Differential mobilization of fatty acids from adipose tissue[1]

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Abstract Are the different fatty acids mobilized into plasma in proportion to their concentrations in adipose tissue triglyceride? To answer this question, we fed weanling rabbits a special diet to label the fat stores with a variety of dietary fatty acids. The release of adipose tissue fatty acids into the plasma was then induced by ACTH-stimulated lipolysis. The compositions of the resulting plasma free fatty acids and of the adipose tissue triglyceride were then compared. Plasma free fatty acids increased from 625 μmol/L at baseline to 2938 μmol/L after ACTH and represented fatty acids released from adipose tissue. The relative mobilization of these fatty acids from adipose tissue was defined as the ratio between their percentage in the plasma free fatty acid fraction to their percentage in adipose tissue triglyceride. For the 24 fatty acids examined, the relative mobilization ranged from 0.11 for 22:1 n-11 to 5.06 for 20:5 n-3, a 46-fold difference. Relative mobilization correlated positively with unsaturation and negatively with chain length. The relative mobilization for essential fatty acids was in the order of 20:5 n-3 > 20:4 n-6 > 18:3 n-3 > 22:6 n-3 > 18:2 n-6. Saturated fatty acids, along with oleic acid, were much less well mobilized than the entire group of polyunsaturated fatty acids. Our data indicate that the mobilization of fatty acids into plasma was not proportional to their content in adipose tissue, but rather was influenced by their molecular structure. Eicosapentaenoic acid 20:5 n-3 (EPA), and arachidonic acid 20:4 n-6, precursors of two different prostaglandins, were the fatty acids with the highest mobilization into the plasma.—**Connor, W. E., D. S. Lin, and C. Colvis.** Differential mobilization of fatty acids from adipose tissue. *J. Lipid Res.* 1996. **37:** 290–298.

Supplementary key words n-3 fatty acids • n-6 fatty acids • eicosapentaenoic acid • docosahexaenoic acid • arachidonic acid

The stores of triglyceride in the adipose tissue are the chief energy source in the body in the post absorptive state. Adipose tissue triglyceride exists in a dynamic state. On the one hand, it is continuously formed by the influx of fatty acids from the triglyceride-rich plasma lipoproteins. On the other hand, it is continuously hydrolyzed to efflux fatty acids into the plasma bound to albumin. Various fatty acids can be incorporated into the adipose tissue from dietary fat, including more unusual fatty acids such as *trans* fatty acids, erucic acid, cetoleic acid, and the n-3 fatty acids (2, 3). In a previous study, we demonstrated that dietary fatty acids were an important source of adipose fatty acids and had a significant effect upon both the composition and quantity of different fatty acids in adipose tissue (4). However, the degree of deposition of different dietary fatty acids into adipose tissue varied greatly (4). Because the deposition of dietary fatty acids was related to their structures rather than to the amounts in the diet, we hypothesized that different fatty acids may also have variable rates of mobilization from adipose tissue. Such a differential mobilization could greatly influence the storage and subsequent utilization of individual fatty acids and the types of fatty acids supplied by adipose tissue as free fatty acids to other tissues.

The important question of whether the release of free fatty acids found in the adipose tissue is a random process or a release favoring certain individual fatty acids has been studied by many investigators. Spitzer et al. (5) and Nakamura, Faludi, and Spitzer (6) reported that the rate of release of each individual fatty acid was proportional to the concentration of the same fatty acid in the adipose tissue. However, the studies of Hunter, Buchanan, and Nye (7), Meinertz (8), and Gavino and Gavino (9) suggested a selective release of fatty acids from adipose tissue. Hollenberg and Angel (10) and Raclot and Groscolas (11) demonstrated that the mobilization of fatty acid from adipose tissue was positively correlated with the unsaturation and negatively with chain length of the fatty acids. For most of these studies, in vitro incubation techniques were used with the rat as the animal model supplying epidymal fat pads. Further-

Abbreviations: ACTH, adrenocorticotropic hormone; IU, international unit; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[1]Presented at the 1994 annual meeting of Experimental Biology (1).

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more, except for the recent in vitro study of Raclot and Groscolas (11), only the release of four to eight different fatty acids was considered in these previous studies. The relative mobilization of very long-chain (20 to 24 carbon atoms) mono- and polyunsaturated fatty acids having two to six double bonds or positional isomers was not examined.

The present study tested the relative mobilization of adipose tissue fatty acids under in vivo conditions. We hypothesized that the different fatty acids stored as triglyceride in adipose tissue represented a wide spectrum of chain lengths and degree of unsaturation. Certain fatty acids might be preferentially released from the triglyceride of the fat cell into the plasma as free fatty acids bound to albumin. Accordingly, the study design was as follows. Weanling rabbits were fed a specially formulated diet to replete their adipose tissue with a variety of fatty acids, including sufficient amounts of essential long chain fatty acids such as linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid, and docosahexaenoic acids, as well as other long chain mono- and polyunsaturated fatty acids. The composition of the plasma free fatty acid fraction before and after hormone-induced lipolysis and the fatty acids of adipose tissue triglyceride were analyzed. From these data, the relative mobilization rate, defined as the ratio between the percentage of released free fatty acids to the fatty acid composition of adipose tissue, was calculated for 24 different fatty acids. As will be shown, the response was most diversified.

