Influence of Highly Purified Eicosapentaenoic Acid Ethyl Ester on Insulin Resistance in the Otsuka Long-Evans Tokushima Fatty Rat, a Model of Spontaneous Non–Insulin-Dependent Diabetes Mellitus

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We investigated the effect of long-term administration of highly purified eicosapentaenoic acid ethyl ester (EPA-E), an n-3 polyunsaturated fatty acid derived from fish oil, in comparison to the effects of lard, olive oil, safflower oil, or distilled water as the control on the development of insulin resistance in Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a model of spontaneous non–insulin-dependent diabetes mellitus (NIDDM) with obesity. After 17 or 18 weeks of treatment, the glucose infusion rate (GIR) in the euglycemic insulin-glucose clamp test only showed a significant increase in EPA-E–treated rats compared with control rats given distilled water alone as the vehicle. The GIR in EPA-E–treated animals was approximately three times greater than in the controls. This is the first report to display the influence of various fatty acids on the development of insulin resistance in OLETF rats. We demonstrated that EPA-E prevents the onset of insulin resistance, whereas olive oil and safflower oil have no effect and lard exacerbates insulin resistance. Fatty acid analysis of phospholipids in skeletal muscle showed a significant increase of the C18:2, C20:5, and C22:5 components in EPA-E–treated rats and, conversely, a significant decrease in C20:4. In addition, EPA-E–treated rats showed a significant increase in GLUT4 mRNA in skeletal muscle when compared with control rats. Our results indicate that the beneficial effect of EPA-E on insulin resistance in OLETF rats is likely to be dependent on modification of the phospholipid components of the skeletal muscle membrane. These findings suggest that dietary fatty acids may play a key role in the development of insulin resistance in patients with NIDDM.

NON–INSULIN-DEPENDENT diabetes mellitus (NIDDM) is an increasingly prevalent disease. It is considered that the insulin resistance of skeletal muscle and the impairment of the action of circulating insulin are important in the onset of NIDDM. Insulin resistance also seems likely to play a key role in the exacerbation of NIDDM. Therefore, treatment and prevention of the insulin resistance are potentially desirable for the management of NIDDM.

It has been shown that crude fish oil prevents insulin resistance induced by a high-fat diet in rats.[1] Additionally, Popp-Snijders et al[2] have reported that dietary supplementation with n-3 polyunsaturated fatty acids improves insulin sensitivity in patients with NIDDM. On the other hand, it has been suggested that fish oil supplements appear to cause a steep increase in glucose and a decrease in insulin secretion in NIDDM.[3] Recently, Storlien et al[4] have demonstrated that dietary fat composition influences the development of insulin resistance in rats. Furthermore, Yokoyama et al[5] have reported that highly purified eicosapentaenoic acid ethyl ester (EPA-E) has a favorable effect on hypertriglyceridemia without a deleterious effect on the glycemic control of diabetic patients.

The purpose of the present study was to investigate the effect of an n-3 polyunsaturated fatty acid, highly purified EPA-E, in comparison to lard, olive oil, or safflower oil on the development of insulin resistance in Otsuka Long-Evans Tokushima Fatty (OLETF) rats,[6-8] a model of spontaneous NIDDM.

We demonstrated that only EPA-E was effective in preventing the development of insulin resistance in OLETF rats, olive oil and safflower oil were ineffective, and lard exacerbated insulin resistance.

MATERIALS AND METHODS

Animals

OLETF rats, a model of spontaneous NIDDM, were obtained from the Tokushima Research Institute of Otsuka Pharmaceutical (Tokushima, Japan) at 5 weeks of age. The cumulative incidence of diabetes mellitus in male OLETF rats reaches 80% to 100% at 24 weeks of age.[9] The animals were housed two per cage in plastic cages (290 × 350 × 175 mm) in an animal room with ventilation by 15 exchanges of room air per hour, a controlled temperature (23° ± 2°C) and relative humidity (55% ± 10%), and a 12-hour light/dark cycle (lights on 7:00 AM to 7:00 PM). They were supplied with rat chow (MB-3; Funabashi Farm, Chiba, Japan) and tap water ad libitum during the experimental period. The fatty acid composition of the rat chow (MB-3) used in this study is shown in Table 1.

Test Substances

Highly purified EPA-E (89.3% pure; Mochida Pharmaceutical, Tokyo, Japan), lard (saturated fatty acid, 40.1%; Funabashi Farm), olive oil (oleic acid, 79.6%; Kishida Chemical, Osaka, Japan), and safflower oil (linoleic acid, 75.0%; Kishida Chemical) were used as the test substances (Table 2).

