Plasma, Platelet, and Aorta Fatty Acids Composition in Response to Dietary $n$-6 and $n$-3 Fats Supplementation in a Rat Model of Non-Insulin-Dependent Diabetes

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Summary Male Sprague-Dawley rats were injected with 90 mg/kg of streptozotocin at 2 days of age. After weaning, they were put on a fat-free diet supplemented with safflower oil (S), a combination of S and linseed oil (L) or a combination of evening primrose oil (E) and L for 8 weeks. Plasma glucose levels and glycosuria were significantly elevated in all 3 groups of diabetic rats in comparison with the corresponding control rats. The percentage of arachidonic acid (20:4n-6) in plasma phospholipids of the S+L and E+L groups was similar to that of the S group and did not differ between control and diabetic rats while adrenic acid (22:4n-6) and docosahexaenoic acid (22:6n-3) changed in proportion to dietary $n$-3 and $n$-6 fats content. Arachidonic acid in aorta phospholipids significantly reduced in all 3 groups of diabetic rats as compared to the corresponding control groups. Dihomo-gamma-linolenic acid (20:3n-6) and arachidonic acid in aorta phospholipids increased by the E+L treatment. These results suggest that arachidonic acid in plasma phospholipids is kept constant regardless of the presence of diabetes of non-insulin-dependent type or dietary $n$-3 and $n$-6 fats supplementation. In aorta phospholipids, arachidonic acid in diabetic animals reduced and this may be compensated by gamma-linolenic acid supplementation, which leads to increase of dihomo-gamma-linolenic acid and arachidonic acid levels.

Key Words essential fatty acids, arachidonic acid, dihomo-gamma-linolenic acid, diabetes, aorta, dietary fat, rat

Linoleic acid (LA; 18:2n-6) is the parent fatty acid for $n$-6 fatty acids synthesis. Its desaturated and elongated metabolites, dihomo-gamma-linolenic acid (DGLA; 20:3n-6) and arachidonic acid (AA; 20:4n-6) act as precursors of 1- and 2-series...
prostaglandins, respectively, and as determinants of membrane physical properties (1, 2). Arachidonic acid level in plasma phospholipids is controlled within a narrow range and its level in human blood is thought to reflect essential fatty acids (EFAs) status of an individual (3). N-3 fatty acids are relatively minor components in plasma, but are found in abundance in retina, brain, and heart phospholipids mainly as docosahexaenoic acid (DHA; 22:6n-3)(4). Their parent fatty acid is alfa-linolenic acid (ALA; 18:3n-3). LA and ALA are thought to compete with each other when metabolized to AA and DHA, respectively (5, 6).

Increased EFAs requirement and defective AA synthesis have been well documented in experimentally induced diabetic rats (7, 8). Decreased activity of delta-6-desaturase, which is a strongly insulin-dependent enzyme and converts LA to gamma-linolenic acid (GLA; 18:3n-6), seems to be responsible (8). Streptozotocin treatment in adult rats is the most frequently used animal model of diabetes. In this model of diabetes, insulin production is completely abolished and glucose metabolism is severely impaired. Animals show extreme hyperlipidemia and weight reduction (9), which themselves might affect EFAs metabolism (10, 11). Recently, an animal model for non-insulin-dependent diabetes was described (12). The animals are moderately hyperglycemic and show impaired insulin biosynthesis (13) but are normolipidemic and show normal weight gain (14). In the present study, the effects of combination of dietary n-6 and n-3 fats on the EFAs composition in plasma, platelet, and aorta phospholipids were examined in this model of diabetes.

MATERIALS AND METHODS

Pregnant Sprague-Dawley rats (Canadian Hybrid Farms, Halls Harbour, Nova Scotia, Canada) had free access to Purina Rodent Chow (No. 5001, Ralston Purina Co., St. Louis, Mo.) and water. Two-day-old male neonates from seven randomly chosen litters were injected intraperitoneally with 90mg/kg streptozotocin (Sigma Chemical Co., St. Louis, Mo.) in 0.1 M citrate buffer, pH 4.5. Control rats were injected with an equivalent volume of citrate buffer. Animals were weaned at 3 weeks of age and then both control and diabetic rats were randomly divided into 3 groups and received a fat-free diet (Teklad Test Diets, Madison, Wis.) supplemented with either 10% by weight of safflower oil (S, commercial source), a combination of each 5% by weight of S and linseed oil (L, commercial source) or a combination of each 5% by weight of evening primrose oil (E; Efamol Ltd., Guildford, England) and L. The fat-free diet contained 70.7% sucrose, 19.3% vitamin-free casein, 5.0% cellulose, 3.5% mineral mix (AIN-76), 1.0% vitamin mix (AIN-76A), 0.3% DL-methionine, and 0.2% choline chloride. The fatty acid compositions of the rodent chow and the supplemented oils are shown in Table 1.

