Tissue Phospholipid Fatty Acid Composition in Genetically Lean (Fa/-) or Obese (fa/fa) Zucker Female Rats on the Same Diet

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The fatty acid composition of serum total lipids, of phospholipids of various organs (liver, heart, kidney), and of nervous structures (brain, retina, sciatic nerve, myelin, synaptosomes) have been compared in lean (Fa/-) and genetically obese (fa/fa) Zucker female rats. Both received a standard commercial diet including 37% of 18:2n-6 and 5% of n-3 polyunsaturated fatty acids (PUFA), 1.7% of which were in the form of 20:5n-3 and 22:6n-3. In comparison with lean rats, the results for the obese rats pointed out (i) no difference in the fatty acid composition of nervous structures; (ii) a decrease of 18:2n-6 (from -8% to -35%) and of 20:4n-6 (from -9% to -49%) in serum, liver and in kidney; this was compensated for by an increase in 20:3n-6 (from +30% to +320%) and in total n-3 PUFA (from +68% to +76%); (iii) a decrease of 20:4n-6 (-18%) and of 22:6n-3 (-24%) in heart compensated for by an increase in 18:2n-6 (+39%) and in 20:3n-6 (+233%); and (iv) constant levels of total PUFA (n-6 and n-3) in the various fractions studied, except in serum where this level decreased (-23%). Finally, except for the nervous structures, tissue phospholipids of obese rats included a lower proportion of 20:4n-6 and a higher proportion of 20:3n-6. This resulted in a significant reduction in the 20:4n-6/20:3n-6 ratio; by contrast, the 20:3n-6/18:2n-6 ratio increased. The results suggest that in Zucker rats, the obese character (fa/fa) affects the desaturation-elongation process of 18:2n-6 to 20:4n-6 by specifically decreasing Δ5-desaturase activity.

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The proportion and nature of polyunsaturated fatty acids (PUFA) acylated in the *sn*-2 position β of phospholipids determine, in part, the physical and functional properties of biological membranes (1-3). PUFA belong to two non-interconvertible series (n-6 and n-3). Due to processes of successive elongation and desaturation, PUFA are obtained from the two key acids, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3).

The conversion process involves liver microsomal desaturases (4,5) and, in particular, two enzymes whose activity, in a defined nutritional context, determines the quantity of long-chain PUFA available to the organism: (i) Δ6-desaturase that permits the conversion of 18:2n-6 to γ-linolenic acid (18:3n-6) and the conversion of 18:3n-3 to stearidonic acid (18:4n-3); this enzyme is generally considered the rate limiting step in PUFA desaturation (6-8); and (ii) Δ5-desaturase that permits the conversion of dihomo-γ-linolenic (20:3n-6) to arachidonic acid (20:4n-6) and of 18:4n-3 to eicosapentaenoic acid (20:5n-3).

Certain factors, such as experimental diabetes (9-12), alcohol and aging (8), and dietary cholesterol (13) more or less specifically inhibit Δ6- and/or Δ5-desaturase activities. Insulin seems to be specifically implicated in the simultaneous regulation of the two enzyme activities (14). Thus, rats made diabetic by streptozotocin injection show physiopathological and biochemical characteristics comparable to those accompanying total PUFA deficiency; insulin administration causes these symptoms to disappear rapidly (15).

Genetically obese Zucker rats (fa/fa) are hyperlipemic and hyperinsulinemic (16,17). In these animals, the proportion of 20:4n-6 in phospholipids (PL) of serum, platelets, liver and heart is significantly decreased as compared to the values seen in lean controls (Fa/-) (18-21). Surprisingly, the reverse process, i.e., an increase in the conversion of 18:2n-6 to 20:4n-6, has been observed in genetically obese mice (ob/ob) (22,23).

In recent studies in Zucker rats, we have confirmed the above observations and shown that in obese rat hearts, phospholipids are low in n-3 PUFA (24) while phospholipids of adipocyte plasma membranes are, by contrast rich in n-3 PUFA (25).

