Effects of Dietary Fatty Acids on Lipid Metabolism in Streptozotocin-Induced Diabetic Rats

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We measured the activity of liver Δ^9- and Δ^6-desaturases and examined plasma and liver microsome phospholipid fatty acid composition in control and diabetic rats fed a basal diet supplemented with 5% (by weight) olive oil (OO), sunflower oil (SO), or fish oil (FO), respectively. Plasma glucose, cholesterol, triacylglyceride, and phospholipid levels were also measured. An increase in plasma and liver microsome oleic acid and a decrease in arachidonic acid were found in diabetes. In the liver, docosahexaenoic acid levels were higher in diabetic versus control rats. Diabetes increased liver Δ^9-desaturase in OO-fed rats and did not modify Δ^6-desaturase activity in OO- or SO-fed rats. Both enzymatic activities were decreased in diabetic rats fed the FO diet. As a main conclusion, it appears that diet-induced alterations in membrane composition provide a mechanism for improving the diabetic condition in animals and overcoming the effect of insulin deficiency on desaturase activities. Plasma cholesterol was not modified either by diabetes or by diet. In diabetes, OO-fed rats showed the lowest levels of triglycerides. Plasma phospholipids were significantly higher in OO-fed versus FO-fed rats. These findings suggest that OO contributes to a better control of the hypertriglyceridemia accompanying diabetes as compared with the other two diets in this rat model.

DIETARY FAT–INDUCED alterations in membrane lipid composition have been repeatedly described,[1-3] although information is more limited for the diabetic state. Diabetes produces specific alterations in the fatty acid profile of several tissues and membranes in both humans and the streptozotocin (STZ)-diabetic rat that have been related to reduced activities of insulin-sensitive hepatic Δ^9- and Δ^6-desaturases.[4-6] These changes in phospholipid fatty acid composition could be an important factor in the development of diabetic complications such as cataracts, neuropathy, or coronary heart disease (CHD).

Dietary advice is the basis of all treatments for diabetes mellitus. The choice of the best dietary treatment for diabetic patients is still an object of debate. While there is no question that a reduction of saturated fat is mandatory, there are still uncertainties about, for example, the role of complex carbohydrates complementing caloric intake versus unsaturated fat. A low-fat diet high in carbohydrates has beneficial effects on plasma cholesterol levels, but it may also have unfavorable effects on other important risk factors for CHD in diabetics such as an increase in plasma glucose, triglycerides, and very–low-density lipoprotein (VLDL)-cholesterol and a decrease in high-density lipoprotein (HDL)-cholesterol.[7,8] Because lipemia in diabetic patients is characterized by increased triglyceride and VLDL-cholesterol levels,[9] any further deterioration by carbohydrate-rich diets may be deleterious.[7] A different approach to decrease plasma lipids without effects on other CHD risk factors could be the fat-modified diet, in which the amount of saturated fat and cholesterol is replaced by either monounsaturated[9-11] or polyunsaturated[12] fat.

The present study examines and compares the effects of three fat-modified diets (5% olive oil [OO], 5% sunflower oil [SO], and 5% fish oil [FO]) rich in monounsaturated, n-6 polyunsaturated, and n-3 polyunsaturated fatty acids, respectively, on plasma and liver microsomal phospholipid fatty acid composition and Δ^9- and Δ^6-desaturase activities in nondiabetic and diabetic rats. Plasma glucose, cholesterol, phospholipids, and triglycerides were also determined.

MATERIALS AND METHODS

Materials

[1-^{14}C]palmitic acid (58 mCi/nmol; 97.1 radiochemical purity) and [1-^{14}C]linoleic acid (59 mCi/nmol; 98.8 radiochemical purity) were purchased from Amersham International (Amersham, England). Unlabeled fatty acids, coenzyme A, bovine fatty acid–free serum albumin, adenosine triphosphate, and NADH were purchased from Sigma Química (Barcelona, Spain). All other chemicals were of analytical grade.

Animals and Diets

Female Wistar rats from the Granada University breeding colony (150 to 160 g) were housed in a temperature (22°C)- and light-controlled (12-hour cycle) animal facility and randomly divided into three groups of 20 animals. Each group was fed for 1 week with the same basal diet with a different (5% wt/wt) fat supplement: OO, SO, and FO, respectively. The composition of the basal diet was 65.0% cornstarch, 20% vitamin-free casein, 5% cellulose, 3.5% mineral mix, 1% vitamin mix, 0.3% DL-methionine, and 0.2% choline bitartrate. The fatty acid composition of the three diets is shown in Table 1.

