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Influence of dietary n-3 fatty acids on the biosynthesis of polyunsaturated fatty acids in STZ-diabetic rats

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Experimental diabetes in rats produces a generalized defect in liver microsomal polydesaturase activity and hence in the total fatty-acid composition. The present study was devised to determine whether or not those enzymic defects in the diabetic animal might be offset by alterations in the profile of fatty acids consumed in the diet. Accordingly, streptozotocin-induced diabetic rats were fed nutritionally adequate basal diets for 2 weeks supplemented with equivalent amounts of free fatty acids from either corn oil, rich in n-6 species, or cod-liver oil, abundant in n-3 acids. Both classes of dietary polyunsaturates were incorporated into hepatic microsomes and caused elevations in the respective levels of those acids in cellular membranes. The unsaturation indices, however, were higher under the latter regime than under the former. Dietary supplementation with the n-3, but not the n-6, fatty acids increased plasma glucose levels in both control and diabetic rats. Neither regime, however, affected microsomal desaturase or fatty acyl-CoA synthetase activity in either experimental group. Therefore, we conclude that the deficiency in hepatic-desaturase activity accompanying diabetes occurs independently of the nature of the fatty acids present in the hepatic membranes. An enhanced dietary intake of n-3 fatty acids thus fails to alleviate the depression in the enzymes involved in hepatic polyunsaturated fatty acid biosynthesis in diabetic rats. (J. Nutr. Biochem. 6:269–274, 1995.)

Keywords: n-6 fatty acids; n-3 fatty acids; acyl-CoA synthetase; Δ9 desaturase; Δ6 desaturase; Δ5 desaturase; eicosapentaenoic acid; docosahexaenoic acid

Introduction

In diabetes mellitus a coordinate set of metabolic changes occurs that severely compromises the utilization of glucose and fatty acids by the cells. The de novo synthesis of fatty acids is diminished, and, at least in rats, this disturbance is accompanied by a combined decrease in the activities of the principal desaturases responsible for double-bond insertion into all three of the long-chain fatty-acid families, i.e., n-9, n-6, and n-3.[1] In this regard, a number of studies both in vitro and in vivo have demonstrated these impairments in the Δ9, Δ6, and Δ5 desaturases in diabetic rats,[1–3] which alterations, in turn, prevent the normal processing to higher order polyunsaturates of the parental species within the n-6 and n-3 essential fatty-acid families. Long-chain fatty-acyl-CoA synthetase activity is also inhibited in the liver and adrenal glands of affected animals.[4]

The metabolic and physiologic complications of human diabetes mellitus (principally the specific microangiopathic type) lead either directly or indirectly to premature mortality. Several sequelae closely related to fatty-acid metabolism, including hypertriglyceridemia, hypercholesterolemia, the hyperaggregativeness of platelets, and abnormalities in lipoprotein homeostasis, are known to occur in

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Received July 1, 1994; accepted November 10, 1994.

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655 Avenue of the Americas, New York, NY 10010

0955-2863/95/$10.00
SSDI 0955-2863(95)00016-S

diabetic patients, thus resulting in a marked proclivity toward atherosclerosis in these individuals.[5]

The low incidence of diabetes and ischemic heart disease among Greenland Eskimos has been attributed to the richness of their diet in fish and other sea foods that provide a high proportion of polyunsaturated fatty acids of the n-3 family. Moreover, the dietary consumption of this class of fatty acids appears to be associated in a direct fashion with a variety of desirable effects on lipoprotein metabolism, platelet function, and eicosanoid production.[6] All of these observations have led to several investigations involving the supplementation of the diet of diabetic patients or experimental animals, with either n-3 fatty acids themselves or with fish oil, after which manipulation concomitant changes in fatty-acid patterns,[7] lipid metabolism,[8–10] and eicosapentaenoid metabolites[11] have been reported.