The aim of the present study was to obtain complete fatty acid data on serum, liver and heart in these animals and to add (in addition to kidney), nervous structures, such as brain, retina, sciatic nerve, myelin and synaptosomes, which are known to contain high amounts of n-3 PUFA in their phospholipids (26).

MATERIAL AND METHODS

Six obese (fa/fa) and six lean (Fa/-) Zucker female rats (three-months-old) were used. They received a commercial standard diet; the lipid content and the fatty acid composition of the diet are shown in Table 1. The diet contained 28% of saturated fatty acids (SFA), 30% of monounsaturated fatty acids (MUFA) and 37% of n-6 PUFA in the form of 18:2n-6 and about 5% of n-3 PUFA, 1.7% of which were in the form of 20:5n-3 and 22:6n-3.

The rats were fasted for 15 hr, and then killed by decapitation. The serum was recovered for assay of total cholesterol, triglycerides and phospholipids (Boehringer methods). Liver, heart, kidneys, brain, retina and sciatic nerves were quickly excised, rinsed and deep-frozen at -80°C. A fraction of the brain was used to prepare synaptosomes and myelin as described previously (27). Serum lipids and various freeze-dried tissues were extracted according to Folch *et al.* (28) in the presence of butylhydroxytoluene (BHT) (0.02%, w/v). The phospholipids of liver, heart, kidney, brain and retina were then separated by thin-layer chromatography on silica gel (60 G Merck) using hexane/diethyl ether/formic acid

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Abbreviations: BHT, butylhydroxytoluene; GLC, gas-liquid chromatography; FFAP, free fatty acid phase; MUFA, monounsaturated fatty acids; PL, phospholipids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TSL, total serum lipids.

TABLE 1

Dietary Fatty Acid Composition[a]

Fatty acids	%
Saturated	
14:0	0.9
16:0	20.0
18:0	6.7
Total	27.6
Monounsaturated	
16:1n-7	3.1
18:1n-9	26.7
Total	29.8
n-6 Polyunsaturated	
18:2n-6	36.6
20:4n-6	0.2
Total	36.8
n-3 Polyunsaturated	
18:3n-3	3.1
20:5n-3	0.9
22:6n-3	0.8
Total	4.8
n-6 plus n-3	41.6
n-6/n-3 ratio	7.67

[a]The dietary lipid content is 7.3%.

(80:20:1, v/v/v) containing 0.02% of BHT (w/v) (29). Finally, the fatty acid composition (% of total fatty acids) of the various lipid fractions was determined by gas-liquid chromatography (GLC) of the methyl esters using a glass capillary column coated with free fatty acid phase (FFAP) (inner diameter, 0.3 mm; length, 50 m; detection by flame ionization).

Statistical analyses were done by Student's t-test. In fatty acid analysis, as percentage data was not normally distributed, statistical analyses were realized using the variance-equalizing transformation (arc-sine transformation) according to Zar (30).

RESULTS

Organ weight and lipid content. For heart, kidney and brain, the weight and total lipid contents were independent of genotype. However, weight and total lipid content were notably increased (+36% and +28%, respectively) in the livers of obese animals; the amount of total liver lipids was increased by 74% in these animals (Table 2).

Serum lipids. As reported previously, the serum of obese rats had a considerably higher lipid content; this was particularly due to the increase in triglyceride levels which were increased seven-fold. The level of phospholipids was doubled, but cholesterol levels were only moderately increased (Table 3).

Total serum lipids (TSL). The level of SFA in TSL was slightly but significantly higher (+8%; $p < 0.05$) in the obese rats due to a marked increase in palmitic acid levels. The difference between total MUFA was quite apparent, since MUFA levels were almost two times higher in obese than in lean rats (23% *vs* 12%), due to the concomitant doubling of palmitoleic and oleic acid levels (Table 4).