METHODS

Six weanling male rabbits were fed laboratory chow (Purina rabbit chow, Ralston Purina Co., St. Louis, MO) for 1 week. They were then switched to a special chow containing laboratory chow and an oil mix (9:1). The standard rabbit chow was obtained from PMI Feeds, Inc. (formerly Purina Co., St. Louis, MO) and was 2–3% fat. The oil mix was composed of 60% menhaden oil (Zapata Haynie Corp., Reedville, VA 22539), 20% safflower oil (Hain pure food Co., Los Angeles, CA 90061) and 20% herring oil (Zapata Haynie Corp. Reedville, VA 22539). Consequently, the special diet contained a wide variety of fatty acids. It contained significant amounts of different essential fatty acids (18:2 n–6, 18:3 n–3, 20:4 n–6, 20:5 n–3, and 22:6 n–3) and other fatty acids, including cetoleic acid from herring oil (22:1 n–11) which is not found in the diet of rabbits. The fatty acid composition of the usual laboratory chow and the special chow is depicted in **Table 1.** To ensure the establishment of a new steady state, these growing rabbits were fed the special diet for 9 weeks before the first experiment.

TABLE 1. Fatty acid composition of the control and experimental diets

Fatty Acids	Control Diet[a]	Experimental Diet[b]
	percent of total fatty acids	
14:0	0.3	3.9
16:0	16.0	12.3
18:0	2.2	2.1
Total saturated	19.7	19.0
16:1n–7	0.7	6.0
18:1n–9	27.2	14.4
20:1n–9	1.8	3.5
22:1n–11	0	5.2
Total monounsaturated	29.7	29.0
20:2n–6	0.2	0.2
18:2n–6	39.3	28.0
20:4n–6	0	0.4
22:5n–6	0.1	0.3
Total n–6	39.7	29.4
18:3n–3	8.4	1.6
18:4n–3	0	1.9
20:4n–3	0	0.7
20:5n–3	0	8.0
22:5n–3	0	1.3
22:6n–3	0	6.0
Total n–3	8.4	19.5
Total polyunsaturated	49.0	51.2

[a]Purina Rabbit Chow.
[b]Purina Rabbit Chow plus special blend of oils to provide for the designated fatty acid composition.

There was a steady gain in weight after consuming the special diet, from 1.4 ± 0.05 kg at the beginning and 2.9 ± 0.36 kg after 9 weeks of feeding. Their weights reached a plateau between 9 to 12 weeks of feeding.

After consuming the special diet for 9 weeks, these rabbits were fasted overnight for 16 h and then were injected with adrenocorticotropic hormone (ACTH) (Sigma Chemical Co., St. Louis, MO) in a dose of 1.0 IU/kg. Blood samples were obtained before and 1 h after injection. The same feeding continued for another 3 weeks. This same experiment was repeated a second time. Rabbits were then killed and their abdominal adipose tissue (retroperitoneal) was collected. The adipose tissue was rinsed with saline and blotted with filter paper before lipid extraction.

Blood samples were centrifuged to separate plasma from red blood cells. The concentration of total plasma free fatty acids was determined according to the method of Hron and Menahan (12). For determining the fatty acid composition of free fatty acid fractions in plasma, the lipids of plasma were extracted by the slightly modified procedure of Bligh and Dyer (13). Freshly separated plasma was first vortexed with chloroform–methanol 1:1 to precipitate the plasma proteins and the supernatant was mixed with chloroform and water to form aqueous and chloroform phases. The lipids in the chlo-

roform were then collected and subjected to thin-layer chromatography as described subsequently. For the analysis of the fatty acid composition of adipose triglyceride, the lipids in the adipose tissue were first extracted by the method of Folch, Lees, and Sloane Stanley (14). The adipose tissue was homogenized with chloroform–methanol 2:1 and the insoluble material was filtered. The lipid extract was washed with 0.58% sodium chloride. Butylated hydroxytoluene (5 mg/100 ml) was added to all lipid extracts as an antioxidant (15).

The lipid extracts of plasma and adipose tissue obtained as described above were separated into four major classes (phospholipids, free fatty acids, triglycerides, and cholesteryl esters) by thin-layer chromatography (16). They were chromatographed on silica gel G plates (500 microns, Analtech, Newark, DE). The solvent system was hexane–chloroform–ethyl ether–acetic acid 80:10:10:1. The fatty acids in the plasma free fatty acid band and in the adipose triglyceride band were scraped off the plate into a screw-capped tube. It was then transmethlyated with trifluoride–methanol (17).

