Effect of Eicosapentaenoic Acid Ethyl Ester on Albuminuria in Streptozotocin-Induced Diabetic Rats

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Summary Wistar rats (4-week-old) were administered with streptozotocin (45 mg/kg) through tail veins. After 3 months, diabetic rats were divided into 2 groups. One group (EPA group, n = 16) was fed a lipid-free diet (90%, w/w) plus lard (8%) and 90% pure eicosapentaenoic acid ethyl ester (2%) for 6 months. The other group (control group, n = 16) was fed in the same way except that eicosapentaenoic acid ethyl ester was replaced by safflower oil. Twenty-four-hour urine was collected just before starting the experimental diets and during the 6-month experimental period at monthly intervals. There were no differences in food intake and body weight between the two groups throughout the experiment. The mean microalbuminuria of the EPA group became significantly lower than that of the control group after 4 months on the diets through the end of the study (6 months). The mean microalbuminuria levels at the end of the study were 1.38 mg/day in the EPA group (n = 9) and 5.19 mg/day in the control group (n = 6) (p < 0.01). Eicosapentaenoic acid administration might retard the progression of diabetic nephropathy by reducing microalbuminuria.

Key Words diabetes mellitus, streptozotocin, eicosapentaenoic acid, diabetic nephropathy, albuminuria, thromboxane B2, fatty acid composition

Diabetic nephropathy is one of the vital complications of diabetes mellitus. Microalbuminuria appears in the early stage of diabetic nephropathy (1). Reduction in microalbuminuria probably prevents or retards the progression of diabetic nephropathy (2). There are many approaches to management of albuminuria. Strict control of blood glucose levels is most important (3), although 12 months of strict control may not reduce microalbuminuria (4). Treatment of hypertension is another important factor to control albuminuria (5). Some anti-platelet drugs are

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also partially effective (6). New kinds of drugs have recently become available; aldose-reductase inhibitors (7) and angiotensin converting enzyme inhibitors (8) may reduce microalbuminuria.

Dietary intervention is an essential part of treatment of diabetes mellitus at all stages of the disease. If dietary intervention also reduces microalbuminuria, this would become a powerful tool because diabetic patients are usually under dietary care. A low protein diet has already been proven effective in reducing microalbuminuria to a certain extent (9).

Apart from renal disorders of diabetic origin, there are reports on beneficial effects of dietary fish oil on proteinuria and renal dysfunction in nondiabetic renal diseases in experimental animals (10). For instance, Prickett et al. reported the beneficial effects of fish oil on experimental nephritis of NZB/NZW F1 mice (11). However, there are only a limited number of reports on effects of fish oil (12,13) or purified n-3 polyunsaturated fatty acids (14), active ingredients of fish oil, on proteinuria or albuminuria of experimental diabetes mellitus. In our previous study, we reported a short-term effect of eicosapentaenoic acid (EPA) administration on proteinuria of diabetic rats (14). In the present study, we examined whether the administration of 90% pure eicosapentaenoic acid (20:5n-3, EPA) ethyl ester for 6 months was able to reduce microalbuminuria in diabetic rats.

**MATERIALS AND METHODS**

*Study design.* Sixty-five 4-week old male Wistar rats were purchased from Sankyo Labo Service (Tokyo). Forty-seven rats were made diabetic by an injection of streptozotocin (STZ, 45 mg/kg body weight, Sigma Chemical Co., St. Louis, MO) through tail veins under light anesthesia with diethyl ester. The remaining rats (n=18) were injected with a vehicle instead of STZ, and were included in the study as nondiabetic rats. One week after the injection, all STZ-treated rats were measured for blood glucose levels. Rats with blood glucose levels below 250mg/dl were excluded from the study. The diabetic rats were not treated with any insulin throughout the study period. For the following 3 months, both diabetic and nondiabetic rats were housed in groups of 4 or 5 and fed a standard pellet diet (CE-1, Clea Japan, Tokyo) ad libitum. Thirty-two diabetic rats survived 3 months on the standard pellet. Then, blood glucose levels of those surviving diabetic rats were examined again. Both diabetic and nondiabetic rats were divided into 2 dietary groups each after matching blood glucose levels and body weight in diabetic rats and matching body weight alone in nondiabetic rats. All 4 groups of rats were placed on either of 2 different experimental diets (as described below) for 6 months. Consequently, there were 4 groups of rats: the diabetic control and EPA groups, and the nondiabetic control and EPA groups. All rats had free access to their respective diets and water throughout the study period. At monthly intervals, diabetic rats were moved into metabolic cages individually from 3 P.M. for 24-h urine collection. The same diets and water were given ad libitum during the urine
collection. Urinary albumin excretion, body weight, and food consumption were measured. In nondiabetic rats, body weight and food consumption were measured at 4 and 6 months after the experimental diet. Blood samples of diabetic rats were collected from tail veins 1, 2, and 6 months after the diets. We did not take blood samples of diabetic rats at monthly intervals to avoid unnecessary stress (sometimes lethal) to rats. Blood pressure was measured with a Rat Sphygmomanometer (Riken Kaihatsu, Tokyo) by the tail cuff method. However, at 4 months after the experimental diets we checked blood pressure of only 4 diabetic rats per each dietary group to avoid unnecessary stress. After collection of the last urine samples at the end of the experiment, i.e. after 6 months on the experimental diets, rats were sacrificed by exsanguination from hearts under ether anesthesia. The blood serum was separated by centrifuge. Both kidneys of each rat were collected. One kidney was stripped of perirenal fat, and stored at $-80^\circ$C until phospholipid fatty acids were analyzed. The other kidney was processed for microscopy.