Experimental Design

Experimental diabetes was induced in half of the rats by a single STZ injection (60 mg/kg intraperitoneally in 50 mmol/L sodium citrate buffer, pH 4 5). Control animals received citrate buffer alone. Diabetes was verified 24 hours later by estimating the hyperglycemia (BM-Test Glycemia; Boehringer Mannheim, Barcelona, Spain). Only animals with nonfasting blood glucose levels greater than 400 mg/dL were considered diabetic. Diabetic rats and matched controls were maintained for 5 additional weeks on the corresponding dietary regimen. Animals were studied in compliance with our institution's guidelines for animal research.

At the end of the experiment, rats were killed by decapitation and blood was collected in heparinized tubes. The blood was centrifuged at 3,000 × *g* for 10 minutes to separate the plasma from the cells. After blood collection, the liver was removed, rinsed immediately with physiological saline, blotted, and weighed

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Submitted March 30, 1998; accepted September 22, 1998.

Supported by the Comision Interministerial de Ciencia y Tecnologia (CICYT) through Project No. PB92-1117.

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0026-0495/99/4804-0008$10.00/0

Table 1. Fatty Acid Composition of the Diets

Fatty Acid	OO*	SO†	FO‡
16:0	15.22	7.02	23.50
16:1 n-7	1.18	0.18	12.83
18:0	3.80	3.90	4.18
18:1 n-9	72.55	34.28	18.77
18:2 n-6	4.87	54.19	1.31
18:3 n-6			0.84
18:3 n-3	0.57	0.29	0.84
18:4 n-3			3.20
20:5 n-3			21.07
22:5 n-3			2.29
22:6 n-3			6.98
Other PUFAs >20 C			4.62

NOTE. Results are expressed as a percentage of total fatty acid methyl esters. The fatty acid composition of the diet was determined by gas-liquid chromatography.

*Carbonell, Carbonell y Cia de Cordoba, Spain.

†Koipesol, Koipe, San Sebastian, Spain.

‡AFAMSA, Vigo, Spain.

Lipid Analysis

All classes of plasma lipids were extracted by a direct transesterification method according to Lepage and Roy.[13] Basically, 100 µL plasma was precisely pipetted into glass tubes and 3 mL methanol:benzene (4:1) was added to the biological samples. While stirring, 200 µL acetylchloride was slowly added to each tube over a period of 1 minute. The tubes were tightly capped and subjected to methanolysis at 100°C for 1 hour. After cooling the tubes, 5 mL 6% K_2CO_3 solution was added to stop the reaction and neutralize the mixture. The tubes were then shaken and centrifuged, and an aliquot of the benzene upper phase was injected into a gas chromatograph (model 5880; Hewlett Packard, Avondale, PA). Fatty acid methyl esters were analyzed as previously described[2] and are expressed as the percent distribution of fatty acid methyl esters.

The livers were homogenized (1:3 wt/vol) in 0.14 mol/L phosphate buffer, pH 7.3, containing 25 mmol/L sucrose, 1 mmol/L EDTA, and 1.5 mmol/L glutathione. Microsomes were obtained as previously described.[1] Microsomal lipids were extracted according to the method of Folch et al.[14] Total phospholipids were separated from neutral lipids on a Silica Gel G 60 plate (Merck, Darmstadt, Germany) developed with *n*-hexane/diethyl ether/acetic acid (80:20:1).[2] The immobile band containing phospholipids was scraped into a tube and *trans*-methylesterified with boron trifluoride.[15] Fatty acid methyl esters were analyzed by gas chromatography as previously described.[2]

Enzyme Assays

The Δ^9- and Δ^6-desaturation of fatty acids from liver microsomes was measured by estimating the percentage conversion of [1-^{14}C]palmitic acid to palmitoleic acid and [1-^{14}C]linoleic acid to γ-linolenic acid, respectively, as previously described.[2,3] Microsomal protein recovery was determined by the method of Bradford.[16]

Other Assays

Plasma glucose levels were measured by a commercial glucose oxidase-peroxidase method. Plasma total cholesterol, triglycerides, and phospholipids were assayed by an enzymatic method followed by a Trinder reaction (BioMérieux, Charbonnières-les-Bains, France).