Experimental Design

At 8 weeks of age, male OLETF rats were randomly assigned to five groups of 12 rats each. They were treated orally with lard, olive oil, safflower oil, or EPA-E at a daily dose of 0.3 g/kg or with distilled water (0.3 mL/kg/d) for 17 or 18 weeks by gavage. The dose used was the same as that previously found to be effective in suppressing the progression of diabetic nephropathy in WBN/Kob rats.[5] Distilled water–treated rats served as the control. Food consumption and body

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Table 1. Fatty Acid Composition of MB-3 Rat Chow

Fatty Acid	Content (%)
14:0	0.5
16:0	16.1
16:1 (n-9)	0.9
17:0	0.2
18:0	8.9
18:1 (n-9)	25.0
18:2 (n-6)	41.9
18:3 (n-3)	3.7
20:0	0.4
20:1 (n-9)	0.3
20:4 (n-6)	0.3
22:0	0.5
22:6 (n-3)	0.3
24:0	0.1
Not identified	0.9

weight were recorded once per week throughout the experimental period.

Plasma Lipid Assay

At 24 weeks of age, after a 4-hour fast, heparinized blood samples were taken from a tail vein without anesthesia and centrifuged at 2,200 × *g* for 15 minutes at 4°C to isolate plasma. Plasma levels of total cholesterol, phospholipids, triglycerides, and free fatty acids were measured enzymatically with an autoanalyzer (Cobas Fara; Roche, Basel, Switzerland).

Euglycemic Insulin-Glucose Clamp Test

At 25 or 26 weeks of age, according to the method of Kergoat and Portha,[10] the hyperinsulinemic-euglycemic clamp test was performed on half of the rats in each group to evaluate insulin-mediated whole-body glucose uptake. After an overnight fast, the rats were anesthetized with an intraperitoneal injection of urethane (640 mg/kg) and α-chloralose (52 mg/kg). Then, a tracheotomy was performed, with a tracheal tube (polyethylene tube, 1.6 mm ID × 2.6 mm OD; Hibiki, Tokyo, Japan) inserted to avoid respiratory problems. Next, three catheters (0.01 in ID × 0.03 in OD, Tygon microbore tubing; Norton, Akron, OH) were inserted into the left carotid artery and the bilateral jugular veins. The carotid catheter was used for blood sampling, and the jugular catheters were used for glucose and insulin infusion. After these procedures, a 20-minute stabilization period was allowed to avoid any immediate effects due to the surgery. Then, porcine insulin (Actrapid; Novo, Copenhagen, Denmark) diluted in 1% bovine serum albumin (fatty acid–free, 97% pure; Sigma, St Louis, MO) was infused at a constant rate (10 mU/min/kg) for about 2 hours following a loading infusion of 20 mU/min/kg for 10 minutes. Five minutes after starting insulin infusion, an infusion of 10% glucose solution was commenced, and the arterial blood glucose concentration was clamped at approximately 6.1 mmol/L by varying the rate of glucose infusion. Blood samples for determination of glucose levels were obtained at 5-minute intervals throughout the study and measured with an electrode-type glucose analyzer (ANTSENSE; Miles-Sankyo, Tokyo, Japan). The total-body glucose uptake was determined as the mean glucose infusion rate (GIR) during the last 20 minutes of the study.

Table 2. Fatty Acid Composition (%) of EPA-E, Lard, Olive Oil, and Safflower Oil

Fatty Acid	EPA-E	Lard	Olive Oil	Safflower Oil
14:0	ND	1.9	ND	0.1
16:0	ND	24.5	10.1	7.0
16:1 (n-9)	ND	3.3	0.9	0.1
18:0	ND	13.5	3.5	2.5
18:1 (n-9)	ND	43.9	79.6	13.9
18:2 (n-6)	ND	8.7	4.5	75.0
18:3 (n-3)	ND	0.8	0.7	0.6
20:0	ND	0.2	0.4	0.3
20:1 (n-9)	ND	0.8	0.3	0.3
20:4 (n-6)	5.6	ND	ND	ND
20:4 (n-3)	2.7	ND	ND	ND
20:5 (n-3)	89.3	ND	ND	ND
Other	2.4	2.4	ND	0.2

Abbreviation: ND, Not detected.