In the obese rats, the total proportion of n-6 PUFA dropped by 36% due to a moderate decrease (-19%) in linoleic acid and a considerable decrease (-48%) in arachidonic acid. There also was a significant increase in 20:3n-6, reaching 0.8% in obese vs 0.3% in lean rats. On the other hand, the level of total n-3 PUFA was higher in obese rats (+68%); this increase was due to all fatty acids of this series with 22:6n-3 (DHA) being by far the major contributor. However, the increase in the level of n-3 PUFA did not compensate for the decrease in the amount of n-6 PUFA, so that the level of total PUFA (n-6 plus n-3) was 23% lower in the obese rats. The decrease in n-6 PUFA and

TABLE 3

Serum: Lipid Class Content in Zucker Female Rats—Comparison of Lean (Fa/-) and Obese (fa/fa) Animals[a]

n=6 Lipids	Cholesterol (g/L)	Triglycerides (g/L)	Phospholipids (g/L)
Lean	0.64 ± 0.08	0.39 ± 0.03	1.15 ± 0.15
Obese	0.82 ± 0.11[b]	2.83 ± 0.58[c]	2.06 ± 0.04[c]

[a]Results are means ± S.D. of six animals.

[b]$p < 0.5$.

[c]$p < 0.05$.

TABLE 2

Weights and Lipid Contents of Different Organs in Zucker Female Rat. Comparison of Lean (Fa/-) and Obese (fa/fa) Animals[a]

	Liver		Heart		Kidney		Brain	
	Lean[b]	Obese[c]	Lean[b]	Obese[c]	Lean[b]	Obese[c]	Lean[b]	Obese[c]
Weight (g)	7.08 ± 1.55	9.61[d] ± 0.67	0.79 ± 0.16	0.85 ± 0.06	1.78 ± 0.44	1.76 ± 0.06	1.62 ± 0.13	1.45 ± 0.05
Lipids/g of weight (mg)	58.9 ± 1.7	75.6[d] ± 4.0	63.0 ± 12.9	51.4 ± 7.3	48.3 ± 17.9	46.1 ± 2.7	109.1 ± 2.7	112.7 ± 15.1
Lipids/organ (mg)	417 ± 54	726[d] ± 80	49.8 ± 9.8	48.7 ± 6.2	86 ± 25.4	81.1 ± 4.7	177 ± 9	163.4 ± 21.9

[a]Results are means ± S.D. of six animals.

[b]Live weight lean: 270 ± 21 g.

[c]Live weight obese: 350 ± 42 g.

[d]$p < 0.01$.

TABLE 4

Serum, Liver, Kidney and Heart: Fatty Acid Composition of Total Serum Lipids and Liver, Kidney and Heart Phospholipids in Zucker Female Rats. Comparison Between Lean (Fa/-) and Obese (fa/fa) Aanimals[a]

