Effect of Highly Purified Eicosapentaenoic Acid Ethyl Ester on Insulin Resistance and Hypertension in Dahl Salt-Sensitive Rats

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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 lard on the development of hypertension and insulin resistance in Dahl salt-sensitive (Dahl-S) rats fed a high-sucrose diet (HSD), a model of salt-sensitive hypertension. After 16 weeks of treatment, the glucose infusion rate (GIR) during the euglycemic insulin-glucose clamp test significantly increased in the HSD-EPA-E group compared with the HSD-water or -lard control group. The GIR was approximately three times higher in the HSD-EPA-E group versus the HSD-water or -lard control group, and it was about 70% of the rate in the calorically deprived control group fed a low-fat–high-fiber diet (LF-HFD). In addition, EPA-E significantly suppressed the elevation of plasma glucose and insulin levels after oral glucose loading. These results indicate that EPA-E prevents the development of insulin resistance in Dahl-S rats fed a HSD. Fatty acid analysis of phospholipids in skeletal muscle showed a significant increase in C18:2, C20:5, and C22:5 components in the HSD-EPA-E group and, conversely, a significant decrease in C16:0, C20:4, and C22:6. The present results indicate that the beneficial effect of EPA-E on insulin resistance in Dahl-S rats fed a HSD is likely dependent on the modification of phospholipid components in the skeletal muscle membrane. These findings suggest that EPA-E might prevent the development of insulin resistance in dietary obesity. In addition, the HSD-EPA-E group showed a significant increase in the level of uncoupling protein (UCP) in brown adipose tissue as compared with the HSD-water or -lard control group. However, EPA-E had no effect on the development of hypertension and obesity in Dahl-S rats fed the HSD.

RECENTLY, attention has focused on the insulin resistance syndrome,[1] in which insulin resistance is regarded as the fundamental pathogenesis of diabetes mellitus, essential hypertension, abnormal lipid metabolism, and obesity. In particular, regarding the relation between insulin resistance and hypertension, it has been shown that hypertensive patients tend to be hyperinsulinemic and resistant to insulin-stimulated glucose uptake.[2-4] Similar changes also have been demonstrated in rats with spontaneous hypertension.[5-7]

We recently reported that long-term administration of highly purified eicosapentaenoic acid ethyl ester (EPA-E), an n-3 polyunsaturated fatty acid from fish oil, is useful for preventing 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.[8] In contrast, lard increased the insulin resistance.[8] We reported previously that Dahl salt-sensitive (Dahl-S) rats, developed by Dahl et al,[9] responded to a high-sucrose (~60%) diet (HSD) with an increase in blood pressure, development of insulin resistance, and obesity, but no such response occurred when a low-fat–high-fiber (~60%) diet (LF-HFD) was given.[10] Thus, in the present study, we investigated the effect of EPA-E on high-sucrose–induced insulin resistance and hypertension in Dahl-S rats, using lard and a LF-HFD as negative and calorically deprived controls, respectively.

We also demonstrated that EPA-E is effective for preventing the development of insulin resistance in dietary NIDDM with hypertension but ineffective for hypertension.

MATERIALS AND METHODS

Animals

Male Dahl-S rats were purchased from Seiwa Experimental Animals (Fukuoka, Japan) at 4 weeks of age. The animals were housed one per cage in stainless steel cages (290 × 350 × 175 mm) in an animal room with controlled temperature (23° ± 2°C) and relative humidity (55% ± 10%) and a 12-hour light/dark cycle. They were supplied with a standard rat chow (CRF-1; Oriental Yeast, Tokyo, Japan) for the first 4 weeks and then with another type of standard rat chow (MB-3; Funabashi Farm, Chiba, Japan) for the next 4 weeks. Thereafter, they were given a commercial HSD containing approximately 60% sucrose (Funabashi Farm) or a commercial LF-HFD containing approximately 60% cellulose (Funabashi Farm; Table 1) and tap water ad libitum during the experiment period.

Test Substances

Highly purified EPA-E (89.3% pure; Mochida Pharmaceutical, Tokyo, Japan) and lard (saturated fatty acid, 40.1%, Funabashi Farm) were used as the test substances (Table 2).