Oral Glucose Tolerance Test

At 26 weeks of age, an oral glucose tolerance test (OGTT) was performed on the other half of the rats in each group. After an overnight fast, glucose (2 g/kg) was administered orally to the animals, and heparinized blood samples were taken from a tail vein without anesthesia at 0, 30, 60, and 120 minutes for measurement of the plasma glucose level with an autoanalyzer (Cobas Fara; Roche) using the hexokinase method. Samples were also taken at 0, 60, and 120 minutes for measurement of the plasma insulin level with a commercial radioimmunoassay kit (Rat insulin [^{125}I] assay system; Amersham, Buckinghamshire, England).

Tissue Lipid Assay and Fatty Acid Composition

A few days after the OGTT, the rats were killed by exsanguination under pentobarbital sodium anesthesia (50 mg/kg body weight intraperitoneally). The left lobe of the liver and left quadriceps muscle of the thigh of each rat were harvested for extraction and derivatization of lipids. The liver and muscle tissue were homogenized in a mixture of chloroform and methanol (2:1 vol/vol), and the total lipid extracts were prepared according to the method of Folch et al.[11] Triglycerides and phospholipids were separated from less polar lipids by solid-phase extraction on Sep-Pak silica cartridges (Waters, Milford, MA). Triglyceride levels were determined enzymatically with a spectrophotometer (U-2000; Hitachi, Tokyo, Japan). Phospholipids from muscle tissue were transmethylated with 14% boron trifluoride at 85°C for 60 minutes. The methylated fatty acids were then separated and quantified by gas chromatography (model 663; Hitachi) using a capillary column (SP-2380). Fatty acids were identified by comparing their retention times against those of authentic standards.

Assay of GLUT4 mRNA in Skeletal Muscle

The right quadriceps femoris muscle was frozen in liquid nitrogen immediately after dissection and stored at −80°C. Total cellular RNA was extracted from the frozen muscle by the guanidinium thiocyanate/CsCl method.[12] mRNA was isolated with oligo-dT Latex (Oligotex-dT 30 super; Takara Biomedicals, Kyoto, Japan), electrophoresed on 1.0% formaldehyde-agarose gel, and blotted and fixed onto nylon membranes (Genescreen plus 0.45 μm; Du Pont, Wilmington, MA). The membranes were hybridized at high stringency using [α^{32}P]dCTP (110 TBq/mmol; Amersham)-labeled cRNA probes specific for GLUT4, followed by washing and analysis by autoradiography. The values obtained were converted to relative amounts of GLUT4 mRNA per unit of glyceraldehyde-3-phosphate dehydrogenase mRNA.

Body Fat Distribution

Following completion of the euglycemic clamp test or the removal of liver and muscle tissue, the mesenteric fat, subcutaneous fat of the left abdominal region, and scapular brown fat were dissected under pentobarbital sodium anesthesia and weighed. The unilateral subcutaneous fat area was calculated by the method of Krotkiewski and

Table 3. Body Weight Gain and Total Food Consumption in OLETF Rats Treated Orally With Lard, Olive Oil, Safflower Oil, or EPA-E for 17 or 18 Weeks

Parameter	Control	Lard	Olive Oil	Safflower Oil	EPA-E
Body weight gain (g)	301 ± 28	336 ± 30	351 ± 21	360 ± 34	356 ± 33
Total food consumption (g/animal)	5,919 ± 174	6,497 ± 285	6,468 ± 293	6,308 ± 459	6,482 ± 379

Bjorntorp.[13] Then, fat weight was converted to a relative value per 100 g body weight.

Histopathological Examination

Following removal of body fat, the pancreas was dissected and immersed in neutralized 10% formalin for a few days. Then, the specimens were embedded in paraffin by routine procedures. Paraffin sections (2 μm) were cut, deparaffinized with xylene, and stained with hematoxylin and eosin or Masson's trichrome for light microscopy.

Statistical Analysis

Data are expressed as the mean ± SD. The significance of differences versus the control group was analyzed by Student's *t* test, and a statistically significant difference was defined at *P* less than .05.

RESULTS

General Description

There were no changes in the general status of the animals during the experimental period. Body weight gain and total food intake are shown in Table 3. Weight gain and total food intake were slightly (not significantly) increased in the lard, olive oil, safflower oil, and EPA-E groups compared with the control group, but were comparable among the four treated groups.

The relative liver weight (g/100 g body weight, n = 12) was 3.50 ± 0.42 in the control group. On the other hand, the value in the lard, olive oil, safflower oil, and EPA-E groups was 3.58 ± 0.39, 3.40 ± 0.36, 3.30 ± 0.29, and 2.94 ± 0.30, respectively. The relative liver weight at the end of the experimental period showed a significant difference between control and EPA-E groups.