In several of the human or animal studies undertaken recently, not only were the specific contributions of the n-6 and n-3 fatty acids not adequately characterized (i.e., with respect to the amount of dietary fat present, the predominance of one polyenoate fatty-acid series over the other, the nature of the individual fatty acid(s) employed), but also the concurrent ingestion of cholesterol was not considered in assessing the possible beneficial influence of the polyunsaturated fatty acids being studied. Furthermore, in view of the metabolic competition between fatty acids of the n-6 and n-3 families within polyunsaturated fatty-acid anabolism,[12] the importance of employing the widely recommended ratio of dietary saturates to unsaturates of ~1.0,[13] and the currently recognized need to clarify conclusively the effect of the addition of essential fatty acids to the diet of diabetic rats on their biosynthesis of polyenoates, we designed experiments in which equivalent amounts of dietary fatty acids obtained from either corn (n-6) or fish (n-3) oil were administered to normal and to diabetic rats for a period of 2 weeks. We then measured polyenoate desaturases as well as long-chain acyl-CoA synthetase activity and characterized the consequent hepatic fatty-acid profile in response to these nutritional manipulations.

Materials and methods

Chemicals

[1-^{14}C]palmitic (59.0 Ci/mol), [1-^{14}C]linoleic (54.7 Ci/mol), [1-^{14}C]α-linolenic (53.0 Ci/mol) and [1-^{14}C]eicosa-8,11,14-trienoic (47.0 Ci/mol) acids were purchased from New England Nuclear Corp. (Boston, MA USA). The following chemicals and cofactors were purchased from the indicated suppliers in the United States: unlabeled acids, Nu-Chek Prep. (Elysian, MN); NADH, ATP and CoA, Sigma Chemicals Co. (St. Louis, MO). Streptozotocin (STZ) was kindly donated by Upjohn Laboratories (Kalamazoo, MI). All other chemicals were of analytical grade.

Treatment of animals

Male Wistar rats, 200–250 g, were maintained on standard rat chow (Cargill type "C", Rosario, Argentina) and water ad libitum before being placed on the experimental diet. Diabetes was induced in nonfasted rats by the intravenous injection of 70 mg/kg of STZ freshly dissolved in sodium citrate-citric acid (0.01 M), pH 4.5. Control rats received an equivalent amount of this vehicle. A week later, STZ-treated rats were fasted for 12 hr, and blood was collected from the orbital venous sinus for glucose determination by a commercial enzymatic method (Wiener Lab. Test, Rosario, Argentina). Only those rats with blood glucose levels higher than 300 mg/dL were considered diabetic.

Control and diabetic animals were then divided into two groups of 5 animals each. One group was fed a basal diet consisting of (in cal) 70% starch and 22% casein with added vitamins and minerals[14] and supplemented with 2% (wt/wt) of free fatty acids prepared from corn oil. The other group was fed on the same regime except that the corn-oil component was replaced by fatty acids prepared and concentrated from cod-liver oil. The preparation of fatty acids from the corn oil or cod-liver oil and the preparation of the concentrate in the latter were done following the procedure of Haagsma et al.[15] Butylated hydroxytoluene was added during extract processing to protect n-6 and n-3 fatty acids from autoxidation. The extraction was undertaken in order to avoid the ingestion of other components of the two oils, particularly tocopherols in the former and the large amount of cholesterol and vitamins in the latter. The cod-liver oil was concentrated so as to enrich the eicosapentaenoic and docosapentaenoic levels in order to make the final mixture contain almost the same proportions of n-3 fatty acids as are represented by the n-6 fatty acids in the corn oil. In addition, the concentrated fatty acids from the marine oil have similar amounts of docosahexaenoic and eicosapentaenoic acids. The fatty acid compositions of the extracted corn oil and the concentrated cod liver oil are given in *Table 1*. The experimental regimen was prepared daily by addition of the required free fatty acids to the powdered diet followed by a thorough mixing and the addition of water. Control and diabetic rats were fed on each of these diets for a total of 2 weeks. All animals were kept in groups of two or three in stainless-steel cages with free access to food and water.

