The mobilization of free fatty acids in relation to adipose tissue triglyceride fatty acids in the rat

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ABSTRACT Three diets, consisting respectively of formulations high in oleic and stearic acid, linolenic acid, and lauric acid, were fed to rats until the adipose tissue TGFA largely reflected the dietary pattern of fatty acids. The composition of the serum FFA under basal conditions and following noradrenaline-stimulated lipolysis, were examined in relation to the respective adipose tissue TGFA. It was found in both in vivo and in vitro studies that lauric acid appeared to be less easily mobilized than longer chain acids. The in vitro studies indicated that this could not be explained either by positional preference of the shorter chain acids for the α-position of esterification or by increased reesterification of the shorter chain acids. The possibility remains that the difference is due to some specificity of tissue lipases for certain ester linkages.

SUPPLEMENTARY KEY WORDS gas–liquid chromatography · lipolysis · noradrenaline · linseed oil · coconut oil · diet

FFA IS THE major form in which fat is transported from adipose tissue TG to the rest of the body via the blood. In the human (1–4) and in other animals (5–7), it has been shown that the fatty acid composition of adipose tissue may be influenced by the nature of dietary fats. The effect of diet upon the fatty acid composition of the plasma FFA is unknown although patterns have been determined in the various following species: humans (8–14); dog (8); rat (14); hen (15); and rabbit (6) fed "normal" or commercial diets. Current interest in attempts to reduce the prevalence of ischemic heart disease by controlling the amount and type of dietary fat emphasizes the need to understand consequences of such dietary manipulation in addition to changes in plasma cholesterol concentrations.

Dole, James, Webb, Rizack, and Sturman (13) have shown that there is no change in plasma FFA composition immediately after ingestion of different fats. Rothlin, Rothlin, and Wendt (8) have also studied the changes in plasma FFA composition after administration of noradrenaline, glucose, insulin, and other agents. They showed that when plasma FFA levels were elevated, the composition approached that of depot fat.

Given that adipose tissue TGFA reflect dietary fatty acids, it does not follow that the processes of lipolysis involved in the mobilization of fat act indiscriminately on the TG ester linkages. Thus, it cannot be assumed that plasma FFA mirrors adipose tissue TGFA under conditions either of basal lipolysis or of enhanced lipolysis mediated through the action of noradrenaline.

The present study is concerned with the effect of feeding diets rich in linolenic acid (linseed oil), lauric acid (coconut fat), and stearic and oleic acids (mutton fat–sucrose mixture) on the fatty acid composition of adipose tissue TG and the serum FFA of the rat, both in the basal condition and following stimulation of lipolysis.

An examination of the behavior of adipose tissue following in vitro stimulation of lipolysis was also made, and the possible influence of the positional preference of esterification site, α or β, of the fatty acids on the glycerol molecule is discussed.

MATERIALS AND METHODS

Experimental Animals and Diet Composition

10-wk-old male Wistar rats with an average starting weight of 233 g were used. The rats were divided into

Abbreviations: FFA, free fatty acids; TG, triglycerides; TLC, thin-layer chromatography; TGFA, triglyceride fatty acids; GLC, gas–liquid chromatography.

TABLE 1 COMPOSITION OF DIETS

	Diet S	Diet L	Diet HC
		% by weight	
Casein	20	20	20
Bran	6	6	6
Salt mixture	4	4	4
Vitamin mixture	2	2	2
Sucrose	58	28	28
Mutton fat	10	—	—
Linseed oil	—	40	—
Hydrogenated coconut oil	—	—	40

three groups and fed one of the diets shown in Table 1.

Diet S was a standard semisynthetic diet used for rats except for the replacement of vegetable oil with mutton fat. The refined linseed oil in diet L was given because of its high linolenic acid content and hydrogenated coconut oil was used in diet HC because of its high content of lauric acid. All rats were fed diets and water ad lib. and were weighed weekly.

Perirenal fat biopsies were taken from six rats from each diet group every 3 wk in order to determine the rate of deposition and the composition of TGFA. All other rats were fed continuously for 26 wk.

