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Review

Selective mobilization of fatty acids from adipose tissue triacylglycerols

Thierry Raclot*

Centre d'Ecologie et de Physiologie Energétiques, CNRS UPR 9010, associé à l'Université Louis Pasteur, 23 rue Becquerel, 67087 Strasbourg Cedex 2, France

Abstract

Adipose tissue triacylglycerols represent the main storage of a wide spectrum of fatty acids differing by molecular structure. The release of individual fatty acids from adipose tissue is selective according to carbon chain length and unsaturation degree in vitro and in vivo in animal studies and also in humans. The mechanism of selective fatty acid mobilization from white fat cells is not known. Lipolysis is widely reported to work at a lipid–water interface where only small amounts of substrate are available. A preferential hydrolysis of a small triacylglycerol fraction enriched in certain triacylglycerol molecular species at the lipid–water interface and enzymological properties of hormone-sensitive lipase could explain the selective mobilization of fatty acids from fat cells. This selectivity could affect the individual fatty acid supply to tissues.

Keywords: Lipid storage; White fat cells; Lipolysis; Fatty acid molecular structure; Lipid–water interface

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* Tel.: +33-3-88-10-69-00; fax: +33-3-88-10-69-06.
E-mail address: thierry.raclot@c-strasbourg.fr (T. Raclot).

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1. Introduction

This review addresses the storage of fatty acids into fat stores with emphasis on the selective incorporation and mobilization of individual adipose tissue fatty acids.

Triacylglycerols (TAG) represent the main lipid storage of a wide spectrum of fatty acids differing by their molecular structure [1–3]. Chain length and unsaturation of most fatty acids range

Nomenclature

ALBP	Adipocyte lipid binding protein
NEFA	Non-esterified fatty acids
HSL	Hormone-sensitive lipase
PUFA	Polyunsaturated fatty acids
TAG	Triacylglycerols
VLC-MUFA	Very long-chain monounsaturated fatty acids
VLC-PUFA	Very long-chain polyunsaturated fatty acids
VLC-SFA	Very long-chain saturated fatty acids

from 12 to 24 carbon atoms and 0–6 double bonds, respectively. However, except fatty acids with 14–18 carbon atoms and 0–3 double bonds, which are widely represented, other fatty acids are usually found in low amounts or even at trace levels.

Special emphasis has been put on the relationships between the molecular structure of fatty acids and their metabolic fate. To allow an accurate comparison of fatty acids with different molecular structure, adipose tissue was first dietarily enriched in very long-chain polyunsaturated fatty acids (VLC-PUFA), in very long-chain monounsaturated fatty acids (VLC-MUFA) and to a lesser extent, in very long-chain saturated fatty acids (VLC-SFA). Since it has already been shown that adipose tissue reaches a new steady-state fatty acid composition after a similar feeding protocol [4], adipose tissue composition has therefore been considered to be in a steady-state.

2. Lipid mobilization from adipose tissue: metabolic pathways

Fatty acids are stored mostly as TAG in adipose depots which represent the lipid storage of mammals and birds [5,6]. The main metabolic functions of adipose tissue are lipid accumulation through lipid synthesis and lipid mobilization via TAG breakdown (Fig. 1). The relative rate of lipid deposition and lipid removal can vary greatly in response to nutritional states and endocrine factors [7,8]. TAG stored in adipose tissue are derived primarily from the action of lipoprotein lipase on circulating lipoproteins but non-esterified fatty acids (NEFA) derived from the circulation can also be taken up by adipose tissue independently of lipoprotein hydrolysis. However in both cases, esterification or reesterification of NEFA requires α-glycerophosphate which originates mainly from glucose conversion, glycerokinase activity being extremely low in adipose tissue [9,10].

In situations of negative energy balance such as fasting, fat mobilization is enhanced to provide fatty acids as metabolic fuel [6,8]. The activation of lipolysis is under acute neural and hormonal control [8]. TAG are hydrolyzed into NEFA and glycerol through the concerted action of numerous proteins involving notably hormone-sensitive lipase (HSL) [11]. Catecholamines increase the activity of HSL through phosphorylation by cAMP-dependent protein kinase, whereas insulin prevents this activation mainly via lowering cAMP levels [12,13]. In adipose tissue, the hydrolysis of the first ester bond of TAG by HSL is the rate-controlling step for lipid

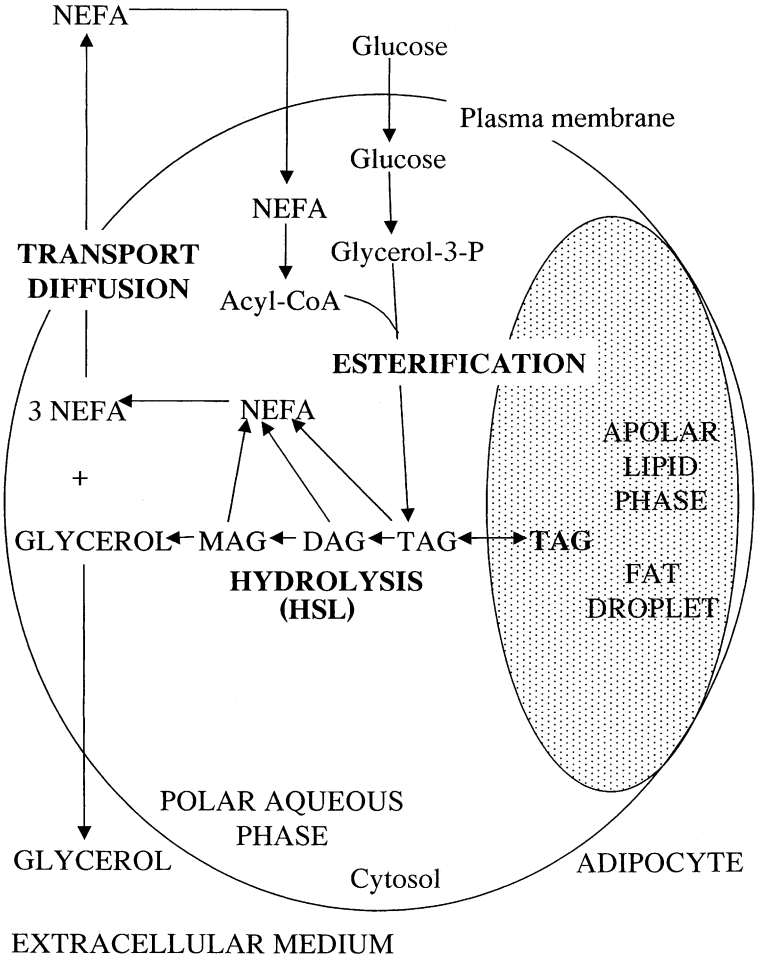

Fig. 1. Simplified scheme for lipid mobilization from and re-uptake into adipose tissue TAG. DAG, diacylglycerols; HSL, hormone-sensitive lipase; MAG, monoacylglycerols; NEFA, non-esterified fatty acids; TAG, triacylglycerols. Further explanation in text.

mobilization [14]. It prevents the accumulation of the intermediary di- and mono-acylglycerols and prevents the NEFA/glycerol ratio from reaching a value higher than three which could result from the partial hydrolysis of TAG.

3. In vivo storage of fatty acids in adipose tissue

3.1. Selective effects of fatty acids on body fat accumulation

The fatty acid composition of adipose tissue is markedly affected by dietary fats [15,16]. High fat feeding leads to the development of dietary obesity, but not only the amount but also the fatty acid composition of dietary lipids is relevant [17]. Indeed, much evidence has now been found

that the nature of the dietary fats can affect lipid homeostasis and body fat accumulation. The n-6 and n-3 PUFA intakes have been shown to affect the development of adipose tissue compared to the consumption of saturated and monounsaturated fatty acids [18]. For instance, feeding fish oil selectively reduces the hypertrophy of retroperitoneal and epididymal adipose tissues in rats compared with a diet containing the same amount of lard in conditions of similar energy intake [19,20]. After this feeding protocol, the differences in the lipid gain in adipose tissues are explained mainly by the limitation of fat cell hypertrophy [20]. The specific effects of fish oil fatty acids have been clearly demonstrated using similar proportions of n-3 polyunsaturated fatty acids (PUFA), n-6 PUFA, saturated and monounsaturated fatty acids in dietary lipids [20,21].

3.2. Relative incorporation of n-3 fatty acids

Little is known about the metabolic fate of individual fatty acids found in adipose tissue. Selectivity of fatty acid incorporation or esterification into adipose tissue has already been reported (Table 1). However, the control of PUFA storage in adipose tissue TAG remains poorly understood. If the fatty acid composition of adipose tissue reflects that of the diet, the proportion of PUFA in adipose tissue is most often lower than that of the diet [16]. Both aspects of the metabolism of the main n-3 PUFA (20:5n-3 and 22:6n-3, and to a lesser extent 22:5n-3 and 18:4n-3) have been recently reported [22,23]. N-3 PUFA are selectively stored in adipose tissue TAG and their in vivo relative incorporation increased in the order: 20:5n-3 < 18:4n-3 < 22:6n-3 < 22:5n-3 (Fig. 2). There was a 3-fold difference between the least (20:5n-3) and the most (22:5n-3) readily stored fatty acids. Whether the preferential release of certain highly PUFA can partly explain their low proportion in adipose tissue TAG as compared to the dietary intake is therefore a question of interest. To answer this question, the net in vivo enrichment of fatty acids in adipose tissue TAG and their net in vitro mobilization have been determined concurrently [23]. N-3 PUFA are selectively mobilized from adipose tissue TAG according to the sequence: 20:5n-3 >

Table 1
Selectivity of fatty acid incorporation or esterification into adipose tissue triacylglycerols

Selectivity of fatty acid	Species	Site	Experimental conditions	Reference
Incorporation/ esterification	Rat	Epididymal	In vivo	[26]
	Rat	Epididymal	In vitro (fat pads)	[24]
	Rat	Epididymal	In vitro (fat pads)	[90]
	Rat	Epididymal	In vitro (adipocytes)	[115]
	Rat	Parametrial	In vitro (adipocytes)	[88]
	Rat	Epididymal	In vitro (fat pads)	[87]
	Rabbit	Intraabdominal	In vivo	[22]
	Angus cattle	Intramuscular/subcutaneous	In vitro (fat pads)	[89]
	Rat	Abdominal/epididymal	In vivo	[116]
	Rabbit	Intraabdominal	In vivo	[117]
	Rat	Retroperitoneal/inguinal	In vivo	[22]
	Human	Subcutaneous	In vivo	[118]
	Rat	Epididymal/omental	In vivo	[119]
	Human	Subcutaneous	In vivo	[120]