Plasma Lipid Levels

The free fatty acid level was significantly increased in all of the treated groups. In addition, triglycerides, total cholesterol, and phospholipids were significantly increased in the olive oil group. Values for these parameters in the lard group were similar to those in the olive oil group. In the safflower oil group, they were slightly or significantly less than the values in the olive oil or lard groups. On the other hand, these parameters were slightly decreased or unchanged in the EPA-E group (Table 4).

Hyperinsulinemic-Euglycemic Clamp

Figure 1 shows the basal blood glucose level before insulin infusion and the metabolic clearance rate of glucose obtained with the euglycemic clamp test. The basal glucose level was significantly decreased in the EPA-E group, but remained unchanged in the lard, olive oil, and safflower oil groups. The GIR in the control, lard, olive oil, safflower oil, and EPA-E groups was 2.8 ± 0.3, 1.8 ± 0.2, 2.9 ± 0.4, 2.8 ± 0.3, and 6.8 ± 0.6 mg/kg/min, respectively. The EPA-E group had a significantly higher value than the others, and the lard group had a significantly lower value.

Glucose Tolerance

The plasma glucose concentration was significantly increased in the lard, olive oil, and safflower oil groups at 0 and 120 minutes after glucose loading (Fig 2). Additionally, it was significantly increased in the olive oil group at 60 minutes after loading. There were no significant differences between the EPA-E and control groups at 0, 60, and 120 minutes after loading, except for a decrease in the former group at 30 minutes. The incidence of NIDDM in OLETF rats used in this study was estimated to be 100% at 26 weeks of age because their average glucose level at 120 minutes in the OGTT was at least 200 mg/dL. The plasma immunoreactive insulin level in the control, lard, olive oil, safflower oil, and EPA-E groups was 7.8 ± 4.3, 8.3 ± 3.1, 8.0 ± 2.3, 7.6 ± 3.5, and 4.5 ± 2.2 ng/dL, respectively, at 120 minutes after loading. The EPA-E group showed the lowest value among the five groups, but the difference was not significant.

Liver and Muscle Triglyceride Levels

The triglyceride level in the liver was significantly decreased in the EPA-E group, and was conversely increased in the lard, olive oil, and safflower oil groups (Fig 3). The triglyceride level in muscle tissue was significantly increased in the lard, olive oil, and safflower oil groups, but remained unchanged in the EPA-E group (Fig 4).

Fatty Acid Composition of Skeletal Muscle Phospholipids

Table 5 documents that the EPA-E group showed a significant increase in C16:0 (palmitic acid), C18:2 (linoleic acid), C20:5

Table 4. Effects of Lard, Olive Oil, Safflower Oil, or EPA-E on Plasma Lipid Levels in OLETF Rats

Plasma Lipids	Control	Lard	Olive Oil	Safflower Oil	EPA-E
Triglycerides (mg/dL)	342 ± 100	433 ± 146‡	425 ± 59*§	368 ± 112	324 ± 92
Total cholesterol (mg/dL)	197 ± 34	226 ± 45§	234 ± 25†§	217 ± 22§	182 ± 27
Phospholipids (mg/dL)	251 ± 41	305 ± 60*‡	315 ± 28†§	287 ± 32*‡	254 ± 34
Free fatty acids (mEq/L)	2.36 ± 0.69§	3.84 ± 0.93†	4.00 ± 1.07†	3.46 ± 0.79†	3.40 ± 0.80†

*$P < .05$, †$P < .01$: *v* control.
‡$P < .05$, §$P < .01$: *v* EPA-E.