The methyl esters of fatty acids were analyzed by gas–liquid chromatography (18) on an instrument equipped with a hydrogen flame ionization detector (Perkin-Elmer Model Sigma 3B, Norwalk, CT) and a 30-meter SP-2330 fused silica capillary column (Supelco, Bellefonte, PA). Temperatures of column, detector, and injection port were 195°, 250°, and 250°C, respectively. Helium was used as the carrier gas; the inlet pressure was 80 psi. The split ratio was 1:170. The retention time and area of each peak were measured by an HP-3390 integrator, and a computer (HP85, Hewlett Packard, Palo Alto, CA) identified and quantified each individual fatty acid. A mixture of fatty acid standards was run daily.

To calculate the net increase of individual fatty acids (mg/L) and its composition (%), we used the concentration of plasma free fatty acid (μmol/L) and the composition of plasma free fatty acid (%) before and after ACTH injection. For example, from the plasma free fatty acid composition (%) before ACTH injection, we calculated the mean molecular weight of these fatty acids (sum of actual molecular weight of each fatty acid times its percentage divided by 100). From the mean molecular weight and the plasma free fatty acid concentration (μmol/L), we obtained the plasma free fatty acid concentration in mg/L. From this value and plasma free fatty acid composition (%), we calculated the concentration of individual fatty acid (mg/L) before ACTH. Using the same method, we calculated the concentration of the plasma individual free fatty acid after ACTH. The net increase of individual fatty acids (representing fatty acid released from adipose tissue) was the difference of the concentration of individual fatty acid after and before ACTH. The percentage of each fatty acid in the total free fatty acids released from adipose tissue after ACTH was also calculated.

Results were expressed as mean ± SD and compared by paired *t* test.

TABLE 2. Fatty acid composition of plasma free fatty acids before and after the injection of ACTH

Fatty Acids	Baseline[a]	Post-ACTH[a]
	percent of total fatty acids	
Saturated		
14:0	4.32 ± 0.69	3.11 ± 0.21[c]
16:0	22.00 ± 0.89	17.60 ± 1.48[d]
17:0	0.42 ± 0.24	0.48 ± 0.07
18:0	5.99 ± 1.82	2.77 ± 0.41[c]
Monounsaturated		
16:1n-7	8.09 ± 1.75	9.90 ± 0.59[b]
18:1n-9	16.7 ± 1.54	15.83 ± 1.40
20:1n-9	1.01 ± 0.44	0.67 ± 0.16
22:1n-11	0.21 ± 0.17	0.14 ± 0.06
Di-unsaturated		
16:2n-4	1.12 ± 0.17	1.41 ± 0.05[c]
18:2n-6	15.14 ± 2.06	16.57 ± 0.84[c]
20:2n-6	0.17 ± 0.12	0.13 ± 0.09
Tri-unsaturated		
16:3n-4	0.88 ± 0.18	0.97 ± 0.11
18:3n-6	0.59 ± 0.29	0.30 ± 0.07[b]
18:3n-3	2.43 ± 0.37	3.36 ± 0.19[d]
20:3n-6	0.16 ± 0.17	0.11 ± 0.04
Tetra-unsaturated		
16:4n-1	1.36 ± 0.26	1.61 ± 0.24
18:4n-3	2.47 ± 0.20	3.06 ± 0.43[b]
20:4n-6	0.78 ± 0.29	0.74 ± 0.12
20:4n-3	0.33 ± 0.21	0.67 ± 0.18[b]
22:4n-6	0.21 ± 0.28	0.32 ± 0.15
Penta-unsaturated		
20:5n-3	6.86 ± 0.38	11.71 ± 1.46[d]
22:5n-6	0.10 ± 0.13	0.15 ± 0.04
22:5n-3	1.05 ± 0.35	1.00 ± 0.36
Hexa-unsaturated		
22:6n-3	3.62 ± 0.66	3.17 ± 0.27

[a]Mean of two experiments.
[b]$P < 0.05$; [c]$P < 0.005$; [d]$P < 0.001$, compared with baseline.

RESULTS

As the results of duplicated experiments were very similar, the data were combined. The plasma free fatty acid concentration was raised drastically by 1 h after the ACTH injection: from a fasting baseline value of 625 ± 343 μmol/L to 2938 ± 362 μmol/L ($P < 0.000$), a 5-fold increase. As indicated in **Table 2,** a significant change of the fatty acid composition in plasma free fatty acids after ACTH injection occurred as well. There was a decrease of myristic (14:0), palmitic (16:0), stearic (18:0),

TABLE 3. Free fatty acids (FFA) released from adipose tissue after ACTH (net increase of plasma FFA) and its composition (n = 6)