At 11 weeks of age, the animals were killed. Blood was collected by cardiac puncture under ether anesthesia into plastic syringes and anticoagulated using 9 vol. of blood with 1 vol. of 3.8% trisodium citrate. The thoracic aorta was removed,
Table 1. Fatty acid composition of the rodent chow and the supplemented oils.

| Fatty Acid | Chow | Oil supplemented |
|------------|------|------------------|
|            | S    | L    | E |
| 14:0       | 1.5  | 0.2  | —  |
| 16:0       | 21.9 | 6.8  | 5.2 | 6.4 |
| 16:1       | 3.1  | —    | 0.4 | —  |
| 18:0       | 9.9  | 2.4  | 3.3 | 1.7 |
| 18:1n-9    | 32.2 | 11.5 | 21.3| 8.6 |
| 18:2n-6    | 24.3 | 78.4 | 20.4| 73.8|
| 18:3n-3    | 3.7  | —    | 48.8| —  |
| 18:3n-6    | —    | —    | —  | 9.1 |
| Others     | 3.4  | 0.7  | 0.5 | 0.3|

The rodent chow contained 4.5% fat.

trimmed, rinsed in saline, blotted, and frozen at −20°C. Platelets and plasma were separated by centrifugation, washed by calcium-free Tyrode’s buffer, and frozen at −20°C (15). Plasma, platelet, and aortic lipids were extracted by the method of Bligh and Dyer (16). The phospholipid fractions were separated by thin-layer chromatography, methylated, and subjected to gas-liquid chromatography for determination of fatty acid composition as previously described (17). A 10% Silar 10C on Gas Chrom Q column was used with a Hewlett Packard 5880A machine with automated integration. Identification was based on retention time with respect to standard methyl ester mixtures (Nu-Chek Preps, Elysian, Minn.).

Plasma and urine glucose concentrations and plasma cholesterol and triglyceride levels were determined enzymatically using a Cobas-Bio centrifugal analyzer (Hoffmann-La Roche, Nutley, N.J.).

Statistical analysis was performed using the Tukey-Kramer test preceded by ANOVA (18) while the difference between control and the corresponding diabetic rats was assessed by Student’s t-test.

RESULTS

General

At 11 weeks of age, body weight and food intake of streptozotocin-treated rats fed on the S (mean ± SD; 315 ± 25 g, 18 ± 3 g/day) or S + L (306 ± 24 g, 18 ± 3 g/day) diet did not differ from those of the corresponding controls (S: 330 ± 20 g, 17 ± 3 g/day; S + L: 329 ± 19 g, 18 ± 3 g/day) whereas diabetic rats fed on the E + L diet showed reduced body weight and increased food intake (291 ± 25 g, 23 ± 4 g/day) as compared to the corresponding controls (326 ± 16 g, 19 ± 3 g/day, p < 0.05). Plasma glucose levels in all 3 groups of diabetic rats were significantly higher than those in the corresponding control groups, but plasma total cholesterol
Table 2. Plasma glucose, cholesterol, and triglyceride concentrations in control and diabetic rats fed for 8 weeks on different lipid diets.

| Diet          | S           | S + L        | E + L        |
|---------------|-------------|--------------|--------------|
| Glucose, mg/100 ml |             |              |              |
| Control       | 147 ± 9*    | 152 ± 15*    | 149 ± 14*    |
| Diabetic      | 176 ± 21    | 184 ± 18     | 200 ± 26     |
| Cholesterol, mg/100 ml |         |              |              |
| Control       | 47 ± 9a,b   | 56 ± 8a      | 38 ± 7b      |
| Diabetic      | 53 ± 9      | 50 ± 6       | 49 ± 10      |
| Triglyceride, mg/100 ml |         |              |              |
| Control       | 126 ± 37a   | 79 ± 19b     | 85 ± 15b     |
| Diabetic      | 110 ± 29    | 104 ± 32     | 96 ± 30      |

Each value represents the mean ± SD of samples from 5–7 rats. Means in a horizontal row that do not share a common superscript letter differ significantly (p < 0.05 or better). *p < 0.05 or better; control vs. diabetic rats.