Statistics

The results are presented as the mean ± SEM. The effects of dietary treatment and diabetes were examined by two-way ANOVA followed by a Tukey test. Analyses were performed with the BMDP program (BMDP Statistical Software, Cork, Ireland).

RESULTS

Table 1 shows the fatty acid composition of the three diets. The OO diet is enriched in monounsaturated fatty acids, mainly oleic acid (72.5%). The SO diet is enriched in n-6 polyunsaturated fatty acids (PUFAs), mainly linoleic acid (54%). The FO diet is enriched in n-3 PUFAs, mainly eicosapentaenoic acid (21%).

Table 2 shows the effect of dietary supplementation on plasma glucose, triglyceride, cholesterol, and phospholipid levels and body and liver weight in control and diabetic rats. Plasma glucose levels were higher in diabetic versus control rats. Plasma cholesterol was not modified either by diabetes or by diet. Triglycerides increased in diabetes, except for OO-fed rats, in which the levels were similar in control and diabetic rats. Phospholipids were significantly higher in OO-fed versus FO-fed rats. The body weight of diabetic rats was significantly decreased and the liver weight was increased in comparison to controls.

The relative fatty acid composition of total plasma lipids in control and diabetic rats fed OO, SO, and FO diets is shown in Table 3. Concerning the saturated fatty acids, diabetes induced an increase in palmitic acid (16:0) in plasma lipids. This difference reached significance in SO- and FO-fed rats. On the contrary, a significant decrease in stearic acid (18:0) was observed in all diabetic groups. Palmitoleic acid (16:1 n-7) showed a significant decrease in diabetic versus control rats. Oleic acid (18:1 n-9) increased significantly with diabetes; the OO group had the highest levels.

SO-fed rats had the highest level of n-6 fatty acids and FO-fed rats had the lowest level. Linoleic acid and 20:3 n-6

Table 2. Effect of Diet and Diabetes on Plasma Biochemical Parameters and Body and Liver Weight

Parameter	OO	SO	FO
Glucose (mg/dL)			
C	148.3 ± 12.6	164.9 ± 18.1	116.0 ± 3.9*‡
D	761.6 ± 62.8¶	710.3 ± 40.9¶	627.0 ± 14.2¶
Cholesterol (mg/dL)			
C	55.5 ± 4.4	64.9 ± 2.9	74.9 ± 11.7
D	73.5 ± 4.3‖	76.2 ± 4.7	77.1 ± 12.0
Triglycerides (mg/dL)			
C	35.0 ± 1.9	30.8 ± 5.1	40.3 ± 7.4
D	39.5 ± 3.7	115.4 ± 11.0†¶	73.6 ± 6.1†§¶
Phospholipids (mg/dL)			
C	120.3 ± 8.2	111.9 ± 10.2	78.7 ± 9.2†
D	106.9 ± 11.3	110.9 ± 6.4	79.1 ± 10.6†
Body weight (g)			
C	201.3 ± 5.2	195.3 ± 10 3	203.2 ± 7.5
D	169.7 ± 9.8‖	174.3 ± 6.1	171.6 ± 4.4¶
Liver weight (g)			
C	4.7 ± 0.1	5.2 ± 0.1	5.0 ± 0.2
D	8.2 ± 0.4¶	8.6 ± 0.3¶	8.9 ± 0.3¶

NOTE. Results are the mean ± SEM for 6-8 animals per group.
Abbreviations: C, control rats; D, diabetic rats.
*$P < .05$, †$P < .001$: OO *v* SO or FO.
‡$P < .05$, §$P < .001$: SO *v* FO.
‖$P < .05$, ¶$P < .001$: control *v* diabetic.