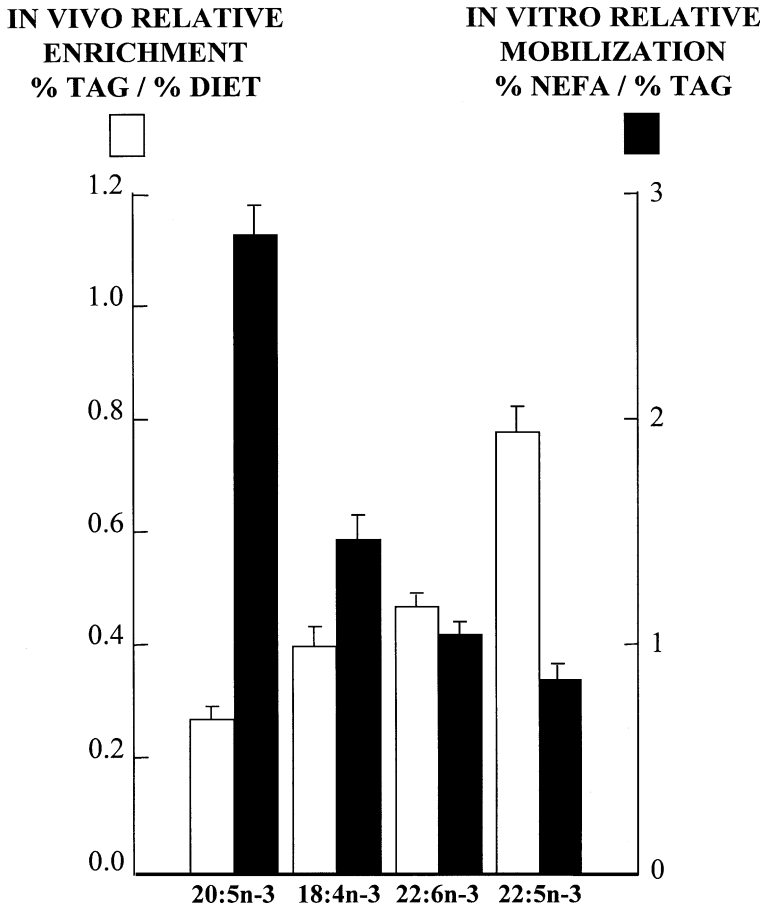

Fig. 2. In vivo relative incorporation into and in vitro relative mobilization from adipose tissue of the four main n-3 polyunsaturated fatty acids. NEFA, non-esterified fatty acids; TAG, triacylglycerols. Data are from [23]. Further explanation in text.

18:4n-3 > 22:6n-3 > 22:5n-3 (Fig. 2), that is to say with opposing facility compared to their relative storage. 20:5n-3 was 3-fold more mobilized than 22:5n-3.

The in vivo relative enrichment of n-3 PUFA into fat reserves was inversely and significantly related to their in vitro relative mobilization. These data provide evidence that the higher mobilization of some fatty acids contributes to explain their lower storage.

4. Mobilization of individual fatty acids

4.1. A selective metabolic process

Until recently, it remained doubtful that fatty acids were selectively released from adipose tissue. How the molecular structure of fatty acids influences their release from fat cells also remained poorly documented. In fact, no strong evidence has yet been reported to support such

an hypothesis. Previous studies support the view of either a selective [24,25] or a random process [26–28]. Only certain fatty acids were considered in these pioneer studies, that is to say, 4–8 fatty acids with chain lengths and unsaturations ranging from 14–18 carbon atoms and 0–3 double bonds, respectively. Therefore, it was not surprising that this question remained open. It is now clear that the mobilization of white fat cell fatty acids is selective (Table 2). For most of the fatty acids, the percentage in NEFA is different from that of the TAG from which they originated [29]. Compared to TAG, released NEFA are enriched in some highly unsaturated fatty acids and depleted in long chain saturated and monounsaturated fatty acids. The mobilization of the most readily mobilized fatty acid (18:5n-3) is about 15-fold higher than that of the least (24:1n-9), whereas among major fatty acids, the mobilization of 20:5n-3 is about 5-times higher than 20:1n-9 (Fig. 3).

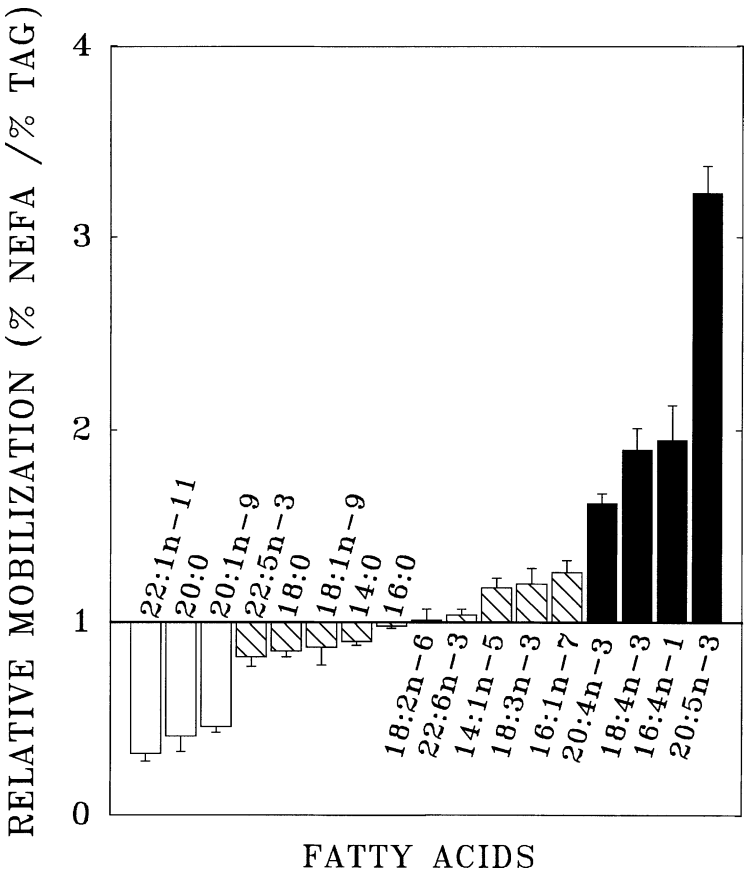

Fig. 3. Relative mobilization of fatty acids from rat white fat cells. The relative mobilization of individual fatty acids from fat cells was calculated as the ratio between their weight percentage in released NEFA to that in fat cell TAG. A ratio more or less than unity indicates that the fatty acid is mobilized, respectively, more or less readily than total fatty acids. Black, hatched and white histobars represent readily, moderately and weakly mobilized fatty acids, respectively. Values for quantitatively major fatty acids are arranged from left to right in increasing order of relative mobilization. Data are partly from [29].

Table 2
Selectivity of fatty acid mobilisation from adipose tissue triacylglycerols

Selectivity of fatty acid	Species	Site	Experimental conditions	Reference
Mobilisation	Rat	Epididymal	In vitro (fat pads)	[24]
	Rat	Epididymal	In vitro (fat pads)	[25]
	Marmot	Gonadal/omental	In vivo	[121]
	Rat	Epididymal	In vitro (fat pads; preadipocytes)	[66]
	Rat	Retroperitoneal	In vitro (adipocytes)	[29]
	Rat	Brown adipose tissue	In vivo	[122,123]
	Rat	Retroperitoneal/inguinal	In vitro (adipose fragments)	[23]
	Rat	Retroperitoneal/epididymal Mesenteric/inguinal	In vitro (adipocytes)	[31]
	Rat	Retroperitoneal	In vivo/in vitro (adipocytes)	[34]
	Rabbit	Mesenteric/inguinal	In vivo	[124]
	Human	Subcutaneous	In vivo	[32]
	Human	Subcutaneous	In vitro (adipocytes)	[33]
	Rat	Brown adipose tissue	In vivo	[35]
	Rat	Epididymal/omental	In vivo	[119]
	Reindeer	Perirenal/abdominal Cardiac/peristernal/caudal	In vivo	[125]

4.2. Relationships with the molecular structure of fatty acids

Recently, the mobilization of white fat cell fatty acids has been demonstrated to be selective, depending on chain length, unsaturation and, to a lesser extent, positional isomerism [29]. For a given chain length, mobilization increases with increasing unsaturation (Fig. 4, left panel). For a given number of double bonds, it decreases with increasing chain length (Fig. 4, right panel). Thus fatty acids are not randomly mobilized, but selectively according to molecular structure. As a practical outcome, fatty acids are more readily mobilized from fat cells when they are short and unsaturated, and when their double bonds are closer to the methyl end of the chain.

4.3. A general metabolic feature

Throughout most of these experiments, animals were fed semisynthetic high fat diets differing by fatty acid composition in order to enrich adipose tissue in a wide spectrum of fatty acids. An enrichment of adipose tissue in specific fatty acids might cause confounding effects on the metabolic fate of some of them due to the "last in– first out" hypothesis [30]. This is the reason why the same experiments have been performed on animals fed on a control diet, i.e. a normal laboratory diet. The relative mobilization of individual fatty acids still depended on molecular structure according to the same relationships described above whatever the dietary treatment, and consequently, whatever the fatty acid composition of adipose tissue [29,31]. Therefore, the selectivity of fatty acid mobilization is independent of recent fatty acid intake. It has now been clearly shown that this selective process is a general metabolic feature of adipose tissue not based on its fatty acid composition or on its location [31]. This result does not lend support for competition

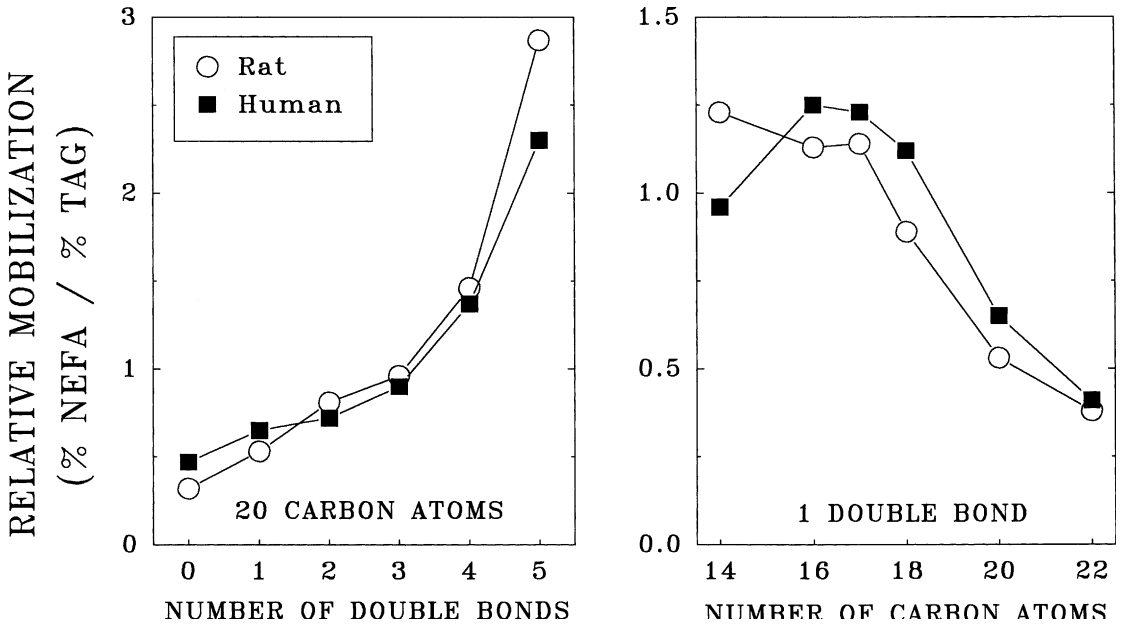

Fig. 4. Relationships between the molecular structure of fatty acids and their in vitro relative mobilization rate from rat and human white fat cells. Influence of unsaturation for fatty acids with 20 carbon atoms (left panel) and of chain length for monounsaturated fatty acids (right panel). When there are several positional isomers, the average value of relative mobilization is plotted. Data are partly from [29].

reactions among fatty acids, at any step of the lipolytic process, as an explanation for such selectivity. It is likely that the selective mobilization of fatty acids represents an intrinsic property, probably due to their molecular structure.