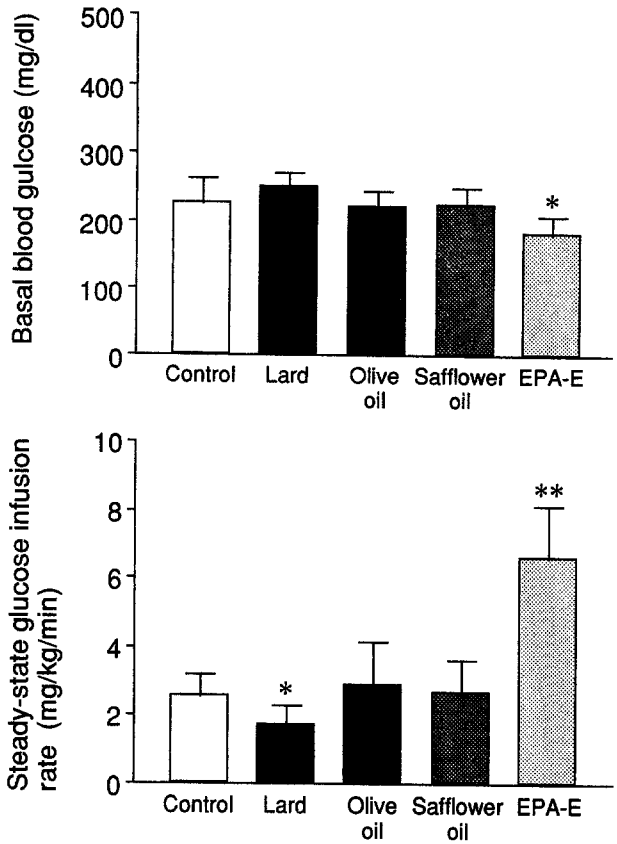

Fig 1. Insulin resistance in the euglycemic insulin-glucose clamp test in OLETF rats. Each column represents the mean ± SD (n = 6). *P < .05, **P < .01: v control group.

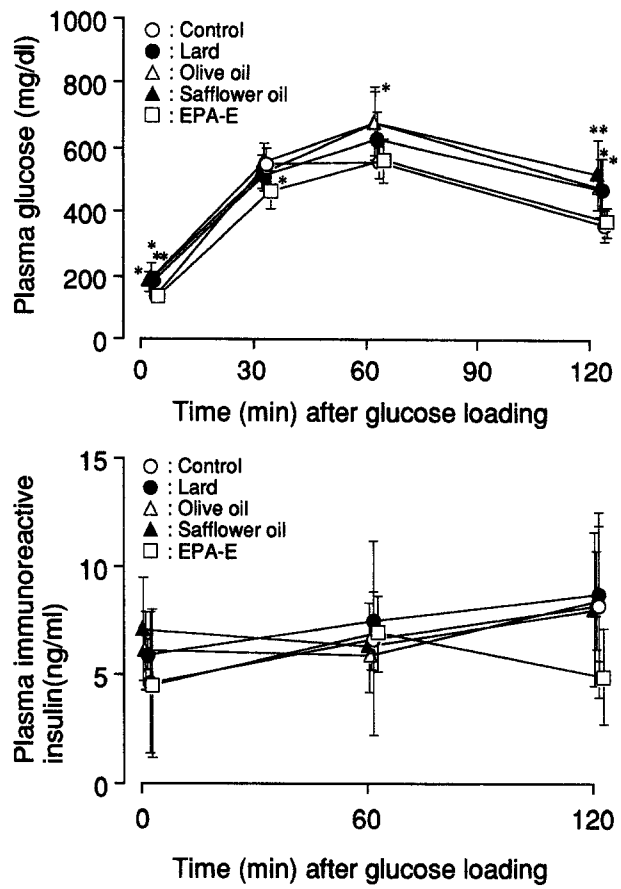

Fig 2. Plasma glucose and insulin levels after an oral glucose load in OLETF rats. Data for each group are expressed as the mean ± SD (n = 6). *P < .05, **P < .01: v control group.

(eicosapentaenoic acid), C22:5 (docosapentaenoic acid), and C22:6 (docosahexaenoic acid) and, conversely, a significant decrease in C20:4 (arachidonic acid). On the other hand, the lard group had a significant increase in C22:5 and C22:6, whereas the olive oil group had significantly elevated levels of C16:0, C18:1, and C22:6. The safflower oil group showed a significant decrease in C18:2 and a significant increase in C22:6. Moreover, the level of C20:4 in the lard, olive oil, and safflower oil groups was significantly higher than in the EPA-E group, and the levels of C18:2, C20:5, and C22:5 in these groups were significantly decreased compared with those in EPA-E–treated rats.

GLUT4 mRNA in Skeletal Muscle

Figure 5 shows that the GLUT4 mRNA level was significantly increased in the EPA-E group compared with the control rats.

Body Fat Distribution

Mesenteric fat weight was slightly but significantly increased in the olive oil and EPA-E groups, whereas brown fat weight was slightly but significantly increased in the lard and safflower oil groups (Table 6). However, fat distribution was comparable among the four treated groups.

