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Arachidonic acid deficiency in streptozotocin-induced diabetes

(polyunsaturated fatty acid)

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ABSTRACT **Fatty acid compositions of phospholipids of heart, liver, kidney, aorta, and serum from rats having streptozotocin-induced diabetes were determined and compared with those of nondiabetic controls. Linoleic and dihomo-γ-linolenic acids were increased whereas arachidonic acid was decreased in most tissues, suggesting an impairment of Δ5-desaturase activity. Acids derived from linolenic acid were increased in some diabetic tissues from diabetic animals although the linolenic content was normal, indicating less impairment in the desaturation of the ω3 series of fatty acids. Diabetes suppressed all polyunsaturated acids in the whole animal, but the competition between ω3 and ω6 acids favored the excessive suppression of long-chain ω6 acids and an increase in the proportion of ω3 acids in lipids of vital tissues. These changes in fatty acid composition of the phospholipids may have significant effects on cellular functions and vasoregulatory control mechanisms in diabetes.**

Involvement of essential fatty acids (EFA) in diabetes was suggested by the observation that alloxan-induced diabetes accelerated and intensified symptoms of EFA deficiency (1). Changes in fatty acid composition have been reported in both humans (2–4) and animals with diabetes (5–8). Decreased arachidonic acid in serum is correlated with hyperglycemia (2, 3) and with increased clinical complications. In diabetic rats, decreased arachidonic, palmitoleic, and oleic acids and increased linoleic and docosahexaenoic acids have been found in liver microsomal lipids (8). Conversely, diabetic rats fed an EFA-deficient diet manifest fewer biochemical changes and a higher level of arachidonic acid than do nondiabetic EFA-deficient controls (9). Many changes in fatty acid patterns of diabetic tissues can also result from dietary changes because mammalian cells require exogenous precursors of the long-chain polyunsaturated fatty acids (PUFA) (10, 11). Linoleic acid (18:2ω6)§ and γ-linolenic acid (18:3ω3) are the precursors of the ω6 and ω3 families of PUFA, which are important structural components of tissue lipids, which in turn are precursors of prostaglandins (PGs) and other products of PUFA oxidation important in the regulation of metabolism.

This report describes the fatty acid composition of phospholipids (PLs) from several organs of rats made diabetic by streptozotocin, emphasizing the fatty acids of the ω6 and ω3 series, both known to be essential in humans (11). These two families of PUFA have parallel pathways of metabolism leading to different vasoactive substances (12), which may be related to the pathogenesis of diabetic vascular disease (13, 14).

METHODS

Male Sprague–Dawley rats, 6 to 7 wk old (Holtzman, Madison, WI), previously maintained on Purina Lab Chow, were used in all experiments. This diet contained 5.3% extractable lipids with the following fatty acid composition: 14:0, 2.7%; 16:0, 19.4%; 16:1ω7, 3.1%; 18:0, 8.8%; 18:1ω9, 28.3%; 18:2ω6, 25.9%; 18:3ω3, 2.4%; 20:4ω6, 0.2%; 20:5ω3, 1.7%; 22:6ω3, 1.2%. The lab chow diet provided approximately 2.7 Cal % of linoleic acid (1 Cal = 4.18 J) and 0.25 Cal % linolenic acid.

In experiment 1, diabetic rats and age-matched nondiabetic controls were fed the chow diet throughout the study to assay whether diabetes influenced the PUFA pattern of rats on a standard rat ration composed of natural feedstuffs. The rats were made diabetic at 49 days of age and killed after 61 days of diabetes. Samples of heart, serum, liver, whole kidney, and abdominal aorta were analyzed.