	Serum		Liver		Kidney		Heart	
Fatty acids n=6	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese
16:0	17.0 ± 1.2	20.3 ± 1.4[c]	16.4 ± 1.5	12.6 ± 0.9[c]	18.5 ± 0.8	19.2 ± 0.6	10.1 ± 0.4	10.5 ± 0.4
18:0	12.1 ± 1.5	11.0 ± 1.1	21.0 ± 2.2	26.2 ± 2.2[c]	17.4 ± 0.7	15.0 ± 2.0	22.1 ± 1.2	19.4 ± 1.8
ΣSFA	30.4 ± 1.4	32.8 ± 1.5[b]	38.5 ± 1.8	39.8 ± 1.8	36.9 ± 0.6	35.5 ± 2.2	32.9 ± 1.2	30.8 ± 1.4
16:1n-7	1.2 ± 0.1	3.2 ± 0.5[c]	0.4 ± 0.1	0.8 ± 0.2[c]	0.6 ± 0.2	0.2 ± 0.1[c]	0.3 ± 0.2	0.5 ± 0.1[b]
18:1n-9	8.8 ± 0.6	17.6 ± 1.4[c]	3.9 ± 0.6	3.0 ± 0.3[b]	7.4 ± 0.4	8.8 ± 0.4[c]	2.7 ± 0.2	4.0 ± 0.3[c]
18:1n-7	1.7 ± 0.2	1.9 ± 0.2	1.9 ± 0.3	1.5 ± 0.2[b]	2.5 ± 0.3	3.0 ± 0.4[c]	3.2 ± 0.2	3.4 ± 0.2
ΣMUFA	12.3 ± 0.8	23.1 ± 1.5[c]	6.4 ± 0.8	5.5 ± 0.4	10.9 ± 0.6	13.5 ± 0.5[c]	6.4 ± 0.4	8.0 ± 0.4[c]
18:2n-6	18.4 ± 1.7	14.8 ± 0.5[c]	14.9 ± 1.2	9.7 ± 0.7[c]	15.3 ± 0.6	14.0 ± 1.2[b]	18.7 ± 0.8	26.0 ± 1.5[c]
20:3n-6	0.3 ± 0.1	0.8 ± 0.2[c]	0.5 ± 0.1	1.6 ± 0.2[c]	0.5 ± 0.2	0.3 ± 0.1	0.7 ± 0.2	0.5 ± 0.1
20:4n-6	30.2 ± 1.6	15.3 ± 1.9[c]	28.7 ± 0.7	24.9 ± 0.7[c]	31.2 ± 1.2	28.4 ± 1.4[b]	22.3 ± 0.5	18.3 ± 0.8[c]
Σn-6 PUFA	50.3 ± 1.2	32.3 ± 1.8[c]	44.7 ± 1.1	36.9 ± 1.1[c]	48.5 ± 1.0	44.3 ± 1.8[c]	42.6 ± 0.8	46.2 ± 0.8[c]
20:5n-3	1.0 ± 0.2	2.3 ± 0.3[c]	0.3 ± 0.1	1.2 ± 0.2[c]	0.6 ± 0.1	1.6 ± 0.2[c]	0.2 ± 0.1	0.5 ± 0.2
22:5n-3	0.7 ± 0.1	1.5 ± 0.2[c]	0.9 ± 0.1	1.2 ± 0.2[c]	0.4 ± 0.1	0.7 ± 0.1[c]	1.9 ± 0.2	2.1 ± 0.1
22:6n-3	4.8 ± 0.4	7.1 ± 0.3[c]	9.1 ± 1.3	15.3 ± 1.0[c]	2.7 ± 0.3	4.3 ± 0.4[c]	16.0 ± 0.6	12.2 ± 0.8[c]
Σn-3 PUFA	6.9 ± 0.6	11.6 ± 0.4[c]	10.4 ± 1.3	17.7 ± 1.1[c]	3.8 ± 0.3	6.7 ± 0.7[c]	18.1 ± 0.4	14.9 ± 0.8[c]
n-6 plus n-3	57.2 ± 0.9	43.9 ± 1.8[c]	55.1 ± 1.1	54.6 ± 1.6	52.2 ± 1.4	51.0 ± 2.4	60.8 ± 0.7	61.1 ± 0.4
n-6/n-3	7.3 ± 0.7	2.8 ± 0.2[c]	4.3 ± 0.6	2.1 ± 0.2	12.9 ± 1.3	6.7 ± 0.4[c]	2.4 ± 0.1	3.1 ± 0.2[c]
20:3n-6/18:2n-6 × 100	1.6	5.4	3.3	16.5	6.5	9.3	1.6	2.7
20:4n-6/20:3n-6	100.6	19.1	57.4	15.6	31.2	21.8	74.3	26.1

[a]Results are means ± S.D. of six animals.

[b]$p < 0.5$.

[c]$p < 0.01$.