Fatty Acids	Net Increase of Plasma FFA[a]	Composition
	mg/L	*% of total fatty acids*
Saturated		
14:0	17.8 ± 2.72	2.89 ± 0.43
16:0	105.18 ± 13.00	17.02 ± 1.98
17:0	3.19 ± 0.32	0.52 ± 0.05
18:0	12.13 ± 3.62	1.96 ± 0.57
Monounsaturated		
16:1n-7	66.59 ± 6.10	10.79 ± 1.14
18:1n-9	100.67 ± 1.54	16.28 ± 1.40
20:1n-9	3.68 ± 1.08	0.59 ± 0.17
22:1n-11	0.75 ± 0.48	0.12 ± 0.07
Di-unsaturated		
16:2n-4	9.55 ± 0.36	1.55 ± 0.08
18:2n-6	108.67 ± 4.83	17.59 ± 0.54
20:2n-6	0.64 ± 0.15	0.13 ± 0.14
Tri-unsaturated		
16:3n-4	6.37 ± 0.81	1.03 ± 0.15
18:3n-6	1.37 ± 0.42	0.22 ± 0.07
18:3n-3	23.13 ± 1.58	3.75 ± 0.27
20:3n-6	0.64 ± 0.15	0.10 ± 0.02
Tetra-unsaturated		
16:4n-1	10.77 ± 1.60	1.74 ± 0.28
18:4n-3	20.58 ± 3.30	3.34 ± 0.36
20:4n-6	4.66 ± 0.96	0.75 ± 0.15
20:4n-3	4.93 ± 1.64	0.80 ± 0.26
22:4n-6	2.26 ± 1.34	0.37 ± 0.22
Penta-unsaturated		
20:5n-3	83.19 ± 12.49	13.48 ± 2.13
22:5n-6	1.13 ± 0.24	0.18 ± 0.04
22:5n-3	6.24 ± 2.47	1.02 ± 0.38
Hexa-unsaturated		
22:6n-3	19.38 ± 2.73	3.39 ± 0.45

[a]Differences between post- and pre-ACTH (see text).

and gamma linolenic (18:3 n-6) acids and increases of 16:1 n-7, 16:2 n-4, 18:4 n-3, 20:4 n-3 eicsosapentaenoic acid (EPA, 20:5 n-3). Palmitic acid decreased from 22.0 to 17.6% of total fatty acids. Eicosapentaenoic acid increased the most of all fatty acids studied, from 6.86 to 11:71%.

The calculated net increase of individual plasma free fatty acid after ACTH injection and its percentage composition are presented in **Table 3** (method of calculation was described in Methods). As the plasma free fatty acids had a much higher concentration after ACTH in comparison to baseline, it is not surprising that the calculated fatty acid composition of the free fatty acids released from the adipose tissue (Table 3) was similar to the plasma free fatty acid composition post ACTH (Table 2).

Table 4 shows the fatty acid compositions of adipose tissue triglyceride and the free fatty acids released from adipose tissue. A total of 24 fatty acids were quantified. A significant difference between adipose tissue triglyceride fatty acid composition and fatty acids released from adipose tissue was found for 18 of these fatty acids. The relative mobilization of these fatty acids from adipose tissue was defined as the ratio between their percent in the plasma free fatty acids to their percent in adipose tissue triglyceride. A ratio greater than, equal to, and lower than one means that an individual fatty acid has, respectively, a greater mobilization, a similar mobilization, or less mobilization than the total fatty acids as a whole. Among the 24 different fatty acids, the relative mobilization ranged from 0.11 for 22:1 n-11 to 5.06 for EPA (20:5 n-3), a 46-fold difference (Table 4). No relationship was found between the content of these fatty acids in the adipose tissue and their relative mobilization from the adipose tissue.

There was a relationship between the structure of the fatty acids and their mobilization. For the fatty acids with mono-unsaturation and tetra-unsaturation, a negative correlation (r = -0.95 and -0.99) was observed between the relative mobilization and the number of carbon atoms (**Fig. 1**). For fatty acids with 16, 18, and 20 carbons, there were positive correlations (r = 0.95, 0.90 and 0.96) between mobilization and unsaturation (**Fig. 2**). However, the effects of positional isomers in n-3 and n-6 fatty acids were variable. For example, 18:3 n-3 had a higher mobilization than 18:3 n-6 (2.38 vs. 1.03). The reverse was true for longer chain fatty acids. The relative mobilization was 2.10 and 3.06 for 20:4 n-3 and 20:4 n-6, respectively, and 0.60 and 1.37 for 22:5 n-3 and 22:5 n-6, respectively, (n-3 fatty acids being lower than n-6 fatty acid chain positional isomers).

The relative mobilization of individual n-3 and n-6 polyunsaturated fatty acids is depicted in **Fig. 3** and **Fig. 4.** The two fatty acids of 20 carbon chain length (20:5 n-3 and 20:4 n-6) stand out with the highest mobilization. In the n-3 group, the relative mobilization for 20:5 n-3 was 5.06 in comparison with 0.60 to 3.14 for other n-3 fatty acids. In the n-6 series, the relative mobilization for 20:4 n-6 was 3.06 in comparison to 0.71 to 1.37 for the other n-6 fatty acids. The relative mobilization for the five essential fatty acids differed greatly: 5.06 (20:5n-3), 3.06 (20:4n-6), 2.38 (18:3n-3), 0.87 (22:6n-3), and 0.71 for 18:2n-6.

