Fasting-induced selective mobilization of brown adipose tissue fatty acids

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Abstract This study was conducted to determine whether there is selective mobilization of fatty acids from brown adipose tissue. Rats were fed a fish-oil diet and then fasted for 0, 7, or 10 days followed by analysis of the fatty acid content and composition of triacylglycerols (TAG) and phospholipid (PL) in interscapular brown adipose tissue (IBAT). Fatty acids were selectively lost from IBAT triacylglycerols, the mobilization following the same structural rules as those previously demonstrated for white adipose tissue. Fractional mobilization increased with unsaturation at a given chain length and tended to decrease with chain length at a given unsaturation. However, linoleic acid (18:2 n-6) was mobilized significantly less than predicted by these structural rules. In IBAT phospholipid, fatty acids were also selectively lost but there was no such relationship between the fractional mobilization of a fatty acid and its structure. The fatty acids of the n-6 series were exceptional in their behavior because they displayed below average fractional mobilization. In fact, linoleic and arachidonic acids actually increased their mass in IBAT phospholipid during the fast. It is concluded that, in IBAT, fatty acids are selectively mobilized during a fast, and that fasting-induced remodeling of the fatty acid composition leads to the selective retention of linoleate in the case of TAG and all the n-6 fatty acids in the case of PL.—**Groscolas, R., and G. R. Herzberg.** Fasting-induced selective mobilization of brown adipose tissue fatty acids. *J. Lipid Res.* 1997. **38:** 228–238.

Supplementary key words fatty acid metabolism and structure • triacylglycerols • phospholipids • n-6 fatty acids

Adipose tissue is a reservoir of fatty acids that are released and used as energy substrates during periods of energy deficit (1-5). The number of individual fatty acids stored as triacylglycerols (TAG) and their molecular structure (chain length, unsaturation, position of double bonds) largely depend on dietary intake (6). In contrast to previous views (7-10) it has been recently demonstrated that the release of individual fatty acids from white adipose tissue (WAT) is highly selective (11-14). As a rule, fatty acids are preferentially mobilized when they are short, more unsaturated, and have double bonds close to the terminal methyl group of the chain. This has been demonstrated for a number of WAT depots, including during fasting (13) and is independent of the relative proportion of individual fatty acids in stored TAG (15).

Brown adipose tissue (BAT) is a highly specialized fat depot because, in contrast to WAT, it is not only a site of fatty acid synthesis and storage but also a site of fatty acid oxidation (16-18). Indeed, BAT is a thermogenic organ that depends on fatty acid oxidation as an energy source for non-shivering thermogenesis (19). The thermogenic activity of BAT may be affected by the nutritional status, including by the level and type of fatty acids in the diet (20, 21) and is decreased during fasting (22). There is also evidence that the essential fatty acid status is important in the ability of the animals to adapt to cold (23, 24), and that BAT function can be related to the fatty acid composition of BAT phospholipids (PL) (25, 26). Long-chain fatty acids have been shown to mimic the calorigenic effects of norepinephrine in BAT although there was no difference in the stimulatory effect of palmitic, oleic, or linoleic acids (27). In addition to their role as a local fuel source, BAT fatty acids can be released from BAT for use by other tissues, as from WAT (28, 29).

Because of the unique and fatty acid-dependent metabolic activity of BAT the question arises whether during fasting fatty acid mobilization from this adipose tissue is selective, and if so, whether the selectivity follows the same pattern as in WAT. Previous studies based on the measurement of fatty acid composition have suggested a selective utilization of some fatty acids from BAT during starvation, cold adaptation, or hibernation

Abbreviations: IBAT, interscapular brown adipose tissue; WAT, white adipose tissue; TAG, triacylglycerols; PL, phospholipids; HSL, hormone-sensitive lipase.

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(30–34). However, no data on the absolute content of fatty acids were obtained and, because a limited range of fatty acids was examined, no relation between fatty acid structure and utilization could be determined. A second question of interest in the understanding of the metabolism of fatty acids in BAT is: is there a relationship between the mobilization of fatty acids from BAT TAG and the fatty acid composition of BAT PL or, in contrast, is the PL composition regulated independently of the release of fatty acids from TAG? Actually, it can be hypothesized that, if selective, the release from TAG would provide specific fatty acids for PL synthesis in BAT itself, which has the potential to affect BAT PL composition.

The present study was undertaken to examine these two questions. It was based on the measurement of the net loss of a large spectrum of individual fatty acids from BAT TAG and PL during prolonged fasting in the rat.

MATERIALS AND METHODS

Animals and diets

The protocol used was identical to that which allowed the demonstration of a selective fasting-induced mobilization of fatty acids from WAT TAG (13). It included a pre-fasting dietary treatment aimed at incorporating a wide variety of fatty acids in BAT lipids. Eighteen 2-month-old male Wistar rats (200 g, IFFA CREDO, L'Arbresle, France) were housed individually in plastic cages with a wire-mesh bottom, at 25 ± 1°C and under a 12-h light/dark cycle. The animals were maintained for 1 week on the same standard laboratory diet (AO4, Usine d'Alimentation Rationnelle, Villemoisson, France) as that fed since weaning. The fatty acid composition of the diet has been described previously (35). Six rats (control group) were killed and used to determine the fatty acid composition of interscapular brown adipose tissue (IBAT) triacylglycerols (TAG) and phospholipids (PL) in animals fed the standard diet. The 12 remaining rats were fed for 3 weeks on the powdered standard diet supplemented with a mixture of MaxEPA oil (RP Scherer, Benheim, France) and herring oil (Norwegian Herring Oil and Meal Industry Research Institute, Bergen, Norway). This dietary manipulation was designed to obtain IBAT with a wide spectrum of individual fatty acids, including very long-chain mono- and n–3 polyunsaturated fatty acids. Based on previous studies (36, 37), we estimated that this duration of fish oil feeding was sufficient for the achievement of a new and steady fatty acid composition of IBAT TAG and PL. The proportions in the fish-oil diet were 100 g standard diet/34 g MaxEPA oil/4 g herring oil. The gross composition of the fish-oil diet by weight was 30% lipid, 15% protein, and 48% carbohydrate, the remaining being cellulose, minerals, and vitamins. Seven and 32%, by weight of its fatty acids were very long-chain mono- and n–3 polyunsaturated fatty acids, respectively. The fish-oil diet was prepared weekly and stored in daily rations at −20°C, with α-tocopherol (40 mg/100 g) as an antioxidant. It was provided ad libitum and replaced daily.