Histopathologic Findings

Histopathologic features of the pancreas are summarized in Fig 6 and Table 7. Enlargement of islets, fibrosis with inflammatory cell infiltration in and around the islets, ductal hyperplasia around the islets, and atrophy of acinar cells were moderate or severe in all groups, including the control group. There were no

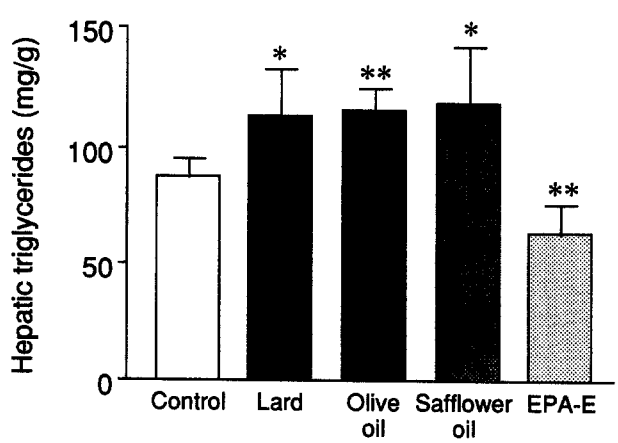

Fig 3. Hepatic triglyceride content in OLETF rats. Each column represents the mean ± SD (n = 6). *P < .05, **P < .01: v control group.

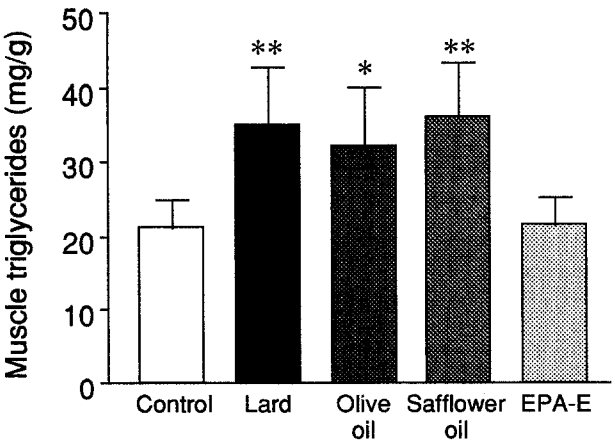

Fig 4. Triglyceride content in skeletal muscle from OLETF rats. Each column represents the mean ± SD (n = 6). *$P < .05$, **$P < .01$: *v* control group.

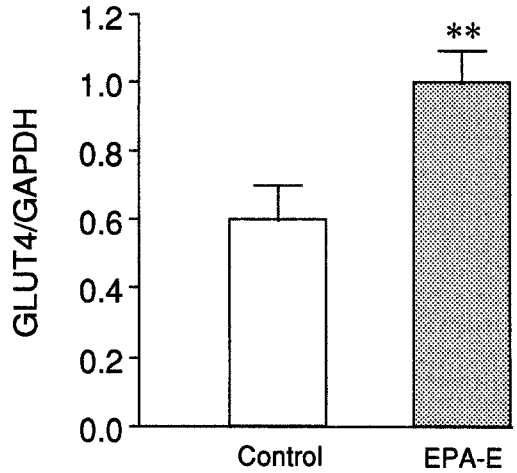

Fig 5. GLUT4 mRNA level in skeletal muscle from OLETF rats. Each column represents the mean ± SD (n = 6). **$P < .01$ *v* control group.

histopathologic differences between the treated and control groups.

DISCUSSION

In this study, we examined the influence of EPA-E on the development of insulin resistance in the OLETF rat, a model of spontaneous NIDDM with obesity. A comparison was made with the effects of lard, olive oil, or safflower oil. In the 17- or 18-week oral administration study, OLETF rats treated with EPA-E showed a decrease in basal blood glucose and an increase in the GIR in the euglycemic insulin-glucose clamp test. The GIR is an essential index of insulin resistance, and it was remarkably improved in rats treated with EPA-E, being approximately three times higher than in the control group. Also, the GIR in OLETF rats that received EPA-E was about 60% of that (10.6 ± 3.1 mg/kg/min) in male nondiabetic control Long Evans Tokushima Otsuka rats (data not shown). The other fatty acids tested (olive oil and safflower oil) had no positive effect and lard had a negative effect on the GIR. These findings suggest that only EPA-E has a preventive effect on the development of insulin resistance in diabetic rats. EPA-E is used for the treatment of hyperlipidemia in Japan, and our results are also supported by previous studies in which crude fish oil containing low levels of EPA-E prevented the development of insulin resistance in diabetic rats.[1,2,14]

In the OGTT, EPA-E–treated rats showed a decreased plasma glucose level at 30 minutes, but afterward their values were comparable to those of the control group. In contrast, plasma glucose levels in the lard, olive oil, and safflower oil groups were significantly higher than in the control group at 120 minutes. However, there were no significant differences in plasma insulin levels between the control and treated groups.