In experiment 2, defined diets were used to provide either high 18:2ω6 or high ω3 acids. The basic diet contained 26 parts vitamin-free casein, 4 parts cellulose, 46 parts purified corn starch, 4 parts American Institute of Nutrition mineral mix 76, and 15 parts fully hydrogenated coconut oil. To a 1-wk supply of this diet was added 5 parts safflower oil and the safflower oil diet was refrigerated to minimize rancidity. All rats were fed the safflower oil diet 1 wk and then half of them were made diabetic at 52 days of age. Half of the diabetic and age-matched nondiabetic rats were continued on the safflower oil diet, and fresh portions were fed daily. The cod liver oil groups, diabetic and age-matched nondiabetic controls, were fed the basic diet and were given 5 parts of cod liver oil by gavage to avoid autoxidation of the oil. The rats were killed after 165 days of diabetes. Heart and liver were analyzed.

In experiment 3, the rats were made diabetic at 54 days and killed after 61 days of diabetes. The dietary regimen was the same as in experiment 2, except that the safflower oil diet was fed 2 days prior to injection. Experiments 2 and 3 were carried out to confirm the findings of experiment 1 using defined diets and exaggerated proportions of dietary ω6 and ω3 fatty acids. Heart and liver PL were analyzed.

In experiment 4, five rats fed the chow diet were made diabetic at 47 days, continued on the chow diet, killed after 57 days of diabetes, and compared with six nondiabetic controls. The total lipids of whole animals were analyzed for PUFA.

Diabetes was induced by intravenous injection of streptozotocin [1.3% in citric acid buffer (pH 4.5)] at 55 mg/kg (14) followed by injection of 2 ml of 30% glucose intraperitoneally. Nonfasting blood glucose was measured monthly and values for diabetic animals at sacrifice were 589–707 mg/dl (experiment 1), 428–666 mg/dl (experiment 2), and 427–724 mg/dl (experiment 3). Control values of the nondiabetic groups were 92–

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Abbreviations: PL, phospholipids; PUFA, polyunsaturated fatty acids; EFA, essential fatty acids; PG, prostaglandin.

§ The ω system of nomenclature for PUFA is as follows—chain length: number of methylene-interrupted double bonds; ω; number of carbon atoms beyond the last double bond, including the methyl group (i.e., 20:4ω6 is arachidonic acid).

167 mg/dl. Blood glucose levels of the safflower oil and cod liver oil groups of experiments 2 and 3 were not significantly different. At termination of experiments 1–3, rats were exsanguinated under anesthesia, blood was allowed to clot, and serum was removed for analysis. Organs were excised and frozen immediately. The numbers of rats per group ranged from four to eight.

Thawed serum was extracted with methanol/chloroform, 1:1 (vol/vol). The lipid extract was filtered, dried under N_2, and dissolved in 0.1 ml of chloroform. One-gram samples of thawed tissues were homogenized with 50 ml of chloroform/methanol, 2:1 (vol/vol), filtered, and reextracted, and the combined extracts were washed with saturated NaCl. The total extracts were dried over sodium sulfate, filtered, and evaporated to dryness, and the lipids were redissolved in chloroform. Aortas were rinsed with distilled water, minced, and homogenized with distilled water and chloroform/methanol, 2:1 (vol/vol). Extracts were centrifuged, water was removed, the chloroform was evaporated under N_2, and the lipids were redissolved. Each lipid extract was applied to silicic acid-impregnated paper (Gelman Instrument) for TLC. The developing solvent was petroleum ether/diethyl ether/acetic acid, 90:10:1 (vol/vol). Papers were sprayed with 0.1% dichlorofluorescein solution and the bands of lipid were made visible under UV light. The PL were cut out and placed in Teflon-lined screw-capped tubes, 2 ml of 14% BF_3/methanol (wt/vol) was added, and the mixture was heated at 85°C for 1.5 hr. After esterification, the methyl esters were isolated by extraction with petroleum ether.

In experiment 4, each rat was killed by ether anesthesia and hair was removed with a clippers and a depilatory. The carcass was frozen in liquid nitrogen, shattered, and ground in a blender with dry ice. The pulverized rat was lyophilized, the powder was mixed thoroughly in a mortar, and a 10-g aliquot was extracted with 20 parts of chloroform/methanol (2:1). Lipid (10 mg) and 100 μg of 17:0 internal standard were methylated with BF_3/methanol and benzene for 1.5 hr.