Minor fatty acids are not reported in the table: 15:0 (0-0.3%); 17:0 (0.4-0.8%); 16:1n-9 (0-1.3%); 18:3n-6 (0-0.4%); 20:2n-6 (0.2-1.3%); 22:4n-6 (0-0.4%); 22:5n-6 (0-0.2%); and 18:4n-3 (<0.1%).

increase in n-3 PUFA resulted in a considerable decrease in n-6/n-3 ratio in the obese animals (2,6). In these animals, the 20:3n-6/18:2n-6 ratio (index of Δ6-desaturase activity) was 3.3 times higher; by contrast, the 20:4n-6/20:3n-6 ratio (index of Δ5-desaturase activity) decreased five-fold (Table 4).

Liver phospholipids. The level of total SFA of liver phospholipids was of the same order (39%) in both types of rat. However, this equivalence resulted from a compensation between palmitic acid (more abundant in lean rats) and stearic acid (more abundant in obese rats). There was no significant difference between total MUFA, and only low proportions of these fatty acids were found.

On the other hand, the levels of n-6 and n-3 PUFA showed marked differences. As was observed for serum total lipids, liver phospholipids of obese rats included less n-6 PUFA than in lean rats (-17%; $p < 0.01$) due to lower levels of 18:2n-6 and 20:4n-6. By contrast, 20:3n-6 increased considerably in the obese animals consistent with what was observed in TSL. The overall decrease in the amount of n-6 PUFA was quantitatively compensated for by an increase in the level of n-3 PUFA (+70%) with a particular contribution from 22:6n-3. Thus, the amount of total PUFA (n-6 plus n-3) was identical in both cases and represented 55% of total fatty acids; however, the n-6/n-3 ratio was two times lower in the obese animals (2.1 vs 4.3). In the obese animals, the 20:3n-6/18:2n-6 ratio increased five times, while the 20:4n-6/20:3n-6 ratio decreased 3.7 times, similar to what was observed in serum lipids (Table 4).

Kidney phospholipids. Kidney phospholipids showed identical levels of total SFA, independent of the genotype. The levels of total MUFA were higher in the PL of obese rats (+26%; $p < 0.01$) as the levels of fatty acids in this series were higher. Kidney PL of the obese animals contained significantly less total n-6 PUFA than controls (-9%) largely due to the decrease of 18:2n-6 and 20:4n-6; as in liver and serum, the level of total n-3 PUFA increased considerably (+76%); this increase compensated for the deficit in total n-6 PUFA, so that the sum of n-6 plus n-3 was very similar in both representing 51-52% of total fatty acids. As in the liver, the n-6/n-3 ratio was decreased two-fold in the obese animals. The 20:3n-6/18:2n-6 ratio was higher and the 20:4n-6/20:3n-6 ratio decreased, but less than in the total serum lipids and liver phospholipids (Table 4).

Heart phospholipids. The levels of total SFA of heart PL were equal in obese and lean rats. As in serum and kidney, the level of MUFA was notably increased in the obese animals (+25%; $p < 0.01$) due to an increase in oleic acid (about +50%) (Table 4). In contrast to what was observed for serum, liver and kidney, the proportion of total n-6 PUFA in the heart was increased in the obese animals in spite of the decrease in arachidonic acid (-18%; $p < 0.01$) observed in heart. The overall gain in the proportion of n-6 PUFA in the liver and the kidney was essentially due to

TABLE 5

Brain, Retina, Synaptosomes, Myelin and Sciatic Nerve: Fatty Acid Composition of Phospholipids in Zucker Female Rats. Comparison Between Lean (Fa/-) and Obese (fa/fa) Animals