These findings suggest that insulin sensitivity may have decreased in all treated groups except the EPA-E group. At 120 minutes after glucose loading in the OGTT, the plasma insulin level (4.5 ± 2.2 ng/mL, equivalent to ~57.7% of the control value) in the EPA-E group was less than that (7.8 ± 4.3 ng/mL) in the control group. Therefore, this result suggests that EPA-E treatment acted to decrease the insulin level in the OGTT. However, because of considerable variation in the data for the control group, we could not clearly demonstrate a preventive effect of EPA-E on the development of insulin resistance in OLETF rats in terms of the insulin level in the OGTT.

Based on the hypothesis that the fatty acid composition of phospholipids in skeletal muscle may influence the action of

Table 5. Effects of Lard, Olive Oil, Safflower Oil, or EPA-E on Fatty Acid Composition of Skeletal Muscle Phospholipids in OLETF Rats

Fatty Acids (μg/g)	Control	Lard	Olive Oil	Safflower Oil	EPA-E
Saturated					
C16:0	1,436 ± 34‡	1,496 ± 67	1,501 ± 53*	1,473 ± 68	1,527 ± 62*
C18:0	700 ± 59	700 ± 30	730 ± 95	675 ± 65	667 ± 34
Monounsaturated					
C18:1	222 ± 13	239 ± 15	271 ± 30†‡	212 ± 21	234 ± 20
n-6 polyunsaturated					
C18:2	817 ± 34§	786 ± 56§	799 ± 66§	767 ± 33*§	954 ± 32†
C20:4	991 ± 60§	1,043 ± 57§	1,076 ± 97§	1,024 ± 50§	726 ± 49†
n-3 polyunsaturated					
C20:5	3 ± 4§	4 ± 5§	6 ± 4§	3 ± 4§	73 ± 9†
C22:5	128 ± 4§	137 ± 10*§	135 ± 6§	125 ± 7§	309 ± 15†
C22:6	711 ± 37‡	795 ± 49†	825 ± 88*	786 ± 73*	777 ± 57*

*$P < .05$, †$P < .01$: *v* control.
‡$P < .05$, §$P < .01$: *v* EPA-E.

Table 6. Effects of Lard, Olive Oil, Safflower Oil, or EPA-E on Body Fat Distribution in OLETF Rats

Body Fat (g%)	Control	Lard	Olive Oil	Safflower Oil	EPA-E
Mesenteric	1.81 ± 0.23	1.93 ± 0.26	2.03 ± 0.24*	1.94 ± 0.15	2.03 ± 0.15*
Subcutaneous	2.47 ± 0.42	2.36 ± 0.32	2.28 ± 0.37	2.32 ± 0.28	2.20 ± 0.25
Brown	0.27 ± 0.05	0.33 ± 0.06*	0.32 ± 0.07	0.34 ± 0.06*	0.32 ± 0.05

*$P < .05$ *v* control.

insulin, we analyzed the skeletal muscle of treated OLETF rats, and found significant differences in fatty acid composition between the control and EPA-E groups or between the EPA-E group and the other treated groups.

In particular, only EPA-E–treated animals showed a prominent increase in C18:2, C20:5, and C22:5 and a decrease in C20:4. Above all, the high C18:2 in muscle cell membrane phospholipids may be attributed to the inhibitory effect of EPA-E on linoleic acid (C18:2 n-6) desaturation and elongation.[15] On the other hand, the lard- and oil-treated animals showed an inverse change in these skeletal muscle phospholipids. However, we could not sufficiently elucidate the relation between the composition of fatty acids ingested and changes in fatty acid composition in the muscle from the results of this study alone. Storlien et al[4] have reported that long-chain n-3 fatty acids in skeletal muscle phospholipids may be important for the efficient action of insulin in rats. In addition, Borkman et al[16] suggested that variations in insulin sensitivity are related to differences in the membrane content of long-chain polyunsaturated fatty acids within skeletal muscle phospholipids in men. Moreover, Storlien et al[1] and Hainault et al[14] have reported the beneficial effect of fish oil on insulin resistance in rats.