Samples from experiment 1 were analyzed on a Packard model 428 gas chromatograph equipped with a flame ionization detector and a 1/8 in × 12 ft (3.18 mm × 3.66 m) aluminum column packed with 10% Silar 10C on 100–120 Gas ChromQ. The temperature was programmed from 160 to 230°C at 3°C/min with a 7-min final hold to separate methyl esters ranging from 12:0 to 22:6ω3. Samples from experiments 2, 3, and 4 were analyzed by capillary gas chromatography on a Packard 428 gas chromatograph using a 50 m × 0.2 mm fused silica column coated with FFAP (Scientific Glass Engineering, Austin, TX) programmed from 190 to 220°C at 2°C/min with a final hold of 30 min. The split ratio was 1:92 and the column flow was 0.77 ml/min. Identification was made by comparisons with known methyl ester standards.

Results were calculated as mean ± SEM, statistical analyses were made by conventional *t* test, and normalcy ratios were constructed by dividing the means for the diabetic groups by the means for the control groups.

RESULTS AND DISCUSSION

Experiment 1. PUFA profiles of PL from heart and liver are shown in Fig. 1, and those of kidney, aorta, and serum are shown in Fig. 2. Because profiles give only ratios of content in diabetic animal and control tissue and do not indicate which fatty acids are major or minor components, the content of individual PUFA that figure in the subsequent discussion are given for two representative tissues. Control heart PL fatty acids contained 18:2ω6, 18.3%; 18:3ω6, <0.01; 20:2ω6, 0.1%; 20:3ω6, 0.3%; 20:4ω6, 22.8%; 22:4ω6, 0.6%; 22:5ω6, 0.2%; 18:3ω3, 0.2%; 20:5ω3, 0.1%; 22:5ω3, 2.2%; 22:6ω3, 8.7%. Control liver PL contained 18:2ω6, 15.8%; 18:3ω6, 0.1%; 20:2ω6, 0.2%; 20:3ω6, 1.0%; 20:4ω6, 26.5%; 22:4ω6, 0.3%; 22:5ω6, 0.1%; 18:3ω3, 0.1%; 20:5ω3, 0.5%; 22:5ω3, 1.6%; 22:6ω3, 6.2%.

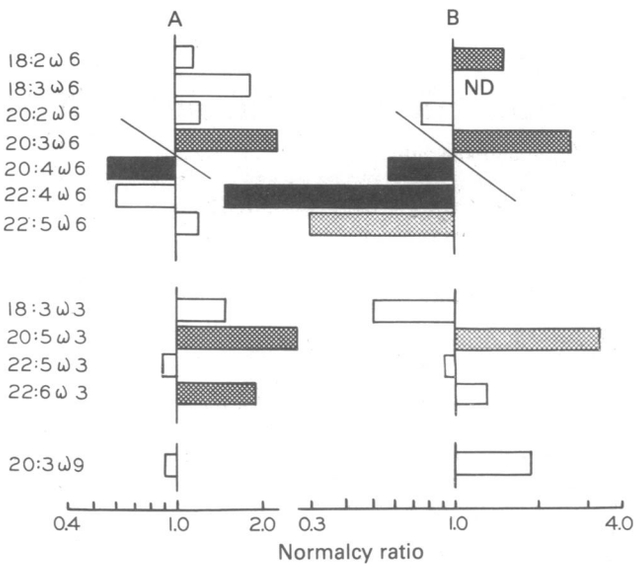

FIG. 1. PUFA profiles of liver (*A*) and heart (*B*) PL of diabetic rats vs. those of nondiabetic controls fed the chow diet (experiment 1). ■, $P < 0.001$; ▩, $P < 0.01$; ▨, $P < 0.05$; □, not significant. ND, not detectable.