In Vivo Studies. After 26 wk on the respective diets, the mean weights of the rats were the following: 414 g (group S); 422 g (group L); 392 g (group HC). 48 rats in each diet group were fasted for 16 hr before anesthetization with sodium pentobarbitone (5 mg/100 g body weight) administered intraperitoneally. 24 rats from each group were exsanguinated through the aorta, and the other 24 were infused through the jugular vein for 30 min with 20 μg of noradrenaline bitartrate (given as a 0.004% [w/v] solution in 0.9% sodium chloride) before exsanguination. Blood was placed in ice and allowed to clot, and the chilled serum was separated by immediate centrifugation at 3000 rpm for 20 min and then kept below 0°C. A sample of perirenal adipose tissue was removed from each rat within 3 min of death and was placed in chloroform–methanol 2:1. Blood and adipose tissue from groups of six rats were pooled in order to provide sufficient material for each study.

Levels of total serum FFA were determined by the method of Dole and Meinertz (16) as modified by Trout, Estes, and Friedberg (17).

Lipids were extracted from both serum and adipose tissue by the method of Folch, Lees, and Sloane Stanley (18) with one modification, i.e. the crude extract was washed with 0.4 vol of 2% monobasic potassium phosphate (KH_2PO_4) (19). Serum FFA were isolated on a 5 g silicic acid column according to the procedure of McCarthy and Duthie (20). Adipose tissue TG were separated on a column (21), I.D. 2 cm, containing 10 g of activated silicic acid and 5 g of Hyflo Supercel. After elution of cholesteryl esters with petroleum ether (bp 60–80°C)–ether 98:2, the TG were eluted with petroleum ether–ether 92:8. The purity of all fractions was checked by TLC, the solvent system being petroleum ether–ether–acetic acid 90:10:1. All extractions and separations were carried out in an atmosphere of nitrogen.

For GLC studies, samples were methylated according to the method of Metcalfe and Schmitz (22) as modified by Morrison and Smith (23) using boron trifluoride–methanol reagent prepared in the laboratory. The methyl esters were analysed on a Packard gas–liquid chromatograph (Model 7820) using columns 183 cm long and 0.44 cm in diameter and an argon ionization detector.

Column packings consisted of 20% polyethylene glycol adipate on 80–100 mesh Chromosorb P and 10% Apiezon-L on 60–80 mesh acid washed Celite. The extraction of the methyl esters allowed only those fatty acids with chain length equal to or longer than 12 carbon chains to be measured accurately.

In Vitro Experiment. Six rats (mean weight 412 g) from each diet group were anesthetized after 18 hr of fasting, and the epididymal fat pads were excised. Each pad was cut into pieces weighing 40–50 mg, and these were distributed among three flasks each containing 15 ml of oxygenated Krebs-Ringer phosphate buffer (pH 7.4) with 2% bovine albumin Fraction V (Armour Pharmaceutical Co., Chicago, Ill.) which had been pretreated by the method described by Hollenberg (24). The albumin contained less than 1% FFA. The albumin fraction was also added to the blank samples in all FFA titrations. 0.1 ml of L-noradrenaline bitartrate (to give a final concentration of 4 μg/ml) was added to half the flasks; 0.1 ml of buffer was added to the remaining half. The flasks were flushed with O_2 and incubated at 37°C for 1 hr with gentle shaking. Incubation started within 40 min of the removal of the epididymal tissue.

After incubation the medium from the three flasks in which one pad had been distributed was pooled, and the total FFA were determined. The remaining medium was extracted with the Dole, et al. mixture (16), and the FFA were isolated, methylated, and identified by GLC as previously described.

Determination of TG Structure

Four rats (mean weight 425 g) on each diet were fasted for 16 hr and anesthetized, and the epididymal fat was removed. 1.5 g of the tissue was immediately extracted with petroleum ether, and the extract was dried over Na_2SO_4. The TG, isolated by silicic acid chromatography, were hydrolyzed with steapsin (Difco Laboratories, Inc., Detroit, Mich.), according to the method of Brockerhoff (25). The monoglycerides were isolated,

and a sample of the original triglyceride was methylated and analyzed by GLC as before.

RESULTS

Fatty Acid Composition of Diets

Analysis of the fatty acid content of each of the semisynthetic diets used is shown in Table 2. In diet S the principal fatty acids were oleic (33.9%), stearic (30.8%), and palmitic (24.8%). In diet L, the principal fatty acid was linolenic (59.8%), and in diet HC, lauric acid (56.4%) was predominant. The distribution of fatty acids was distinctly different in the three diets. Unsaturated fatty acids were present in amounts less than 1% in diet HC and in amounts greater than 90% in diet L. In diet S there was a more even distribution of saturated and unsaturated acids with stearic and oleic acids in approximately equal proportions.