Table 3. Effect of Diet and Diabetes on Plasma Fatty Acid Composition

Fatty Acid	OO	SO	FO
16:0			
C	14.7 ± 0.7	12 9 ± 0 2	15.9 ± 0.2§
D	16.7 ± 0.6	15.1 ± 0.4¶	18.6 ± 0.7§‖
18:0			
C	20.0 ± 1.1	19.7 ± 1.2	16.9 ± 0.3*
D	12.1 ± 0.7¶	14.3 ± 0.5¶	12.8 ± 0.5¶
16:1 n-7			
C	0.9 ± 0.3	1.4 ± 0.1	2.8 ± 0.2†§
D	0.3 ± 0.1	0.3 ± 0.1¶	1.4 ± 0.1†‡‖
18:1 n-9			
C	15.7 ± 1.8	7.2 ± 0.2†	11 6 ± 0 4*§
D	28.0 ± 1.0¶	11.7 ± 0.5†¶	15.5 ± 0.8†‡‖
18:2 n-6			
C	5.8 ± 0.5	10.6 ± 0.3†	7.1 ± 0.4§
D	7.5 ± 0.5‖	18.5 ± 0.9†¶	8.8 ± 0.4§
20:3 n-6			
C	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.1
D	0.4 ± 0.0‖	0.4 ± 0.0¶	0.4 ± 0.0
20:4 n-6			
C	32.1 ± 3.2	39.3 ± 0.8	17.6 ± 0.5†§
D	22.0 ± 1.3¶	27.0 ± 1.1¶	13.0 ± 1.0†§‖
22.5 n-6			
C	1 1 ± 0.2	1 7 ± 0.1*	0.1 ± 0.1†§
D	2.1 ± 0.2¶	4.2 ± 0.3†¶	0.2 ± 0.1†§
PUFA n-6 >18 C			
C	34.4 ± 3.2	42.0 ± 0.9*	18 1 ± 0.6†§
D	26.3 ± 1.3‖	33.8 ± 0.9†¶	13.9 ± 1.1†§‖
20·5 n-3			
C	0.4 ± 0.1	0.4 ± 0.3	16.4 ± 0.8†§
D	0.2 ± 0.0	0.3 ± 0.2	9.7 ± 0.6†§‖
22:6 n-3			
C	4.9 ± 0.6	1.7 ± 0.1†	6.3 ± 0.3†§
D	4.9 ± 0.3	2.2 ± 0.1†	13.6 ± 0.6†§¶
PUFA n-3 >18 C			
C	5.5 ± 0.6	2.4 ± 0.3†	24 2 ± 0.9†§
D	5.5 ± 0.3	2.7 ± 0.3†	26.3 ± 0.8†§

NOTE. Results are the mean ± SEM for 6-8 animals per group.
Abbreviations· C, control rats; D, diabetic rats.
*$P < .05$, †$P <$ 001: OO *v* SO or FO.
‡$P < .05$, §$P < .001$: SO *v* FO
‖$P < .05$, ¶$P < .001$: control *v* diabetic.

increased significantly with diabetes in OO- and SO-fed rats. Arachidonic acid was significantly higher in control versus diabetic rats in every dietary group. The most unsaturated fatty acid, 22:5 n-6, was higher in the SO group than in the other two groups and significantly increased in diabetic compared with control rats.

Eicosapentaenoic acid, 20:5 n-3, was mainly present in the FO group, being higher in control versus diabetic rats. The major n-3 fatty acid in plasma lipids was 22:6 n-3. This fatty acid was similar in control and diabetic rats in OO and SO groups and was significantly increased in FO-fed rats by diabetes.

Table 4 shows the fatty acid composition of liver microsomal phospholipids in control and diabetic rats. With respect to saturated fatty acids, palmitic and stearic acids increased in diabetic FO-fed rats versus controls. Oleic acid was increased by diabetes; this increase was significant in SO- and OO-fed groups. The levels were highest in the latter group. Linoleic acid was twice as high in the SO group versus the other two groups. Arachidonic acid decreased in diabetic animals versus controls. This decrease was minimal in FO-fed rats, which had the lowest levels, as occurs in plasma lipids. 22:5 n-6 was significantly increased by diabetes. PUFA n-6 longer than 18 C were not changed in diabetes. They were highest in SO groups and lowest in FO groups. 20:5 n-3 was not detected in OO-fed or SO-fed groups. In FO-fed rats, diabetes caused a decrease in the 20:5 n-3 percentage. Levels of 22:6 n-3 were higher in diabetic versus control rats in all dietary groups, with FO groups having the highest levels and SO groups the lowest levels.