After 3 weeks of fish oil feeding, the 12 rats were randomly matched as groups of 3 and within each group an individual rat was randomly killed in the fed (day 0, post-absorptive) state, or fasted for 7 or 10 days (4 animals at each time). The durations of fasting were chosen from Belkhou et al. (38) who reported that rats of the same strain and of similar body mass can tolerate a 13-day fast and a 40% body mass loss without detrimental effects, as demonstrated by the capacity of the animals to be successfully refed. In the present study, a safety margin was provided by limiting the duration of fasting to 10 days. Tap water was given ad libitum and body mass was measured daily. This protocol was approved by the local Ethical Committee, and followed the CNRS's (Centre National de la Recherche Scientifique) guide for the care and use of laboratory animals.

Tissue sampling and lipid extraction

Rats were killed by cervical dislocation. IBAT was rapidly and totally dissected, stripped of adherent white adipose tissue, and weighed to the nearest mg. It was minced with scissors and totally extracted according to Folch, Lees, and Sloane Stanley (39). The lipid content of IBAT was determined gravimetrically after solvent evaporation under vacuum. Lipid extracts were dissolved in chloroform–methanol 2:1 (v/v) and kept at −20°C under nitrogen until analysis. Analytical grade solvents supplemented with butylated hydroxytoluene as an antioxidant were used throughout the experiment.

Lipid analysis

Thirty mg of IBAT total lipids was mixed with known amounts of triheptadecanoin and L-α-phosphatidylcholine-diheptadecanoyl (Sigma, L'Isle d'Abeau Chesnes, France) as internal standards. Then, TAG and PL were separated from other lipids using column chromatography on silicic acid. TAG and PL were further purified by thin-layer chromatography (plates coated with Kieselgel 60, Merck, Darmstadt Germany). The developing solvent system was hexane–diethyl ether–acetic acid 70:30:1 (v/v/v). Fatty acids in TAG and PL were converted to methyl esters using 14% boron trifluoride in methanol (Sigma), according to Morrison and Smith (40). Fatty acid methyl esters were separated and quantified

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by gas–liquid chromatography using a Chrompack CP 9000 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 (60 m × 0.25 mm I.D.; 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 (12).

Calculations and statistics

The total mass of fatty acids in the IBAT lipid pools (i.e., TAG and PL) was calculated from the weight % of the internal standard. The total mass of a given fatty acid in the different lipid pools was given by weight % in the pool × total mass of fatty acids/100. To evaluate and compare the loss of fatty acids between 0 and 7 d and between 0 and 10 d of fasting, the fractional loss of each fatty acid from the two lipid pools was calculated and called fractional mobilization. It corresponded to the fraction of the initial mass of the fatty acid which was lost, and was calculated as: (initial − final mass)/initial mass. Initial and final values were taken from paired animals (see above). Statistical significance of differences between means was assessed using the Peritz' F-test for multiple comparison, or the Student's *t*-test for unpaired or paired values, as appropriate after transforming the percentage values into arcsin. Linear regression analyses with the F-test were performed for statistical analysis of correlations (SIGMASTAT, Jandel Corporation, Erkrath, Germany). In all cases the criterion of significance was $P < 0.05$.

RESULTS

Body weight and lipid masses

Body weight at the onset of fasting was 325 ± 6 g. Fasting induced a 17 and 28% loss in body weight after 7 and 10 days of fasting, respectively. The wet and the lipid masses of IBAT declined respectively by 33 and 37% after 7 days of fasting, and by 64 and 83% after 10 days **(Table 1).** After 7 and 10 days of fasting, the mass of fatty acids in TAG declined by 39 and 84%, respectively. Previous results suggested that an approximately 50% reduction in TAG fatty acid mass is appropriate for the characterization of the selectivity of fatty acid release from WAT but that at higher reductions this selectivity is blunted (13). Accordingly, the reduction in IBAT TAG mass after a fast of 7 days in the present study were considered as appropriate for the study of selective fatty acid release, and the mobilization after 10 days of fasting was not examined in detail. The loss in the PL fatty acids that decreased by only 25 and 50% after 7 and 10 days of fasting was less than the respective losses from TAG ($P < 0.05$). The mass of the lipid pools was not determined in control rats.

Changes in fatty acid composition of the lipid pools of IBAT during fish oil feeding

The fatty acid compositions of TAG and PL before and after fish oil feeding are presented in **Table 2** and **Table 3.** In TAG, fish oil feeding causes a marked increase in the proportion of n−3 and very long chain monoene fatty acids and to a lesser extent an increase in the n−6 fatty acids. These increases were at the expense of fatty acids that are endogenously produced, i.e., 16:0, 18:0, 16:1 n−7, and 18:1 n−9.

A somewhat different pattern emerged for the effect of fish oil feeding on the proportion of various fatty acids in PL. As expected and similar to the changes in TAG, there were increases in all n−3 and very long chain monoenes. In contrast to TAG, this increase was at the expense of 18:2 n−6 and 20:4 n−6 and there were no major changes in the endogenously produced fatty acids.

Changes in fatty acid composition during fasting

TAG. The weight percentage of all but six fatty acids significantly changed during the 10-day fast (Table 2). As a rule and with the main exception of 22:5 n−3, the weight percentage of very unsaturated fatty acids (3 or more double bonds) decreased during fasting and that of very long chain fatty acids (20 or more carbon atoms) with 0 or 1 double bond increased. The 30-fold decline in the weight percentage of 20:5 n−3 was particularly dramatic. Among other saturated fatty acids, the weight percentage of those with 16 or fewer carbon atoms declined during fasting whereas that of 18:0 increased. The weight percentage of monounsaturated fatty acids with fewer than 20 carbon atoms either increased (18:1 n−9), decreased (16:1 n−7), or was unchanged (18:1 n−7). The weight percentage of 18:2 n−6 increased during the fast.