4.4. Mobilization of fatty acids of the n-6 and n-3 series

In humans, the mobilization of fatty acids was also believed to be a random process [28] but the systemic plasma pattern of NEFA has been recently reported to differ from their content in adipose tissue TAG [32]. Thus, an emerging picture is that the selectivity of fatty acid mobilization, which is well-demonstrated in animal studies, could also occur in humans. Additionally, the composition of NEFA released by white fat cells from humans in their normal dietary state was compared to that of subcutaneous mammary adipose tissue TAG [33]. The weight percentage in released NEFA was different from that in TAG for half of the 34 well-identified fatty acids. NEFA were enriched in certain highly PUFA and depleted in long-chain saturated and monounsaturated fatty acids. For a given unsaturation, the relative mobilization decreased as the number of carbon atoms increased, whereas for a given chain length, the relative mobilization increased with the number of double bonds (Fig. 4). Thus, individual fatty acids are selectively mobilized from human fat cells according to their molecular structure so that the rules demonstrated in animal studies are valid in humans. Among essential fatty acids of the n-3 and n-6 series, 20:5n-3 and 20:4n-6 were preferentially released (Fig. 5).

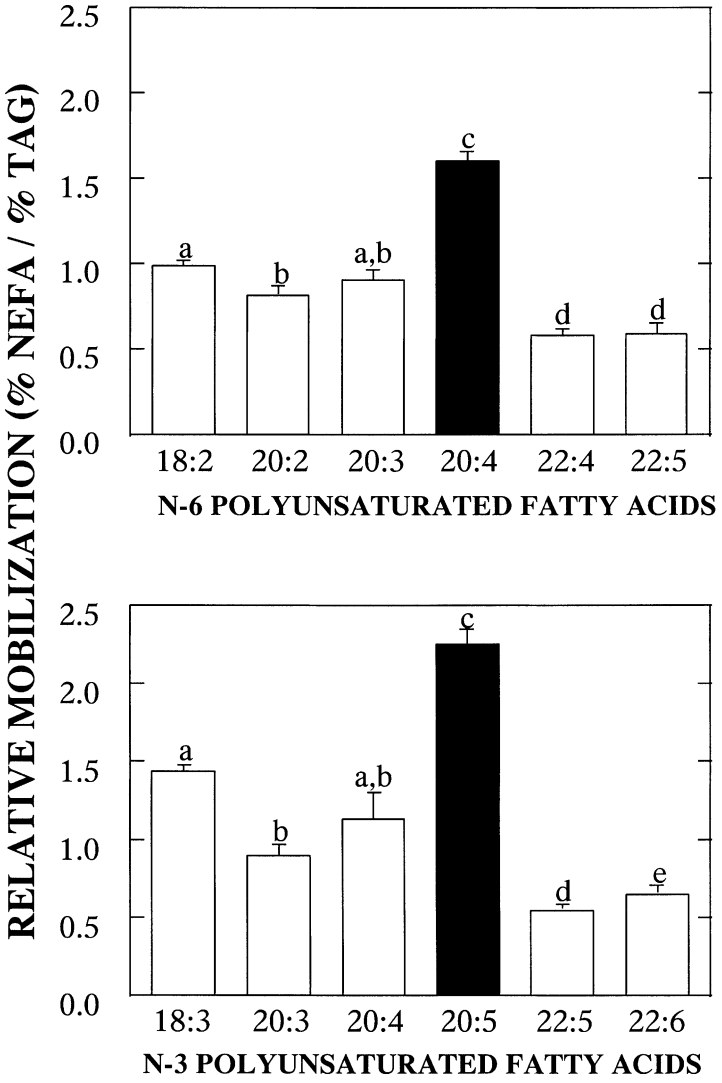

Fig. 5. Relative mobilization of fatty acids of the n-6 and n-3 series from human white fat cells. Data are from [33].

4.5. *In vivo selective mobilization of individual fatty acids*

Whether individual fatty acids are released in vivo according to the same selective pattern described in in vitro studies needed also to be considered. Throughout an experimental fast, the fatty acid composition of adipose tissue TAG was clearly affected, indicating a selective in vivo fatty acid mobilization [34]. During fat store depletion, adipose tissue was relatively enriched in VLC-SFA and VLC-MUFA, and depleted in some highly unsaturated fatty acids (16–20 carbon atoms and 4–5 double bonds). Thus, the selectivity of fatty acid mobilization from adipose tissue operates in vivo. On the whole, similar trends have been obtained in vivo on interscapular brown adipose tissue [35]. This means that the selective mobilization of fatty acids is a general metabolic

feature of adipose tissue. The relationships between the molecular structure of fatty acids and the selectivity of their mobilization are valid in vivo. A direct relationship was found between in vitro and in vivo relative mobilization that entirely accounts for the selective removal of fatty acids during fat store depletion [34].

4.6. Classification of fatty acids according to mobilization rate

The mobilization of fatty acids from white fat cells is selective and depends mainly on fatty acid chain length and unsaturation [29]. Among fatty acids usually found in adipose tissue TAG (12–24 carbon atoms and 0–6 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 [31]. 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. Moderately mobilized fatty acids include all the others.

5. Possible role of the substrate in the selectivity of fatty acid mobilization

5.1. Positional distribution of fatty acids in TAG

The mechanism of the selective mobilization of white fat cell fatty acids remains unclear. It has already been reported that the explanation does not lie in their relative proportions in fat cell TAG, since there is no support for a competition process among fatty acids [31]. During lipolysis, the breakdown of stored TAG by HSL has been shown to be the rate-limiting step [14]. Therefore, a selective hydrolysis of adipose tissue TAG can be reasonably proposed. Among known lipase specificities, HSL has been reported to preferentially cleave sn-1 and sn-3 positions of TAG [14]. Fatty acids are not randomly distributed among the three positions of the glycerol moiety (Fig. 6). The hypothesis predicts that VLC-SFA and-MUFA (weakly mobilized) would be located mainly in the inner position, whereas highly unsaturated fatty acids with 18–20 carbon atoms (highly mobilized) would be found mostly in the outer positions. However, the fact that VLC-SFA and-MUFA are mainly found in the outer positions does not agree with this hypothesis. Thus, the selectivity of fatty acid mobilization from fat cells seems unrelated to the positional distribution of fatty acids on the glycerol backbone [36].

5.2. Hydrolysis of fat cell TAG by lipases: the role of the lipid–water interface

Mature adipocytes contain a large unilocular lipid droplet [37] which presents a small surface-to-volume ratio and thus a limited surface availability of substrate. The neutral lipids are insoluble in water and lipases are water-soluble, and the enzymes are maximally activated when they are adsorbed at the water-lipid interface [37,38]. This seems 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 [39]. Therefore, substrate availability is a limiting step during lipolysis. It strengthens the concept that HSL works at the cytosol (water)—TAG droplet (neutral lipid) interface where

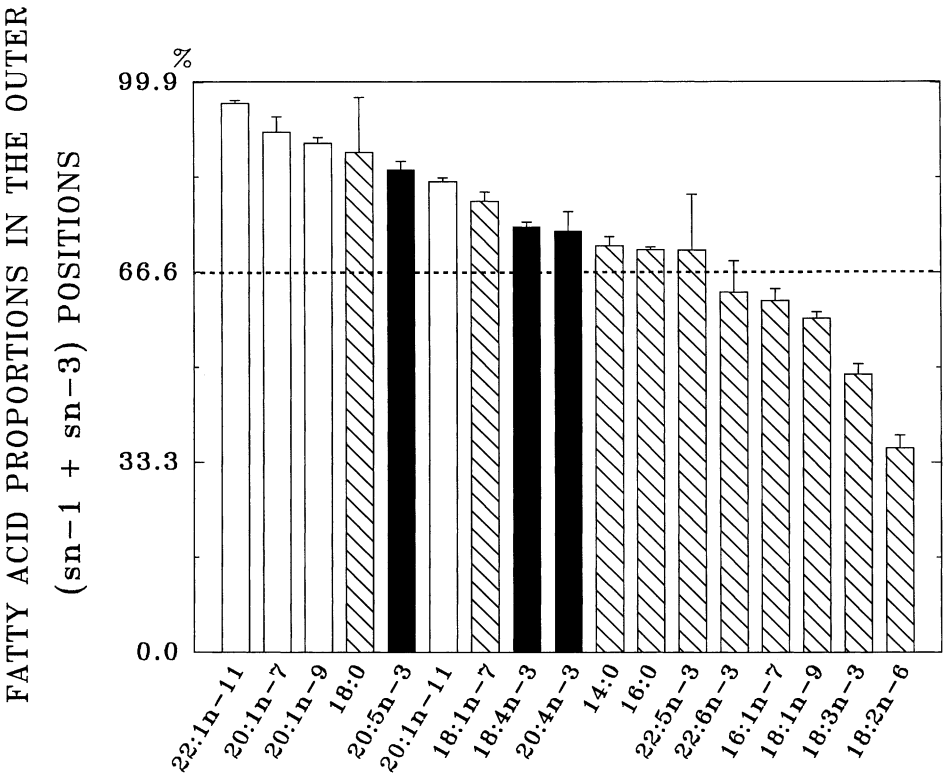

Fig. 6. Positional distribution of fatty acids in TAG of rat white fat cells. Black, hatched and white histobars represent readily, moderately and weakly mobilized fatty acids, respectively. From left to right, major fatty acids are arranged in decreasing order of proportions in the outer (sn-1 + sn-3) positions. The dotted line at 66.6% indicates a random distribution of fatty acids between outer and inner positions. Data are from [36].

only small amounts of substrate are accessible. On the other hand, it has been suggested that some TAG species can be preferentially hydrolyzed under stimulated lipolysis [40]. Thus, a qualitative release of fatty acids through the known limited substrate availability at the lipid–water interface during lipolysis by the selective hydrolysis of only certain TAG species can be considered.

5.3. The substrate: more than a passive compartment during lipolysis?

HSL is active at a lipid–water interface and its ability could be modulated by the availability and/or the reactivity of the substrate and also by the distribution of the reaction products which can act as surface-active compounds [37]. Therefore, the specificity of a lipase may reflect the physical availability of substrate to the enzyme at the lipid–water interface. In addition, the substrate concentration at this interface may influence the lipolysis rate. The hydrolysis of non-polar TAG molecules yields more polar reaction products such as diacylglycerol, monoacylglycerol and finally glycerol, while fatty acids are generated at each step. Apolar lipids as TAG are then largely stabilized by more polar lipolysis products which are also efficient as emulsifiers of TAG [37]. Modification of the lipolysis products leads to a redistribution of components among phases and the interfacial availability of TAG for hydrolysis changes during lipolysis. It has already been

suggested that substrate availability could be an activated process concomitant with the lipase activation [41,42]. Lipolysis arises in a medium containing at least two distinct phases and frequently more. Lindstrom et al. [43] reported that the products of intestinal lipolysis could potentially form at least four phases in addition to the oil substrate phase and the micellar product phase more commonly admitted. The physical and conformational form of the substrate is then a relevant parameter of lipase activity through adsorption of water soluble lipases at the interface.