Isolation of microsomes and enzyme assays

At the end of the dietary treatment, the animals were decapitated; blood was drained off and collected into a test tube containing an anticoagulant EDTA solution (Wiener Lab., Rosario, Argentina), plasma was separated from erythrocytes by centrifuging, and livers were excised, weighed, and homogenized in an ice-cold solution (1:3 wt/vol) containing 0.25 M sucrose, 62 mM phosphate buffer (pH 7.0), 0.15 M KCl, 5 mM $MgCl_2$ and 100 μM EDTA. The microsomal fraction was separated by differential centrifugation at 105,000 *g*, as described previously.[16] Protein content was

Table 1 Composition of the fatty acids extracted from corn oil and cod-liver oil

Fatty acid	Corn oil	Cod-liver oil concentrate
14:0	—	4.9
16:0	10.7	13.1
16:1 (n-7)	—	7.2
18:0	2.5	2.9
18:1 (n-9)	34.2	18.0
18:2 (n-6)	51.5	1.5
18:3 (n-6)	1.1	—
18:3 (n-3)	—	7.6
20:4 (n-6)	—	6.0
20:5 (n-3)	—	18.3
22:5 (n-3)	—	3.1
22:6 (n-3)	—	17.4

The quantity of each fatty acid is given as a percent of the total fatty acids.

measured by the method of Lowry et al.[17] using bovine albumin as standard.

The desaturation of fatty acids from liver microsomes was measured by estimation of the percentage conversion of [1-^{14}C]palmitic acid to palmitoleic acid, [1-^{14}C]α-linolenic acid to octadeca-6,9,12,15-tetraenoic acid and [1-^{14}C]eicosa-8,11,14-trienoic acid to arachidonic acid. Four nanomoles of the labeled acid plus 96 nmol of the corresponding unlabeled acid were incubated with 5 mg of liver microsomal protein in a metabolic shaker at 37°C for 10 min. The incubation medium contained 4 μmol of ATP, 0.1 μmol of CoA, 1.25 μmol of NADH, 5 μmol of $MgCl_2$, 2.42 μmol of N-acetyl cysteine, 62.5 μmol of NaF, 0.5 μmol of nicotinamide, and 62.5 μmol of phosphate buffer (pH 7) in a total volume of 1.5 mL of 0.15 M KCl, 0.25 M sucrose solution per tube. The reaction was stopped by the addition of 2 mL of 10% (wt/vol) KOH in ethanol. The fatty acids were recovered by saponification of the incubation mixture (45 min at 85°C), followed by acidification and extraction with petroleum ether (boiling point 30 to 40°C). The free fatty acids were first separated by reverse phase high pressure liquid chromatography after the technique of Narce et al.[18] as modified by Garda et al.,[19] and finally quantitated in a liquid scintillation counter (LKB-Wallac Rakbeta 1214, Turku, Finland).

The assay to measure the long chain fatty-acyl-CoA synthetase activity from liver microsomes determined in triplicate was similar to that described by Tanaka et al.[20]

Total fatty acid analysis

Liver microsomal lipids were extracted by the method of Folch et al.[21] After conversion of the fatty acids to their corresponding methyl esters, the latter were analyzed by a Hewlett-Packard Model 5840-A gas-liquid chromatograph (Hewlett-Packard, Palo Alto, CA USA) equipped with a flame-ionization detector, as described previously.[22]

Statistical analysis

The results were tested statistically through a one-way analysis of variance (ANOVA). The statistical differences among the experimental groups were determined using Newman–Keuls multiple range test.[23]