Comparison of the changes in the fatty acid composition of IBAT TAG induced by fish oil feeding (day 0 vs. control) and fasting (day 7 or 10 vs. day 0) indicates that these changes were not related. That is, the changes during fasting were not simply the reversal of the changes during fish oil feeding. For example, the weight percentage of the long chain monounsaturates (20:1 and 22:1) and of linoleic acid increased during fish oil feeding and during fasting. The weight percentage of 16:0 and 16:1 n−7 decreased during fish oil feeding and during fasting while that of a number of fatty

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TABLE 1. Intercapsular brown adipose tissue mass and lipid content

	Wet Mass	Total Lipid Mass	Mass of Fatty Acids in TAG	Mass of Fatty Acids in PL
			mg	
Fed	420 ± 14^{a}	249 ± 15^{a}	218 ± 13^{a}	3.41 ± 0.26^{a}
Fasted 7 days	280 ± 7^{b}	156 ± 8^{b}	133 ± 7^{b}	2.55 ± 0.15^{b}
Fasted 10 days	153 ± 9^{c}	42 ± 1^{c}	34 ± 1^{c}	1.69 ± 0.06^{c}

Values are means ± SEM (n = 4). Values in a column not sharing the same superscript letter are significantly different ($P < 0.05$).

TABLE 2. Fatty acid composition (weight %) of IBAT TAG before and after fish oil feeding (day 0 of fasting) and after 7 and 10 days of fasting

Fatty Acids*	Control	Day 0	Day 7	Day 10
			weight %	
Saturated				
12:0	0.12 ± 0.01^{a}	0.21 ± 0.01^{b}	0.20 ± 0.01^{b}	0.14 ± 0.03^{a}
14:0	2.81 ± 0.14^{a}	4.17 ± 0.15^{b}	3.22 ± 0.25^{a}	2.05 ± 0.02^{c}
15:0	0.07 ± 0.01^{a}	0.37 ± 0.00^{b}	0.34 ± 0.02^{b}	0.23 ± 0.00^{c}
16:0	32.85 ± 1.20^{a}	18.97 ± 0.82^{b}	18.41 ± 0.70^{b}	12.10 ± 0.46^{c}
18:0	8.98 ± 0.51^{a}	5.42 ± 0.38^{b}	6.32 ± 0.06^{b}	7.49 ± 0.06^{a}
20:0	0.11 ± 0.01^{a}	0.19 ± 0.01^{b}	0.22 ± 0.01^{b}	0.45 ± 0.02^{c}
22:0	0.00 ± 0.00^{a}	0.04 ± 0.00^{b}	0.04 ± 0.00^{b}	0.11 ± 0.01^{c}
24:0	0.05 ± 0.01^{a}	0.05 ± 0.01^{a}	0.06 ± 0.01^{a}	0.14 ± 0.01^{b}
Mono-unsaturated				
14:1 n-5	0.17 ± 0.02^{a}	0.07 ± 0.00^{b}	0.06 ± 0.01^{b}	0.06 ± 0.00^{b}
16:1 n-9	0.15 ± 0.04^{a}	0.46 ± 0.01^{b}	0.51 ± 0.02^{b}	0.51 ± 0.01^{b}
16:1 n-7	5.97 ± 0.37^{a}	3.75 ± 0.13^{b}	2.46 ± 0.30^{c}	0.75 ± 0.04^{d}
16:1 n-5	0.04 ± 0.00^{a}	0.10 ± 0.01^{b}	0.07 ± 0.00^{b}	0.03 ± 0.00^{a}
17:1 n-8	0.11 ± 0.01^{a}	0.44 ± 0.00^{b}	0.32 ± 0.02^{c}	0.15 ± 0.03^{a}
18:1 n-9	37.67 ± 1.02^{a}	19.75 ± 0.63^{b}	23.59 ± 0.61^{c}	24.42 ± 0.69^{c}
18:1 n-7	0.78 ± 0.60^{a}	3.27 ± 0.11^{b}	3.79 ± 0.08^{c}	3.33 ± 0.08^{b}
18:1 n-5	0.06 ± 0.01^{a}	0.15 ± 0.00^{b}	0.13 ± 0.01^{bc}	0.11 ± 0.01^{c}
20:1 n-11	0.09 ± 0.04^{a}	1.68 ± 0.04^{b}	2.24 ± 0.12^{c}	4.59 ± 0.26^{d}
20:1 n-9	0.26 ± 0.07^{a}	3.16 ± 0.04^{b}	3.46 ± 0.11^{c}	4.95 ± 0.16^{d}
20:1 n-7	0.20 ± 0.04^{a}	0.46 ± 0.05^{b}	0.52 ± 0.06^{b}	0.78 ± 0.02^{c}
22:1 n-11	0.00 ± 0.00^{a}	1.90 ± 0.17^{b}	2.22 ± 0.14^{b}	4.54 ± 0.25^{c}
22:1 n-9	0.00 ± 0.00^{a}	0.30 ± 0.00^{b}	0.35 ± 0.02^{b}	0.71 ± 0.02^{c}
Di-unsaturated				
18:2 n-6	8.29 ± 0.83^{a}	10.51 ± 0.31^{b}	14.59 ± 0.64^{c}	15.93 ± 0.66^{c}
18:2 n-4	0.01 ± 0.01^{a}	0.21 ± 0.02^{b}	0.14 ± 0.00^{c}	0.05 ± 0.01^{d}
20:2 n-9	0.05 ± 0.01^{a}	0.60 ± 0.04^{b}	0.57 ± 0.11^{b}	0.89 ± 0.04^{c}
20:2 n-6	0.08 ± 0.01^{a}	0.28 ± 0.01^{b}	0.29 ± 0.01^{bc}	0.34 ± 0.02^{c}
Tri-unsaturated				
18:3 n-6	0.02 ± 0.00^{a}	0.09 ± 0.03^{b}	0.09 ± 0.00^{b}	0.05 ± 0.00^{c}
18:3 n-4	0.04 ± 0.01^{a}	0.33 ± 0.02^{b}	0.25 ± 0.01^{c}	0.20 ± 0.01^{d}
18:3 n-3	0.29 ± 0.03^{a}	0.73 ± 0.03^{b}	0.52 ± 0.03^{c}	0.22 ± 0.00^{a}
20:3 n-6	0.04 ± 0.01^{a}	0.36 ± 0.03^{b}	0.30 ± 0.02^{b}	0.38 ± 0.04^{b}
Tetra-unsaturated				
16:4 n-1	0.07 ± 0.02^{a}	0.16 ± 0.01^{b}	0.07 ± 0.01^{a}	0.02 ± 0.00^{c}
18:4 n-3	0.02 ± 0.00^{a}	0.72 ± 0.05^{b}	0.35 ± 0.04^{c}	0.13 ± 0.01^{d}
18:4 n-1	0.00 ± 0.00^{a}	0.15 ± 0.05^{b}	0.06 ± 0.02^{c}	0.02 ± 0.01^{ac}
20:4 n-6	0.22 ± 0.03^{a}	1.10 ± 0.11^{b}	0.71 ± 0.04^{c}	0.61 ± 0.05^{c}
20:4 n-3	0.00 ± 0.00^{a}	0.65 ± 0.06^{b}	0.31 ± 0.02^{c}	0.15 ± 0.02^{d}
Penta-unsaturated				
20:5 n-3	0.01 ± 0.01^{a}	2.38 ± .28^{b}	0.47 ± 0.13^{c}	0.08 ± 0.01^{c}
22:5 n-6	0.07 ± 0.02^{a}	0.87 ± 0.04^{b}	1.06 ± 0.23^{b}	1.44 ± 0.14^{b}
22:5 n-3	0.13 ± 0.03^{a}	3.60 ± 0.35^{b}	3.08 ± 0.14^{b}	3.73 ± 0.52^{b}
Hexa-unsaturated				
22:6 n-3	0.15 ± 0.03^{a}	12.35 ± 1.31^{b}	8.66 ± 0.19^{c}	8.13 ± 1.08^{c}