Diets. The EPA groups were fed a lipid-free powder diet, plus lard (8%) and 90% pure EPA ethyl ester (2%). The control groups were fed the same diet as the EPA groups except that EPA ethyl ester was replaced with safflower oil. EPA ethyl ester was kindly provided from Nippon Suisan (Tokyo), and safflower oil was purchased from a local supermarket. These two kinds of diets were equal in energy content. The fatty acid composition of the control and EPA diets, and the composition of the lipid-free powder diet are shown in Table 1. The dietary components were mixed with lard and oil every other day. Before mixing the dietary components, $\alpha$-tocopherol (Wako, Tokyo) was added to EPA ethyl ester and safflower oil at a concentration of 0.2% to prevent peroxidation of oil. If the mixed diet was not used on the same day, it was stored at $-20^\circ$C for not more than 1 day. Diet was given in clean serving cups every afternoon and old cups were

| Fatty acids | Control diet | Experimental diets |
|------------|--------------|---------------------|
| 16:0       | 16.7         | 15.5                |
| 16:1       | 2.2          | 2.2                 |
| 18:0       | 7.1          | 6.9                 |
| 18:1n-9    | 42.4         | 41.0                |
| 18:2n-6    | 30.3         | 13.5                |
| 18:3n-3    | 0.9          | 0.9                 |
| 18:4n-3    | 0.4E         | 0.4E                |
| 20:4n-6    | 1.0E         | 0.4E                |
| 20:4n-3    |              | 18.2E               |

| 20:5n-3(EPA)|
|-------------|

The composition of lipid-free powder (wt%) was 21.3% crude protein, 0.1% crude lipids, 2.1% crude fiber, 4.2% mineral, 62.0% soluble non-nitrogenous materials, and 10.3% water. $^E$Provided in the form of ethyl ester.

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removed to prevent peroxidation of diets and contamination with urine and feces. Lipid peroxides in diet did not increase appreciably during storage (data not shown).

**Laboratory methods.** Blood glucose levels were measured by the glucose oxidase method \((15)\). Microalbuminuria was measured by immunonephelometry with an anti-rat albumin antibody (Organon Teknika Co., West Chester, PA) using a Boehringer nephelometer (Hoechst Japan, Tokyo) after examination of overt proteinuria by the dip-stick method (Multistix SG, Miles Sankyo, Tokyo). Serum concentrations of total cholesterol, triglycerides, HDL-cholesterol, total protein, albumin, urea nitrogen and creatinine, and urinary concentrations of glucose were determined with an autoanalyzer (Hitachi 736, Hitachi). Thromboxane \((TX)B_2\) and 6-keto prostaglandin \((PG)F_1a\) in urine were measured by RIA using \(^{125}\text{I}-TXB_2\) and \(^{125}\text{I}-6\text{-keto PGF}_{1a}\) kits (New England Nuclear, Boston, MA), respectively.