Table 3. Fatty acid composition (mg/100 mg total fatty acids) of plasma phospholipids from control and diabetic rats fed for 8 weeks on different lipid diets.

| Diets       | S           | S + L        | E + L        |
|-------------|-------------|--------------|--------------|
| Control     |             |              |              |
| 16:0        | 22.6 ± 1.6  | 23.5 ± 1.4a  | 24.0 ± 2.2   | 18.8 ± 1.9b  |
| 18:0        | 22.9 ± 1.7  | 21.3 ± 1.8a  | 22.5 ± 1.6   | 24.9 ± 1.0   | 25.0 ± 1.3b  |
| 18:1        | 6.1 ± 1.1a  | 6.5 ± 0.8    | 5.9 ± 0.9a   | 5.7 ± 1.0    | 4.6 ± 0.6b   | 5.6 ± 0.5    |
| 18:2n-6     | 15.0 ± 2.4ab| 16.5 ± 0.9a  | 16.5 ± 1.9a  | 13.7 ± 1.4b  | 19.3 ± 2.3b* |
| 18:3n-3     |             |              |              |              |              |
| 20:3n-6     | 0.7 ± 0.3   | 0.6 ± 0.3a   | 0.7 ± 0.2    | 0.8 ± 0.2    | 1.2 ± 0.4b   |
| 20:4n-6     | 26.4 ± 1.3  | 26.4 ± 2.3   | 24.4 ± 2.7   | 26.8 ± 1.6   | 24.2 ± 2.0   |
| 20:5n-3     |             |              | 0.5 ± 0.1a   | 0.4 ± 0.1a   | 0.4 ± 0.1b   | 0.3 ± 0.1b   |
| 22:4n-6     | 1.1 ± 0.1a  | 1.2 ± 0.1a   |             |             | 0.3 ± 0.1b   |
| 22:5n-6     | 4.2 ± 0.8   | 2.8 ± 0.6    |             |             | 2.8 ± 0.6    |
| 22:5n-3     |             |              | 1.6 ± 0.2    | 1.5 ± 0.2    | 1.1 ± 0.2    | 1.4 ± 0.3    |
| 22:6n-3     | 0.3 ± 0.1a  | 0.3 ± 0.0a   | 5.3 ± 0.6b   | 4.6 ± 0.6b   | 5.0 ± 0.2b   | 3.6 ± 1.1b   |
| 18:2n-6/metabolites | 0.46 | 0.53 | 0.66 | 0.67 | 0.49 | 0.75 |

Each value represents the mean ± SD of samples from 5–7 rats. Means in a horizontal row that do not share a common superscript letter differ significantly (control: a, b; diabetic: a', b', p < 0.05 or better). *The sum of 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6. *p < 0.05 or better; control vs. diabetic rats.

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and triglyceride levels did not differ between control and diabetic rats on any dietary regimen (Table 2). In control rats, plasma total cholesterol in the E+L group was lower than in the S+L group and plasma triglyceride in the S+L and E+L groups was lower than in the S group, but these effects were not observed in diabetic rats.

**Fatty acid analysis**

**Plasma phospholipids.** AA content did not differ between control and diabetic rats, or between different fat diets (Table 3). Minor differences were observed for palmitic acid (16:0) and stearic acid (18:0) composition among the diabetic groups and for oleic acid (18:1) composition among the control groups. Dietary ALA supplementation (the S+L and the E+L groups) increased eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid (22:5n-3), and DHA and led to an absence or remarkable reduction of adrenic acid (22:4n-6) and docosapentaenoic acid (22:5n-6) content. In diabetic rats fed the E+L diet, compositions of LA and DGLA were significantly higher than those in the S and the S+L groups.

**Aorta phospholipids.** Palmitic acid was lower and stearic acid and LA were higher in the E+L fed diabetic rats as compared to the corresponding controls (Table 4). On all 3 dietary regimens, AA content significantly decreased in diabetic rats in comparison with the controls. Adrenic acid and docosapentaenoic acid

### Table 4. Fatty acid composition (mg/100mg total fatty acids) of aorta phospholipids from control and diabetic rats fed for 8 weeks on different lipid diets.