Values are means ± SEM (n = 4). Values in a row not sharing the same letter are significantly different ($P < 0.05$). * Number of carbon atoms: number of double bonds, position of the first double bond from the methyl end of the molecule.

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TABLE 3. Fatty acid composition (weight %) of IBAT PL before and after fish oil feeding (day 0 of fasting) and after 7 and 10 days of fasting

Fatty Acids	Control	Day 0	Day 7	Day 10
		weight %		
Saturated				
12:0	0.05 ± 0.01^{a}	0.21 ± 0.03^{b}	0.18 ± 0.04^{b}	0.27 ± 0.06^{b}
14:0	0.24 ± 0.03^{a}	2.56 ± 0.46^{b}	1.03 ± 0.26^{c}	0.76 ± 0.24^{c}
15:0	0.05 ± 0.01^{a}	0.40 ± 0.03^{b}	0.28 ± 0.05^{b}	0.25 ± 0.06^{b}
16:0	16.42 ± 0.67	18.19 ± 0.58	17.27 ± 1.05	15.34 ± 1.67
18:0	17.05 ± 1.08	14.77 ± 1.41	15.96 ± 0.22	16.39 ± 0.68
20:0	0.06 ± 0.00^{a}	0.23 ± 0.02^{b}	0.16 ± 0.02^{b}	0.24 ± 0.05^{b}
22:0	0.06 ± 0.01^{a}	0.16 ± 0.01^{b}	0.11 ± 0.01^{b}	0.21 ± 0.07^{b}
24:0	0.07 ± 0.02^{a}	0.33 ± 0.07^{b}	0.23 ± 0.01^{b}	0.28 ± 0.04^{b}
Mono-unsaturated				
14:1 n−5	0.10 ± 0.02^{ab}	0.07 ± 0.01^{ab}	0.06 ± 0.01^{b}	0.11 ± 0.02^{a}
16:1 n−9	0.00 ± 0.00^{a}	0.57 ± 0.02^{b}	0.34 ± 0.09^{c}	0.27 ± 0.04^{c}
16:1 n−7	2.73 ± 0.30^{a}	2.17 ± 0.32^{b}	0.98 ± 0.13^{c}	0.61 ± 0.03^{c}
16:1 n−5	0.05 ± 0.01^{a}	0.18 ± 0.02^{b}	0.17 ± 0.02^{b}	0.16 ± 0.05^{b}
17:1 n−8	0.07 ± 0.02^{a}	0.28 ± 0.07^{b}	0.26 ± 0.02^{b}	0.19 ± 0.02^{b}
18:1 n−9	18.46 ± 1.33	15.37 ± 1.22	14.90 ± 0.47	14.19 ± 1.60
18:1 n−7	1.98 ± 0.07^{a}	2.59 ± 0.10^{b}	2.41 ± 0.10^{b}	2.22 ± 0.11^{ab}
18:1 n−5	0.03 ± 0.00^{a}	0.14 ± 0.02^{b}	0.09 ± 0.01^{b}	0.05 ± 0.01^{ac}
20:1 n−11	0.19 ± 0.02^{a}	0.84 ± 0.18^{b}	0.64 ± 0.11^{b}	0.67 ± 0.04^{b}
20:1 n−9	0.00 ± 0.00^{a}	1.84 ± 0.33^{b}	1.14 ± 0.12^{b}	1.12 ± 0.08^{b}
20:1 n−7	0.09 ± 0.01^{a}	0.26 ± 0.07^{b}	0.20 ± 0.03^{b}	0.20 ± 0.03^{b}
22:1 n−11	0.02 ± 0.01^{a}	1.13 ± 0.16^{b}	0.84 ± 0.14^{b}	0.81 ± 0.02^{b}
22:1 n−9	0.03 ± 0.00^{a}	0.25 ± 0.04^{b}	0.14 ± 0.03^{b}	0.15 ± 0.02^{b}
Di-unsaturated				
18:2 n−6	17.29 ± 1.14^{a}	9.37 ± 0.09^{b}	15.31 ± 0.54^{a}	16.87 ± 0.65^{a}
18:2 n−4	0.00 ± 0.00^{a}	0.17 ± 0.02^{b}	0.13 ± 0.04^{b}	0.16 ± 0.04^{b}
20:2 n−9	0.11 ± 0.09^{a}	0.34 ± 0.07^{b}	0.25 ± 0.02^{ab}	0.28 ± 0.04^{ab}
20:2 n−6	0.27 ± 0.02	0.29 ± 0.02	0.32 ± 0.08	0.35 ± 0.04
Tri-unsaturated				
18:3 n−6	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.00	0.05 ± 0.01
18:3 n−4	0.02 ± 0.01^{a}	0.16 ± 0.05^{b}	0.12 ± 0.01^{b}	0.09 ± 0.02^{b}
18:3 n−3	0.16 ± 0.01^{a}	0.43 ± 0.08^{b}	0.17 ± 0.03^{a}	0.12 ± 0.01^{c}
20:3 n−6	1.03 ± 0.05^{a}	0.41 ± 0.03^{b}	0.63 ± 0.03^{c}	0.71 ± 0.06^{c}
Tetra-unsaturated				
16:4 n−1	0.00 ± 0.00^{a}	0.08 ± 0.01^{b}	0.06 ± 0.01^{b}	0.06 ± 0.01^{b}
18:4 n−3	0.00 ± 0.00^{a}	0.46 ± 0.10^{b}	0.25 ± 0.11^{b}	0.25 ± 0.10^{b}
20:4 n−6	17.10 ± 0.96^{a}	6.27 ± 1.30^{b}	9.72 ± 0.93^{c}	10.72 ± 0.80^{c}
20:4 n−3	0.00 ± 0.00^{a}	0.38 ± 0.06^{b}	0.17 ± 0.03^{c}	0.18 ± 0.03^{c}
Penta-unsaturated				
20:5 n−3	0.71 ± 0.05^{a}	4.40 ± 0.80^{b}	1.23 ± 0.20^{b}	1.02 ± 0.07^{b}
22:5 n−6	0.35 ± 0.04^{a}	0.52 ± 0.05^{b}	0.54 ± 0.09^{b}	0.70 ± 0.17^{c}
22:5 n−3	0.94 ± 0.09^{a}	2.65 ± 0.20^{b}	2.23 ± 0.16^{b}	2.29 ± 0.34^{b}
Hexa-unsaturated				
22:6 n−3	4.24 ± 0.52^{a}	11.49 ± 0.58^{b}	11.35 ± 0.71^{b}	11.64 ± 1.69^{b}