5.4. Lipid partition according to polarity during the lipolytic process

Selective solubility or a partition of substrates (from which fatty acids originate through lipolysis) could occur between two (or more) phases at the lipid–water interface. In other words, the two phases between which substrate fatty acids partition according to their molecular structure, i.e. their polarity, would be the lipid droplet containing the substrates and the cytoplasm containing the lipases. The physicochemical state of the substrate that conditions its availability has not been extensively studied during investigations of HSL substrate specificity. However, classifications of biologically active lipids based on their interactions with water have been reported [44,45]. Lipids have been classified by physical properties and lipid partition coefficients between several phases differing by their polarity [45]. According to this dynamic concept, an apolar phase includes TAG and diacylglycerols, and the interfacial phase contains protonated long-chain NEFA, monoacylglycerols and soaps including ionized long-chain NEFA [45]. Hydrolysis of apolar lipid molecules, i.e. TAG, generates products which move from the apolar phase to the interfacial phase and thereafter to the polar aqueous phase only as ionized NEFA. The optimum catabolic process for apolar lipids is a function of the lipid–water interface where most lipases act. Lipolysis mediated through enzymatic activities modulates the volume of the interfacial phase by an increase of monoacylglycerol and fatty acid content, and therefore the hydrolysis products migrate from the interfacial phase to the aqueous polar phase. It has been reported for lipoproteins [45] that apolar lipids, mainly TAG enriched in PUFA, display a higher partition coefficient between an interfacial phase and an apolar phase than TAG enriched in saturated fatty acids. As a result, lipolytic enzymes would be more efficient towards PUFA. Hence, the relative amount of released PUFA would be higher compared to saturated fatty acids. In addition, a low molecular weight TAG molecule in a heterogeneous TAG mixture facilitates hydrolysis by the lipase. A higher hydrophilicity or diffusional mobility of the lower weight TAG has been proposed as an explanation [46]. The accumulation of these molecules in the surface of the oil droplets, that may partly account for a more rapid hydrolysis of TAG of lower molecular weight, can also be suggested.

5.5. Fatty acid composition of the most polar TAG: methodological considerations

A selective enrichment in certain TAG species, according to a physicochemical property like polarity, at the lipid–water interface where HSL acts can be proposed. The strong analogy of the retention of fatty acids in fat cells (reverse of their mobilization) with that on a nonpolar gas–liquid chromatography column illustrates the role of the physicochemical properties of fatty acids in this metabolic process [29]. Liquid–liquid partition chromatography has been widely used to analyze TAG [47], and the separation is based both on the combined chain length of the fatty acid and on the total number of double bonds, as is their selective mobilization from fat cells [47]. Very often, natural

lipid samples are too complex to yield an adequate separation of TAG into single components. The case of adipose tissue TAG enriched with a wide spectrum of fatty acids is even more complex because at least of highly unsaturated fatty acids with five or six double bonds which contribute to generating overlapping components. Since the purpose was to examine the fatty acid composition of the most polar adipose tissue TAG, a total separation into individual spots was not needed. As expected, fat cell TAG were not separated by distinct molecular species but were nevertheless sufficiently spread out into TAG fractions according to their polarity [48]. The fatty acid compositions of the most polar fat cell TAG were determined and compared to fatty acid mobilization.

5.6. *Relative enrichment of individual fatty acids in the most polar TAG*

The relative enrichment of some fatty acids is shown in Fig. 7 where the fatty acid composition of the most polar TAG are compared to that of total TAG by calculating the ratios (% in polar

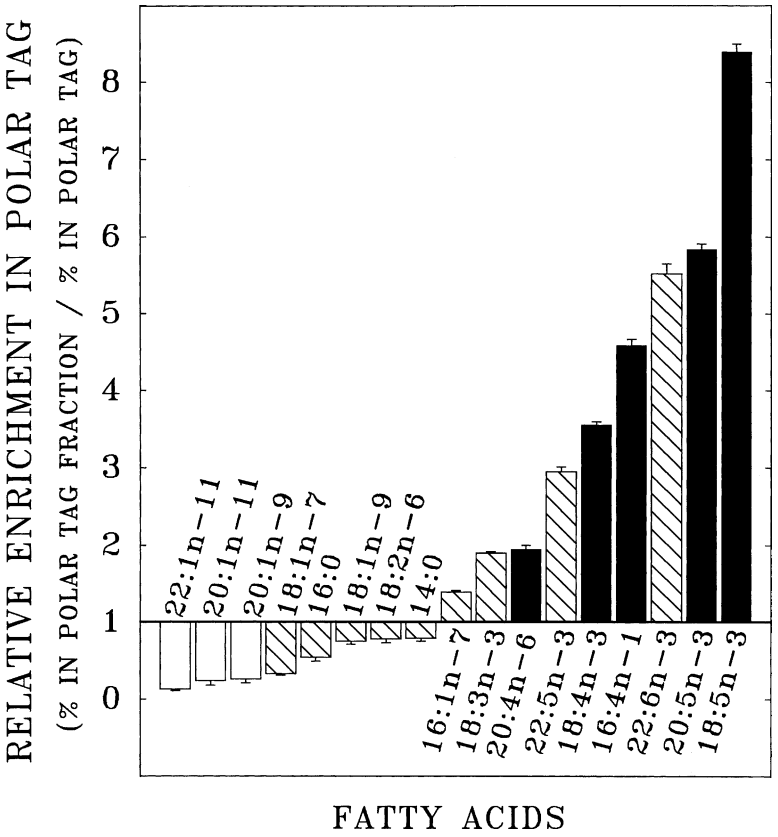

Fig. 7. Relative enrichment of fatty acids in the most polar adipose tissue TAG. The relative enrichment of individual fatty acids in polar TAG was calculated as the ratio between their weight percentage in the most polar TAG fraction to that in fat cell TAG. A ratio greater than, equal to, or lower than unity shows that the fatty acid proportion is, respectively, more, equally, or less important than in the total adipose tissue fatty acids. Black, hatched and white histobars represent readily, moderately and weakly mobilized fatty acids, respectively. Values for fatty acids are arranged from left to right in increasing order of relative enrichment in polar TAG. Data are from [48].

TAG fraction)/(% in total TAG). Most of the ratios are either higher or lower than unity, indicating selective enrichment or depletion [48]. As an average, the ratios show a trend to be higher than one for highly unsaturated fatty acids and lower than one for VLC-SFA and-MUFA.

Fatty acids have already been roughly classified into three categories as a function of their mobilization rates [31]. Highly mobilized fatty acids, found to be those with 16–20 carbon atoms and 4–5 double bonds and illustrated by 20:5n-3 or 18:4n-3, were preferentially represented in the most polar TAG fraction. The relative enrichment of this class of fatty acids is high in the most polar TAG fraction. Weakly mobilized fatty acids, i.e. those with 20–24 carbon atoms and 0–1 double bond, that are illustrated by 20:1n-9 or 22:1n-11, were found at very low levels in the most polar TAG fraction, corresponding to a depletion. Hence, the relative enrichment of this class of fatty acids is low in the most polar TAG fraction. 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 and illustrated by 16:0, 18:1n-9, or 18:2n-6 were represented in about the same proportions in the most polar TAG fraction compared with total TAG. The relative enrichment of this class of fatty acids was close to one. Thus, the hydrolysis of the most polar TAG could contribute towards explaining the selective mobilization of fatty acids from fat cells [48].

6. Release of individual fatty acids by HSL

6.1. Molecular mechanisms controlling the hydrolysis of TAG

Different steps and proteins in the overall lipolytic process could account for selective mobilization of fat cell fatty acids, notably access of the enzyme to its lipid substrate, hydrolysis of TAG, and transfer and transport of released fatty acids. During lipolysis, the breakdown of TAG by HSL is thought to represent the rate-limiting step [14]. By analogy with other lipases, HSL may exist in two conformational states: one with an active open form with exposure of the catalytic site and a hydrophobic area that interacts with lipids and another inactive closed form. Whether or not phosphorylation of HSL is required to trigger the transition from the closed to the open form [49], a simple conformational change of phosphorylated HSL leading to a higher affinity for its substrate does not fully account for the stimulation of fat cell lipolysis. An important step in lipolysis activation seems to be the translocation of HSL from a cytosolic compartment to the surface of the lipid droplet [39,50,51]. HSL is diffusively distributed throughout the cytosol in unstimulated fat cells, whereas upon stimulation, the enzyme translocates concomitantly with the onset of lipolysis. Interestingly, activation of lipolysis may also rely upon proteins that are not directly involved in the catalytic process and two proteins have recently been shown to interact with HSL: adipocyte lipid binding protein (ALBP) and lipotransin [52,53]. ALBP is an intracellular fatty acid-binding protein highly expressed in adipocytes and its interaction with the HSL N-terminal region would avoid local accumulation of NEFA during lipolysis. It would also allow the NEFA to be shuttled out of adipose tissue. Consistent with such a role for ALBP is the observation that ALBP-null mice exhibit decreased lipolytic capacity [54,55]. Lipotransin may dock the protein to the surface of the lipid droplet but the exact contribution of lipotransin to catecholamine lipolytic and insulin antilipolytic effects will have to be the subject of further studies. Access to the fat droplet constitutes another potential mechanism for the control

of lipolysis. Perilipins, which are proteins covering the large lipid droplets in adipocytes, shield stored triglycerides from cytosolic lipases [56,57]. Upon phosphorylation, perilipins would allow access to the lipid droplet and thereby allow HSL interaction with its substrates [58]. 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 [59,60]. Other factors and mechanisms, such as substrate availability [61] and intermediary lipid metabolites [62], also seem to be involved in the control of HSL activity.

6.2. Role of HSL

Lipolysis is done mainly by HSL which has a critical role in the control of energy homeostasis and catalyzes the rate-limiting step in the breakdown of adipocyte TAG [14]. Among the mechanisms that might affect the selective mobilization of fatty acids, a differential hydrolysis of adipose tissue TAG by HSL can be proposed. To determine whether HSL itself plays a role in the selectivity of fatty acid release, the in vitro hydrolysis of stable lipid emulsions by recombinant rat and human HSL has been studied. The use of a stable lipid emulsion and recombinant HSL allowed investigating the direct contribution of HSL to the selective hydrolysis of fatty acids from triglycerides. The substrate specificity of HSL has been thoroughly investigated using enzymes from both rat adipose tissue and recombinant sources [63,64]. These studies have shown that HSL hydrolyzes emulsions of tri-, di-, and monoacylglycerols and cholesterol esters. In contrast, very little is known regarding whether HSL shows selectivity towards the fatty acids contained in these neutral lipids. Whether pure HSL selectively hydrolyzes TAG containing a wide range of fatty acids and whether this selectivity is related to fatty acid molecular structure remains to be determined.