	Brain[a]		Retina[b]		Synaptosomes[c]		Myelin[c]		Sciatic nerve[c]	
Fatty acids	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese	Lean	Obese
16:0	20:6 ± 0.8	21.6 ± 1.6	15.8	17.3	22.2	22.9	15.6	16.8	28.6	29.0
18:0	19.1 ± 0.4	18.3 ± 0.8	17.7	17.8	20.7	19.8	17.4	17.6	8.2	9.5
Σ SFA	40.6 ± 0.6	41.0 ± 1.5	37.7	37.3	43.9	44.3	35.1	36.5	41.2	43.1
16:1n-7	0.4 ± 0.1	0.5 ± 0.1	1.5	1.6	0.8	1.0	1.2	1.4	9.0	8.1
18:1n-9	19.9 ± 0.6	20.0 ± 0.8	11.5	11.9	14.8	15.4	34.5	35.6	33.6	32.9
18:1n-7	4.3 ± 0.3	4.3 ± 0.2	3.3	3.6	3.8	3.3	5.2	4.9	2.9	3.2
20:1n-9	1.9 ± 0.3	1.8 ± 0.3	0.4	0.2	0.6	0.5	2.6	2.6	0.5	0.4
20:1n-7	0.6 ± 0.1	0.5 ± 0.1	—	—	0.2	0.2	0.9	0.8	0.2	0.1
Σ MUFA	27.7 ± 1.2	27.7 ± 1.2	17.6	17.7	20.6	21.2	44.8	44.6	47.2	45.4
18:2n-6	0.8 ± 0.1	0.8 ± 0.1	5.6	5.0	1.1	1.0	0.8	0.9	2.3	2.3
20:3n-6	0.4 ± 0.1	0.5 ± 0.1	0.4	0.5	0.3	0.4	0.7	0.8	0.4	0.3
20:4n-6	11.0 ± 0.5	10.4 ± 0.7	12.0	11.8	13.5	15.1	10.1	9.0	3.7	4.0
22:4n-6	3.4 ± 0.3	2.8 ± 0.3	1.4	1.6	3.3	2.5	3.8	3.6	1.0	0.8
22:5n-6	0.6 ± 0.1	0.5 ± 0.1	0.3	0.4	1.4	0.8	0.8	0.5	1.0	0.8
Σ n-6 PUFA	16.2 ± 0.6	15.1 ± 0.8	19.9	19.5	19.8	20.1	16.3	15.0	8.8	8.4
22:5n-3	0.2 ± 0.1	0.2 ± 0.1	1.1	1.4	—	—	0.2	0.3	0.4	0.3
22:6n-3	15.2 ± 0.4	15.7 ± 1.3	23.2	23.6	15.7	14.4	3.6	3.6	1.8	2.0
Σ n-3 PUFA	15.4 ± 0.4	16.1 ± 1.3	24.6	25.3	15.7	14.4	3.8	3.9	2.8	3.1
n-6 plus n-3	31.6 ± 0.9	31.2 ± 2.1	44.5	44.8	35.5	34.5	20.1	18.9	11.6	11.5
n-6/n-3	1.1 ± 0.1	0.9 ± 0.1	0.81	0.77	1.26	1.40	4.29	3.85	3.14	2.70
20:3n-6/18:2n-6 × 100	50.0	62.5	7.1	10.0	27.3	40.0	87.5	88.9	17.4	13.0
20:4n-6/20:3n-6	27.5	20.8	30.0	23.6	45.0	37.7	14.4	11.2	9.2	13.3

[a]Measurements carried out on six rats (values are means ± S.D.).

[b]Measurements carried out on a pool of six rats.

[c]Measurements carried out on two pools of three rats.