Values are means ± SEM (n = 4). Values in a row not sharing the same letter are significantly different ($P < 0.05$).

acids including 18:1 n−7 and 22:5 n−3 increased during fish oil feeding and was unchanged during fasting. By day 10 of the fast the proportion of all but six fatty acids was significantly different from that in control rats indicating that for almost all fatty acids the fasting-induced changes were different from those induced by fish oil feeding.

PL. The weight percentage of only 10 fatty acids significantly changed during the fast (Table 3). Moreover, these changes showed no clear pattern in relation to unsaturation or chain length. With the exception of 14:0 the weight percentage of which declined, that of the saturated fatty acids was unchanged during the fast. The weight percentage of the majority of the monounsaturated fatty acids was not significantly altered during the fast. The notable exception was 16:1 n−7 the weight percentage of which declined. The proportion of the major fatty acids of the n−6 series increased during the fast. The weight percentage of PUFA of the n−3 series with 20 or fewer carbons declined while that of the 22

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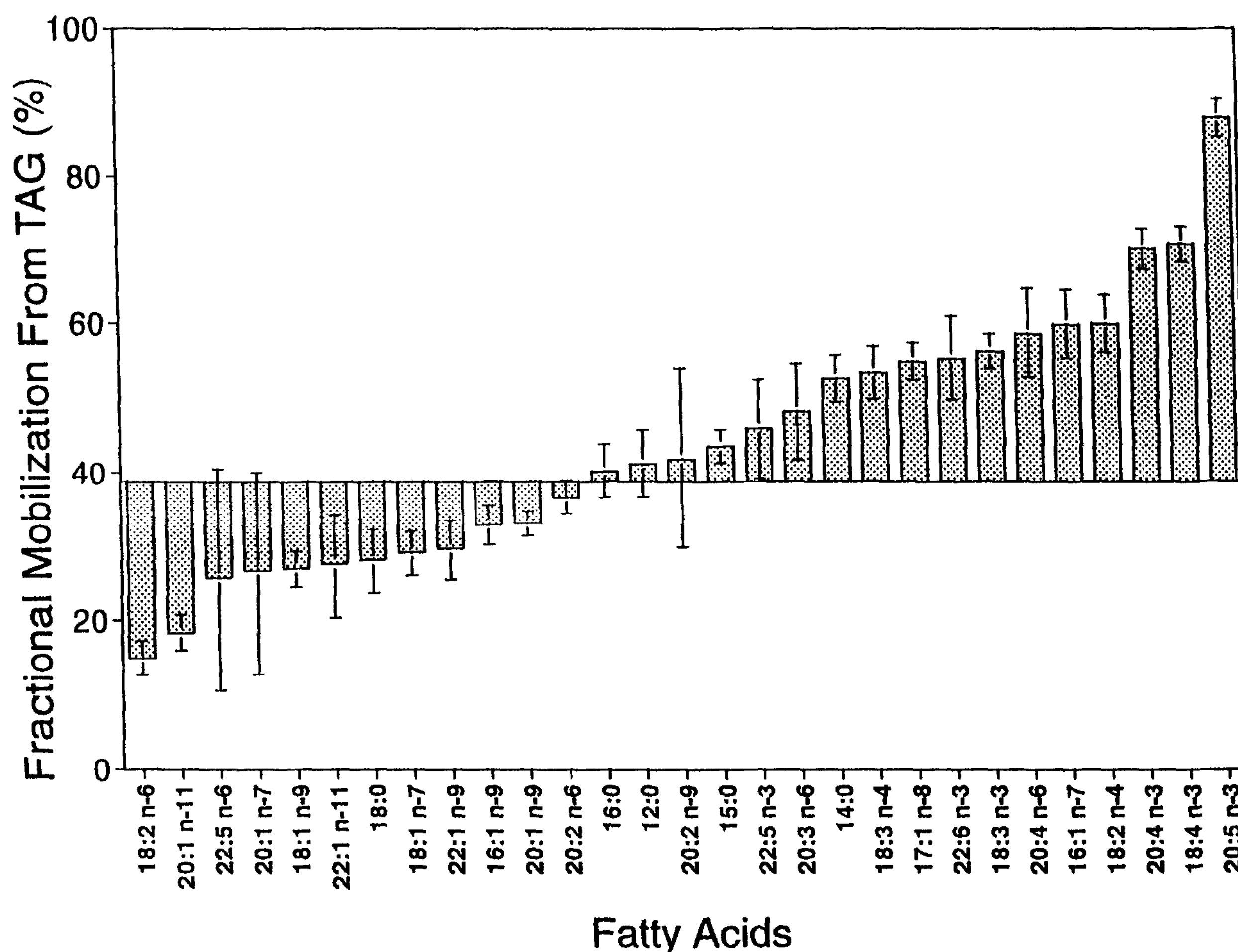

Fig. 1. Fractional mobilization of major fatty acids from IBAT TAG between days 0 and 7 of the fast. Fractional mobilization is the fraction of the initial mass of the fatty acid that was lost from IBAT TAG during the fast. Only fatty acids whose initial percentage weight was ≥0.2% are considered. The horizontal line at 38.7% shows the fractional mobilization of total TAG fatty acids (See Table 1). Values are means ± SEM.

carbon n-3 fatty acids did not change. In fact, after the 7-day fast, 20:5 n-3 had returned to the proportion in the control animals.