6.3. A selective metabolic process

Only a few fatty acids with chain length ranging from 12 to 24 carbon atoms and degree of unsaturation from 0 to 3 double bonds could be confidently identified and quantified in the lipid emulsion TAG (Intralipid) and the released NEFA [65]. The reasons for the use of such a stable lipid emulsion for the experiments had been discussed previously in detail [65]. The relative hydrolysis (% NEFA/% TAG) differed greatly among fatty acids (Fig. 8). After hydrolysis with HSL and comparison with TAG, NEFA were slightly enriched in some fatty acids such as 18:1n-7 and 18:3n-3 and markedly depleted in VLC-SFA (20–24 carbon atoms). The percentages of almost all VLC-SFA were significantly 2–5 times lower in NEFA than in TAG [65]. Other fatty acids differed less in weight percentage between NEFA and TAG. There was a 5-fold difference between the most (18:1n-7; 1.10) and the least (24:0; 0.22) readily released fatty acid. Thus, HSL selectively releases individual fatty acids from lipid emulsion TAG.

6.4. Relationship with carbon chain length of fatty acids

The relative hydrolysis of fatty acids from Intralipid emulsion was related to their molecular structure [65]. The influence of chain length is shown in Fig. 9 (left panel). For saturated fatty acids, the relative hydrolysis of fatty acids by rat and human HSL tended to decrease as the number of carbon atoms increased above 18. Using rat and human HSL, a 5-fold and 3-fold difference was observed when the chain length of saturated fatty acids increased from 12 to 24

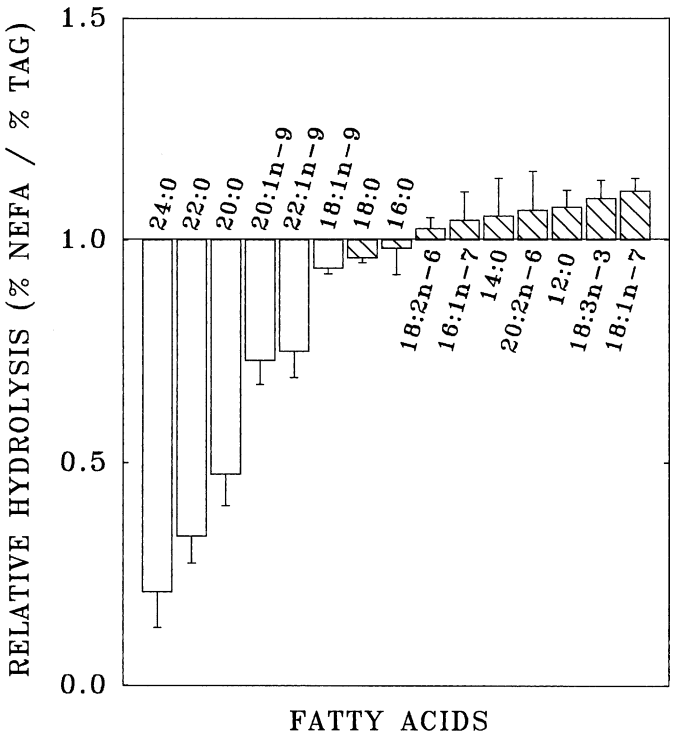

Fig. 8. Relative hydrolysis of individual fatty acids from the Intralipid emulsion by recombinant rat HSL. The relative hydrolysis of each fatty acid was calculated as the ratio between its weight percentage in released NEFA to that in lipid emulsion TAG. Hatched and white histobars represent moderately and weakly mobilized fatty acids, respectively. Values for major fatty acids are arranged from left to right in increasing order of relative hydrolysis by HSL. Data are partly from [65].

carbon atoms, respectively. These results clearly demonstrate that HSL-mediated release of fatty acids from lipid emulsion is selective and decreases with chain length for a given degree of unsaturation. From the comparison of the release of a few fatty acids by crude preparations of HSL acting in vitro on synthetic TAG, a higher hydrolysis rate of 18:3n-3 compared to 18:1n-9 was observed [66] and it was suggested that the lipase preferentially releases PUFA. The effect of unsaturation on the fatty acid release by HSL does not seem to be very marked since the difference reported above between 18:3n-3 and 18:1n-9 was not found here, although there was a slight preference for the release of 18:3n-3 (Fig. 9). Fatty acids are selectively hydrolyzed from TAG by HSL according to carbon chain length [65]. Whether HSL shows a selectivity towards highly unsaturated fatty acids remains to be clarified. Notably, the release of various fatty acids, including highly PUFA such as 20:4n-6, 20:5n-3 and 22:6n-3, should be investigated to confidently relate hydrolysis rate to degree of unsaturation.

6.5. Relationship with degree of unsaturation of fatty acids

To see if HSL preferentially releases PUFA from TAG the in vitro hydrolysis of individual fatty acids from a lipid emulsion containing a wide spectrum of fatty acids was examined [67].

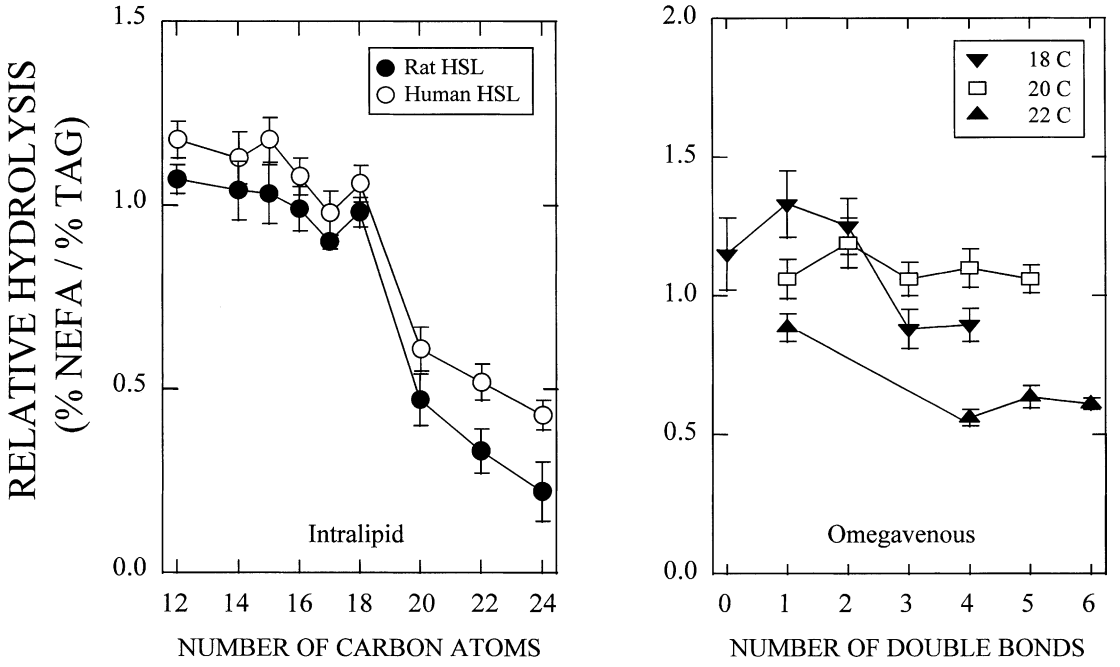

Fig. 9. Comparison of the relative hydrolysis of saturated fatty acids by recombinant rat and human HSL (left panel) and of 18-, 20-, and 22-carbon atom fatty acids by recombinant rat HSL (right panel). Data are from [65] and [67].

The fatty acid chain length and unsaturation ranged from 12 to 24 carbon atoms and from 0 to 6 double bonds, respectively. In particular, 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. The relative release of individual fatty acids ranged from about 0.4–0.5 to 1.5–1.7 with recombinant HSL. The least and the most readily released fatty acids were 24:1n-9 and 18:1n-7, respectively. Among polyunsaturated fatty acids, 16:4n-3, 22:4n-6, 22:5n-3 and 22:6n-3 were about 1.5–2 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. The influence of fatty acid molecular structure on hydrolysis by HSL can be visualized by plotting relative release versus unsaturation at given chain lengths (Fig. 9, right panel). For a given chain length, the relative release tended to decrease with the number of double bonds for fatty acids with 18, 20, and 22 carbon atoms. PUFA are not readily released compared to total fatty acids (Fig. 9). Conversely, most of PUFA were resistant to the action of the lipase. Considering all fatty acids, the effect of carbon chain length on HSL selectivity was greater than that of unsaturation degree.

6.6. *Possible mechanisms*

The mechanism underlying the selectivity of fatty acid release by HSL is unclear. An explanation based on the selective hydrolysis of a small pool of substrate fatty acids or the positional specificity of HSL is not supported by previous studies [34,36]. It has been suggested that some TAG species are preferentially hydrolyzed during lipolysis [40]. A selective enrichment in some

TAG species at the lipid–water interface where HSL acts has already been suggested [29,48] and discussed in Section 5.4 with regard to physicochemical properties. Lipoprotein apolar lipids (mainly TAG) enriched in PUFA show a higher partition coefficient between an interfacial phase and an apolar phase than TAG enriched in saturated fatty acids [45]. Hence, the relative amount of released very-long-saturated fatty acids by lipolytic enzymes would be lower compared to other fatty acids. The accessibility and/or the orientation of the ester group at the lipid–water interface may differ [46] and could also be considered as another factor influencing the hydrolysis of fatty acids. HSL selectivity may be related to different rates of substrate hydrolysis. HSL is active at a lipid–water interface and its ability might be modulated by the availability and/or the reactivity of the substrate and also by the distribution of the reaction products. In this regard, it is possible that the molecular structure of fatty acids is a determinant in the physical properties of the substrate and lipase activation.

It has been shown that the catalytic domain of HSL adopts the α/β-hydrolase fold [68] and furthermore that it is structurally related to the carboxylesterase B family of lipases and esterases [69]. The validity of the three-dimensional model of HSL has been confirmed through the crystal structure of a bacterial homologue of HSL, the Brefeldin A esterase [70]. Structural comparisons of these two enzymes have identified a substrate binding pocket into which the fatty acid to be hydrolyzed accommodates. The exact length of this pocket in HSL is not known, but it is clear that it can accommodate fatty acids. It is likely that the length and overall shape of the substrate binding pocket in HSL is a major determinant in the selective release of fatty acids in HSL-catalyzed TAG hydrolysis. Further studies, using different synthetic homoacid TAG (composed of the same three fatty acids) as substrates, are required to test this hypothesis. Hence, the selective hydrolysis of fatty acids might also be influenced by the enzymatic properties of HSL.

6.7. *Selectivity of other lipases*

Selectivity in fatty acid release has also been investigated for several other mammalian lipases (acylglycerol acylhydrolase) [71–74]. For instance, some studies reported that lipoprotein lipase does not show fatty acid specificity [71,72]. This view has however recently been challenged [73]. Using racemic diacid TAG as substrates, gastric lipase has been shown to be selective for shorter chain fatty acids (8–12 carbon atoms) but not for longer chain fatty acids [74]. Pancreatic lipase exhibits differences in the rate of fatty acid hydrolysis in vitro [75] with a marked resistance of PUFA, supporting previous work [76]. In addition, there was a clear effect of double bond position upon hydrolysis by pancreatic lipase [77]. Concerning the effect of chain length, maximal rates of pancreatic lipolysis are roughly similar among long-chain monoenoic fatty acid esters [78]. Taken together, these data support an effect of unsaturation degree rather than of chain length in the fatty acid selectivity of mammalian lipases. As for the fatty acid selectivity of microorganism lipases, some data are also available [79,80]. For instance, TAG containing medium-chain saturated fatty acids are good substrates for chromobacterium lipase B while those containing longer chain fatty acids are poorly hydrolyzed, indicating a selective effect of the fatty acid chain length [79]. Indeed, among the fatty acids with 10–18 carbon atoms, the longer the carbon chain, the lower the hydrolysis rate. Similarly, the stereoselective hydrolysis of TAG by some microbial lipases depends on the fatty acid length of the substrate [80]. As a whole, this view would be in line with the fact that HSL shares no homology with other mammalian lipases but shares amino acid sequence homology with a few bacterial lipases [64].