TGFA Composition of Adipose Tissue

TGFA composition of adipose tissue samples obtained from the three groups of rats is shown in Table 3. There were different patterns for each diet group, the predominant fatty acids being oleic (59%) in diet group S, linolenic (44.4%) in diet group L, and lauric (41.4%) in diet group HC. A preliminary feeding experiment revealed that the composition of adipose tissue TGFA for each group after 26 wk was achieved after only 6–8 wk of feeding and remained constant thereafter irrespective of which sampling site was examined, i.e. perirenal, epididymal, subcutaneous, or mesenteric adipose tissue.

Serum Concentrations of FFA before and after Noradrenaline Infusion

As shown in Table 4, total serum FFA levels were significantly higher after infusion of 20 μg noradrenaline in each of the three groups ($P < 0.001$). Between the groups there were no significant differences in pre- and postinfusion levels. In a preliminary experiment, it had been found that total FFA levels as well as serum FFA composition (as shown by GLC) were no different with doses of noradrenaline varying from 10–30 μg.

Serum FFA Composition before and after Noradrenaline Infusion in relation to TGFA of Adipose Tissue

The fatty acid composition of the serum FFA before and after noradrenaline infusion is shown in Table 3. These

TABLE 2 FATTY ACID COMPOSITION OF THREE SEMISYNTHETIC DIETS FED TO RATS

Fatty Acid		Diet S	Diet L (Linseed Oil 40%)	Diet HC (Coconut Oil 40%)
			% by weight	
Shorter than	12:0*	—	—	20.3
Lauric	12:0	0.6	0.2	56.4
Myristic	14:0	4.2	0.2	11.9
Palmitic	16:0	24.8	6.7	5.4
Palmitoleic	16:1	2.2	1.2	—
Stearic	18:0	30.8	2.7	5.7
Oleic	18:1	33.9	14.2	0.7
Linoleic	18:2	3.8	15.9	0.2
Linolenic	18:3	—	59.8	—

* Number of carbon atoms: number of double bonds.

TABLE 3 ANALYSIS OF SERUM FFA IN RATS BEFORE AND AFTER NORADRENALINE INFUSION COMPARED WITH THE FATTY ACID COMPOSITION OF ADIPOSE TISSUE TRIGLYCERIDES

Fatty Acid	Serum FFA Preinfusion	Serum FFA Postinfusion of Noradrenaline	Adipose Tissue TGFA
		% ± SEM*	
Diet S			
12:0	0.5 ± 0.1	0.4 ± 0.1	0.8 ± 0.3
14:0	2.3 ± 0.1	2.2 ± 0.2	2.2 ± 0.2
16:0	33.8 ± 1.2	31.8 ± 1.8	24.3 ± 1.1
16:1	4.2 ± 0.7	5.8 ± 0.5	7.9 ± 1.4
18:0	10.5 ± 0.9	8.2 ± 1.0	3.6 ± 0.5
18:1	37.3 ± 1.6	42.0 ± 1.4	59.0 ± 2.5
18:2	4.8 ± 1.7	5.4 ± 1.2	2.3 ± 0.3
18:3	—	—	—
20:4	5.9 ± 0.9	4.8 ± 0.8	—
Diet L			
12:0	1.0 ± 0.6	0.5 ± 0.3	0.9 ± 0.6
14:0	1.0 ± 0.1	0.7 ± 0.5	0.2 ± 0.2
16:0	19.7 ± 0.7	15.9 ± 0.6	10.1 ± 0.9
16:1	1.5 ± 0.5	1.2 ± 0.3	0.8 ± 0.4
18:0	9.9 ± 0.9	7.0 ± 0.9	1.4 ± 0.5
18:1	17.7 ± 1.9	17.1 ± 1.2	22.9 ± 2.6
18:2	17.5 ± 1.5	17.2 ± 2.2	19.2 ± 2.2
18:3	28.1 ± 0.9	36.0 ± 1.1	44.4 ± 3.7
20:4	3.6 ± 0.6	4.3 ± 0.7	—
Diet HC			
12:0	14.2 ± 1.2	14.8 ± 1.7	41.4 ± 4.6
14:0	9.7 ± 0.9	9.0 ± 0.4	12.8 ± 0.5
16:0	25.7 ± 1.3	25.2 ± 0.8	12.7 ± 1.7
16:1	5.5 ± 1.0	5.9 ± 0.4	6.8 ± 0.7
18:0	7.1 ± 0.6	7.6 ± 0.6	0.9 ± 0.6
18:1	29.5 ± 1.3	29.5 ± 0.6	22.4 ± 2.5
18:2	3.8 ± 1.2	3.8 ± 0.8	2.3 ± 0.3
18:3	—	—	—
20:4	4.7 ± 0.7	4.1 ± 1.6	—