Linoleic acid was increased in most tissues from diabetic animals whereas ω6 acids beyond 20:3ω6 in the metabolic sequence were markedly decreased in heart, liver, kidney, aorta, and serum from such animals, suggesting impaired utilization of linoleate. In heart PL from diabetic animals, the ω6 metabolites constituted 14.2 ± 1.4% of total fatty acids compared with 24.0 ± 0.4% in nondiabetic controls. In liver PL, the comparable values were 18.0 ± 1.1% and 28.2 ± 1.1%. Although proportions of γ-linolenic acid (18:3ω3) were not significantly increased in diabetes (except in kidney), at least one of its metabolites was significantly increased in each tissue analyzed, indicating little impairment in utilization of γ-linolenic acid. Oleic acid decreased in heart, aorta, and liver and increased in kidney from diabetic animals. No significant differences in 20:3ω9, stearic acid (18:0), or palmitic acid (16:0) were found in any of the tissues.

Δ6-Desaturation products, 16:2ω7 and 18:3ω6, were unaffected by diabetes except for a decrease in serum. Δ5-Desaturase causes the desaturation of 20:3ω6 to 20:4ω6, of 20:4ω3 to 20:5ω3, and of 20:2ω9 to 20:3ω9. The content of the 20:3ω6 substrate for Δ5-desaturation was found to be increased, and the product, arachidonic acid, was significantly suppressed in the PL of all five tissues. For example, in heart PL, diabetes increased 20:3ω6 to 0.7 ± 0.1% of fatty acids from a control value of 0.3 ± 0.1% ($P < 0.01$), and arachidonic acid was suppressed to 13.3 ± 1.3% of fatty acids from a control value of 22.8 ± 0.6% ($P < 0.001$). In contrast, contents of comparable products in the ω3 series were increased but not consistently nor significantly in the several tissues. Thus, the metabolic defect in streptozotocin-induced diabetes was limited mainly to the Δ5-desaturation of 20:3ω6 to 20:4ω6, consistent with previous reports (5–8), suggesting that the metabolic defect in streptozotocin-induced diabetes may be governed by the ω-structure of the PUFA. The Δ4-desaturation product 22:5ω6 mostly decreased, and the product 22:6ω3 increased in the PL of tissues studied. The C_{20} elongation product 20:3ω6 was in-

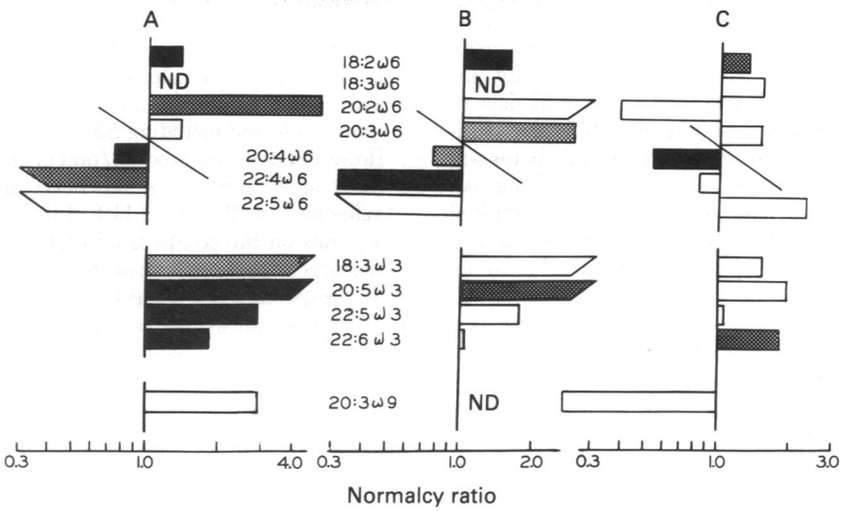

FIG. 2. PUFA profiles of kidney (*A*), aorta (*B*), and serum (*C*) PL of diabetic rats vs. those of nondiabetic controls fed the chow diet (experiment 1). Bars are as in Fig. 1.

creased in all the tissues, indicating the ability to elongate to C_{20} acids was unimpaired, confirming the suppression of the Δ5-desaturase for which it is the substrate.