Because albumin-bound free fatty acids from adipose tissue are the major source of the plasma free fatty acids, the composition of the plasma free fatty acids under steady state conditions reflected the fatty acids released from adipose tissue. We compared the relative mobilization rate of fatty acids released from the adipose tissue after ACTH and after fasting (**Table 5**). The overall pattern of relative mobilization of different fatty acids calculated under these two different conditions, lipolysis

TABLE 4. Relative mobilization of individual fatty acid from adipose tissue after ACTH injections

Fatty Acids	Adipose Triglyceride (TG)	Free Fatty Acids (FFA) Released from Adipose Tissue	Relative Mobilization[a]
	%	%	
Saturated			
14:0	5.31 ± 0.17	2.89 ± 0.43[d]	0.54 ± 0.08
16:0	19.45 ± 0.28	17.02 ± 1.98[b]	0.86 ± 0.11
17:0	0.57 ± 0.10	0.52 ± 0.05	0.94 ± 0.23
18:0	3.15 ± 0.37	1.96 ± 0.57[d]	0.63 ± 0.23
Monounsaturated			
16:1n-7	6.73 ± 0.20	10.79 ± 1.14[d]	1.61 ± 0.18
18:1n-9	19.90 ± 0.68	16.28 ± 1.40[d]	0.82 ± 0.06
20:1n-9	3.42 ± 0.33	0.59 ± 0.17[d]	0.17 ± 0.06
22:1n-11	1.09 ± 0.07	0.12 ± 0.07[d]	0.11 ± 0.06
Di-unsaturated			
16:2n-4	0.80 ± 0.04	1.55 ± 0.08[d]	1.94 ± 0.11
18:2n-6	24.7 ± 0.47	17.59 ± 0.64[d]	0.71 ± 0.02
20:2n-6	0.13 ± 0.02	0.13 ± 0.14	1.15 ± 1.28
Tri-unsaturated			
16:3n-4	0.34 ± 0.07	1.03 ± 0.15[d]	3.05 ± 0.39
18:3n-6	0.22 ± 0.01	0.22 ± 0.07	1.03 ± 0.31
18:3n-3	1.58 ± 0.11	3.75 ± 0.27[d]	2.38 ± 0.14
20:3n-6	0.08 ± 0.02	0.10 ± 0.02	1.36 ± 0.35
Tetra-unsaturated			
16:4n-1	0.36 ± 0.05	1.74 ± 0.28[d]	4.91 ± 0.63
18:4n-3	1.07 ± 0.15	3.34 ± 0.36[d]	3.14 ± 0.66
20:4n-6	0.25 ± 0.02	0.75 ± 0.15[d]	3.06 ± 0.60
20:4n-3	0.38 ± 0.03	0.80 ± 0.26[c]	2.10 ± 0.60
22:4n-6	0.30 ± 0.05	0.37 ± 0.22	1.23 ± 0.74
Penta-unsaturated			
20:5n-3	2.67 ± 0.20	13.48 ± 2.13[d]	5.06 ± 0.84
22:5n-6	0.13 ± 0.03	0.18 ± 0.04[b]	1.37 ± 0.26
22:5n-3	1.73 ± 0.16	1.02 ± 0.38[c]	0.60 ± 0.24
Hexa-unsaturated			
22:6n-3	3.70 ± 0.31	3.39 ± 0.45	0.87 ± 0.16

[a]The relative mobilization of these fatty acids from adipose tissue was defined as the ratio between their percent in the plasma free fatty acid fraction to their percent in adipose triglyceride.
[b]$P < 0.05$; [c]$P < 0.005$; [d]$P < 0.001$, adipose TG versus FFA released from adipose tissue.

induced by fasting and after ACTH injection, were similar. During fasting, the three prostaglandin precursors, homogamma-linolenic acid, arachidonic acid, and EPA had relative mobilizations of 2.26, 3.19, and 2.58, respectively, which were among the highest mobilization rates. Linoleic and linolenic acid had relative mobilizations 0.61 and 1.45, respectively. It is interesting to note that both palmitic and stearic acid had relatively high mobilizations, 1.13 and 1.89, respectively. Despite the similar pattern of relative mobilization of various fatty acids under lipolysis induced by two different conditions (ACTH stimulated and fasting), differences did exist for certain fatty acids. Most notably, the relative mobilization of stearic acid and gamma-linolenic acid (18:3 n-6) decreased from 1.89 and 2.79, respectively, during fasting compared to 0.63 (-67%) and 1.03 (-63%) after ACTH. On the other hand, EPA and 22:5 n-6 increased from 2.58 and 0.82 to 5.06 (+96%) and 1.37 (+67%) in comparing the fasting state to lipolysis after ACTH.