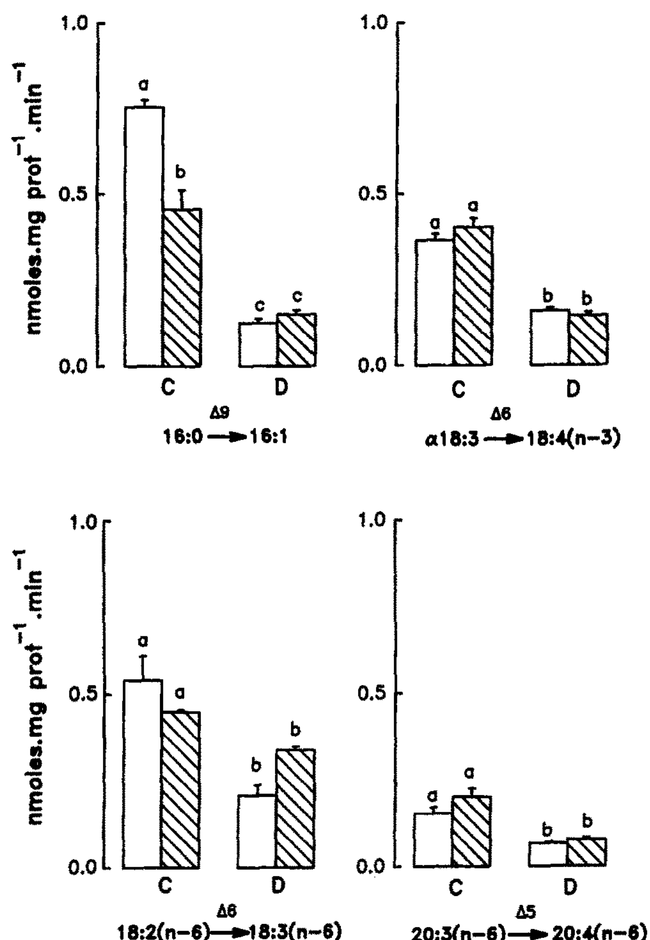

Figure 1 Fatty acid desaturase activity in liver microsomes of control (C) and diabetic (D) rats fed the balanced diet supplemented with n-6 (open columns) and n-3 (hatched columns) fatty acids. Results are the means ± SEM from 5 animals, with each having been analyzed in duplicate. Values not bearing the same superscript letter are significantly different at $P < 0.05$ or less.

Results

Table 2 gives values for body weight and plasma-glucose levels determined both at the beginning of the dietary treatment and at the time of sacrifice (2 weeks later). The body weights of the diabetic rats, 1 week after the injection of STZ (start) were similar to those of the controls. The untreated rats showed a normal increase in weight, which was highly significant at the end of the experiment, regardless of the nature of the diet. By contrast, diabetes was accompanied by a marked weight loss that was similar in both dietary groups.

Table 2 Changes in body weight and blood glucose in control and diabetic rats fed n-6 and n-3 fatty acid supplemented diets for 2 weeks

Body weight (g)	Start	After n-6 consumption	After n-3 consumption
Control	237 ± 12^a	265 ± 9^b	278 ± 11^b
Diabetic	221 ± 13^a	157 ± 9^c	165 ± 10^c
Blood glucose (mg/dL)			
Control	31 ± 7^a	26 ± 7^a	67 ± 4^b
Diabetic	336 ± 14^c	279 ± 27^c	509 ± 24^d

Values (mean ± SEM) not bearing the same superscript letter are significantly different at $P < 0.05$ or less.

Glucose values exceeded 300 mg/dL in the diabetic groups at the beginning of the dietary treatment. The addition of fatty acids from the marine oil produced over a 50% increase in plasma glucose in both control and diabetic rats. No significant changes were observed in those animals fed with fatty acids from corn oil over the experimental period.

Figure 1 illustrates the activity of liver microsomal Δ9, Δ6, and Δ5 desaturases, as measured by the transformation of palmitic to palmitoleic acid, linoleic to γ-linolenic acid, α-linolenic to octadecatetraenoic acid, and eicosa-8,11,14-trienoic to arachidonic acid. Dietary treatment did not significantly alter the activity of the desaturases in the nondiabetic animals except in the instance of Δ9-desaturation activity where the values were found to be lower in the rats fed the fatty acids from cod-liver oil. Diabetes produced a significant decrease in the activity of all desaturases, and

these diminished values were not further affected by the diet.