* Means of four groups, six rats per group.

TABLE 4 SERUM CONCENTRATIONS OF TOTAL FFA BEFORE AND AFTER NORADRENALINE INFUSIONS

	Preinfusion	Postinfusion (20 μg Noradrenaline)	*P*
		meq/liter ± SEM*	
Rats on diet S	0.52 ± 0.04	1.32 ± 0.15	<0.001
Rats on diet L (linseed oil)	0.53 ± 0.01	1.34 ± 0.11	<0.001
Rats on diet HC (coconut oil)	0.52 ± 0.03	1.23 ± 0.31	<0.001

* Means of four groups, six rats per group.

results are compared with the TGFA composition of perirenal adipose tissue in the three diet groups.

In diet groups S and HC no significant differences were found in the individual fatty acids between the pre- and postinfusion sera. However, in diet group L the percentage of 18:3 increased ($0.025 < P < 0.05$) and that of 16:0 decreased significantly ($0.001 < P < 0.01$).

In rats fed diets S and L, the fatty acids found in highest concentrations in the serum both before and after noradrenaline infusion were also the fatty acids found in highest concentrations in the adipose tissue TG. These were 18:1 and 16:0 for rats on diet S, and 18:3 and, to a lesser extent, 18:1 and 18:2 for rats on diet L. In the rats fed diet HC, however, the principal fatty acids in both the pre- and postinfusion samples were 18:1 and 16:0, while 12:0 and, to a lesser extent, 18:1 were the predominant adipose tissue TGFA.

In Vitro Study of FFA Mobilization from Adipose Tissue

This experiment was undertaken to show if the difference in serum FFA response noted between rats on diet HC on the one hand, and those on diets S and L on the other, was due to a difference in mobilization from adipose tissue rather than to differences in the utilization of FFA. Observations were made on the concentration and composition of FFA released into the medium after noradrenaline was added to the medium (Krebs phosphate buffer with albumin) in which the epididymal fat pads from the rats of each diet group were incubated. As shown in Table 5, there were significant increases of FFA levels in the medium after incubation with noradrenaline. Although in the three diet groups there was no significant difference in the basal release of FFA, there were differences after noradrenaline stimulation. FFA release was significantly greater from the fat pads from rats on diet L than from those from rats on diet S ($0.001 < P < 0.01$), and there was a slight increase of release in pads from rats on diet L over those from rats on diet HC ($0.05 < P < 0.1$). The fatty acid composition of the FFA in the medium before and after the addition of noradrenaline and of the TGFA of the incubated epididymal fat pad is shown for each diet in Table 6. For rats on diet S, it is noted that the major fatty acids in the medium before and after noradrenaline stimulation are those which are present in greatest amount in the adipose tissue, 18:1 and 16:0. Similarly for rats on diet L, the major FFA in the medium after noradrenaline stimulation reflect the composition of TGFA in the adipose tissue in which 18:3 predominates. However, in this group it is to be noted that before noradrenaline stimulation, the relative amount of 18:3 in the medium was low in contrast to the level after noradrenaline stimulation.

For rats on diet HC, the major fatty acid in the adipose tissue TG (12:0) was not the major component of the FFA in the medium although there was some increase in its level after noradrenaline. As with the in vivo experimental observations, this finding contrasted with the pattern noted for the FFA in the medium in relation to TGFA of adipose tissue for rats on diets S and L.