DISCUSSION

Our data demonstrated that the mobilization of individual fatty acids was not proportional to their content in adipose tissue but was rather influenced by their molecular structure. The range from low to high mobilization was more than 46-fold. Saturated fatty acids were mobilized the least and polyunsaturated fatty acids were mobilized the most. Monounsaturated fatty acids were intermediate. Especially notable of all the polyunsaturated fatty acids were the mobilizations of arachidonic acid in the n-6 series of fatty acids and eicosapentaenoic acid from the n-3 series. Both of these very long chain polyunsaturated fatty acids are eicosanoid precursors. Arachidonic acid is the precursor for the 2 series prostaglandins, of which thromboxane A_2 is a characteristic example. Eicosapentaenoic is the substrate for the 3 series prostaglandins, prostacyclin (PGI_3). These two prostaglandins have antagonist actions upon plate-

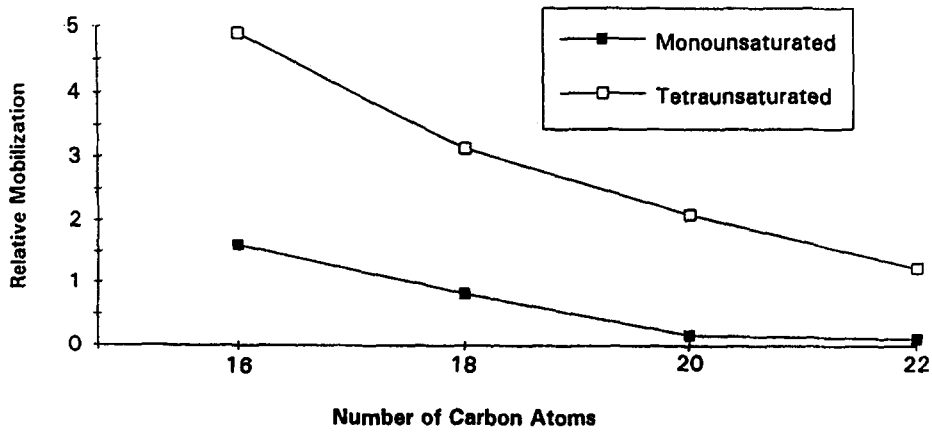

Fig. 1. Negative correlation between the fatty acid chain length and relative mobilization.

let adhesion and vascular tone. Thromboxane A_2 enhances platelet aggregation and vasoconstricts, whereas prostacyclin has exactly opposite actions (19).

However, the most polyunsaturated fatty acid, docosahexaenoic (DHA, 22:6) of the n–3 series, was among the most poorly mobilized fatty acids. Characteristically, this fatty acid is incorporated into the membrane phospholipids of cells, and would not be expected to be utilized for energy purposes or in prostaglandin synthesis. Such sparing of this fatty acid from oxidation has already been noted in its transfer into the central nervous system where it serves as the most abundant fatty acid in the phospholipid membranes of the brain (20, 21). Herzberg and Skinner (22) switched fish oil-fed rats to an n–3-free diet and observed that the turnover of EPA in adipose tissue was two times greater than DHA.

Cetoleic acid is a 20 carbon monounsaturated fatty acid and of medical interest because it is a common constituent of fish and fish oil, being particularly abundant in herring oil. It is the isomer of erucic acid found in rapeseed oil. Cetoleic acid has been implicated in possible toxicity of fish oil (23). It is readily incorporated into adipose tissue (3). In this study, it was found to be the least mobilized fatty acid among the 24 fatty acids we examined. Because of this slow release, this fatty acid might accumulate in adipose tissue. However, the adipose tissue of patients given menhaden oil, which contains cetoleic acid, for over a year did not contain any cetoleic acid (24). It was also absent in their plasma fatty acids. We conclude that the slow mobilization of cetoleic acid is not a problem for humans because it is not stored in adipose tissue.

As the plasma free fatty acids constitute the major form in which fat is transported from adipose tissue triglyceride to the liver, muscle, and other tissues, the release of selected fatty acids from adipose tissue may be an important mechanism by which essential fatty acids are made available for utilization by the body.