Table 5 shows that diabetes increased liver microsomal Δ^9-desaturase in the OO group and did not modify Δ^6-desaturase activity in OO and SO groups. Both enzymatic activities were decreased in diabetic rats fed the FO diet. OO-fed rats had the highest Δ^9-desaturase activity, and control FO-fed rats had the highest Δ^6-desaturase activity.

Table 4. Effect of Diet and Diabetes on Fatty Acid Composition of Liver Microsomal Phospholipids

Fatty Acid	OO	SO	FO
16:0			
C	15 0 ± 0.7	13.2 ± 0 4	16.2 ± 0.7‡
D	15.5 ± 0.6	14 6 ± 0.6	21.3 ± 0.5†§¶
18:0			
C	33.7 ± 0.6	31.4 ± 0.6	24.8 ± 0.6†§
D	28.2 ± 0.8¶	29.8 ± 0.6	31.4 ± 0.5¶
18:1 n-9			
C	9.0 ± 0.7	3.8 ± 0.1†	6.7 ± 0.4§
D	12.1 ± 1.0‖	6.2 ± 0.7†¶	7 6 ± 0 4†
18:2 n-6			
C	3.8 ± 0.3	7.1 ± 0.2†	3.6 ± 0.2§
D	2.4 ± 0.2‖	6 8 ± 0 4†	4.3 ± 0.1†§
20:3 n-6			
C	0.4 ± 0.03	0.3 ± 0.02	0.3 ± 0.04
D	0.5 ± 0.1	0.3 ± 0.03	0.3 ± 0.02
20:4 n-6			
C	29.1 ± 1.1	30.9 ± 0.3	16.3 ± 0.5†§
D	25.6 ± 0.9‖	26.3 ± 0.4¶	15.1 ± 0.2†§
22:5 n-6			
C	1.8 ± 0.2	5.6 ± 0.4	0.0 ± 0.0†§
D	3.2 ± 0.3¶	8.0 ± 0.4†¶	0.3 ± 0.01†§¶
PUFA n-6 >18 C			
C	31.4 ± 1.4	37.9 ± 0.8*	16.7 ± 0.5†§
D	30.2 ± 1 3	36.3 ± 0.9*	18.2 ± 0.2†§
20:5 n-3			
C	0.0 ± 0.0	0.0 ± 0.0	7.5 ± 0.5†§
D	0.0 ± 0.0	0.0 ± 0.0	2.2 ± 0.3†§¶
22:6 n-3			
C	7.4 ± 0.6	3.2 ± 0.2†	14.6 ± 0.7†§
D	10.1 ± 0.5¶	5.0 ± 0.3†¶	20.2 ± 0.7†§¶
PUFA n-3 >18 C			
C	7.6 ± 0.6	3.3 ± 0.3†	23.3 ± 1.3†§
D	10 5 ± 0.4¶	5.1 ± 0.3†¶	23.8 ± 1.1†§

NOTE. Results are the mean ± SEM for 6-8 animals per group.
Abbreviations: C, control rats; D, diabetic rats.
*$P <$ 05, †$P < .001$: OO *v* SO or FO.
‡$P < .05$, §$P < .001$: SO *v* FO.
‖$P < .05$, ¶$P < .001$: control *v* diabetic

Table 5. Effect of Diet and Diabetes on Δ^9- and Δ^6-Desaturase Activity (pmol/min/mg)

Enzyme	OO	SO	FO
Δ^9-desaturase			
C	152.0 ± 14.0	82.4 ± 8.3†	69.2 ± 6.9†
D	171.1 ± 12.8§	86.5 ± 8.4	20.3 ± 1.4†‡‖
Δ^6-desaturase			
C	71.1 ± 7.4	78.3 ± 7.7	131.1 ± 10.7†‡
D	86.3 ± 9.7	100.6 ± 7.4	46.2 ± 5.5*‡‖

NOTE. Results are the mean ± SEM for 6-8 animals per group.
Abbreviations: C, control rats; D, diabetic rats.
*$P < .05$, †$P < .001$: OO *v* SO or FO.
‡$P < .001$, SO *v* FO.
§$P < .05$, ‖$P < .001$: control *v* diabetic.