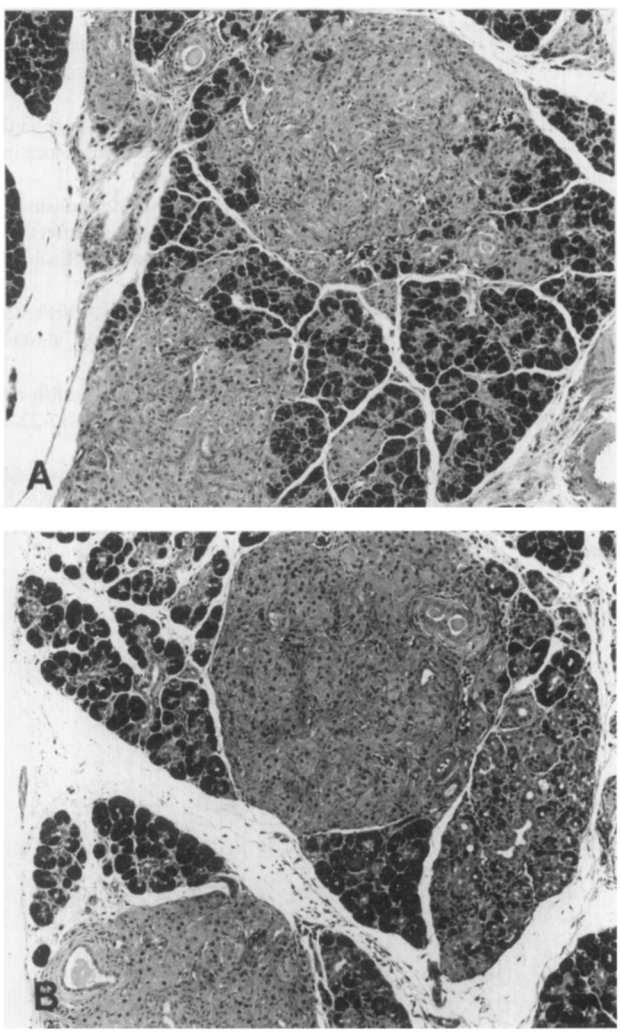

Fig 6. Representative histologic appearance of the pancreas from a control rat (A) and an EPA-E–treated rat (B). Enlargement of islets, fibrosis with inflammatory cell infiltration in and around the islets, ductal hyperplasia around the islets, and atrophy of acinar cells are evident in both organs. (Original magnification ×88.)

Our results are supported by these previous studies and provide more detailed data regarding the effect of EPA-E on skeletal muscle phospholipids in rats. The data we obtained suggest that polyunsaturated fatty acids, especially C20:5, may play a role in the management of NIDDM.

Jackson et al[17] and Sohal et al[18] suggested that high levels of dietary n-3 fatty acids, which alter muscle membrane composition, also increase muscle glucose transport in response to insulin in diabetic rats. Therefore, we determined the GLUT4 mRNA level in skeletal muscle from EPA-E–treated rats. The EPA-E group showed a significant increase in GLUT4 mRNA when compared with the control group, suggesting that the preventive action of EPA-E on the development of insulin resistance in OLETF rats is likely to depend on increased GLUT4 expression mediated by alterations in muscle membrane phospholipid composition.

Regarding the effects of fatty acids on lipid metabolism in OLETF rats, there was no significant difference in body fat distribution between control and treated rats. On the other hand, muscle triglyceride content was comparable in the control and

Table 7. Histopathologic Findings in the Pancreas of OLETF Rats Treated Orally With Lard, Olive Oil, Safflower Oil, or EPA-E

Findings	Rating Scale	Control	Lard	Olive Oil	Safflower Oil	EPA-E
Enlargement of islets	–	0	0	0	0	0
	+	4	3	4	4	3
	++	2	3	2	2	3
Fibrosis with inflammatory cell infiltration in or around islets	–	0	0	0	0	0
	+	6	5	6	6	6
	++	0	1	0	0	0
Ductal hyperplasia around islets	–	0	0	0	0	0
	+	5	6	5	6	6
	++	1	0	1	0	0
Atrophy of acinar cells	–	0	0	0	0	0
	+	4	5	4	4	4
	++	2	1	2	2	2

NOTE. Rating scale: –, no change; +, moderate; ++, severe. Results are the number of animals with pancreatic lesion from the number of animals examined (n = 6).

EPA-E groups, but was significantly higher in the lard or oil groups. Moreover, the hepatic triglyceride level was significantly lower in EPA-E–treated rats than in the controls and, conversely, significantly higher in rats given the other fatty acids. The plasma free fatty acid level was significantly higher in fatty acid–treated rats than in control rats, whereas EPA-E–treated animals were comparable to the controls in terms of plasma triglycerides, total cholesterol, and phospholipids. In contrast, the other treated groups showed significant increases in these values. These findings suggest that EPA-E is likely to be superior to the other tested fatty acids with respect to the effect on lipid metabolism in diabetic rats.

In conclusion, our study provides evidence that EPA-E prevents the development of insulin resistance in OLETF rats without a deleterious effect on the blood glucose level or lipid metabolism. Our findings also suggest that modulation of the fatty acid composition of skeletal muscle may be important for the management of NIDDM.

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