**Fatty acid analysis.** Frozen kidneys were thawed, minced, and homogenized with a Polytron (Kinematica, Luzern, Switzerland) \((10\,\times\,3)\), and total lipids were extracted with chloroform/methanol \((2:1, \, v/v)\) \((16)\). Total phospholipids were isolated by thin-layer chromatography on Silica Gel 60-plates (Merek, Darmstadt, Germany) using petroleum ether/diethyl ether/acetic acid \((80:20:1, \, v/v/v)\) as a solvent. Methanolicysis of phospholipids was done at \(70^\circ\text{C}\) for 45 min with 6% sulfuric acid in anhydrous methanol without prior extraction of the lipids from silica gel. Methylated fatty acids were analyzed with a gas chromatograph (GC-14A, Shimadzu, Kyoto) equipped with a 30-m Supelcowax 10 column (Supelco, Bellefonte, PA) essentially as described by Ackman \((17)\).

**Renal histology.** Tissue was fixed in a 15% formaldehyde solution and stained with HE, PAM, PAS, or diastase digestive PAS. Glomerular exudative lesions and mesangial sclerosis were expressed as none \((-)\), slight \(\pm\), mild \((+)\), or moderate \((++)\). The presence of tubular changes (glycogen deposition) was also rated similarly.

**Statistical analysis.** Data are expressed as \(M\pm SD\). The data on urinary albumin, urinary eicosanoids, and serum triglycerides are expressed as means \((M-SD, \, M+SD)\) after log transformation. For comparison between the two dietary groups of either diabetic or nondiabetic rats, data were analyzed by \(t\)-test; in the case of the comparison of microalbuminuria, a \(t\)-test was performed with Bonferroni's adjustment because of multiple comparison. Mortality rate was analyzed by \(\chi^2\)-test. Statistical comparison between diabetic and nondiabetic rats within the same dietary groups were also made by \(t\)-test.

**RESULTS**

**Survival**

Six out of 16 diabetic rats survived the 6-month experimental period in the control group, whereas 9 out of 16 diabetic rats in the EPA group survived. There were no significant differences in mortality rates between the 2 diabetic groups. The
cause of death was presumably dehydration. Nondiabetic rats did not die throughout the experimental period.

**Food intake and weight gain**

Dietary intake of diabetic rats was measured while they were in metabolic cages at monthly intervals. The mean intake after one month on the diets were 30.9 ± 4.2 g for the control group and 29.2 ± 5.7 g for the EPA group (p > 0.10). There were no differences in the mean intake between the 2 groups at any other month either (data not shown). Body weight of diabetic rats remained much lower than that of nondiabetics (Fig. 1). There were no differences in body weight between the 2 dietary groups in either diabetic or nondiabetic rats (Fig. 1).

**Metabolic status (Table 2)**

Blood glucose concentrations of diabetic rats were high throughout the experimental period. There were no significant differences between the 2 dietary groups of diabetic rats after 1, 2, or 6 months on diets. There were also no differences in urinary glucose concentrations at the end of the experiment.

**Microalbuminuria (Fig. 2)**

All diabetic rats showed microalbuminuria, but no rats showed overt proteinuria detectable by the dip-stick method. Albumin excretion in EPA group was reduced significantly compared with that in the control group after 4, 5, and 6 months on diets (Fig. 2). At the end of the experiment, the mean excretion levels were 1.38 (0.90, 2.07) mg/day in the EPA group (n = 9) and 5.19 (2.45, 11.0) mg/day in the control group (n = 6) (p < 0.01).

![Fig. 1. Changes in body weight of the control and EPA groups. ---○---, the nondiabetic control group; ---○---, the nondiabetic EPA group; ---, the diabetic control group; ---○--- the diabetic EPA group. Data are expressed as M±SD.](image-url)
Table 2. Glycemic status of diabetic rats.

| Measured items | Diabetic groups |
|----------------|----------------|
|                | Control        | EPA            |
| Blood glucose (mg/dl) |               |                |
| Before         | 263±52         | 250±33         |
| (n = 16)       | (n = 16)       |                |
| At 1 month     | 330±32         | 341±41         |
| (n = 16)       | (n = 16)       |                |
| At 2 months    | 326±53         | 293±36         |
| (n = 14)       | (n = 15)       |                |
| At 6 months    | 441±106        | 494±116        |
| (n = 6)        | (n = 9)        |                |
| Urinary glucose (mg/day) |     |                |
| At 6 months    | 11.5±4.0       | 11.4±3.4       |
| (n = 6)        | (n = 9)        |                |

Diabetic rats were fed either the control or EPA-supplemented diet for 6 months. Blood glucose concentrations were measured before diets and at 1, 2, and 6 months thereafter. Urinary glucose concentrations were measured at the end of the experiment. Data are expressed as M±SD.