Minor fatty acids are not reported in the table: 15:0 (0–0.4%); 17:0 (0–0.4%): 20:0 (0.1–0.9%); 22:0 (0–0.4%); 16:1n-9 (0–1.0%); 22:1n-9 (0–0.9%); 24:1n-9 (0–0.3%); 18:3n-6 (0–0.4%); 18:3n-3 (<0.1%).

an increase in linoleic acid (+39%; $p < 0.01$). As in serum, liver and kidney, the level of 20:3n-6 was very high in obese rats. Total n-3 PUFA (particularly 22:6n-3) was lower in obese fats resulting in total PUFA (n-6 plus n-3) being maintained at essentially the same level (21%) in both types of rats. The n-6/n-3 ratio was higher in obese animals, contrary to what was observed in serum, liver and kidney. The 20:3n-6/18:2n-6 ratio was higher, in spite of the considerable amounts of 18:2n-6 present, and the 20:4n-6/20:3n-6 ratio was decreased three-fold in the hearts of obese animals (Table 4).

Lipids of nervous structures. No differences between obese and lean rats were observed in the complex (brain, retina, sciatic nerve) or subcellular (synaptosomes, myelin) neural structures studied by any of the criteria used (Table 5).

DISCUSSION

As previously reported (21,31,32), we observed hyperlipemia in genetically obese Zucker female rat (fa/fa) mostly due to an increase in triglyceride levels. The hypertriglyceridemia was accompanied by a marked increase in the total amount of liver lipids. There was also an accumulation of saturated fatty acids, and especially of monounsaturated fatty acids (mainly 18:1n-9 and 16:1n-7), in serum, indicating an increase in Δ9-desaturase activity and in *de novo* lipogenesis (18,19,33). These perturbations in the *de novo* synthesis of fatty acids in obese rats were expressed by an increase in the levels of 18:0 in liver phospholipids and of 18:1n-9 in kidney and heart phospholipids.

n-6 PUFA. The present study shows a dwindling of the desaturation processes which permit the conversion of 18:2n-6 to 20:4n-6. Except for the neural structures, in which the n-6 PUFA levels were remarkably independent of genotype, the tissue phospholipids of obese rat typically included much less 20:4n-6 (from -9% to -49%) than in controls.

Contrary to what has been reported in experimental diabetes (12), 18:2n-6 was accumulated only in heart phospholipids of obese rats. On the other hand, the accumulation of 20:3n-6 was more general and relatively high. Thus, in obese rats, the 20:3n-6/18:2n-6 ratio, which is an index of Δ6-desaturase activity, was always higher (including in the heart), while the 20:4n-6/20:3n-6 ratio, an index of Δ5-desaturase activity, was always lower (Table 6). This suggests that inhibition of the conversion

TABLE 6

Comparisons of the 20:3n-6/18:2n-6 Ratio (Index of Δ6-Desaturase Activity) and of the 20:4n-6/20:3n-6 Ratio (Index of Δ5-Desaturase Activity) in Tissues of Lean and Obese Zucker Female Rats

Tissue	Serum	Liver	Kidney	Heart	Brain
Δ6-Desaturase index (20:3n-6/18:2n-6 × 100)					
• Lean	1.6	3.3	6.5	1.6	50.0
• Obese	5.4	16.5	9.3	2.7	62.5
• Obese/lean	3.37	5.00	1.43	1.68	0.80
Δ5-Desaturase index (20:4n-6/20:3n-6					
• Lean	100.6	57.4	31.2	74.3	27.5
• Obese	19.1	15.6	21.8	26.1	20.8
• Obese/lean	0.19	0.27	0.70	0.35	1.32

of 18:2n-6 to 20:4n-6 did not result from a decrease of Δ6-desaturation, but rather of Δ5-desaturation. The results confirm the recent data of Blond *et al.* (34) obtained by direct measurement of Δ6- and Δ5-desaturase activities in liver microsomes of Zucker rats. In heart phospholipids, the accumulation of 18:2n-6 occurred concomitantly with a reduction in 22:6n-3 levels. The increase in 18:2n-6 may suggest a change in the proportion of different phospholipids in favor of cardiolipins. These diphosphatidylglycerols, which occur mainly in mitochondrial membranes (35), contain remarkably high levels of this fatty acid (60–90%) (36,37). In the artificially diabetic rat, the heart is also the organ in which the highest levels of 18:2n-6 are observed (12). The fact that the differences seen between obese and lean rats do not extend to nervous tissue emphasizes the ability of these structures to incorporate the various PUFA in a very controlled fashion because neural tissues do not have Δ6- or Δ5-desaturase activity (38). Liver is known as the major site of Δ6- and Δ5-desaturation in mammals (39).