| Diets      | Control | Diabetic | Control | Diabetic | Control | Diabetic |
|------------|---------|----------|---------|----------|---------|----------|
| 16:0       | 20.4 ± 1.5 | 19.0 ± 1.0 | 20.6 ± 0.9 | 19.1 ± 1.6 | 21.9 ± 1.9 | 17.9 ± 1.2* |
| 18:0       | 20.0 ± 2.8 | 22.1 ± 3.0<sup>b</sup><sup>v</sup> | 21.9 ± 0.7 | 21.6 ± 1.3<sup>a</sup> | 22.5 ± 1.1 | 24.7 ± 0.8<sup>b</sup><sup>.*</sup> |
| 18:1       | 10.7 ± 1.2<sup>a</sup> | 10.8 ± 0.6<sup>v</sup> | 12.9 ± 1.4<sup>b</sup> | 13.0 ± 1.3<sup>b</sup> | 11.6 ± 1.1<sup>a</sup><sup>b</sup> | 10.9 ± 1.2<sup>a</sup> |
| 18:2n-6    | 12.9 ± 2.6<sup>a</sup> | 15.1 ± 3.2<sup>v</sup> | 13.1 ± 1.7<sup>a</sup> | 17.6 ± 1.5<sup>b</sup> | 5.5 ± 0.8<sup>b</sup> | 12.3 ± 2.1<sup>b</sup><sup>.*</sup> |
| 18:3n-3    | —       | —        | —       | —        | —       | —        |
| 20:3n-6    | 1.0 ± 0.2<sup>a</sup> | 1.0 ± 0.1<sup>a</sup> | 1.4 ± 0.2<sup>b</sup> | 1.6 ± 0.2<sup>b</sup> | 1.8 ± 0.2<sup>c</sup> | 2.1 ± 0.2<sup>c</sup> |
| 20:4n-6    | 25.9 ± 1.6<sup>a</sup> | 23.2 ± 1.5<sup>.*</sup> | 20.6 ± 1.4<sup>b</sup> | 17.9 ± 0.9<sup>b</sup><sup>.*</sup> | 25.5 ± 2.1<sup>c</sup> | 22.2 ± 1.1<sup>c</sup><sup>.*</sup> |
| 20:5n-3    | —       | —        | 0.8 ± 0.1<sup>a</sup> | 0.8 ± 0.1<sup>a</sup> | 0.6 ± 0.1<sup>b</sup> | 0.5 ± 0.1<sup>b</sup> |
| 22:4n-6    | 5.3 ± 0.7<sup>a</sup> | 5.9 ± 0.5<sup>a</sup> | 2.6 ± 0.3<sup>b</sup> | 2.3 ± 0.3<sup>b</sup> | 4.1 ± 0.4<sup>a</sup> | 3.5 ± 0.4<sup>a</sup> |
| 22:5n-6    | 3.7 ± 0.7 | 3.0 ± 0.3 | —       | —        | —       | —        |
| 22:6n-3    | —       | —        | 3.1 ± 0.2 | 3.1 ± 0.2 | 3.0 ± 0.3 | 3.3 ± 0.4 |
| 18:2n-6/metabolites<sup>1</sup> | 0.36   | 0.46     | 0.53     | 0.81     | 0.18     | 0.44     |

Each value represents the mean ± SD of samples from 5–7 rats. Means in a horizontal row that do not share a common superscript letter differ significantly (control: a, b, c; diabetic: a', b', c'). <sup>1</sup>The sum of 20:3n-6 + 20:4n-6 + 22:4n-6 + 22:5n-6. <sup>.*</sup>p < 0.05 or better; control vs. diabetic rats.
(22:5n-6) in the S groups were replaced with EPA, docosapentaenoic acid (22:5n-3) and DHA in the S+L and the E+L groups. Levels of DGLA were significantly higher both in control and diabetic rats of the E+L groups than those in the S or the S+L groups.

**Platelet phospholipids.** There were no significant differences in fatty acid compositions between control and diabetic rats, except for increased LA and decreased oleic acid and DHA in the E+L fed diabetic rats. As similarly observed in aorta phospholipids, adrenic acid and docosapentaenoic acid (22:5n-6) in the S groups were replaced by EPA, docosapentaenoic acid (22:5n-3), and DHA in the S+L and the E+L groups. DGLA in the E+L groups was significantly higher than that in the S and the S+L groups both in control and diabetic rats.

**DISCUSSION**

It has been documented that conversion of LA to AA is reduced in experimental diabetic rats induced by injecting diabetogenic chemicals (streptozotocin, alloxan, etc.) to adult animals (7, 8). Consequently, AA deficiency has been shown in the phospholipid fraction of plasma, liver, aorta, platelet, and so on (19–21). Decreased delta-6-desaturase activity seems to be at least partly responsible for