The kind and proportions of PUFA in a metabolic pool strongly influence the metabolism of 18:2ω6 to 20:4ω6 and of 18:3ω3 to 22:6ω3, and the influence is in the sequence ω3 > ω6 > ω9 (15). Saturated acids have a minor influence, and isomeric monoenoic acids perturb the metabolism of PUFA significantly (16). Even if diabetes suppresses Δ5-desaturation, the competition between ω3 and ω6 acids should favor the synthesis of long-chain ω3 PUFA, accounting for their general increase in the tissues studied. Decreased Δ6- and Δ9-desaturase activities in tissues from diabetic animals have been reported (6, 17, 18) and the Δ9-desaturase was more severely depressed in diabetic liver microsomes and was more responsive to insulin than was Δ6-desaturase (17). The increased proportions of 22:6ω3 found in this study confirm the report that 22:6ω3 increased in diabetic animals before insulin treatment (8). The altered fatty acid composition in diabetic rats was not due to starvation, because fasting of nondiabetic control animals neither induced changes in fatty acid composition nor decreased fatty acid desaturation (8). Insulin therapy reverses and overcorrects the diminished desaturase activities and restores the fatty acid composition, except for the diminished arachidonic acid (8). However, the fatty acid changes are diet related because limiting food intakes to the amounts ingested by control animals diminished the magnitude of the fatty acid changes (8). Thus, fatty acid changes in tissues from diabetic animals result from a complex relationship between diet and insulin deficiency. The arachidonic acid deficiency could likewise result in part from excessive phospholipase A_2 activity and consequent conversion of arachidonate to PG (13, 19).

Assessment of EFA status of humans has been by analysis of serum PL fatty acids (11) assuming that serum PL reflects the EFA status of tissue membranes. This was confirmed by analysis of tissues of one EFA-deficient human taken at autopsy (20). In diabetic rats, the fatty acid profile of serum PL confirms the EFA status of the tissues, although the magnitude of the suppression of arachidonic acid and the buildup of its precursors is less in serum than in other tissues.

Experiments 2 and 3. Significant increases of some ω3 acids in the PL of several tissues prompted comparison of the effects of diabetes on PUFA profile in rats fed diets rich in 18:2ω6 (safflower oil) or in ω3 acids (cod liver oil). Fig. 3 shows that, for the safflower oil diet, the ω6 acids of heart PL prior to arachidonic acid in the metabolic sequence were increased by diabetes and that there were deficiencies of arachidonic and subsequent acids, indicating a metabolic block at the Δ5-desaturation step. On the safflower oil diet, which suppresses the metabolism of γ-linolenic acid, diabetes additionally increased the content of the precursor γ-linolenic acid (not significant, experiment 2; $P < 0.05$, experiment 3) and suppressed the long-chain PUFA products derived from it.