DISCUSSION

Diet is the cornerstone therapy for patients with diabetes mellitus. The main aim of the diet is to normalize the derangements in intermediary metabolism in patients with diabetes mellitus such as hyperglycemia and lipemia. Another objective of diet therapy is to prevent long-term complications of diabetes mellitus such as atherosclerotic vascular disease, nephropathy, retinopathy, and neuropathy.[6-8,10,12]

The fatty acid composition of plasma lipids and liver microsomal phospholipids and the Δ^9- and Δ^6-desaturase activity in control and diabetic rats were measured. Animals were fed diets differing in fatty acid composition. Diets supplemented with OO, SO, and FO prevent the characteristic hypercholesterolemia accompanying diabetes. Only the OO diet corrected the high plasma triglyceride levels present in the other diabetic groups. In liver microsomes, OO and SO diets induce alterations in membrane composition that could explain the effect of both diets to overcome the decrease in desaturase activity due to insulin deficiency in diabetic rats.

It has been reported that supplementation with long-chain n-3 fatty acids is more effective for reducing triglyceride levels than an equivalent amount of n-6 PUFAs.[12] The increase in triglycerides induced by diabetes in the FO group (182%) was smaller than in the SO group (373%). In contrast to these favorable effects, a mild deterioration of glycemic control following n-3 PUFA administration to diabetics is usually observed, including an increase in fasting glucose and a deterioration in glucose tolerance.[8,12] Most of these studies have been performed on rats using diets supplemented with 12% FO by weight.[17] Our results showed that rats fed a diet supplemented with 5% FO had the lowest fasting glucose levels, although the difference with respect to the other dietary groups did not reach significance. Collier and Sinclair[12] hypothesized that the detrimental effects of dietary n-3 PUFAs were only the result of large doses of n-3 PUFAs. From our results, we observe that low doses of these fatty acids have beneficial effects on glycemic control but not on the increase in triglyceride content accompanying diabetes.

Garg[9] performed a study comparing diets rich in monounsaturated fatty acids versus diets rich in carbohydrates in patients with diabetes mellitus. Their results showed that the high–monounsaturated-fat diet decreased plasma triglyceride and VLDL-cholesterol and increased plasma HDL-cholesterol compared with the carbohydrate-rich diet. Our results show that a diet supplemented with only 5% OO prevented the increase in triglycerides observed in diabetic FO- and SO-fed rats. Moreover, recent studies have shown a reduced susceptibility to LDL oxidation in subjects consuming monounsaturated fatty acid–enriched diets, thus adding another dimension to the ongoing debate over the most appropriate diet for diabetic patients.[10,11]

Plasma cholesterol was not modified either by diabetes or by diet in our study. Similar results have been found by Seigneur et al.[6] Plasma phospholipids were significantly higher in OO-fed versus FO-fed groups.

The fatty acid composition of any tissue reflects both the activity of esterifying enzymes and the quantity of the different fatty acids available to the enzymes.[18] Many alterations in these processes have been reported in diabetes mellitus. Fatty acyl transferase and desaturase activities are altered in the liver of STZ-induced diabetic rats, accounting for the observed changes in fatty acid composition in diabetic animals. It has been reported that in untreated STZ-induced diabetic rats, the major consistent changes in the liver were an increase of stearic and linoleic acids associated with a decrease of oleic and arachidonic acids[4,17] and other PUFAs.[5,19] These modifications were mainly due to a depressed activity of Δ^9-, Δ^6-, and Δ^5-desaturases.[4,11] Insulin treatment of diabetic rats for only 1 to 2 days[17,20] corrected and even overcorrected Δ^9- and Δ^6-desaturase activities. It has also been reported that diet overcomes the effect of insulin deficiency on desaturase activities.[21]

Fatty acid profiles are similar in plasma and liver microsomes. As a general rule, in terms of the fatty acid composition of the different oils, the levels of oleic acid, linoleic acid, and n-3 PUFAs were highest in OO, SO, and FO groups, respectively.

In the plasma, diabetes is characterized by an increase in oleic and linoleic acids and a decrease in arachidonic acid. Data related to the plasma are a result both of serum increases in triglycerides in diabetics, which reflect dietary fatty acid composition, and of free fatty acids from adipose tissue.[4] In poorly controlled diabetics, the overall rate of lipolysis is accelerated and a considerable portion of the circulating fatty acids originate from recently mobilized adipose tissue fat containing high amounts of oleic and linoleic acids.[6]

In liver microsomal phospholipids, saturated fatty acids are similar in diabetic and control rats for OO- and SO-fed groups. Similar results have been found in the liver of spontaneously diabetic Bio-Breeding rats.[22] In contrast to these results, saturated fatty acids are increased in diabetic FO-fed rats, which may be partly a response to the high levels of 22:6 n-3 in these animals.

