b}	$C_{20:3,n-3}$	0.90 ± 0.06^{b}
$C_{20:3,n-6}$	$0.90 \pm 0.05^{a,b}$	$C_{20:4,n-3}$	$1.13 \pm 0.16^{a,b}$
$C_{20:4,n-6}$	1.60 ± 0.04^{c}	$C_{20:5,n-3}$	2.25 ± 0.08^{c}
$C_{22:4,n-6}$	0.58 ± 0.03^{d}	$C_{22:5,n-3}$	0.55 ± 0.02^{d}
$C_{22:5,n-6}$	0.59 ± 0.05^{d}	$C_{22:6,n-3}$	0.65 ± 0.04^{e}

increased from 0 to five double bonds. Moreover, the influence of the degree of unsaturation on relative mobilization was less pronounced for the highest chain length. Thus both chain length and degree of unsaturation significantly affect relative mobilization, but in an opposing way. There was also a slight effect of positional isomerism at a given chain length and unsaturation. For example, among monounsaturated fatty acids with 16, 18 and 20 carbon atoms, the relative mobilization tends to increase as the double bond moves towards the methyl end of the chain (e.g. from the 9 to the 7 position). The same applies for $C_{18:3}$ and $C_{20:2}$ fatty acids. However, this trend is not very consistent since no differences were found between positional isomers of $C_{20:1}$, $C_{20:3}$ and $C_{22:5}$, whereas the relative mobilization of $C_{20:4,n-6}$ was higher than that of $C_{20:4,n-3}$.

A special emphasis was put on essential fatty acids of the n−6 and n−3 series (Table 2). In the n−6 series, the relative mobilization of $C_{20:4,n-6}$ (1.6) was 1.3–2.8 times higher ($P < 0.05$) than those of other n−6 PUFAs. The $C_{18:2,n-6}$ had a relative mobilization very close to unity, whereas those of $C_{22:4,n-6}$ and $C_{22:5,n-6}$ were close to 0.6. In the n−3 series, the relative mobilization of $C_{20:5,n-3}$ (2.25) was 1.6–4.1 times higher ($P < 0.05$) than those of other n−3 PUFAs. Among other n−3 fatty acids, $C_{18:3,n-3}$ was preferentially released (relative mobilization close to 1.5) whereas, in contrast, $C_{22:5,n-3}$ and $C_{22:6,n-3}$ were preferentially retained (relative mobilization close to 0.6).

DISCUSSION

A recent study *in vivo* based upon the comparison between veno-arterial concentration differences of NEFA and fatty acid content in human adipose tissue TAG, suggests a selective mobilization of individual fatty acids [5]. The results from the present study clearly demonstrate that the mobilization of fat-cell fatty acids from mammary adipose tissue from eight young and healthy non-obese women is selective and depends on molecular structure, according to the same rules as those found for laboratory animals [6–9]. There is no evidence that this selectivity is oriented towards a special demand by tissues. For all fatty acids, the relative mobilization is as could be expected from their structure. This was notably the case for $C_{18:0}$, which has a relative mobilization close to unity, so that its recently reported high concentration in human plasma would reflect its low clearance rather than its preferential mobilization [5]. The relative mobilization ranged from 0.37 ($C_{22:1,n-11}$) to 2.25 ($C_{20:5,n-3}$), i.e. a 6-fold difference. This value compares well with the 6- to 7.5-fold difference measured *in vitro* [6,7] and *in vivo* [8] for these two fatty acids in fish-oil-fed rats. On the whole, the content of mammary adipose tissue TAG in fatty acids subject to selective release, from women in their normal dietary state, is close to 7 %, whereas the main fatty acids (e.g. $C_{16:0}$, $C_{18:1,n-9}$, $C_{18:2,n-6}$), which represent approx. 90 % of total adipose tissue fatty acids, are not selectively released.

The mechanism that determines the selectivity of fatty acid mobilization is not known. In animal studies, we previously reported that this metabolic property is a general feature of white adipose tissue that is not restricted to a single fat depot and not related to the lipolytic agents used [7]. The selectivity does not depend on the fatty acid composition of adipose tissue, giving no support for a competition between fatty acids during lipolysis [7]. The lipolytic process is widely described to take place at the lipid–water interface [22]. This applies for hormone-sensitive lipase (HSL) during hormone-stimulated lipolysis in fat cells. Thus the selective release of fatty acids could be related to their selective accessibility to HSL, which is based on the selective partition of TAG fatty acids according to physicochemical properties at the lipid–water interface [6,23]. This process does not exclude other steps, such as HSL and monoacylglycerol lipase selectivities. A preferential release of linolenate compared with oleate was shown using a crude preparation of HSL [24]. Selective mobilization could also originate from differential binding of fatty acids to fatty acid binding proteins and albumin during transport and transfer.