Two further in vitro experiments were conducted to determine whether the differences in fatty acid mobilization (between 12:0 and other fatty acids) were due to preferential mobilization or to positional differences in

TABLE 5 CONCENTRATIONS OF TOTAL FFA AFTER NORADRENALINE STIMULATION OF RAT EPIDIDYMAL ADIPOSE TISSUE IN VITRO

Source of Epididymal Adipose Tissue	Before Noradrenaline	After Noradrenaline (4 μg/ml)	P
	μeq ± SEM*		
Rats on diet S	2.88 ± 0.29	9.11 ± 0.74	<0.001
Rats on diet L	2.93 ± 0.26	13.59 ± 0.86	<0.001
Rats on diet HC	2.57 ± 0.26	10.29 ± 0.63	<0.001

* Means of six rats per group. The values are μeq of fatty acids (per g of adipose tissue) in the media after an incubation for 1 hr.

TABLE 6 FFA COMPOSITION BEFORE AND AFTER NORADRENALINE STIMULATION OF RAT EPIDIDMYAL ADIPOSE TISSUE AS COMPARED WITH THE FATTY ACID COMPOSITION OF THE ADIPOSE TISSUE TRIGLYCERIDES

Fatty Acid	FFA in Medium before Noradrenaline	FFA in Medium after Noradrenaline (4 μg/ml)	Epididymal Adipose Tissue TGFA
		% ± SEM*	
Diet S			
12:0	0.5 ± 0.1	0.3 ± 0.2	0.9 ± 0.1
14:0	2.5 ± 0.3	2.0 ± 0.1	2.6 ± 0.2
16:0	26.2 ± 1.2	24.0 ± 1.2	23.8 ± 0.7
16:1	5.7 ± 0.3	5.8 ± 1.2	10.5 ± 1.9
18:0	15.4 ± 0.8	17.0 ± 2.1	5.6 ± 0.7
18:1	43.1 ± 0.4	43.4 ± 1.8	51.7 ± 1.1
18:2	6.6 ± 0.7	6.3 ± 0.7	4.8 ± 0.4
18:3	—	—	—
Diet L			
12:0	0.4 ± 0.1	0.1 ± 0.1	0.6 ± 0.2
14:0	1.0 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
16:0	18.5 ± 1.1	14.3 ± 0.9	12.0 ± 0.3
16:1	1.9 ± 0.6	1.3 ± 0.5	1.2 ± 0.1
18:0	14.1 ± 0.1	7.8 ± 0.7	3.5 ± 0.2
18:1	30.8 ± 2.1	23.4 ± 0.8	24.0 ± 0.6
18:2	13.3 ± 0.5	15.4 ± 0.2	21.2 ± 1.1
18:3	19.8 ± 1.6	37.1 ± 2.0	37.1 ± 0.8
Diet HC			
12:0	12.8 ± 0.4	17.6 ± 0.9	31.2 ± 0.8
14:0	8.5 ± 0.2	10.0 ± 0.2	12.2 ± 0.9
16:0	22.2 ± 0.1	21.7 ± 0.3	18.1 ± 0.5
16:1	5.9 ± 0.2	8.9 ± 0.5	6.3 ± 0.4
18:0	14.9 ± 0.6	8.5 ± 0.3	4.7 ± 0.9
18:1	31.4 ± 0.9	28.3 ± 1.4	26.2 ± 1.8
18:2	4.4 ± 0.3	5.0 ± 0.5	1.3 ± 0.3
18:3	—	—	—

* Means of six rats per group.

TABLE 7 FATTY ACID AND GLYCEROL RELEASE FROM RAT EPIDIDYMAL ADIPOSE TISSUE AFTER NORADRENALINE STIMULATION IN VITRO

Source of Epididymal Adipose Tissue	FFA Release	Glycerol* Release	Ratio FFA:Glycerol
	μeq/g per hr	*μmole/g per hr*	
Rats on diet S	6.37 ± 0.41	2.21 ± 0.27	3.15 ± 0.45
Rats on diet L	9.96 ± 0.23	3.52 ± 0.15	2.83 ± 0.10
Rats on diet HC	10.06 ± 0.98	3.27 ± 0.49	3.35 ± 0.47

All values are means of six rats per group ± SEM.
* Glycerol was analyzed by the method of Laurell and Tibbling (37).

the fatty acids of the TG. Firstly, the concentrations of FFA and glycerol in the incubation medium were measured after noradrenaline stimulation. The results are shown in Table 7. No difference in ratio of FFA: glycerol was noted in the three groups. Secondly, the adipose tissue TG were hydrolyzed with pancreatic lipase, and the fatty acid composition of the β-monoglycerides was determined. The results of these analyses as well as the compositions of the original TG are shown in Table 8.