Considering the substrate specificity of lipases, a preference for some fatty acids remains a possible selective step. For pancreatic lipase-resistant PUFA, it has been proposed that there might be a limited accessibility of the lipase to the ester bonds of the substrate, due to the presence either of a double bond near the carboxyl group or the terminal methyl group close to the carboxyl end [76]. 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 for TAG-fatty acid hydrolysis by HSL since considering eicosapentaenoic, docosahexaenoic and docosapentaenoic acids, the first is about twice more readily released than the two others (Fig. 9) [67].

6.8. Implication of HSL in the selective mobilization of fat cell fatty acids

The question of whether or not the selective release of fatty acids from lipid emulsion by HSL is consistent with their mobilization rate from adipocytes needed close examination. It is of particular interest to determine if the relationships between the molecular structure of fatty acids and their relative mobilization rates are found in vitro for the relative hydrolysis of fatty acids by HSL, and whether the most readily mobilized fatty acids are readily released by HSL, and conversely, whether the least readily mobilized fatty acids are resistant to hydrolysis.

The relative mobilization decreases from 1.05 to 0.3 in saturated fatty acids when the chain length increases from 12 to 22 carbon atoms [29], and one notes that the release of saturated and monounsaturated fatty acids according to chain length by HSL varies in the same way as their mobilization from adipocytes [29,31,34]. Thus, the selective release of fatty acids according to chain length observed in fat cells may be explained by HSL enzymatic activity. The relative mobilization of fat cell fatty acids increases with increasing unsaturation for a given chain length. For instance, the relative mobilization increases from 0.75–0.8 to 1.75 in 18C-fatty acids when unsaturation increases from 0 to 4 double bonds and from 0.5 to 2.7 in 20C-fatty acids when unsaturation increases from 0 to 5 double bonds [4]. The release of 18C- and 20C-fatty acids according to unsaturation by HSL does not vary in the same way as their mobilization from adipocytes. Hence, 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 enzymatic properties of HSL.

Fatty acids have previously been roughly classified into three categories according to their mobilization rates [31]. The relative release of moderately mobilized fatty acids by HSL was close to unity, in agreement with their mobilization rate. However, the relative release of highly and of weakly mobilized fatty acids also close to unity is in sharp contrast with their mobilization rates. At first glance, it would appear that the release of fatty acids by HSL does not account for the broad trends of selective fatty acid mobilization. However, this release supports a role for HSL in the mobilization of certain individual fatty acids, notably of 20:5n-3 compared with 22:5n-3 and 22:6n-3. The selectivity of fatty acid hydrolysis by HSL does not fully account for the selective pattern of fatty acid mobilization but could contribute to explaining the mobilization of specific individual fatty acids compared to others.

6.9. Relationship with the fatty acid content in fat cell TAG

The relative hydrolysis or mobilization is based on a comparison of the composition of released NEFA to that of the TAG from which they originated. The spectrum of fatty acids of the Intralipid emulsion is close to that usually found in adipose tissue of animals [16] and humans [32,33] in their normal dietary state. Adipose tissue TAG of most mammals including humans contain a mixture of fatty acids differing by molecular structure [16,32,33], chain length and unsaturation of most fatty acids ranging from 12 to 24 carbon atoms and 0–6 double bonds, respectively. However, except fatty acids with 14–18 carbon atoms and 0–3 double bonds which are present in high proportions, other fatty acids are usually found in low amounts or even at trace levels. In rats and humans, adipose tissue TAG contain up to 96.5–97% fatty acids with 12–18 carbon atoms and 0–3 double bonds [29,33]. It has been previously shown that HSL selectively releases TAG-fatty acids according to carbon chain length. Viewed in another way, it can be suggested that HSL shows selectivity towards quantitatively important fatty acids, and this can be seen as an adaptation of the lipase to the adipose tissue TAG composition [29,33] as suggested for a seed lipase and vernolic acid [81]. This view is further supported by the fact that among the monounsaturated fatty acids arranged in Fig. 10 (upper panel) from left to right in increasing order of chain length or in decreasing order of amount in adipose tissue TAG (Fig. 10, lower panel), 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. The fatty acid specificites of HSL do not seem to be oriented towards a special demand by tissues or a preferential sparing of particular or essential fatty acids. The fatty acid selectivity of HSL is related to the fatty acid composition of adipose tissue TAG. In other words, HSL preferentially releases fatty acids usually stored in high amount in adipose tissue from rats and humans in their normal dietary state. Moreover, no marked effects on the selectivity of fatty acid mobilization from fat cells have been obtained following dietary manipulation [29,31].

7. Mechanisms for the selective control of fatty acid mobilization from adipose tissue

7.1. Possible mechanism

A possible mechanism involved in the selective mobilization of fatty acids from adipose tissue is proposed through a model taking into account a heterogeneous distribution of the TAG between the fat droplet and the lipid–water interface (Fig. 11). The rate-limiting step of lipolysis being TAG hydrolysis [14,63], diacylglycerol and monoacylglycerol do not accumulate. The hydrolysis by HSL of a substrate fraction enriched in the most polar TAG at the lipid–water interface would yield transient intermediate polar products and NEFA high in polyunsaturated fatty acids (including highly mobilized fatty acids). Fatty acids are selectively hydrolyzed from TAG by HSL. VLC-SFA, -MUFA and-PUFA are resistant to hydrolysis. According to our model, the hydrolysis of the most polar TAG by HSL would be preferential but not stringent, thus allowing also the release of the moderately mobilized fatty acids and also to a lesser extent the weakly mobilized ones (e.g. VLC-MUFA). Taken as a whole, this model is in line with the selective

mobilization of fatty acids from rat and human fat cells reported in vitro [29,33] and in vivo [32,34]. Such a mechanism would lead the lipolytic products (NEFA) to reflect mainly the differential partition of TAG molecules according to their polarity at the lipid–water interface. These data provide new insight on the mechanism by which fatty acids are selectively mobilized from fat cells.

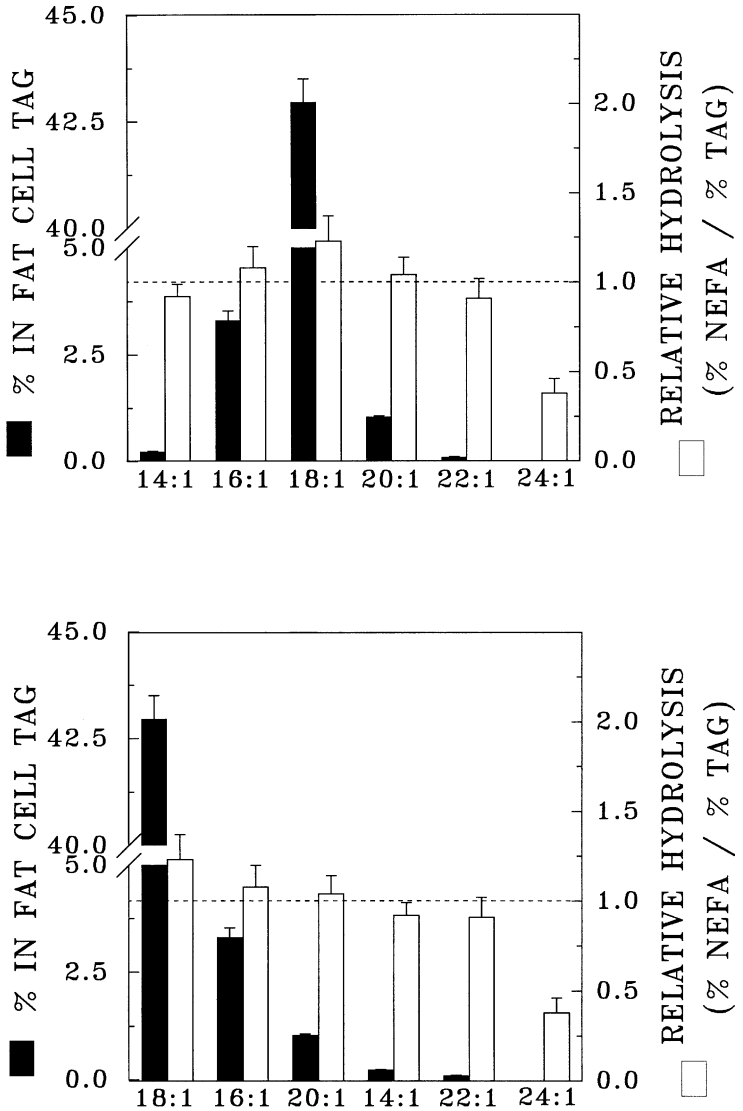

Fig. 10. Relationships between the relative hydrolysis of fatty acids from lipid emulsion by HSL and their molecular structure (upper panel) or their amount in fat cell TAG (lower panel). From left to right, fatty acids are arranged in increasing order of carbon chain length (upper panel) or decreasing order of amount in adipose tissue TAG (lower panel). Fatty acid composition in fat cell TAG and relative mobilization of fatty acids from adipocytes are computed from the results of previous studies in humans [33].

7.2. Other putative steps for the selectivity of fatty acid mobilization from fat cells

The physicochemical properties of the substrate could influence phase behavior of TAG molecular species [37,44–46]. It is known that TAG containing fatty acyl chains with different degrees of unsaturation have a complicated phase behavior. It is widely reported that TAG occur in a