As with the TAG, it is clear that the changes in PL fatty acid composition during fasting were not simply the reversal of changes during fish oil feeding. This is illustrated by the saturated and monounsaturated fatty acids, especially those with 20–24 carbons, which generally increased during fish oil feeding but were mainly unchanged during fasting. A notable exception to this is seen for 18:2 n-6, 20:4 n-6, and 20:5 n-3, the weight percentage of which returned towards or up to control levels during the fast.

Quantitative mobilization of individual fatty acids from IBAT

TAG. From day 0 to 7, the fractional mobilization (the fraction of the initial mass that was lost) of 23 of the 38 fatty acids was significantly different ($P < 0.05$ or less) from 38.7%, that is from the fraction of total fatty acids that was lost during this period (see Table 1). For fatty acids that made up more than 0.2% of the fatty acids present at day 0, the fractional mobilization ranged from 15% for 18:2 n-6 to 88% for 20:5 n-3, i.e., a 6-fold difference ($P < 0.001$). This is illustrated in **Fig. 1.** Relationships were found between fractional mobilization and the structure of the fatty acids **(Fig. 2).** With the notable exception of 18:2 n-6, it can be seen that fractional mobilization increased with unsaturation at a given chain length and tended to decrease with chain length at a given unsaturation. The same pattern of loss of fatty acids from TAG was observed at both fasting periods which differed markedly in the extent of depletion of the TAG pool (38.7% after 7 days, 84.5% after 10 days). There was a highly significant relationship ($r^2 = 0.71$, $P < 0.001$) between the fractional mobilization

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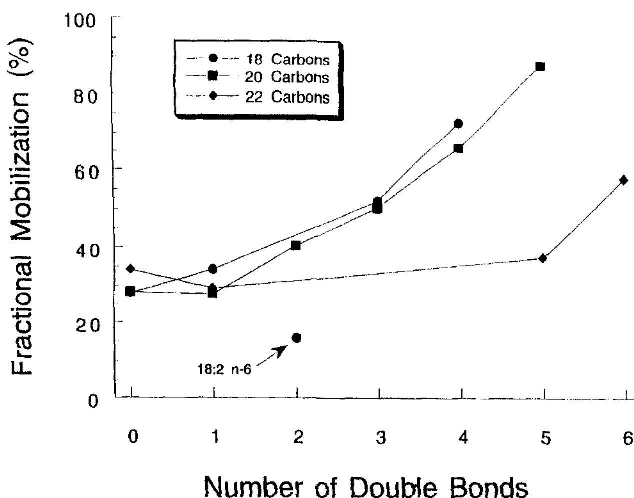

Fig. 2. Relationship between fractional mobilization of fatty acids from IBAT TAG during days 0–7 of the fast and their unsaturation at a given chain length. For clarity, SEM not shown.

of each fatty acid at day 7 versus day 10 (plot not shown).

PL. Fractional mobilizations for PL fatty acids from day 0 to 7 are presented in **Fig. 3** for fatty acids that contributed more than 0.2% to the total fatty acid content at day 0. Similar to TAG, there was a wide range of fractional mobilizations for PL fatty acids from −22 to 79%, and 18:2 n−6 was the least mobilized fatty acid and 20:5 n−3 was the most highly mobilized. On the other hand, there were several differences between the patterns of fatty acid mobilization from PL and TAG. First, there were several PL fatty acids with negative fractional mobilizations, indicating there was actually a gain in the mass of these fatty acids during the 7-day fast. This gain was 22 ± 4% for 18:2 n−6 and 16 ± 3% for 20:4 n−6. Each of the fatty acids with a negative fractional mobilization from PL is a fatty acid of the n−6 series, while in TAG there were no fatty acids with negative fractional mobilization. Second, the distribution of fatty acids with above or below average mobilization is highly skewed in the case of PL. For TAG, 15 fatty acids had numerically below average mobilization, 22 above average. For PL, only six had below average mobilization and of these six, five were fatty acids of the n−6 series. In PL, all the fatty acids of the n−6 series had below average mobilization while for TAG, fatty acids of this series had fractional mobilizations of both above and below average. Unlike TAG, there was no relationship between the fractional mobilization and the structure of the fatty acid mobilized (graph not shown). These differences between TAG and PL are further illustrated by the finding that there was no relationship between the fractional mobilization of fatty acids from TAG and PL ($r^2 = 0.10$, $P > 0.05$).

DISCUSSION

Changes in BAT mass and lipid content

As previously observed (30, 34), fasting caused a significant reduction in the total mass of BAT. The decline in total mass was principally due to loss of TAG but there was also a significant reduction in PL mass. The percentage decline of TAG was greater than PL, reflecting their different roles in the tissue. TAG serves as an energy reserve that is mobilized during fasting. PL serves a structural role in cells and plays a role in cell signaling by the release of specific fatty acids for eicosanoid synthesis and the release of DAG. As the size of BAT cells would be reduced as the TAG is utilized, it is not surprising to find a decrease in PL as it is the major lipid of the plasma membrane whose area would be reduced in parallel with the reduction in cell size.