Fig. 2. Changes in urinary albumin excretion of diabetic rats. The albumin excretion was significantly reduced in the EPA group compared with that in the control group at 4 (p<0.05), 5 (p<0.05), and 6 (p<0.01) months on the experimental diets. ●, the control group; ○, the EPA group. Numbers in parentheses indicate those of surviving rats. Data are expressed as M±SD after log transformation.

Urinary eicosanoids (Fig. 3)

Urinary excretion of TXB₂ was significantly reduced in the diabetic EPA group as compared with that in the diabetic control group, whereas there was no
Fig. 3. Urinary excretion of 6-keto PGF$_{1 \alpha}$ and TXB$_2$ of diabetic rats at the end of the experiment. ●, the control group ($n=6$); ○, the EPA group ($n=9$). Data are expressed as M±SD after log transformation. *$p<0.05$.

difference in 6-keto PGF$_{1 \alpha}$ excretion between the 2 groups. The mean ratios of TXB$_2$ to 6-keto PGF$_{1 \alpha}$ in individual rats were larger in the control group (2.23±2.10, $n=6$) than in the EPA group (1.10±0.81, $n=9$). However, the difference was not significant.

**Blood chemistry (Table 3)**

There were no differences between the 2 diabetic groups in serum concentra-

| Measured items of serum | Diabetic groups | Nondiabetic groups |
|-------------------------|-----------------|-------------------|
|                         | Control ($n=6$) | EPA ($n=9$)       | Control ($n=9$) | EPA ($n=9$)       |
| Total protein (g/dl)    | 6.4±0.4         | 6.4±0.5           | 6.7±0.4         | 7.0±0.5           |
| Albumin (g/dl)          | 4.1±0.6         | 4.1±0.5           | —               | —                 |
| Creatinine (mg/dl)      | 0.48±0.17       | 0.44±0.11         | 0.50±0.07       | 0.57±0.09         |
| Urea nitrogen (mg/dl)   | 23.2±5.9        | 26.5±5.0          | 14.4±2.0        | 14.4±2.9          |
| Total cholesterol (mg/dl)| 138±32         | 102±36            | 146±18          | 106±18*           |
| HDL cholesterol (mg/dl) | 98±39           | 74±20             | 116±32          | 70±20*            |
| Triglycerides$^1$ (mg/dl)| 186            | 87                | 68              | 47*               |
|                         | (102,338)       | (45,170)          | (55,83)         | (37,59)           |

Blood samples were taken after 6 months on diets. Data were compared within the diabetic or nondiabetic groups. $^1$Data are expressed as geometric means (-SD, +SD). *$p<0.05$
Serum lipids (Table 3)

There were no differences in serum concentrations of total cholesterol, HDL-cholesterol, and triglycerides between the 2 diabetic groups. In nondiabetic rats, serum concentrations of total cholesterol, HDL-cholesterol, and triglycerides in the EPA group were all significantly lower than those in the control group.

Blood pressure

After 4 months on the diets, the mean blood pressure of the control (n=4) and EPA (n=4) groups was 154±20 and 148±35 mmHg, respectively, in diabetic rats, and 136±14 and 136±15 mmHg, respectively, in nondiabetic rats. There were no significant differences between the 2 dietary groups in either diabetic or nondiabetic groups.

Kidney weight and its fatty acid composition

The relative kidney weight to total body weight of diabetic rats (0.50±0.12 and 0.53±0.10% in the control and EPA groups, respectively) was markedly larger than that of nondiabetic rats (0.23±0.02 and 0.22±0.02% in the control and EPA groups, respectively), but there were no differences between the 2 dietary groups in either diabetic or nondiabetic rats. As for the fatty acid composition of the total phospholipid fraction of kidneys at the end of the experiment (Table 4), the EPA group had a marked increase in EPA and docosapentaenoic acid (22:5n-3), and a marked reduction of arachidonic acid (20:4n-6) in diabetic rats, compared with

Table 4. The fatty acid composition (mol %, M±SD) of the total phospholipid fraction of kidneys of both diabetic and nondiabetic rats.