Experimental Design

At 8 weeks of age, male Dahl-S rats were randomly assigned to four groups of 12 rats each. Animals that were fed the HSD were treated with EPA-E or lard at a daily dose of 0.3 g/kg or with distilled water (0.3 mL/kg/d) for 16 weeks by gavage with microsyringes (Gastight; Hamilton, Reno, NV). The EPA-E dose was previously found to be effective for suppressing the development of insulin resistance in OLETF rats.[8] The lard dose also was used in the previous study.[8] The remaining animals were fed the LF-HFD and treated with distilled water (0.3 mL/kg/d) for 16 weeks by gavage. Rats treated with distilled water or lard and fed the HSD and rats treated with distilled water and fed the LF-HFD served as the water or lard control and the calorically deprived control, respectively. Food consumption and body weight were recorded once per week throughout the experimental period.

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Table 1. Ingredients, Calculated Nutrient Composition, and Fatty Acid Composition of HSD and LF-HFD

Parameter	HSD	LF-HFD
Ingredient (% by weight)		
Casein	23.7	23.5
DL-Methionine	0.3	0.3
Soybean oil	8.0	2.0
α-corn starch	0.0	9.0
Cellulose powder	3.0	60.2
Sucrose	60.0	0.0
USP17-vitamin	1.0	1.0
USP17-mineral	4.0	4.0
Calculated nutrient composition (% by weight)		
Moisture	2.75	8.0
Crude protein	20.48	20.22
Crude fat	8.06	2.35
Crude fiber	1.97	38.18
Ash	3.91	4.04
Nitrogen-free extract	62.82	27.21
Na	0.22	0.22
K	0.45	0.45
Metabolizable energy (kcal/g)	406	140
Fatty acid (%)		
14:0	0.3	1.0
16:0	10.9	12.9
16:1 (n-9)	ND	0.2
18:0	4.4	5.2
18:1 (n-9)	23.7	24.3
18:2 (n-6)	51.5	45.8
18:3 (n-3)	7.0	6.1
20:0	0.3	0.4
20:1 (n-9)	0.2	0.2
22:0 (n-6)	0.4	0.5
24:0 (n-3)	ND	0.3
Other	1.3	3.1

Abbreviation: ND, not detected (defined as <0.1%).

Blood Pressure Measurement

Each rat was kept warm for 5 minutes with a preheated plate at 40°C and then moved to a cylindrical box for immobilization and heating. Blood pressure was measured every 2 weeks for 16 weeks in unanesthetized rats by a tail-cuff apparatus (BP-98A; Softron, Tokyo, Japan).

Table 2. Fatty Acid Composition (%) of EPA-E and Lard

Fatty Acid	EPA-E	Lard
14:0	ND	1.9
16:0	ND	24.5
16:1 (n-9)	ND	3.3
18:0	ND	13.5
18:1 (n-9)	ND	43.9
18:2 (n-6)	ND	8.7
18:3 (n-3)	ND	0.8
20:0	ND	0.2
20:1 (n-9)	ND	0.8
20:4 (n-6)	5.6	ND
20:4 (n-3)	2.7	ND
20:5 (n-3)	89.3	ND
22:5 (n-3)	0.2	ND
22:6 (n-3)	0.1	ND
Other	2.1	2.4

Abbreviation: ND, not detected (defined as <0.1%).

Analytical Methods

Heparinized blood samples were taken from a tail vein by a butterfly needle without anesthesia every 4 weeks during the experimental period for measurement of the plasma glucose level with an autoanalyzer (Cobas Fara; Roche, Basel, Switzerland) using the hexokinase method. Urine samples were collected at weeks 8 and 16 of administration for measurement of urinary volume and urinary catecholamine levels in 24-hour urine samples by high-performance liquid chromatography method.[11]

Oral Glucose Tolerance Test

After 16 weeks of administration, an oral glucose tolerance test (OGTT) was performed on 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 plasma glucose by the method already described. Samples were also taken at 0, 60, and 120 minutes for measurement of plasma insulin with a commercial radioimmunoassay kit (Rat insulin [^{125}I] assay system; Amersham, Buckinghamshire, England).

Euglycemic Insulin-Glucose Clamp Test

After the 16-week administration period, a hyperinsulinemic-euglycemic clamp test was performed, according to the method of Kergort and Portha,[12] on the other half of the rats in each group to evaluate insulin-mediated whole-body glucose uptake. After an overnight fast, the surgical treatment required for the test was performed on animals anesthetized with an intraperitoneal injection of urethane (640 mg/kg) and α-chloralose (52 mg/kg). 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 (3 mU/min/kg) for about 2 hours following a loading infusion at 6 mU/min/kg for 10 minutes. Five minutes after the start of the 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). Total body glucose uptake was determined as the mean glucose infusion rate (GIR) during the last 20 minutes of the study. The plasma insulin level during the clamp was comparable in all groups, with a mean level of 167 ± 3 μU/mL in the HSD-water control group and 169 ± 2, 168 ± 2, and 168 ± 4 μU/mL in the HSD-lard control, HSD-EPA-E, and LF-HFD control groups, respectively.