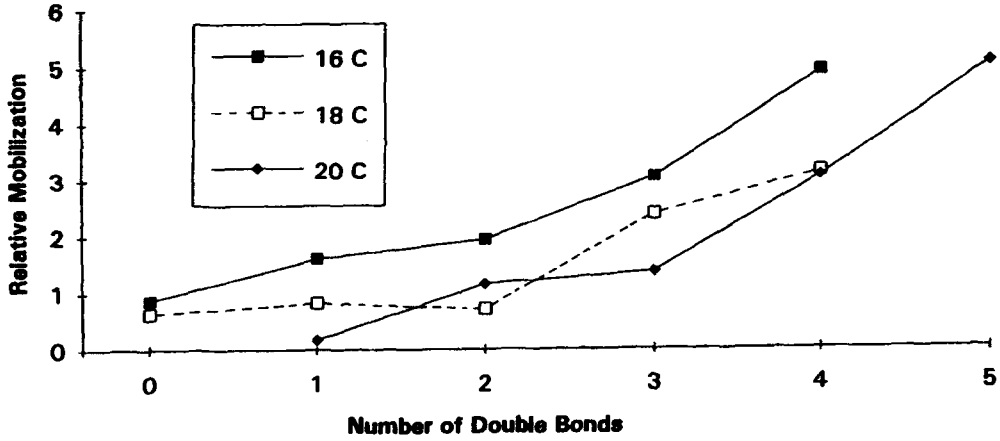

Fig. 2. Positive correlation between the number of double bonds of fatty acid and relative mobilization.

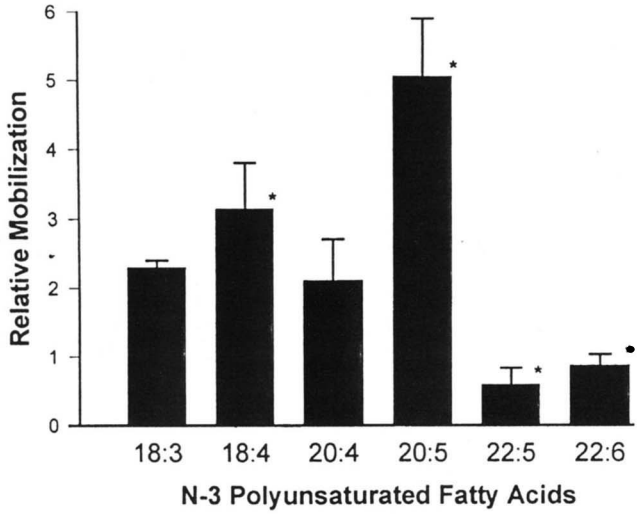

Fig. 3. Relative mobilization of n–3 fatty acids from adipose tissue (*significantly different from linolenic acid 18:3n–3 with $P < 0.05$ or less).

Furthermore, this selectivity may also have significant implications. For example, in view of the preferential release of the polyunsaturated fatty acids, especially EPA, arachidonic acid, and linolenic acid from adipose tissue during hormone-induced lipolysis, it is possible that the same phenomenon may occur during negative caloric intake. Indeed, high mobilization of these fatty acids occurred in rabbits after overnight fasting. Phinney et al. (25) found that there was a decrease of EPA and linolenic acid (18:3 n–3) in the adipose tissue of subjects consuming weight-reducing diets for 3–5 months. In a study of the fatty acid composition of adipose tissue of eight obese subjects, Hudgins and Hirsch (26) found a 15% decrease in 18:3 n–3 in both abdominal and gluteal fat during weight loss. These observations, suggest that essential fatty acid content in weight-reducing diets may be inadequate. The high mobilization of palmitic and stearic acids during fasting is also worth noting. These saturated fatty acids have been implicated in atherogenesis. Stearic acid causes hypercoagulability of the blood by activation of factor XII and aggregates the blood platelets (27, 28), and palmitic acid is hypercholesterolemic (29).

The release of individual fatty acids from adipose tissue has been studied by many investigators (5–11). Most studies have been in vitro. The present study is the only in vivo study that examined 24 different fatty acids with a wide range of chain length and degree of unsaturation. The only previous in vivo study is that of Hunter et al. (7) in which only the mobilization of eight different fatty acids with up to three double bonds was considered. These authors concluded from their data that lauric acid (12:0) appeared to be less easily mobilized than longer chain fatty acids.

Although in vivo studies are more physiological, the interpretation of the data is less straight forward because of the complexity of the system. We assume that the composition of the increased plasma free fatty acids represented fatty acids released from adipose tissue. The plasma free fatty acids have a rapid turnover, typically only a few minutes (30). The possible effect of different turnover rates of individual fatty acids must be evaluated. After giving isotopically labeled fatty acids (16:0, 18:1, 18:2) intravenously to humans, Fredrickson and Gordon (30, 31) found no detectable differences in the turnover of these fatty acids. Unfortunately, all three of these fatty acids had a low rate of mobilization from adipose tissue with the relative mobilization well under one; those with a high mobilization were not investigated for turnover. However, similar results were obtained in our in vivo study and the in vitro study of Raclot and Groscolas (11). As the in vitro study was a closed system without the possibility of clearance, we suspect that a differential clearance of fatty acids, if any, is unlikely to have significantly influenced our results.