The selective mobilization of fatty acid from human fat cells could have important consequences for the use of adipose tissue fatty acids as biomarkers of dietary intake in epidemiological studies. While it is true that the fatty acid composition of adipose tissue TAG roughly reflects that of the diet [2], it is unlikely that fatty acids with high or low relative mobilization would also have respectively high or low relative uptakes, so one would expect that their turnover in adipose tissue would be unselective [3,5]. Actually, several studies argue against such an unselective metabolic fate of fatty acids [4,25,26], and suggest that the interpretation of adipose tissue fatty acid composition as a marker of dietary fatty acid intake be taken cautiously. For instance, we previously reported that in the rat the relative incorporation of the four major n−3 PUFAs in adipose tissue is selective, increasing in the following order: $C_{20:5,n-3} < C_{18:4,n-3} < C_{22:6,n-3} < C_{22:5,n-3}$, whereas their relative mobilization decreases in the reverse order [25]. This indicates a direct effect of the selectivity of mobilization on the adipose tissue content of these fatty acids. The same seems to apply in humans. Indeed, the very preferential mobilization of $C_{20:5,n-3}$ compared with $C_{22:6,n-3}$ observed here probably explains why a stronger association between dietary intake and adipose tissue content of $C_{22:6,n-3}$ rather than of $C_{20:5,n-3}$ was observed previously in humans [11,27]. This led to the recommendation to use the adipose tissue content of $C_{22:6,n-3}$ rather than $C_{20:5,n-3}$ as a biomarker of choice for the assessment of long-term dietary intake of n−3 PUFAs.

A selective mobilization of adipose tissue fatty acids could also have implications for human physiology and health. The preferential mobilization of certain essential PUFAs and, in contrast, the retention of long-chain saturated and monounsaturated fatty acids could explain the selective depletion of $C_{18:3,n-3}$ [28–30] and the slight relative enrichment of $C_{20:1,n-9}$ [3] that have been observed in various situations of negative energy balance (e.g. very-low-calorie dieting, weight cycling or breast cancer). As shown in long-term fasting rats [8], a depletion of $C_{20:4,n-6}$ and $C_{20:5,n-3}$ could also be expected. Consequently, it can be hypothesized that the supply of several essential fatty acids is not sufficient in situations of prolonged weight loss. The selective release of fatty acids from adipose tissue could influence their qualitative supply to tissues and organs in situations where lipolysis is enhanced. This could affect the fatty acid composition of cellular phospholipids, thus leading to modified membrane fluidity, which in turn could modulate membrane protein or receptor activity [15,16,31]. The preferential mobilization of $C_{20:5,n-3}$, $C_{20:4,n-6}$ and $C_{18:3,n-3}$ probably contributes to their maintenance in the plasma at a higher level than predicted by their proportion in adipose tissue [5]. The idea is worth considering since these PUFAs have been described as potential anticancer drugs via their cytotoxic actions [32]. The two most readily mobilized fatty acids, $C_{20:5,n-3}$ and $C_{20:4,n-6}$, are both precursors of eicosanoids, which have many biological effects on lipolysis [33], on factors involved in atherothrombosis and inflammation [10,34], and on adipocyte differentiation [33,35,36]. A selective mobilization and thus selective supply of fatty acids could also have metabolic effects through modulation of gene expression [37,38] or insulin release [39]. It is also interesting to consider that some n−3 PUFAs are able to suppress immune cell functions in humans [40]. Thus by affecting local or circulatory concentrations of $C_{18:3,n-3}$, $C_{20:4,n-6}$

and $C_{20:5,n-3}$, the preferential release of these fatty acids from adipose tissue could greatly modulate these various biological effects.

In conclusion, this study demonstrates that in humans fatty acids are selectively mobilized from fat cells according to their chain length and degree of unsaturation. This probably contributes towards explaining why the proportions of most individual fatty acids in circulating NEFA differ greatly from those in adipose tissue [5]. An elevated whole-plasma NEFA level could represent a risk marker for cardiovascular and other chronic diseases [41]. Because they are affected by a selective release, we suggest that plasma fatty acid composition, with reference to individual fatty acids, should also be taken into account in clinical studies.

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