On the cod liver oil diet, which suppresses ω6 metabolism,

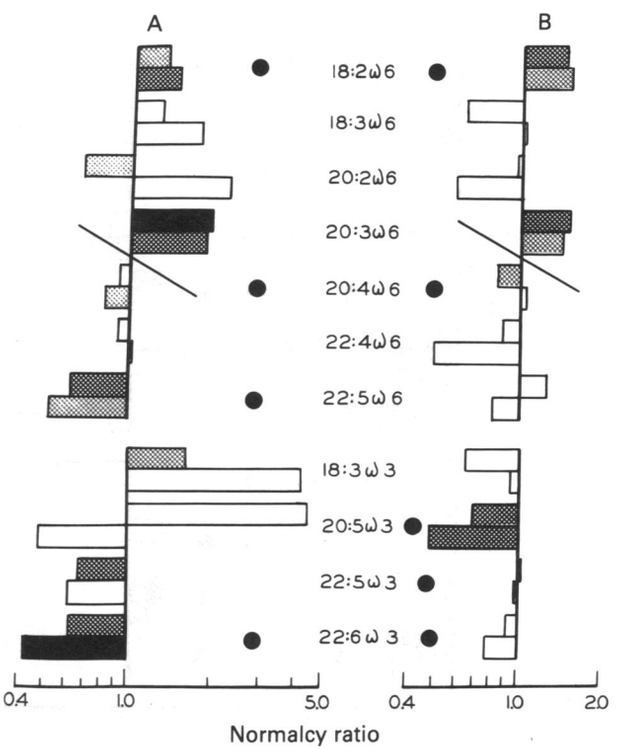

FIG. 3. PUFA profiles of heart PL of diabetic rats vs. those of nondiabetic controls in groups fed either the safflower oil diet (*A*) or the cod liver oil diet (*B*). The upper bar of each pair is from experiment 3 (61 days of diabetes) and the lower bar is from experiment 2 (165 days of diabetes). ●, Major constituent (>2%) in control samples. Bars are as in Fig. 1.

18:2ω6 in heart PL was increased significantly by diabetes and, with the exception of 20:3ω6 and 20:4ω6, all other ω6 acids were not significantly affected. 20:3ω6 was significantly increased by diabetes, and 20:4ω6 was suppressed at 61 days of diabetes but was not significantly abnormal at 165 days of diabetes. In many instances, the effect of duration of diabetes was to exaggerate the abnormalities but, in others, the abnormalities appeared to be transitory. The cod liver oil diet diminished the effect of diabetes on the ω6 acids in heart PL. On the ω3-rich diet, diabetes significantly suppressed 20:5ω3, the Δ5-desaturase product in the ω3 series.

In diabetic rats fed the linoleate-rich diet (safflower oil), liver PL had slightly increased ω6 acids through 20:3ω6 but suppressed arachidonic acid (Fig. 4). Elongation to 22:4ω6 and Δ4-desaturation to 22:5ω6 were enhanced. The ω3 acids were also generally enhanced by diabetes, but significantly so for the final product 22:6ω3, a major PUFA. In rats on the cod liver oil diet, 18:3ω6 and 20:3ω6 were enhanced significantly only after 165 days of diabetes. Arachidonate was decreased very significantly at 61 days but was within normal range by 165 days. Diabetes suppressed all metabolites of γ-linolenic acid except 22:6ω3 at 61 days, suggesting that Δ5-desaturation was suppressed in the ω3 sequence.

The effect on ω3 and ω6 metabolism can also be illustrated by product/precursor ratios. In rats on the linoleate-rich diet, the 20:4ω6/20:3ω6 ratio was decreased by diabetes to 20.4 ± 1.2% compared with 32.4 ± 2.6%, 63% of the control value ($P < 0.01$) in liver PL. In heart PL, diabetes of 165 days duration (experiment 2) decreased the ratio to 44% of the control value, 26.0 ± 4.1% and 59.3 ± 5.5% ($P < 0.01$). In rats on the ω3-rich diet, this ratio was 67% of the control value for liver ($P < 0.05$) and 72% of the control value (not significant) for heart.