The effect of molecular structure of fatty acid on its release from adipose tissue observed in our in vivo experiment was similar to the in vitro data of both Hollenberg and Angel (10) and Raclot and Groscolas (11). This phenomenon appeared not to be specific for the lipolysis induced by hormone induction. A similar release pattern was also observed during mild fasting. The mechanism that controls this specificity is unknown. Intrinsically, there may be a need for rapid

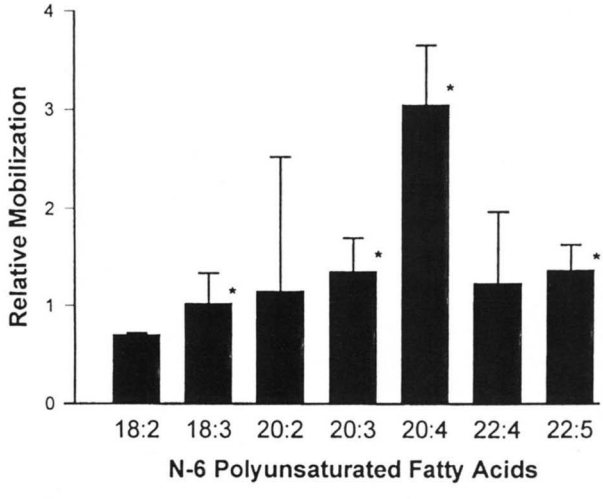

Fig. 4. Relative mobilization of n–6 fatty acids from adipose tissue (*significantly different from linoleic acid 18:2n–6 with $P < 0.05$ or less).

TABLE 5. Comparison of the relative mobilization of adipose fatty acids after a 16-h fast and after lipolysis induced by adrenotrophic hormone (ACTH)

Fatty Acids	Sixteen Hour Fast	ACTH-Induced Lipolysis;	Changes
Saturated			
14:0	0.81 ± 0.12	0.54 ± 0.08[a]	-33%
16:0	1.13 ± 0.04	0.86 ± 0.11[c]	-24%
17:0	0.83 ± 0.43	0.94 ± 0.23	-
18:0	1.89 ± 0.51	0.63 ± 0.23[c]	-67%
Monounsaturated			
16:1n-7	1.20 ± 0.26	1.61 ± 0.18[a]	+34%
18:1n-9	0.82 ± 0.07	0.82 ± 0.06	-
20:1n-9	0.29 ± 0.12	0.17 ± 0.06	-
22:1n-11	0.21 ± 0.15	0.11 ± 0.06	-
Di-unsaturated			
16:2n-4	1.40 ± 0.21	1.94 ± 0.11[c]	+39%
18:2n-6	0.61 ± 0.07	0.71 ± 0.02[a]	+16%
20:2n-6	1.42 ± 0.62	1.15 ± 1.28	-
Tri-unsaturated			
16:3n-4	2.64 ± 0.69	3.05 ± 0.39	-
18:3n-6	2.79 ± 1.45	1.03 ± 0.31[a]	-63%
18:3n-3	1.45 ± 0.36	2.38 ± 0.14[c]	+64%
20:3n-6	2.26 ± 1.36	1.36 ± 0.35[a]	-40%
Tetra-unsaturated			
16:4n-1	3.80 ± 0.45	4.91 ± 0.63[b]	-29%
18:4n-3	2.35 ± 0.51	3.14 ± 0.66[b]	+34%
20:4n-6	3.19 ± 1.23	3.06 ± 0.60	-
20:4n-3	1.09 ± 0.41	2.10 ± 0.60	-
22:4n-6	1.45 ± 0.43	1.23 ± 0.74	-
Penta-unsaturated			
20:5n-3	2.58 ± 0.21	5.06 ± 0.84[d]	+96%
22:5n-6	0.82 ± 0.18	1.37 ± 0.26[a]	+67%
22:5n-3	0.61 ± 0.22	0.60 ± 0.24	-
Hexa-unsaturated			
22:6n-3	0.98 ± 0.17	0.87 ± 0.16	-

[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.005$; [d]$P < 0.001$, versus fasting (paired t test).

release of essential fatty acids to meet the needs of the body. It is possible that the preferential release of these essential fatty acids, especially EPA and arachidonic acid, is to respond to the imposed stress, i.e., the need for prostaglandins. Biochemically, shorter chain and polyunsaturated fatty acids are more polar and thus more water soluble. Raclot and Groscolas (11) suggested that more polar shorter chain and unsaturated fatty acids may be more accessible to hormone-sensitive lipase for hydrolysis and membrane fatty acid binding protein for transport. Therefore, the differential mobilization of adipose fatty acids may result from a differential hydrolysis of triglyceride and/or from a differential transfer of free fatty acids into the plasma (11). Binding to albumin does differ for different fatty acids with the polyunsaturated fatty acids having greater affinity for the two tight binding sites of albumin (32). Another possibility would be the greater specificity of adipose tissue lipase for certain fatty acids (33).

Supported by grants from the Oregon Health Sciences University Foundation and DL-40566 (Clinical Nutrition Research Unit) from the National Institutes of Health.

Manuscript received 17 July 1995 and in revised form 2 November 1995.

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