The long-chain acyl-CoA synthetase activity is shown in *Figure 2*. In the control animals, the thioesterification of labeled eicosa-8,11,14-trienoic acid was higher than that of linoleic acid, and neither value was modified by the diet. In the diabetic state, the synthesis of linoleyl-CoA and eicosatrienoyl-CoA were significantly depressed (by 40 and 50%, respectively) and were likewise not affected by the diet.

The fatty acid composition of liver microsomal lipids from the rats after 14 days of feeding are shown in *Table 3*. Whether normal or diabetic, animals fed the fish-oil fatty acids evinced a significant enrichment in n-3 fatty acids, together with a reciprocal decline in n-6 polyenes, compared with the rats fed the corn-oil polyunsaturates. This reduction in the relative percentages of n-6 fatty acids occurred mainly at the expense of arachidonic acid. Whereas diabetes was accompanied by a decrease in arachidonic acid (25%) in rats fed the vegetable oil, this diminution was considerably larger (38%) and statistically significant in the animals fed the marine-oil diet. Moreover, within these experiments the diabetic rats evinced the following overall changes relative to the control group: in spite of an elevation in oleic-acid levels in those animals, their unsaturation index was significantly lower under either dietary regime. By contrast, upon ingestion of the marine oils, the diabetic animals manifested increases in stearic acid as well as the polyunsaturates 20:5, 22:5, and 22:6. Finally, both the diabetic and the normal rats responded to the marine-oil diet with an enrichment in acids of the n-3 family within their hepatic membranes and a small enhancement of unsaturation index (*Table 3*).

Discussion

Animal growth during the 2-week experimental period, as measured by the increase in total body weight, was significantly less in the diabetic rats than in the controls. That this decrease was a consequence of the diabetic state per se was shown by the lack of any compensatory effect by the diet (i.e., the feeding of n-6 versus n-3 fatty acids) on this parameter. This observation furthermore served to corroborate the findings from an earlier report by others in which no changes in weight gain were observed in normal versus diabetic rats followed by dietary manipulations with n-6 and n-3 fatty acids after even longer periods of experimental treatment.[24]

An elevation of plasma-glucose levels in normal and diabetic rats alike by dietary n-3 fatty acids had been previously noted by several authors.[25] Considering the results that associate glucose homeostasis with macrovascular complications are controversial,[26,27] the usefulness of fish oil to prevent cardiovascular disease would not be discarded.

A significant decrease in the desaturation of palmitic acid to palmitoleic acid observed in nondiabetic rats after n-3 fatty acid feeding had been previously shown by Garg et al.,[28] using fish-oil dietary treatments. In our experiments, this effect was also observed in the control group and did not affect the total fatty-acid pattern in the microsomes.

In the control rats, neither the Δ6 nor the Δ5 desaturation activity in liver microsomes was affected by the type of dietary fatty acid fed. These results are in accordance with Mahfouz et al.[29] who reported no changes in liver microsomal Δ6 desaturase activity following feeding of a diet rich in linoleic acid. However, their observations differ from those in which an inhibitory effect of dietary n-3 fatty acids on Δ6 and Δ5 desaturases activities was shown in isolated microsomes.[30–32] In all of these studies fish oils were used. When, however, rather than these marine lipids, the diet contained highly purified eicosapentaenoic acid and docosahexaenoic acid, the inhibitory effect on the desaturase activities was either reduced or else abolished entirely.[33]

In STZ-diabetic rats, before the onset of dietary treatment, a significant depression in microsomal desaturation and in polyunsaturated fatty-acids thioesterification, was observed as compared with control values. These results are in accordance with previous observations in which an impairment of desaturase[1–3] and long-chain fatty acyl CoA synthetase[4] activities were described. The supplementation of n-3 fatty acids to diabetic rats suppressed the activity of linoleyl desaturase (24.5%) to a lesser extent than did the addition of n-6 fatty acids (61.1%).