Fatty acid mobilization from TAG

The mobilization of fatty acids from IBAT TAG was highly selective and there was a 6-fold difference between the most and the least mobilized fatty acid. Moreover, there was a clear relationship between the structure of the fatty acids and their mobilization. Fractional mobilization increased with unsaturation at a given chain length and tended to decrease with chain length at a given unsaturation. This relationship between structure and mobilization was the same as that previously reported for the mobilization of fatty acids from WAT in vitro and in vivo (12, 13). This similarity between BAT and WAT is further demonstrated by the fact that the mobilization of fatty acids from BAT (this study) and mobilization from WAT (13) were significantly correlated **(Fig. 4).**

In IBAT, linoleic acid (18:2 n−6) was a notable exception to the rules that govern the mobilization of fatty acids from TAG. Based on the structural relationships, it could be predicted that linoleic acid should have had a fractional mobilization of approximately 45% when, in fact, its mobilization was only 17%, i.e., 2.5-fold less than predicted. This is consistent with the previous findings that in fasting rats the percentage of 18:2 n−6 increases in BAT TAG (34) and total lipids (30). An increase in the percentage of linoleic acid in BAT TAG has also been observed in rats subjected to treatments that stimulate the turnover of BAT TAG, e.g., cold acclimation and exercise (26). Interestingly, Chen and Cun-

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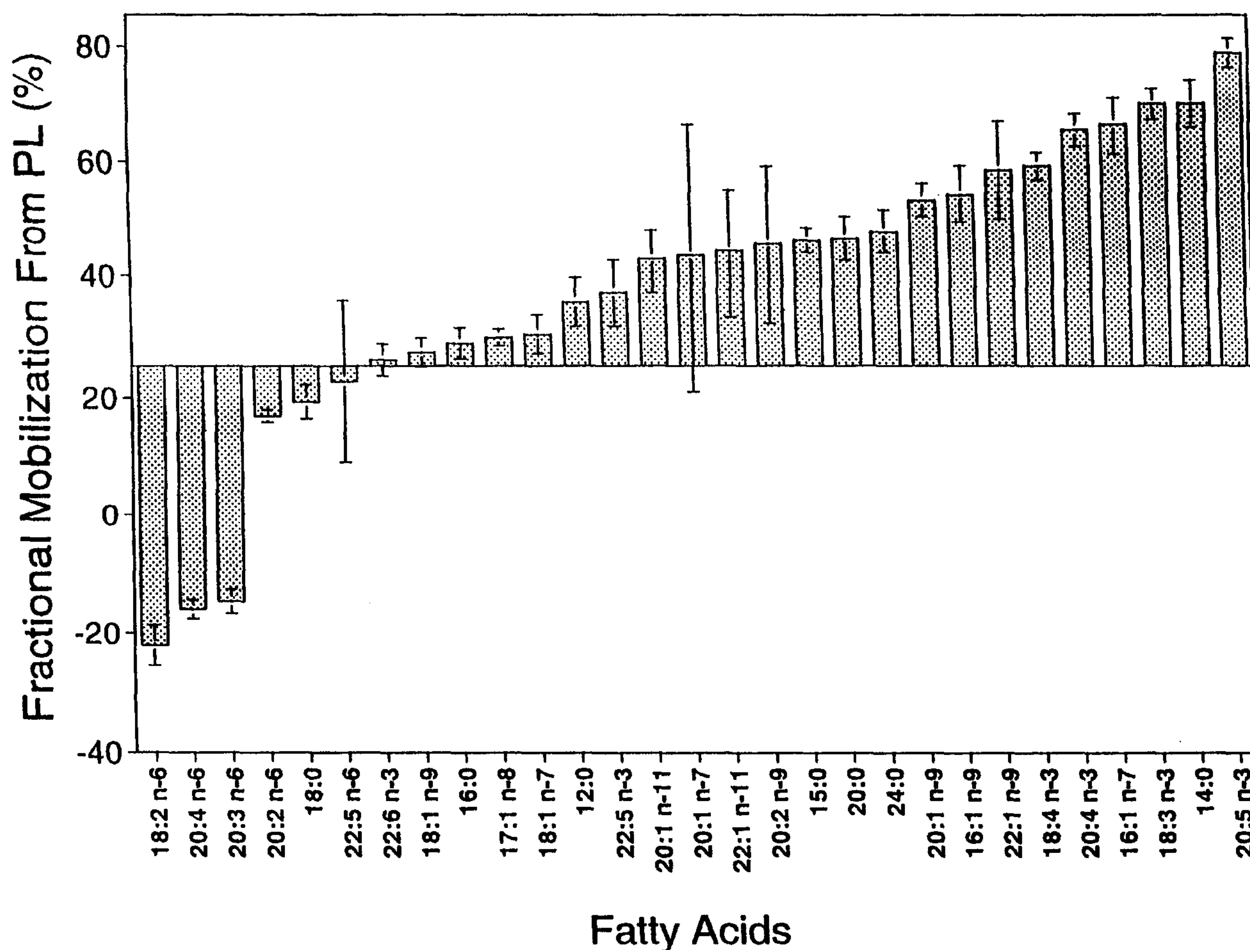

Fig. 3. Fractional mobilization of major fatty acids from IBAT PL between days 0 and 7 of the fast. Only fatty acids whose initial percentage weight was ≥0.2% are considered. The horizontal line at 25.2% shows the fractional mobilization of total PL fatty acids (See Table 1). Values are means ± SEM.

nane (41, 42) also found a preferential retention of linoleic acid enriched triacylglycerols in the liver of rats consuming 25% of their normal intake or fasted for 24 or 48 h. Unlike WAT, both BAT and liver are capable of both fatty acid synthesis and oxidation, and perhaps linoleic acid plays a particular role in such tissues that requires that it be retained in oxidative metabolic states.

Loss of fatty acids from PL

The loss of individual fatty acids from PL was very selective, the fractional mobilization between day 0 and 7 ranging from −20 to 80%. However, and in contrast to TAG, there were no general relationships between the number of carbons or number of double bonds in fatty acids and their mobilization from PL. Also, in contrast to BAT TAG from which there was a significant loss of all fatty acids during the fast, in BAT PL there were three fatty acids (18:2 n−6, 20:3 n−6, and 20:4 n−6) that had negative mobilizations, indicating that their mass actually increased during the fast. Thus, there are clear differences in the mobilization of fatty acids from IBAT TAG compared to PL, suggesting that very different mechanisms are involved in the mobilization of fatty acids from these two pools during a fast.