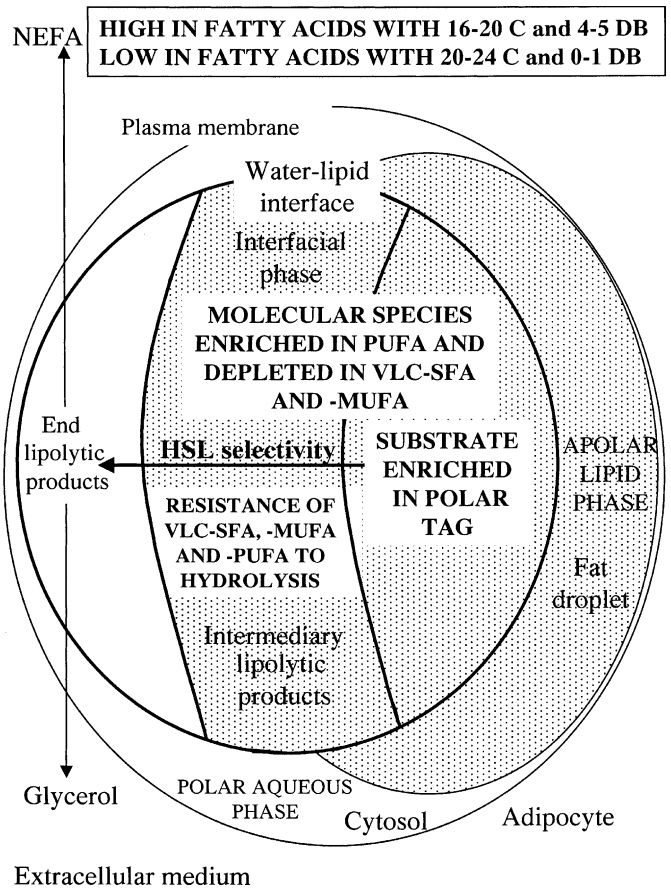

Fig. 11. Possible mechanism for the selective control of fatty acid mobilization from white fat cells. An isolated adipocyte is drawn to represent the present model where the thickness of the interfacial phase is magnified. We propose that the basis of selective fatty acid mobilization from fat cells is a selective partition of TAG according to their polarity at the lipid–water interface where HSL acts. First, the most polar TAG (high in PUFA) would be more abundant at the interface than in the droplet core and, as a consequence, they would be preferentially hydrolyzed. Second, HSL-mediated release of fatty acids is selective and partly depends upon fatty acid molecular structure, decreasing with chain length for a given degree of unsaturation, so that VLC-fatty acids are resistant to hydrolysis. Hence, the released NEFA would be enriched in some polyunsaturated fatty acids (including highly mobilized fatty acids which have 16–20 C and 4–5 DB) and depleted in very long-chain saturated and monounsaturated fatty acids (i.e. weakly mobilized fatty acids which have 20–24 C and 0–1 DB). On this model, the apolar phase includes TAG and diacylglycerols, and the interfacial phase contains monoacylglycerols, protonated NEFA and soaps including ionized NEFA. It is commonly stated that the lipolytic enzymes, if water-soluble, must penetrate through an interfacial layer of polar lipids to reach substrate molecules. C, carbon atom; DB, double bond; VLC-SFA,-MUFA, and-PUFA, very long-chain-saturated,-monounsaturated and-polyunsaturated fatty acids. Further explanation in text.

liquid or crystalline (partial or total) form as a function of temperature and constituent fatty acids. On chylomicrons isolated at low temperatures, lipoprotein lipase seems to preferentially hydrolyze unsaturated TAG compared to saturated TAG. This selective hydrolysis could result in a selective crystallization of the most saturated TAG in the remnant core which would be therefore inaccessible to the lipase. Thus, the changes in the physical state of TAG species may influence their metabolic fate. Conceptually, such an explanation does not seem to apply to the mobilization of fatty acids from fat cells because of similar selectivity in spite of big differences in the fatty acid composition, and thereby physical state, of TAG species in adipose tissue. Moreover, among substrate specificities of HSL, we already exclude its positional specificity as well as its putative stereospecificity as an explanation for the selective mobilization of fatty acids. But its preference for certain fatty acids and its dependence on different rates of substrate hydrolysis remain possible selective steps, as previously argued [36].

We already reported that the selectivity of mobilization is not based on the proportion of stored fatty acids since there is no support for a competition process among fatty acids based on relative proportion in fat cell TAG [31]. Another explanation could lie in a partition of released fatty acids between two (or more) phases, although the existence of a NEFA pool is known to inhibit HSL activity [62]. In addition, this NEFA pool would be progressively enriched in the least readily mobilized fatty acids (e.g. VLC-SFA and-MUFA) whereas the most readily fatty acids should be shuttled out of the adipose tissue. To sustain an intense lipolysis, an active reesterification should occur within the cell rather than by the more common extracellular pathway [82], and this improbable intracellular reesterification should mainly concern the less readily mobilized fatty acids by acyltransferases and/or acylCoA synthetase selectivities [83].

While other selectivities could also occur, they would be subsequent to the proposed partition of TAG at the lipid–water interface. Indeed, enzymes or transfer proteins are capable of exhibiting hydrolytic or transport selectivities for substrates or reaction end products that might contribute to explain the pattern of fatty acids released. The rate of fatty acid hydration increases with decreased fatty acid chain length and increases with degree of unsaturation [84]. While these rates could account for intracellular transfer between different compartments, their influence remains uncertain at least because of carrier proteins [52,55,85,86] and also because NEFA do not accumulate within the fat cell [62]. It seems clear at present that the physicochemical properties of fatty acids play a key role in the selective mobilization, but more information is needed to clearly demonstrate the mechanisms by which the molecular structure of fatty acids affects their metabolic fate.

8. Individual fatty acid re-uptake by adipose tissue

During lipolysis, adipose tissue TAG undergo concurrent breakdown and synthesis because a part of the newly hydrolyzed and released NEFA can be subsequently taken up and re-esterified. Until recently, it was believed that the turnover of fatty acids in adipose tissue was unselective [28]. We and others have contributed to ruling out this hypothesis by demonstrating that fatty acids are selectively mobilized from adipose tissue [29,31–35]. Among studies dealing with the incorporation [26,87], the esterification [24,88,89], and the rates of uptake [90] of NEFA by adipose tissue, no conclusive evidence was found for a selectivity of these metabolic processes. Except for the pioneer study of Hollenberg and Angel [24], who considered only a few fatty acids,

no clear data has been provided for a differential re-uptake of fatty acids by adipose tissue in vitro on the basis of molecular structure. Whether the re-uptake of any newly hydrolyzed NEFA by adipose tissue is selective during lipolysis, and whether it could affect or partly explain the selectivity of fatty acid mobilization, remained to be elucidated.

8.1. Net release of individual fatty acids for low and high re-uptake rates

Using various incubation conditions allowed manipulating the lipolytic rate, the re-uptake rate, or both could be manipulated [91]. The molar NEFA/glycerol ratio ranged from 3.0 (isolated adipocytes incubated without glucose) to about 1.5 (high concentration of adipose fragments incubated with glucose). This indicates that the rate of re-uptake of NEFA was extremely low in the former case, whereas in the latter case, about half the newly released fatty acids were taken up by adipose tissue after TAG hydrolysis.

The relative mobilization of various fatty acids ranged from 0.4 to 2.4 (i.e. a 6-fold change) when the re-uptake of fatty acids was very low (isolated adipocytes minus glucose) and from 0.25 to 3.7 (i.e. almost a 15-fold change) when the re-uptake of fatty acids was very high (adipose fragments plus glucose; Fig. 12). In both cases, the least and the most readily mobilized fatty acids were 22:1n-11 and 20:5n-3, respectively, and fatty acids with 16, 18 or 20 carbon atoms and 4 or 5 double bonds had the highest relative mobilization, whereas fatty acids with 20 or 22 carbon atoms and 0 or 1 double bond had the lowest. Whatever the incubation conditions, including those of very low and high re-uptake rates, the relative mobilization of individual fatty acids was selective and closely related to chain length and unsaturation. However at the high re-uptake rate, there was a widening of the range of the relative mobilization [91]. In agreement with previous results [29,31,34], the mobilization of adipose tissue fatty acids is selective and the rules relating the relative mobilization to molecular structure are still valid whatever the re-uptake rate. This demonstrates that the re-uptake rate of fatty acids by adipose tissue affects their individual relative mobilization values but not the overall selectivity of their mobilization. Therefore, the selectivity of fatty acid mobilization is a general feature of adipose tissue which is still valid when part of newly hydrolyzd fatty acids is taken up.

8.2. Evidence for selective fatty acid re-uptake

The relative re-uptake of fatty acids (% NEFA at very low re-uptake)/(% NEFA at high re-uptake) by adipose tissue is shown in Fig 13. As expected, the relative re-uptake of some fatty acids tends to be different from unity, indicating a selective metabolic process. Among all fatty acids, the relative re-uptake ranged from about 0.65–0.7 (18:4n-3 and 20:5n-3) to 1.55-1.8 (20:1n-11 and 22:1n-11), i.e. a 2.5-fold difference. Fatty acids with 16, 18, 20 or 22 carbon atoms and 4, 5, or 6 double bonds had the lowest relative re-uptake, while fatty acids with 20 or 22 carbon atoms and 0 or 1 double bond had the highest relative re-uptake. Other fatty acids had a relative re-uptake rather close to unity.

Based on the relative re-uptake, carbon chain length, and degree of unsaturation, the fatty acids can be roughly divided into three categories. During lipolysis, fatty acids with 16–20 carbon atoms and 4–5 double bonds are weakly taken up in adipose tissue, whereas fatty acids with 20–24 carbon atoms and 0–1 double bond showed the reverse trend. On the average, the other fatty acids are rather moderately taken up.

8.3. Relationship between the molecular structure of fatty acids and their relative re-uptake

The relative re-uptake of fatty acids by adipose tissue is also related to molecular structure. For a given chain length (Fig. 14, left panel), it decreased progressively with increasing unsaturation, whereas for a given unsaturation (Fig. 14, right panel), it tended to increase with increasing chain

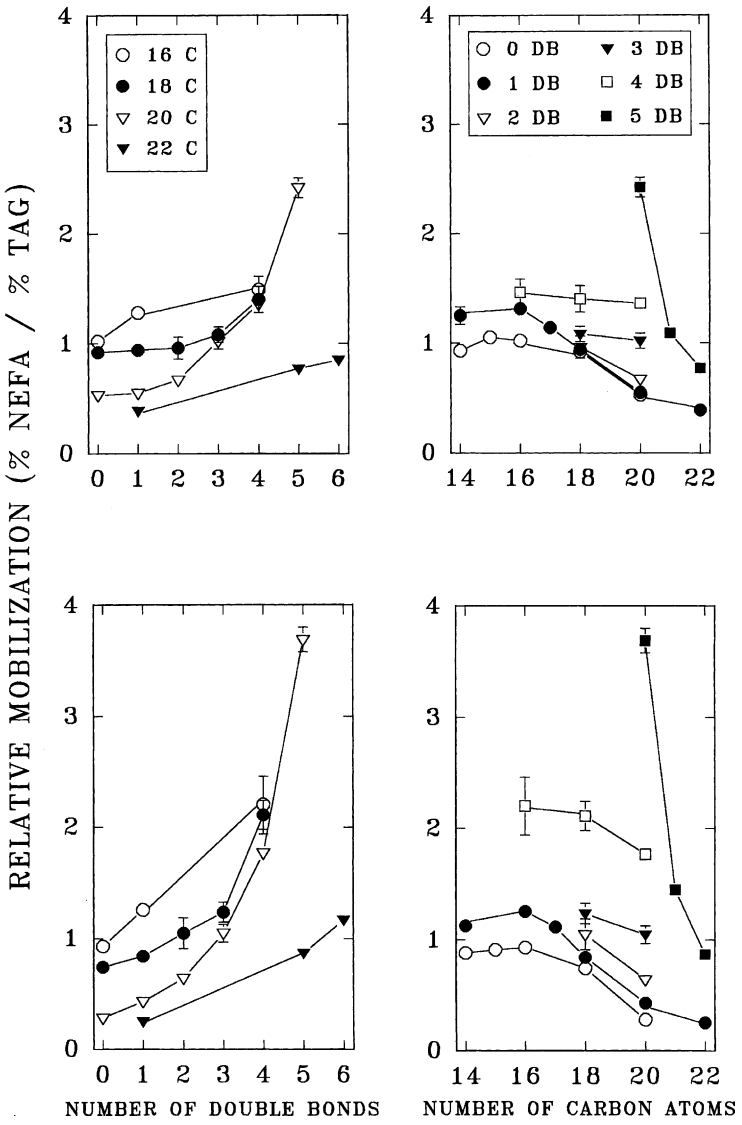

Fig. 12. Relationships between individual fatty acids and their in vitro relative mobilization from rat white adipose tissue incubated under various conditions involving different lipolytic and re-uptake rates. Influence of unsaturation at given chain lengths (left panels) and of chain length at given unsaturations (right panels). From top to bottom, plots are arranged in increasing order of fatty acid re-uptake. NEFA present in the medium at the end of the incubation represented the net efflux of NEFA, i.e. production from TAG hydrolysis less the re-uptake. C, carbon atom; DB, double bond. Data are from [91].