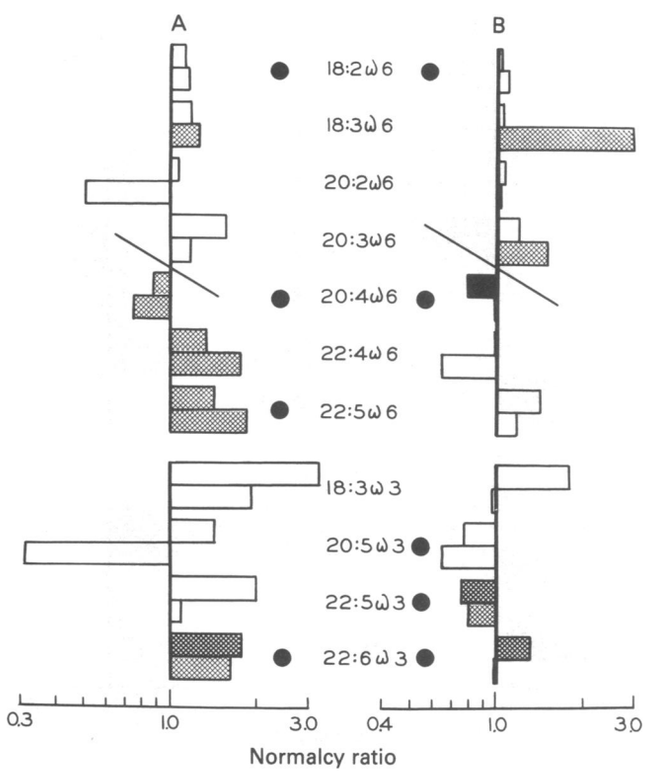

FIG. 4. PUFA profiles of liver PL of diabetic rats vs. those of nondiabetic controls in groups fed either the safflower oil diet (*A*) or the cod liver oil diet (*B*). The upper bar of each pair is from experiment 3 (61 days of diabetes) and the lower bar is from experiment 2 (165 days of diabetes). Symbols are as in Figs. 1 and 3.

Thus, despite the type of diet, the Δ5-desaturation of 20:3ω6 to 20:4ω6 was suppressed. The comparable ratio, 20:5ω3/20:4ω3, cannot be calculated because 20:4ω3 was not detectable, but the ratio, 20:5ω3/18:3ω3, evaluates overall reactions, up to and including Δ5-desaturation. For rats on the safflower oil diet, the product/precursor ratio of 20:5ω3/18:3ω3 (calculated from means) was decreased to 17% of the control value for liver PL and to 11% of the control value for heart PL. For rats on the cod liver oil diet, this ratio was decreased by diabetes to 65% and 52% of the control values for liver and heart PL, respectively. Irrespective of the ω3 and ω6 nutritional status of the animals, the product/precursor ratio 20:5ω3/18:3ω3 was decreased by diabetes.

Rats that had had diabetes for 165 days had increased total ω3 acids, from 2.2 ± 0.2% in controls to 3.3 ± 0.6% of liver PL, and reduced ω3 acids, from 2.8 ± 0.1% to 1.4 ± 0.2% of heart PL, in the group fed the safflower oil diet. In the groups fed the cod liver oil diet, 28.0 ± 2.4% of the liver PL in controls and 24.2 ± 0.5% in the diabetic animals were ω3 acids. In heart, the values were 25.3 ± 1.7% and 19.1 ± 2.6%. In experiment 1, which represents a more typical diet for the rat and provides a more optimal dietary ratio of ω3 to ω6 acids, the percentages of total fatty acids represented by ω3 acids in control and diabetic animals respectively, were 8.6 ± 0.6 and 14.9 ± 1.2 for liver PL and 11.3 ± 0.7 and 13.4 ± 1.1 for heart PL. Clearly, the magnitude and direction of effect of diabetes upon the proportions of ω3 acids is mediated by diet.

The changed proportions of the ω6 and ω3 fatty acids in tissues from diabetic animals may have significant biologic implication because they are precursors of different PGs and other oxidation products of the PUFA having different biological activities (12, 21). Linoleic acid is converted to 20:4ω6, which is the precursor of the common prostacyclin, PGI_2. When ω3 fatty acids predominate over ω6 fatty acids, thromboxane synthesis is diminished (12, 21). Thus, the changed proportion of the ω3 fatty acids in diabetes may influence the magnitude of thromboxane and prostacyclin synthesis.