| Diets   | S       | S+L     | E+L     |
|---------|---------|---------|---------|
| 16:0    | 26.6±4.7| 30.8±3.0| 30.2±2.0|
| 18:0    | 17.3±1.9| 17.6±1.4| 18.2±2.2|
| 18:1    | 6.9±0.8 | 7.4±0.5 | 7.2±0.4 |
| 18:2n-6 | 9.2±1.4 | 9.5±0.6 | 6.7±0.5 |
| 18:3n-3 | 20:3n-6 | 1.0±0.2 | 1.5±0.1 |
| 20:4n-6 | 30.3±2.9| 26.2±1.3| 29.2±2.7 |
| 20:5n-3 | 1.5±0.2 | 2.8±0.1 | 3.9±0.3 |
| 22:4n-6 | 6.4±0.6 | 3.6±0.3 | 4.7±0.3 |
| 22:5n-6 | 6.8±0.5 | 3.5±0.6 | 3.9±0.3 |
| 18:2n-6/metabolites | 2.0±0.1 | 0.7±0.0 | 1.6±0.1 |
|         | 0.24    | 0.31    | 0.19    |

Each value represents the mean ± SD of samples from 5–7 rats. Means in a horizontal row that do not share a common superscript letter differ significantly (control: a, b, c; diabetic: a', b', p<0.05 or better). *p<0.05 or better; control vs. diabetic rats.

**Table 5. Fatty acid composition (mg/100 mg total fatty acids) of platelet phospholipids from control and diabetic rats fed for 8 weeks on different lipid diets.**
this (8, 11, 22). In the present study, we observed no significant changes of AA in plasma phospholipids in a rat model for insulin-independent diabetes. It is, however, noteworthy that ratios of LA to its desaturated and elongated products are increased (especially in the E+L treatment) in diabetic rats, suggesting slow metabolic rate of LA in these animals (Tables 3 to 5). Our previous study in human non-insulin-dependent diabetes showed no significant changes of fatty acid composition in plasma total lipid (23), and similar results were also reported on liver lipid analysis (24). Hypercholesterolemia is common in severe diabetes. Both endogenous and diet-induced hypercholesterolemia have been demonstrated to lower AA in plasma and liver lipids (10, 25). In addition, our recent study indicated a possibility that distribution of AA among various lipid fractions is disturbed by metabolic acidosis, which commonly complicated with severe diabetes (26). In the present study, plasma total cholesterol and triglyceride levels (Table 2) and blood pH values (data not shown) were not different between control and diabetic rats. AA deficiency in plasma and liver phospholipids of severe diabetic animals might be induced by these factors.

AA levels in plasma phospholipids were hardly altered by the 8-week treatment of various combinations of n-6 and n-3 fats. Although ALA inhibits the conversion of LA to AA (6, 27), its inclusion in the diet does not change AA content in the liver if dietary ALA/LA ratio does not exceed 0.5 and a basic requirement of LA is provided (28). This may explain our results (in the present study, ALA/LA+GLA ratios were 0.49 and 0.47 in the S+L and E+L diet, respectively, and caloric intake of LA was more than 9%) and agrees with other studies in rats (6, 27, 29) and in humans (30).

In contrast, compositions of C-22 polyenoic fatty acids were substantially affected by dietary n-6 and n-3 fats replacing each other according to the type of fats available. Moreover, ratios of arachidonic acid/docosapentaenoic acid (22:5n-6) or docosapentaenoic acid (22:5n-3)/DHA (indices of delta-4-desaturase activity) were markedly different between plasma phospholipids and aorta and platelet phospholipids (in plasma, 0.22–0.30; in aorta, 0.83–1.43; in platelet, 2.29–5.82; data from controls). Little is known about the pathophysiological role of delta-4-desaturase in EFAs metabolism. These results might also be explained by the preferential incorporation of arachidonic and docosapentaenoic acid (22:5n-3) into aorta and platelet phospholipids (31).

AA in aorta phospholipids was reduced in diabetic rats regardless of the different fat diets (Table 4). The same results have been shown in insulin-requiring ketoacidotic diabetic rats induced by streptozotocin treatment in adult animals (19, 21) and may be related to diminished aortic prostacyclin production in both types of diabetic animals (14, 32). Recently, it has been shown that AA has a protective effect against hyperglycemia-induced teratogenesis in vitro and in vivo (33, 34), suggesting the importance of reduced AA content in the pathological events of diabetes.

DGLA content in plasma, aorta, and platelet phospholipids was significantly
raised in the E+L groups as compared to the other 2 diet groups. GLA, which is included in the former diet, is the direct precursor of DGLA. Undesirable effects of AA deficiency in aorta from diabetic rats may be neutralized by dietary GLA supplementation, partly because possible reduction of prostacyclin production seems to be compensated by increase of DGLA (substrate for anti-aggregatory prostaglandin E₁ synthesis) content and partly because GLA treatment facilitates incorporation of AA into aorta phospholipids (Table 4)(35).

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