Plasma Lipid Assay

After the OGTT and euglycemic insulin-glucose clamp test, heparinized blood samples were taken from the aorta abdominalis under pentobarbital sodium anesthesia (50 mg/kg body weight intraperitoneally), and centrifuged at 2,200 × *g* for 15 minutes at 4°C to isolate the plasma. Plasma levels of total cholesterol, phospholipids, triglycerides, and free fatty acids were measured enzymatically with an autoanalyzer (Cobas Fara; Roche).

Body Fat Distribution

At autopsy after the OGTT and euglycemic clamp test, 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 Bjorntorp.[13] Then, the fat weight was converted to a relative value per 100 g of body weight.

Western Blot Analysis of Uncoupling Protein in Brown Adipose Tissue

Uncoupling protein (UCP) in the scapular brown adipose tissue was measured by Western blot analysis and semiquantified by densitometry.[14] Briefly, the brown adipose tissue was homogenized in a solution (pH 7.5) containing 1 mmol/L $NaHCO_3$, 0.5 mmol/L $CaCl_2$, and 0.2 mmol/L $MgSO_4$ using a homogenizer, and the homogenate was then centrifuged at 15,000 × *g* for 10 minutes at 4°C. The protein level in the supernatant was measured with the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL). The supernatant was boiled for 5 minutes in sample buffer (pH 6.8) containing 125 mmol/L Tris, 4.6% sodium dodecyl sulfate, 20% glycerol, 50 mmol/L dithiothreitol, and 0.025% bromophenol blue. The sample (100 μg protein per lane) was applied to 15% polyacrylamide gels and subjected to electrophoresis at a constant current of 15 mA. After electrophoresis, the developed pattern was transferred to a polyvinylidene difluoride membrane (Immobilon; Millipore, Bedford, MA) by a semidry blotting method. The lane containing molecular markers was separated from the blotted membrane and subjected to protein staining with amido black 10 B. The remaining sample lanes were blocked with phosphate-buffered saline ([PBS] pH 7.4) containing 1% skim milk overnight at 4°C. After blocking, the membrane was washed with PBS containing 0.05% Tween 20 three times. Anti–rat UCP rabbit serum,[15] was diluted 1:1,000 with PBS containing 1% skim milk. The diluted serum was added to the membrane, which was then incubated at room temperature for 2 hours. After the membrane was washed again with Tween 20–PBS, peroxidase-labeled anti–rabbit immunoglobulin G goat antibody (TAGO Immunologicals, Burlingame, CA) was diluted 1:1,000 with PBS containing 1% skim milk and added to the membrane, which was then incubated for 1 hour at room temperature. Next, the membrane was washed with PBS containing 0.05% Tween 20 and with 50 mmol/L Tris hydrochloride (pH 7.4) three times and one time, respectively. After washing, the membrane was exposed to Hyperfilm ECL (Amersham Life Science) for 15 seconds to record the chemiluminescence induced by ECL (Amersham Life Science). Following exposure, color development was performed with Konica Immunostain HRP-1000 (Konica, Tokyo, Japan) The UCP band on the film was confirmed by the molecular marker and the blotted membrane, and its concentration was measured by densitometry (Atto Densitograph; Atto, Tokyo, Japan).

Measurement of Fatty Acid Composition of Skeletal Muscle Phospholipids

At autopsy, the left quadriceps muscle of the thigh of each rat was harvested for extraction and derivatization of lipids. The muscle tissue was 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.[16] Phospholipid extraction and fatty acid identification were performed according to the method of Borkman et al.[17] Phospholipids were separated from less polar lipids by solid-phase extraction on Sep-Pak silica cartridges (Waters, Milford, CT) and then transmethylated with 14% boron trifluoride at 85°C for 60 minutes. The methylated fatty acids were subsequently separated and quantified by gas chromatography (model 663; Hitachi, Tokyo, Japan) using a capillary column (SP-2380). The fatty acids were identified by comparing their retention times with those of authentic standards.

Histopathological Examination

Following the removal of body fat, the pancreas, kidneys, and liver were dissected, weighed, 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 HSD-water or -lard control group was analyzed by Student's *t* test. A statistically significant difference was defined at a P level less than .05.