In the group of rats fed fish oil, the decreased content of 20:4 (n-6) and the increased levels of 20:5 (n-3) and 22:6 (n-3) would indicate that the fatty-acid composition of the liver microsomes is sensitive—especially in the diabetic animals—to modifications of the dietary lipids for a moderate period of time (i.e., 2 weeks). Although these changes are of a statistically significant magnitude in the diabetic group, they fail to attain that degree in the normals. Moreover, in neither set of animals were the linoleic acid contents altered to a significant extent; thus indicating that, especially within the nondiabetic cells, there exists a precise homeostatic control of the polyunsaturated fatty-acid spectrum, regulation of which may become somewhat relaxed at the onset of diabetes. Thus, within the normal cells, for example, a certain constitutive quantity of essential fatty acid, i.e., linoleic and arachidonic, is retained despite the extremely low percentage of the first of these acids in the marine diet.

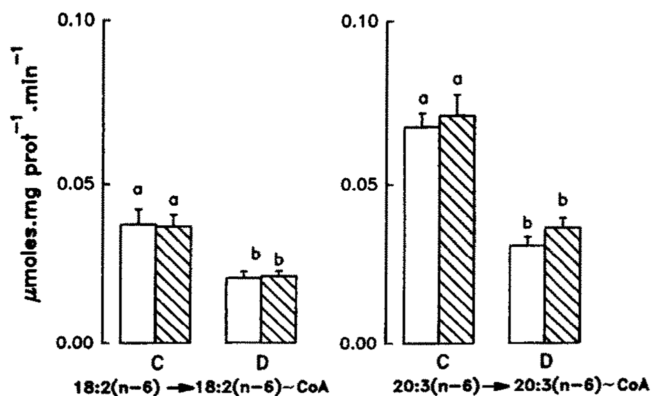

Figure 2 Fatty acyl-CoA-synthetase activity in liver microsomes of control (C) and diabetic (D) rats fed the balanced diet supplemented with n-6 (open columns) and n-3 (hatched columns) fatty acids. Results are the means ± SEM from 5 animals, which each having been analyzed in triplicate. Values not bearing the same superscript letter are significantly different at $P < 0.05$ or less.

Table 3 Effect of dietary fatty acids on the fatty acid composition of liver microsomes in control and diabetic rats

	Control		Diabetic	
Fatty acid	n-6	n-3	n-6	n-3
16:0	21.3 ± 0.2[a]	23.7 ± 0.7[b]	17.4 ± 1.0[a,c]	19.8 ± 0.3[c]
16:1	1.3 ± 0.2	1.6 ± 0.3	0.9 ± 0.1	1.2 ± 0.1
18:0	23.1 ± 0.9[a]	16.2 ± 0.7[b]	23.8 ± 2.3[a]	22.6 ± 1.1[a]
18:1	7.5 ± 0.5[a]	10.3 ± 0.2[b]	11.0 ± 0.6[b,c]	12.9 ± 0.5[c]
18:2 (n-6)	9.8 ± 0.4	10.0 ± 0.4	12.8 ± 1.1	9.2 ± 0.6
18:3 (n-6)	0.4 ± 0.03	0.5 ± 0.06	0.9 ± 0.2	0.6 ± 0.06
18:3 (n-3)	0.7 ± 0.1[a]	1.0 ± 0.2[a,b]	1.5 ± 0.3[b]	1.1 ± 0.1[a,b]
20:3 (n-6)	1.3 ± 0.1	2.1 ± 0.5	2.1 ± 0.3	1.5 ± 0.08
20:4 (n-6)	22.4 ± 1.8[a]	17.6 ± 1.5[a]	16.7 ± 2.2[a]	10.9 ± 0.5[b]
20:5 (n-3)	tr	2.9 ± 0.1[a]	tr	3.6 ± 0.2[b]
22:4 (n-6)	3.9 ± 0.8[a]	2.9 ± 0.4[a]	4.9 ± 0.9[a]	1.5 ± 0.08[b]
22:5 (n-6)	1.1 ± 0.1[a]	0.6 ± 0.2[a,b]	1.8 ± 0.2[c]	0.8 ± 0.04[b]
22:5 (n-3)	2.0 ± 0.4[a,b]	2.3 ± 0.0[a]	2.0 ± 0.3[a]	3.3 ± 0.2[b]
22:6 (n-3)	5.2 ± 0.8[a]	8.3 ± 0.8[a]	4.2 ± 1.7[a]	11.0 ± 0.4[b]
20:4 (n-6)/18:2 (n-6)	2.29	1.76	1.30	1.18
Total n-6	38.9	33.7	39.2	24.5
Total n-3	7.9	14.5	7.7	19.0
U.I.	1.88	2.04	1.82	1.95