Table 9 shows the percentage of each fatty acid in the β-position of adipose tissue TG for each diet group. These values were calculated from the data in Table 8, using the method of Mattson and Volpenhein (26). Applying the "concept" (27) that if there is less than 28% of a particular fatty acid esterified at the β-position then that acid is located preferentially at the α-position and, conversely, if more than 38% of an acid is found at the β-position that acid is attached preferentially at the β-position, it is assumed that the fatty acids preferentially at the β-position of the TG were 18:1 for rats fed diet S, 18:2 in rats fed diet L, and 18:1 and 16:1 in rats fed diet HC.

Neither 18:3 in the case of rats fed diet L nor 12:0 in the case of rats fed diet HC, were preferentially esterified at the β-position. This indicates that the pattern of release of these fatty acids was not determined by the position of the fatty acid in the TG molecule.

DISCUSSION

The foregoing observations compared the release of certain fatty acids from the adipose tissue of rats fed diets high in (*a*) oleic and stearic acids, (*b*) linolenic acid, and (*c*) lauric acid.

The most striking observation, namely the difference between the fatty acid profiles of the serum FFA and the adipose tissue TGFA in diet group HC as compared with those observed for the other two groups, is one which has not been demonstrated by previous investigators. In other studies of the correlation between release of individual FFA and the fatty acid composition of rat adipose tissue (28–33), rats fed large amounts of coconut oil or linseed oil were not studied, and hence little attention has been directed to the behavior of either the shorter chain saturated lauric acid or the longer chain polyunsaturated linolenic acid, both of which normally constitute a negligible fraction of the TGFA in rat adipose tissue.

In interpreting the difference in the patterns of fatty acid release among the three diet groups in the in vivo study, three possibilities should be considered. Firstly, FFA released during lipolysis might reflect the adipose tissue TGFA pattern but because of more rapid removal by reesterification of lauric or myristic acids these acids appear disproportionately reduced compared to longer chain acids. Secondly, increased oxidation of lauric and myristic acids could cause an apparent decrease in serum levels of these acids. Finally, selective mobilization of fatty acids from adipose tissue could occur with preferential release of certain individual acids.

In order to eliminate the first two possibilities, the in vitro study was performed in the absence of glucose so that mobilization of fatty acids without reesterification could take place into the medium. Results were similar

TABLE 8 FATTY ACID PATTERN IN TRIGLYCERIDES OF RAT EPIDIDYMAL ADIPOSE TISSUE IN TERMS OF CHAIN LENGTH AND PERCENTAGE OF FATTY ACID ESTERIFICATION AT THE β-POSITION

	TGFA as Percentage of Total ± SEM					
	Rats on Diet S		Rats on Diet L		Rats on Diet HC	
Fatty Acid	Original Triglyceride	β-Position	Original Triglyceride	β-Position	Original Triglyceride	β-Position
12:0	0.29 ± 0.11	0.03 ± 0.02	0.20 ± 0.06	0.03 ± 0.01	22.83 ± 0.54	7.27 ± 0.52
14:0	1.91 ± 0.20	0.83 ± 0.23	0.36 ± 0.11	0.29 ± 0.10	12.21 ± 0.84	9.92 ± 0.35
16:0	22.50 ± 1.80	11.40 ± 0.73	12.24 ± 0.30	6.41 ± 0.43	19.30 ± 0.15	10.67 ± 0.65
16:1	9.08 ± 1.27	7.40 ± 0.83	1.60 ± 0.12	1.32 ± 0.26	7.48 ± 0.50	9.55 ± 0.27
18:0	4.36 ± 0.57	0.92 ± 0.54	1.62 ± 0.55	—	2.98 ± 0.26	1.34 ± 0.61
18:1	59.97 ± 0.57	74.25 ± 1.89	28.55 ± 0.87	27.55 ± 1.18	32.85 ± 0.85	58.57 ± 2.35
18:2	3.86 ± 1.16	5.21 ± 0.75	20.57 ± 0.20	30.62 ± 1.87	2.60 ± 0.54	2.51 ± 0.61
18:3	—	—	34.95 ± 0.45	33.70 ± 1.91	—	—