Experiment 4. All the phenomena reported above were changes in relative proportions (percentages) of fatty acids of PL of selected tissues. Changes in profile represent local deficiencies or excesses of PUFA and, for the functions of these tissues, these alterations may have profound consequences, especially with respect to synthesis of the PGs and other oxidation products of PUFA. However, the phenomena discussed above could be merely disproportions of PUFA between lipid classes or between tissues, rather than deficiencies of PUFA in the whole animal. To test whether diabetic rats were arachidonate-deficient as a whole, analyses were made of the PUFA of the total lipids of the entire rat (Fig. 5, experiment 4). The diabetic rats contained 1.94 ± 0.23 g of total PUFA and the control rats contained 6.44 ± 0.49 g. When normalcy ratios were constructed from milligrams of each PUFA per rat it was clear that the individual and composite ω6 and ω3 acids were suppressed by diabetes of 57 days duration. Diabetic rats are smaller than control rats but, even when PUFA were expressed as milligrams per gram of rat, the deficiencies of PUFA in diabetic rats remained, although with somewhat lesser significance. The diabetic rats contained 6.33 ± 0.75 mg of PUFA/g of tissue and the controls contained 13.12 ± 1.16 mg. Thus, the diabetic rats developed general deficiencies of ω6 and ω3 fatty acids, upon which was superimposed a suppression of Δ5-desaturase products to different degrees in vital tissues. The most striking deficiency was a relative arachidonic acid deficiency of significant proportions in several vital tissues. Considering that arachidonic acid is a major acid in PL of those tissues, the mass change of that acid is considerable in those tissues.

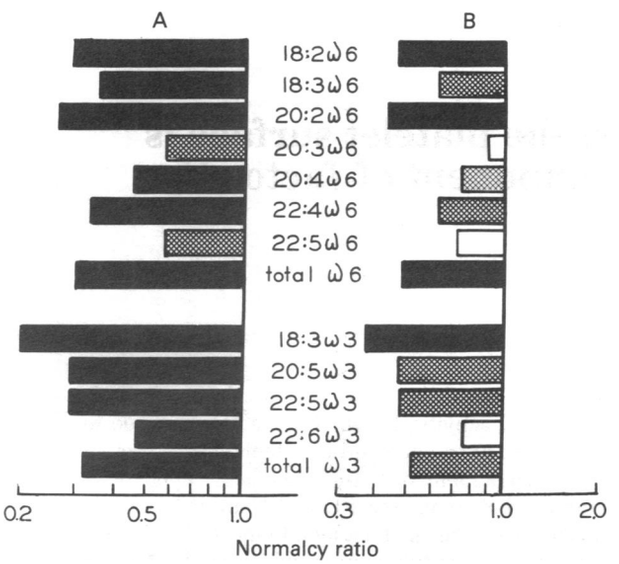

FIG. 5. Effect of diabetes on PUFA profiles for total rat lipids expressed as normalcy ratios calculated from mg of fatty acid per rat (*A*) and from mg of fatty acid per g of tissue (*B*).

Taken in the context of altered PG metabolism in tissues of diabetic animals (19, 22) and of altered physical properties of the cell membranes of such animals (23), changes in fatty acid composition of the PL in these tissues could have significant effects on pathogenic mechanisms. The recent report of altered membrane fluidity of lymphocytes from diabetic subjects, measured by the fluorescence polarization technique, suggests that factors that influence fluidity of lipid membranes (24) may operate in diabetes. These may include alterations of cholesterol content, the proportions of PUFA in PL, the ratios of PL classes, and the nature of proteins in the lipid layer. Altered fatty acid composition of cell membrane lipids can influence membrane-associated phenomena such as lectin-mediated mitogenesis and immunoglobulin capping in lymphocytes (25) and properties of neuroreceptors of brain (26). Altered lipid content and organization of lymphocytes may contribute to the altered immune functions found in cells of diabetic animals (27).

If defective Δ5-desaturation is a fundamental feature of diabetes, perhaps the disease may yield to dietary treatments involving PUFA.

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