Results are the means ± SEM expressed as μg% of total fatty acids. Values not bearing the same superscript letter are significantly different at $P < 0.05$ or less. U.I.: unsaturation index = (en^1x^1/FA), where n^1 = number of double bonds in each fatty acid, x^1 = moles of each fatty acid, FA = total moles of fatty acids.

In the diabetic rats fed fatty acids from cod-liver oil, the failure to accumulate linoleic acid despite depressed Δ6 and Δ5 desaturase activities relative to normal animals might well be explained by the decreased amount of 18:2 (n-6) in the diet itself. Nevertheless, according to our data, the 20:4 (n-6)/18:2 (n-6) ratio, still used by some authors as an indirect index of relative Δ6 and/or Δ5 desaturation activity, indicated a reduction of 23% in the control group and 9% in the diabetic rats in the presence of the fish-oil diet. Since, however, the dietary manipulation examined here produced no changes in the enzymic activities of Δ6 and Δ5 desaturases, we would conclude that a depression by as great as 23% in the 20:4 (n-6)/18:2 (n-6) ratio does not necessarily indicate with certainty an inhibition of these desaturases.

In conclusion, the most significant effect observed in these studies as a result of the dietary consumption of high levels of n-3 polyunsaturates was a modification of the lipid composition of the hepatic membranes. These changes, however, were not accompanied by alterations in the specific activities of microsomal membrane-bound enzymes implicated in polyunsaturated fatty-acid biosynthesis, either in control or in diabetic rats. For this reason, the impairment in the desaturation activities observed in the diabetic rats could not be said to depend on the nature of the fatty acids inherent in the lipid membranes. Furthermore, a more direct verification of this notion is found in the similarity between the fatty-acid profiles of the normal and diabetic animals on the n-6 polyunsaturated diet. Indeed, of the four merely marginal differences (i.e., 16:0, 18:1, 22:5 (n-6), and 22:5 (n-3)), only two either attained statistical significance (18:1 and 22:5 (n-6)) or involved species generated from the essential fatty-acid families (22:5 (n-6) and 22:5 (n-3)). In this regard, Pugh et al.[34] proposed that the activity of the desaturases was inversely related to the degree of membrane-lipid unsaturation. Considering that, in the present work, the unsaturation index was lower in the groups fed n-6 than in those fed n-3 fatty acids, we are forced to conclude that the Pugh et al.[34] relation does not hold since in our example the desaturase activities are the same for both sets of groups; and on the basis of this and all the above data, we would infer that membrane unsaturation per se cannot be responsible for the drop in either the desaturase or the thioesterase values accompanying diabetes.

Finally, the results from these experiments conclusively demonstrate that a moderate dietary intake of essential fatty acids of the n-3 family, as obtained and purified from cod-liver oil, had no beneficial effects on the enzymatic activities involved in polyunsaturated fatty-acid biosynthesis in the livers of diabetic rats. Whether or not dietary n-3 fatty acids might favorably influence other pathways of lipid metabolism otherwise adversely affected by diabetes is a possibility requiring further investigation.

Acknowledgments

The authors thank M.C.P. de Stringa for excellent technical assistance. Casein was kindly provided as a gift by SanCor Cooperativas Unidas Ltd., Santa Fe, Argentina. This work was supported by grants from CONICET, Argentina and EFAMOL Research Inc., Canada.

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