Of the six fatty acids in BAT PL with below average fractional mobilization during the fast, five were fatty acids of the n−6 series, including the three the mass of which increased during the fast. Thus, similar to 18:2 n−6 in TAG, the fatty acids of the n−6 series behaved in a manner that distinguishes them from the rest of PL fatty acids. This strongly suggests that n−6 fatty acids have a special metabolic role in IBAT. This role does not seem to be played through the regulation of uncoupling protein, the key protein involved in BAT thermogenesis that is activated by fatty acid binding. Indeed, it has been shown that the fatty acid specificity of the

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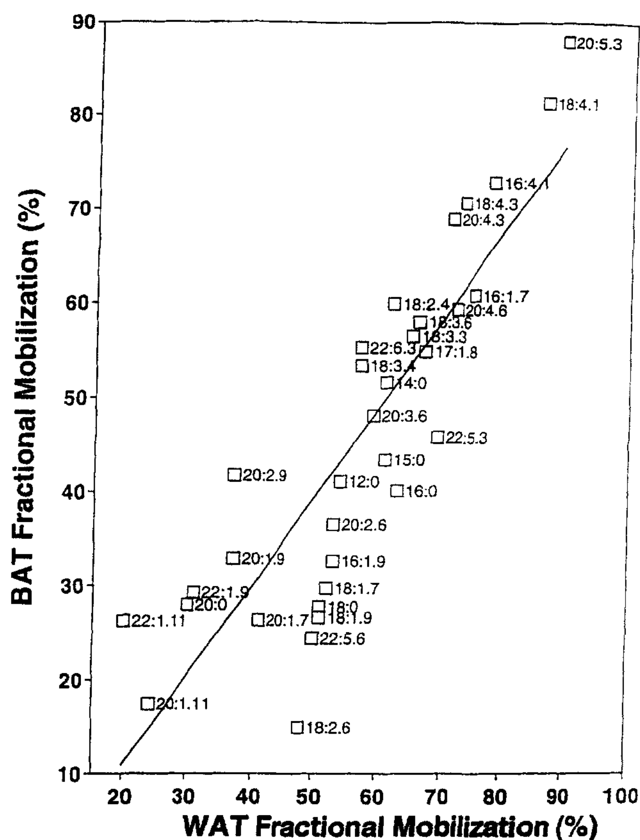

Fig. 4. Relationship between the fractional mobilization of individual fatty acids from TAG of white (WAT) and brown adipose tissue (IBAT). The equation of the line of best fit is: $Y = 0.94\ X - 7.77$ ($r^2 = 0.73$, $P < 0.001$). Data on WAT fractional mobilization are from Raclot and Groscolas (ref. 13 and unpublished data) and were obtained under identical experimental conditions. Fractional mobilization of total WAT fatty acids was 55.7%. The fatty acid abbreviations are in the form a:b.c, where a is the number of carbons, b is the number of double bonds, and c is the position of the first double bond relative to the methyl end. Data for 18:1.5 and 16:1.5 are not shown because of overlap of data points but all values were included in the calculation of the regression equation.

binding site is very broad (43). On the other hand, several studies suggest that n–6 fatty acids stimulate the thermogenic function of BAT. Notably, it has been shown that the polyunsaturation index (mainly linoleate and arachidonate) in BAT mitochondrial PL increases during cold exposure, even in essential fatty acid-deficient rats (44, 45), and that a diet high in polyunsaturated fatty acids, including n–6, increases BAT thermogenic activity (46). Provided n–6 fatty acids have such a stimulatory effect, a decrease rather than an increase in their content in BAT lipids would have been expected during fasting, in parallel with the well-known decrease in BAT activity (22). Thus, the functional significance of the increase in n–6 fatty acids in BAT lipids during fasting remains unclear.

Mechanisms of the selective mobilization and retention of fatty acids in IBAT TAG and PL

A selective hydrolysis of TAG by hormone-sensitive lipase (HSL) has been previously suggested as the origin of the selective mobilization of fatty acids from WAT TAG (13). This likely applies for BAT TAG, which, as in WAT, are stored in droplets and are hydrolyzed by HSL. Among putative mechanisms, a preferential location and thus accessibility to HSL of TAG with the highest polarity at the lipid–water interface (TAG droplet–cytoplasm) has been proposed (12). This view is supported by the recent demonstration that the more polar WAT TAG are actually enriched in the more readily mobilizable fatty acids, the reverse being true for the less polar TAG and the less mobilizable fatty acids (47). However, other selectivities should not be discarded. This includes HSL and monoacylglycerol lipase selectivities, and selective transport of fatty acids to the extracellular compartment. Similarly, the selective loss or retention of fatty acids in PL could be the result of selectivities of phospholipases and acyl transferases for individual fatty acids. Unfortunately, we are not aware of data in this field concerning BAT. However, evidence for such a highly specific acyltransferase has been demonstrated in guinea pig heart mitochondria by Arthur et al. (48) who described an acyl-CoA:1-acyl-GPC transferase that only used linoleoyl-CoA as the acyl donor.

In contrast to WAT, where the remodelling of the composition of TAG fatty acids during fasting does not seem directed towards the preferential retention of particular fatty acids (13), in BAT the retention of 18:2 n–6 in TAG and the retention or even accumulation of n–6 fatty acids in PL suggest that there are mechanisms that prevent or reduce the loss of some (TAG) or all (PL) of the n–6 fatty acids. It is important to note that such retentions exist in fasting rats previously maintained on a standard laboratory diet, as suggested by an increase in the percentage weight of 18:2 n–6 in BAT total lipids (30) or TAG (34), and of 20:4 n–6 in PL (34). This supports the view that here the retentions of n–6 fatty acids are not related to the preliminary fish-oil feeding. It also appears that neither the retention nor accumulation of linoleate in the lipids of IBAT is a result of selective release of this fatty acid from WAT which would make it more available to IBAT. Indeed, Raclot and Groscolas (13) showed that linoleate is not preferentially released from WAT. However, we cannot overlook the possibility of selective uptake of n–6 fatty acids from plasma lipids during fasting, including from WAT-derived non-esterified fatty acids. Another mechanism would be a selective re-uptake and reesterification of linoleate by brown fat cells after TAG hydrolysis, making this fatty acid available for conversion into longer

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n-6 fatty acids and for incorporation into PL. Lastly, the possibility that the selective retention of 18:2 n-6 in IBAT TAG could be a function of the TAG subclass into which this fatty acid is acylated should be considered. It could notably be hypothesized that 18:2 n-6 is preferentially acylated into TAG molecules rich in very-long chain saturated and monounsaturated fatty acids, that is, in TAG having a low polarity and thus a low accessibility to HSL. This hypothesis could be tested by analyzing IBAT TAG molecular species throughout the course of a fast.

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