| Fatty acids | Diabetic groups | | Nondiabetic groups |
|-------------|----------------|----------------|------------------|
|             | Control (n=6)  | EPA (n=9)      | Control (n=9)    | EPA (n=9)        |
| 16:0        | 18.4±1.9       | 17.2±1.0       | 17.3±0.4         | 18.2±0.5         |
| 18:0        | 19.6±1.2       | 19.4±0.8       | 19.2±0.7         | 20.2±0.4         |
| 18:1n-9     | 7.2±0.6        | 9.1±0.6*       | 7.5±0.6          | 9.1±0.5*         |
| 18:2n-6     | 9.5±2.0*       | 8.8±1.1        | 7.1±0.5          | 8.8±0.3*         |
| 20:4n-6     | 28.7±2.1       | 19.2±18*       | 31.7±0.1         | 19.3±1.3*        |
| 20:5n-3 (EPA)| 0.08±0.04     | 7.6±1.6*       | 0.06±0.01        | 7.3±0.5*         |
| 22:5n-3     | 0.21±0.03      | 1.9±0.2*       | 0.20±0.04        | 1.6±0.1*         |
| 22:6n-3     | 1.6±0.2        | 1.9±0.2        | 1.7±0.1          | 1.7±0.04         |

Kidneys were taken after 6 months on diets. The fatty acid composition of the total phospholipid fraction was analyzed by gas chromatography. *Significantly different (p<0.05) from respective control groups. †Significantly different (p<0.05) from the nondiabetic group on the same diet.
Table 5. Histological changes in kidneys.

| Histological changes | Rating | Diabetic groups | Nondiabetic groups |
|----------------------|--------|-----------------|--------------------|
|                      |        | Control (n = 6) | EPA (n = 9)        |
|                      |        | Control (n = 9) | EPA (n = 9)        |
| Glomerular exudative lesion | − to ±  | 1 | 0 | 9 | 9 |
|                       | +      | 5 | 8 | 0 | 0 |
|                       | ++     | 0 | 1 | 0 | 0 |
| Mesangial sclerosis   | − to ± | 2 | 0 | 9 | 9 |
|                       | +      | 4 | 9 | 0 | 0 |
|                       | ++     | 0 | 0 | 0 | 0 |
| Tubular lesion        | −      | 1 | 3 | 9 | 9 |
| (glycogen deposition) | +      | 5 | 6 | 0 | 0 |

Kidneys were taken for microscopic examination after 6 months on diets.

the control group. The nondiabetic EPA group showed a tendency similar to that of the diabetic EPA group. There were significantly larger amounts of linoleic acid (18:2n-6) and lesser amounts of arachidonic acid in the diabetic control group than in the nondiabetic control group.

Renal histology (Table 5)

There were no significant changes in histological findings such as glomerular exudative lesions, mesangial sclerosis, and tubular lesions between the 2 dietary groups of diabetic rats.

DISCUSSION

In the present study, rats in which diabetes was induced by STZ administrations showed diabetic nephropathy at its very early stage. In both control and EPA groups of diabetic rats, relative kidney weight was twice as big as that of nondiabetic rats fed the same diets. This feature is one of the typical early signs of diabetic nephropathy (18). Moreover, all diabetic rats showed microalbuminuria (Fig. 2) without overt proteinuria detectable with a dip-stick. In addition, all diabetic rats had one or more diabetes-specific histological changes that were not shown in nondiabetic rats (Table 5).

The most important finding in the present study is that dietary supplementation with EPA reduced microalbuminuria of diabetic rats compared with the control diet. Dietary intake, body weight (Fig. 1) and serum glucose concentrations (Table 2) were not different between the 2 dietary groups of diabetic rats throughout the experimental period. Consequently, this effect appeared to be unrelated to the differences in caloric intake or in metabolic status.

The mechanism of action of EPA is not very clear from the present study. However, the reduction of TXB₂, the stable metabolite of TXA₂, in urine of the
diabetic EPA group may be important. Although there is no direct proof that \(\text{TXA}_2\) formation deteriorates diabetic nephropathy, several pieces of circumstantial evidence for this phenomenon are available. \(\text{TXA}_2\) formation is said to be associated with deterioration of renal function in severe diabetes as well as in nondiabetic renal diseases (19). The administration of \(\text{TXA}_2\) synthetase inhibitor induces the protection of renal function of diabetics (20). Thus, it is highly likely that depression of \(\text{TXA}_2\) formation is beneficial for preventing progress of diabetic nephropathy.