TABLE 9 PERCENTAGE OF INDIVIDUAL FATTY ACIDS OF RAT ADIPOSE TISSUE TRIGLYCERIDES ESTIMATED TO BE IN β-POSITION

Fatty Acid	Rats on Diet S	Rats on Diet L	Rats on Diet HC
		%	
12:0	3.4	5.0	10.6
14:0	14.5	25.8	14.4
16:0	16.9	17.4	30.0
16:1	27.2	27.5	42.6
18:0	7.0	—	22.5
18:1	41.2	32.2	59.5
18:2	44.8	49.6	32.2
18:3	—	32.2	—

to the in vivo study and showed that although the first two possibilities might still occur, there is indeed a difference in mobilization between rats fed the linseed oil and those fed the hydrogenated coconut oil.

The concept that release of FFA is not just a random process is a view which has been presented by previous investigators (30, 32). Hollenberg and Douglas (30) found higher concentrations of palmitic and palmitoleic acids, and lower concentrations of oleic and linoleic acids in the released FFA than were present in the TGFA of rat epididymal fat pads after exposure to adrenaline and corticotrophin. Similarly, Meinertz (32) found a difference between the relative release of palmitic and oleic acids, again in the presence of adrenaline. On the other hand, Spitzer, Nakamura, Gold, Altschuler, and Lieberson (33), using rat epididymal fat pads incubated without the addition of glucose or any hormones which might enhance lipase activity found that for the six major individual fatty acids (myristic, palmitic, oleic, palmitoleic, stearic, and linoleic), mobilization rates appeared to be proportional to the concentration of each in the adipose tissue. Previously, Stein and Stein (29) using an in vivo incubation technique, had observed also that palmitic, oleic, and linoleic acids were mobilized from adipose tissue at similar rates. In the present study, which differs essentially from others in that the behavior of two additional fatty acids, lauric and linolenic, was also observed, only in diet group L did the serum FFA profile alter significantly in vivo after noradrenaline infusion and then only with respect to the relative behavior of linolenic and palmitic acid release. As earlier investigators have pointed out (33), it is difficult to compare the many observations which have been made on fatty acid release from rat adipose tissue because of the different experimental conditions used. Notwithstanding, with or without noradrenaline infusion, the relatively poor release of lauric acid when it is the major fatty acid constituent of rat adipose tissue after coconut oil feeding, would support the concept that at least under certain circumstances fatty acid release is not just a random process.

The similarity in the ratio of glycerol to FFA (in the in vitro studies) in the three groups of rats, showed that the apparent mobilization differences observed were probably not due to differences in partial or complete hydrolysis of the adipose tissue TG, since hydrolysis of TG yielded the expected molar ratio of fatty acids to glycerol of 3:1, and thus appeared to be complete. However, no direct chemical analysis has been undertaken to confirm this interpretation.

It is possible that, as suggested by Hollenberg et al. (30), differences in release may be due to the specificity of fatty acid distribution in the TG molecule or alternatively to the specificity of the tissue lipases for certain ester linkages.

At least two tissue lipases which preferentially hydrolyze fatty acids esterified in the α-position have been isolated (34). Pancreatic lipase attacks preferentially the two α-ester bonds (35). Brockerhoff (36) has formulated a set of empirical rules to forecast the positional distribution of fatty acids in the TG. Briefly stated these rules are that for acids with a given chain length there is a tendency for the unsaturated acids to occupy the β-position, while for acids of equal unsaturation those with the shorter chain length are preferred in this position. Pancreatic lipase hydrolysis of the adipose tissue TG of the three dietary groups show that these tendencies are followed although it is apparent that if both unsaturated and short-chain acids are present then it is the unsaturated acids, rather than the shorter chain acids which occupy the β-position. Based on Brockerhoff's assumptions and on our observations, there would be a tendency for lauric and myristic acids to occupy the α-positions and for linoleic and linolenic acids to occupy the β-position. Therefore, if the differences in mobilization were due to the specificity of fatty acid distribution in the TG molecule, a result opposite to what was observed would have been expected, namely lauric and myristic acids would have been liberated more freely into the serum FFA fraction than some of the longer chain and more unsaturated acids.

We are left with the possibility that the difference in mobilization between lauric and the longer chain fatty acids may be caused by some specificity of the tissue lipases for certain ester linkages.

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