Oleic acid levels are highest in OO-fed groups both in plasma lipids and in liver microsomal phospholipids. This is due to its content in the diet and also to the higher Δ^9-desaturase activity with respect to the other groups. In a previous study, we repeatedly found this effect of an OO diet on Δ^9-desaturase activity.[21] Moreover, it has been reported that unsaturated fatty acid supplementation reduces the activity of Δ^9-desaturase.[23] In liver microsomal phospholipids, oleic acid levels in diabetics are significantly increased in OO and SO groups, but not in the FO group, in which Δ^9-desaturase is depressed. Our data confirm that the fatty acid composition of liver microsomal

lipids does not necessarily correlate with changes in desaturase activities, particularly Δ^9-desaturase, in agreement with other studies.[20,22]

Arachidonic acid is significantly decreased in diabetes in all cases in plasma lipids and liver microsomal phospholipids. Alterations of n-6 PUFAs in diabetics have been repeatedly described.[4,17] Decreased levels of arachidonic acid have been observed in diabetic rats,[24] but unchanged[25] and increased[17] levels have also been reported. Decreased levels are usually attributed to an impairment of liver Δ^6-desaturase activity.[21] In our study, Δ^6-desaturase was only diminished in diabetic FO-fed rats. The type of dietary fat affects the enzyme activity. There are data in the literature describing increases[26] in response to unsaturation in the diet, as well as decreases.[27] In our experimental groups, OO- and SO-fed animals had similar enzyme activity, in agreement with our previously reported data in rats and dogs.[1-3] The decrease in enzyme activity in FO-fed rats is not reflected in long-chain n-6 PUFAs in microsomal phospholipids.

Our results show elevations of 22:6 n-3 in liver microsomal phospholipids in diabetic rats under every dietary regimen. Studies with diets supplemented with unsaturated fatty acids of either fish or vegetable origin have raised the possibility of a competitive mechanism among the long-chain fatty acids for respective incorporation into membrane phospholipids.[4] Thus, when n-3 fatty acid intake is increased as in FO-supplemented diets, these fatty acids (20:5 n-3 and 22:6 n-3) are highly incorporated into cell membranes at the expense of n-6 fatty acids such as linoleate and arachidonate.[4] Data concerning the elevation in 22:6 n-3 levels in diabetic FO-fed rats, in which Δ^6-desaturase is greatly diminished, point to peroxisomal fatty acid metabolism and agree to some extent with the suggested absence of Δ^4-desaturase.[28] This absence supposes that 20:5 n-3 is first elongated into 22:5 n-3 and later Δ^6-desaturated into 22:6 n-3 in peroxisomes. Peroxisomal desaturation and β-oxidation systems cooperate with microsomal synthesis in the metabolism of PUFAs.[29] We can then appreciate that the increase of PUFAs in diabetic animals may have multiple origins, such as the effect of diabetes on peroxisomal metabolism, insufficient metabolic control, or even hepatomegaly, which is characteristic of these diabetic animals.

Our results show an increase of 18:1 n-9 and 22:6 n-3 and a decrease of 20:4 n-6 in microsomal phospholipids in diabetes. There is no change in long-chain n-6 PUFAs. These results do not correlate with the in vitro desaturase activities measured. Actually, factors that alter membrane lipid composition independently of desaturation, eg, acyl exchange, increased triglyceride synthesis, fatty acid β-oxidation, and prostaglandin synthesis, may also have an effect on the altered lipid metabolism observed in diabetes.[17]

As a main conclusion, we observe that diet-induced changes in the fatty acid profile of plasma and microsomal phospholipids in control and diabetic rats show a similar tendency. With respect to the specific diets, OO-fed animals show levels of oleic, n-6, and n-3 fatty acids that are more adequately compensated compared with SO-fed rats, in which there is an increase of n-6 PUFAs to the detriment of n-3 PUFAs, or with FO-fed rats, in which the same can be observed for n-3 PUFAs. These results, in conjunction with the better control of plasma triglycerides, allow us to propose OO as a dietary treatment in the prevention of cardiovascular complications in diabetes.

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