Jensen et al. administered fish oil or olive oil to diabetic patients for 8 weeks in a double-blind crossover manner and observed a significant reduction of the increased transcapillary escape rate of albumin during intake of fish oil compared with olive oil (21). Although they did not find any improvement in proteinuria, the same beneficial effect on the glomerulus may be expected if fish oil is administered longer. Indeed, we recently reported that the administration of 1.8 g of pure EPA ethyl ester/day for 6 months to diabetic patients reduced microalbuminuria (22).

Fish oil intake is also known to reduce blood pressure (23). In the present study we could not measure the blood pressure of every diabetic rat because we thought diabetic rats could hardly tolerate the high temperature necessary for pressure measurement. For 8 diabetic rats of both groups (4 each) there was no significant difference in blood pressure.

A change in blood rheology is one of the most important factors that affect the renal vasculature, since the hematocrit of the blood in the glomerulus is very high due to ultrafiltration. Kamada et al. observed that dietary supplementation with fish oil in diabetics increased erythrocyte membrane fluidity (24). Blood viscosity and erythrocyte filterability were shown to improve by EPA administration in healthy volunteers (25) and patients including diabetics (26) and renal allograft recipients (27). In the present study, beneficial effects of EPA on blood rheology seemed to have reduced micro-injury of the glomerulus exposed to rheological stress induced by a high hematocrit. Reduction in urinary \(\text{TXB}_2\) that was produced by renal tissue and platelets circulating in the kidney should also be considered in the same context; the reduction in \(\text{TXB}_2\) was probably favorable for microcirculation of the kidney.

Barcelli et al. (12) made rats diabetic with STZ and divided them into 4 groups on different diets: beef tallow-, evening primrose oil-, safflower oil-, and fish oil-supplemented diets. After 38 weeks on these diets, proteinuria levels in the safflower oil- and evening primrose oil-supplemented groups were significantly lower than those of the beef tallow-supplemented group. Proteinuria levels of the fish oil-supplemented group tended to be reduced compared with those of the beef tallow-supplemented group, although the difference was not statistically significant. It is rather difficult to compare their study with ours because the measured parameters were different, i.e. proteinuria vs microalbuminuria, and because our control diet contained 3\% (w/w) linoleic acid which was just between their beef tallow oil-supplemented (0.4\% linoleic acid) and safflower oil-supplemented (8.5\%...
linoleic acid) diets. Moreover, there were major differences between their studies and ours: 1) Their rats suffered from more severe diabetes; rats were treated with insulin, but blood glucose levels of their rats were nevertheless as high as those of our rats without insulin treatment. 2) As a result, diabetic nephropathy of their rats was more advanced, showing about 35 mg protein/16 h in the safflower oil- and evening primrose oil-supplemented groups and about 90 mg in the beef tallow-supplemented group, whereas our rats did not show overt proteinuria.

Sinha et al. (13) examined the effect of a fish oil-based diet on proteinuria of diabetic rats compared with a beef tallow-based diet. Both diets contained about 1% (w/w) linoleic acid. After 20 weeks on the diets, the fish oil group showed significantly reduced proteinuria compared with the beef tallow group. It is again difficult to compare their study with ours because of the same reasons as in the study of Barcelli et al. (12). However, considering the results together, it is highly likely that n-3 fatty acid-supplemented diets are better in terms of proteinuria/albuminuria than control diets which do not contain extremely large amounts of linoleic acid.

The triglyceride-lowering effects of n-3 fatty acids are well documented (28), and these effects were produced in our nondiabetic rats. However, the difference in serum triglycerides between the control and EPA groups of diabetic rats was not statistically significant (p = 0.06) due to wide variations of values. There was a significant reduction in arachidonic acid in the EPA group of both diabetic and nondiabetic rats (Table 4). This reduction was roughly inversely correlated with the increase in EPA and docosapentaenoic acid, an elongation product of EPA. In the diabetic control group, linoleic acid increased and arachidonic acid decreased compared with those of the nondiabetic control group. This was probably due to the lower desaturase activity in the control diabetic rats, considering that the enzyme activity was necessary for the conversion of linoleic acid to arachidonic acid and was insulin dependent (29).

In conclusion, EPA administration reduces microalbuminuria of diabetic rats partly due to a reduction of kidney-related TXA₂ formation. This regimen may be useful for the control of the early phase of diabetic nephropathy and for its progression.

We are grateful to Mrs. M. Tabayashi for her technical assistance and Ms. A. Takashima for her editorial help.

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