n-3 PUFA. The obese genotype is characterized by higher than normal levels of n-3 PUFA in serum lipids and in liver and kidney phospholipids, thus compensating for the drop in the level of n-6 PUFA and maintaining the sum n-6 plus n-3. This increase in n-3 PUFA, particularly in respect to 22:6n-3 and 20:5n-3, may result from preferential acylation of these acids as compared to n-6 PUFA due to their presence in dietary lipids rather than to activation of α-linolenic acid conversion (21). These C_{20} and C_{22} fatty acids represent about 2% of the total fatty acids in the diet (118 mg/100 g of diet) and alone cover a large portion of n-3 PUFA requirements in the rat (40,41). In order to elucidate this point, further, it would be necessary to experiment with a diet containing n-3 PUFA in the form of α-linolenic acid only.

As noted above, preferential incorporation of n-3 PUFA into phospholipids is not a general mechanism in obese rats because there is also a decrease in the relative level of 22:6n-3 in heart phospholipids. The drop in the level of 20:4n-6 and rise in 20:3n-6, which was already reported for obese Zucker rats at one month of age (24), could cause lowered production of PGI_2 and PGE_2, representing physiological conditions which favor the cardiovascular complications typical of obesity.

In respect to the nervous structures, and in parallel with n-6 PUFA, the level of n-3 PUFA was maintained in all the cellular or subcellular structures considered. The n-6/n-3 ratio was unchanged and remained between 1 and 2 in the brain, retina and synaptosomes; it remained close to 4 for myelin and sciatic nerve (27,42). This homeostasis of n-3 PUFA composition in nervous structures has already been noted in rats, even when receiving long-chain n-3 PUFA in the form of fish oil supplied in the diet (43,44), except when excessive amounts of fish oil were provided for the animals (45).

The obese genotype in Zucker rats is characterized by abnormalities in PUFA composition of tissue phospholipids. These anomalies may correspond to changes in the proportion of phospholipid classes, as reported in some membranes (25). The whole of the changes of a genetic disorder occur in obese mice (ob/ob) (22,23) or in some cases of human obesity (46), even if they are different from those which characterize Zucker rats. The changes cause biophysical (fluidity) and functional (activities of Na^+K^+ATPase and adenylate cyclase, number of insulin receptors, glucose transport) alterations in cellular membranes (46).

According to Horrobin (8), decreased Δ6-desaturase activity could be one of the keys to the problem of aging. To compensate for this decrease, dietary lipids could be supplemented with 18:3n-6, which would bypass this metabolic deficiency. However, Δ5-desaturase activity seems to also decrease in certain physiological states, such as experimental diabetes (12) and hypercholesterolemia artificially induced by an excessive intake of dietary cholesterol (13). Takahashi and Horrobin (47) have recently shown that such a process is characteristic of aging in mammals. According to these authors, the level of 20:3n-6, as well as the 20:3n-6/20:4n-6 ratio, increases with age in liver phospholipids and platelets in rats. Thus, supplementing dietary lipids with γ-linolenic acid would not always permit a normal level of 20:4n-6 in tissue phospholipids and would lead to an increase of 20:3n-6 (48). Therefore, it seems that a diet directly supplying C_{20} and C_{22} PUFA (20:4n-6, 20:5n-3, 22:6n-3) would be advisable, as soon as the relative requirements of these two families of fatty acids have been defined.

In any case, the genetically obese Zucker rat (fa/fa) is a good model for studies aimed at preventing or nutritionally treating enzyme deficiencies in regard to fatty acid desaturation.

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