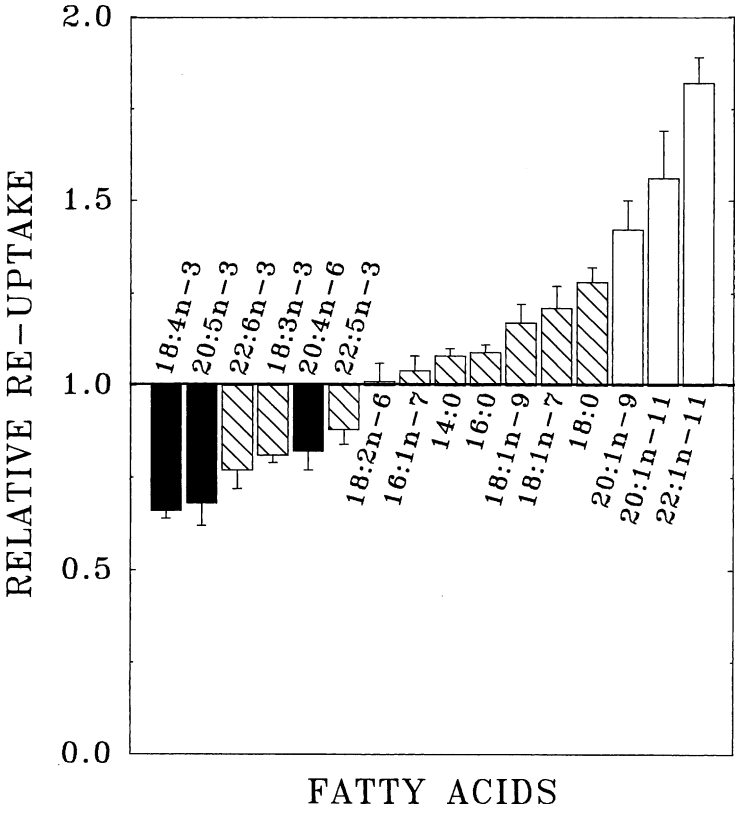

Fig. 13. Relative re-uptake of fatty acids by rat white adipose tissue. The relative re-uptake of each fatty acid was calculated as the ratio of its weight percentage in released NEFA when they were very slightly taken up by adipocytes (NEFA/glycerol ratio close to 3) to that in released NEFA when they were highly taken up (NEFA/glycerol ratio close to 1.5). A ratio greater or lower than unity indicates that the fatty acid is taken up, respectively, more or less readily than total fatty acids. Black, hatched and white histobars represent readily, moderately and weakly mobilized fatty acids, respectively. Values for quantitatively major fatty acids are arranged from left to right in increasing order of relative re-uptake. Data are partly from [91].

length. This increase was more apparent for fatty acids with 0 or 1 double bond and with 18–22 carbon atoms. These rules are approximately the reverse of those observed for relative mobilization. Highly PUFA had the lowest relative re-uptake while VLC-SFA and-MUFA had the highest. Hence, fatty acids are selectively taken up by adipose tissue during lipolysis according to molecular structure, which modulates but does not explain the selectivity of their mobilization.

These results are at variance, at least partly, with those of Hollenberg and Angel [24]. In accordance with these authors, we found similar relationships between fatty acid molecular structure and mobilization rate. In contrast, however, we did not find that at a given unsaturation shorter fatty acids were more readily taken up and thereby esterified, nor that at a given chain length that the more unsaturated ones were more readily taken up. In other words, the more polar NEFA (the shorter and highly unsaturated) are more readily mobilized than total fatty acids whereas they are less readily taken up. Conversely, the less polar NEFA are less readily mobilized than the total fatty acids and are more readily taken up. Thus, a given physicochemical

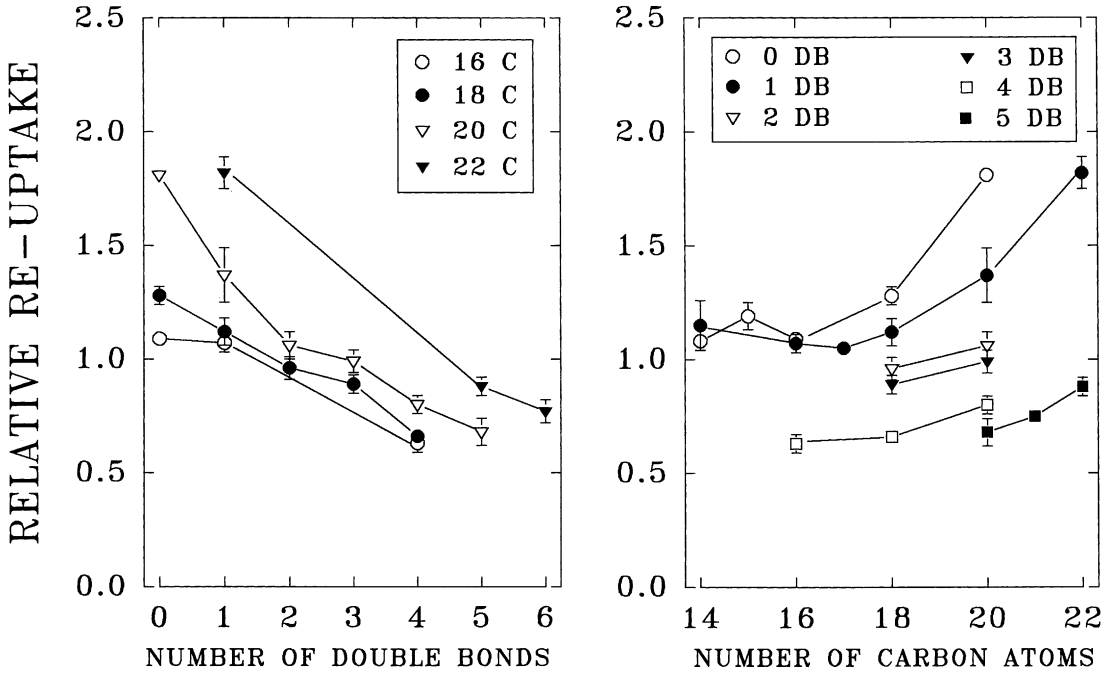

Fig. 14. Relationships between the molecular structure of fatty acids and their in vitro relative re-uptake by rat white adipose tissue. Left panel, influence of unsaturation at given chain lengths; right panel, influence of chain length at given unsaturations. When there were several positional isomers, the average value of relative re-uptake is plotted. C, carbon atom; DB, double bond. Data are partly from [91].

property of fatty acids (such as differential aqueous solubility [84]) does not account for the selectivity of mobilization and re-uptake because the two selectivities vary in opposite ways. This would be consistent with the fact that the overall re-uptake process (reesterification) is not the reverse of lipolysis for the enzymes involved. Therefore, the basis of the fatty acid selectivity observed during mobilization differs from that found during re-uptake.

Whether or not it is reasonable to propose that the selectivity of fatty acid mobilization lies, at least partly, in the selective hydrolysis of TAG, the selective steps involved in fatty acid uptake and subsequent reesterification are presently unclear. It is likely that the different relative re-uptakes of individual fatty acids are due to differences in fatty acid availability through competitive reactions between fatty acids at the transfer or transport level, or selectivity of fatty acid binding proteins and/or of enzymes involved in the esterification process.

9. Conclusions and perspectives

The data discussed in this review have implications for methodology, epidemiology, physiology and health. The choice of a tracer fatty acid in metabolic studies should be made carefully to be representative of total fatty acids [29]. The method of a fatty acid signature based on the fatty acid composition of adipose tissue TAG as a biomarker of dietary intake has to be used cautiously since individual fatty acids are selectively metabolized.

The selective mobilization and re-uptake of individual fatty acids could affect their amounts in adipose tissue. The control of PUFA storage is unclear. The fatty acid composition of adipose tissue TAG largely reflects that of the diet but the proportion of PUFA in adipose tissue is lower [16]. The preferential release and the low re-uptake of some highly PUFA can partly explain their low proportions in adipose tissue TAG as compared to the diet. For example, 18:3n-3, 20:4n-6 and 20:5n-3 are preferentially mobilized and their proportions in released NEFA tend to increase as the rate of fatty acid re-uptake increases, whereas that of 18:2n-6 is not significantly affected. Obviously, the composition of adipose tissue TAG does not necessarily reflect the different rates of fatty acid mobilization from, and re-uptake by, adipose tissue. Fatty acids found in the diet (possibly a wide spectrum) and those originating from lipogenesis (mostly 16- and 18-carbon atoms and 0–1 double bond) contribute to explain the fatty acid profile of adipose tissue [16,20,23]. Additionally, fatty acid availability and enzyme selectivity rather than, or in addition to, rate of mobilization and re-uptake probably affect the composition of adipose tissue. Differential oxidation of saturated and unsaturated fatty acids in vivo can also contribute to their selective storage in adipose tissue [92,93].

Other implications will be related to the selective supply of fatty acids to tissues, notably in the post-absorptive state and in situations of negative energy balance. The selective storage and mobilization of fatty acids could affect their maintenance in the circulation and their long-term storage in adipose tissue. Fatty acids are not only used as fuels and as membrane components, but some of them are also precursors of eicosanoids and mediators of gene expression. The two most readily mobilized fatty acids, 20:5n-3 and 20:4n-6, are both precursors of eicosanoids which have many biological effects [94–96]. PGE_2 and PGI_2, which are two important prostanoids produced in adipose tissue, could affect lipolysis [97]. Recently, PGI_2 and PGJ_2 have been shown to induce adipocyte differentiation [98,99] whereas PGE_2 and $PGF_{2\alpha}$ are described as inhibitors of the adipogenic process [97,100]. The regulation of gene expression by fatty acids [101–103], and particularly PUFA of the n-6 and n-3 series, has been extensively reported in the liver and adipose tissue in vitro and in vivo [104–112]. Indeed, PUFA are potent regulators of mRNA levels of various proteins involved in lipid and glucose metabolism [112–114]. It is interesting to note that the PUFA which have the strongest biological effects are among those readily mobilized from adipose tissue.

The observation that the molecular structure of fatty acids seems to govern their release does not support the idea of a particular demand of the body for specific fatty acids. Because of the physiological relevance of a selective fatty acid supply to tissues, the extent that the mobilization and the re-uptake rates of fatty acids in vivo affects their release needs close examination. In order to further address the molecular mechanism by which fatty acids are selectively mobilized, new insights and other lines of evidence need to be found in future studies. A better knowledge of the selectivity of individual fatty acid storage and of the underlying mechanisms is of major importance for understanding the regulation of fat cell hypertrophy by the amount and the type of dietary lipids.

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