RESULTS

General Description

There were no changes in the general status for any of the groups during the experimental period. The HSD-water control, -lard control, and -EPA-E groups were comparable in body weight gain, total food intake, and food efficiency. However, the LF-HFD control group showed significantly decreased body weight gain and food efficiency and increased total food intake (Table 3). In addition, there were no macroscopic abnormalities in any group. The relative weights of the liver, heart, kidney, spleen, and pancreas were comparable in the HSD-water control, -lard control, and -EPA-E groups (data not shown).

Blood Pressure

The HSD-water control, -lard control, and -EPA-E groups showed a gradual elevation from 130 to 180 mm Hg in systolic blood pressure for 16 weeks, and blood pressure was comparable among these groups. In contrast, the LF-HFD control group showed a constant blood pressure of 140 to 150 mm Hg after 2 weeks of treatment, and the values were significantly lower versus the HSD-water or -lard control group from weeks 4 or 6 to 16 of treatment. As well as the values for the HSD-water or -lard control group, values for the HSD-EPA-E group were apparently higher versus the LF-HFD control group (Fig 1).

Table 3. Body Weight Gain, Total Food Intake, and Food Efficiency in Male Dahl-S Rats Treated Orally With EPA-E for 16 Weeks

Parameter	HSD-Water Control	HSD-Lard Control	HSD-EPA-E	LF-HFD Control
Body weight gain (g)	268 ± 33	253 ± 23	263 ± 28	164 ± 28*†
Total food intake (g)	2,320 ± 113	2,217 ± 79	2,361 ± 184	4,828 ± 220*†
Food efficiency (body weight gain/100 g food)	11.5 ± 0.9	11.4 ± 0.7	11.1 ± 0.5	3.4 ± 0.5*†

NOTE. Values are the mean ± SD (n = 12).

*$P < .01$ *v* HSD-water control.

†$P < .01$ *v* HSD-lard control.

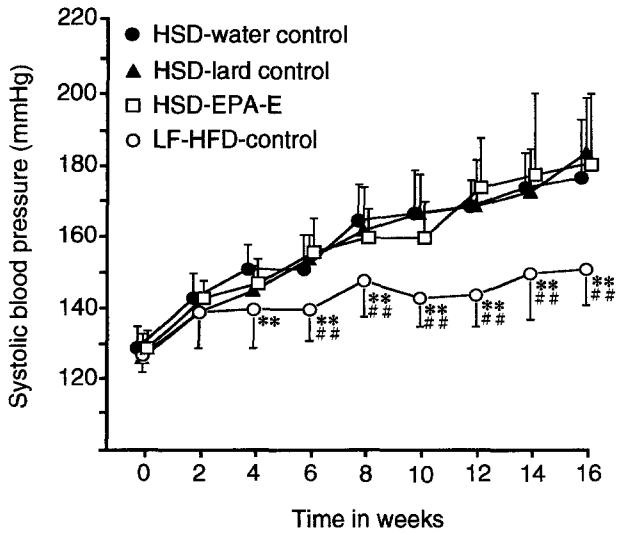

Fig 1. Changes in systolic blood pressure of Dahl-S rats. Data are the mean ± SD (n = 12). **$P < .01$ *v* HSD-water control. ##$P < .01$ *v* HSD-lard control.

Urinary Catecholamine Levels

The HSD-water control, -lard control, and -EPA-E groups were comparable for the urinary output of epinephrine, norepinephrine, and dopamine and the urinary volume after 8 and 16 weeks of treatment. In contrast, the LF-HFD control group showed significantly decreased urinary output of epinephrine after 8 and 16 weeks (only *v* HSD-water control) of treatment, decreased norepinephrine after 16 weeks (only *v* HSD-lard control), and decreased dopamine after 8 weeks compared with the HSD-water or -lard control group (Table 4).

Glucose Tolerance

The HSD-EPA-E and LF-HFD control groups showed significantly decreased plasma glucose before (except HSD-EPA-E *v* HSD-water control) and at 30 minutes (only LF-HFD control *v* HSD-lard control), 60, and 120 minutes after glucose loading compared with the HSD-water or -lard control group. In addition, the HSD-EPA-E group showed significantly decreased plasma immunoreactive insulin at 60 minutes (only *v* HSD-lard control) and 120 minutes after glucose loading, as did the LF-HFD control group before and at 60 and 120 minutes, compared with the HSD-water or -lard control group (Fig 2).

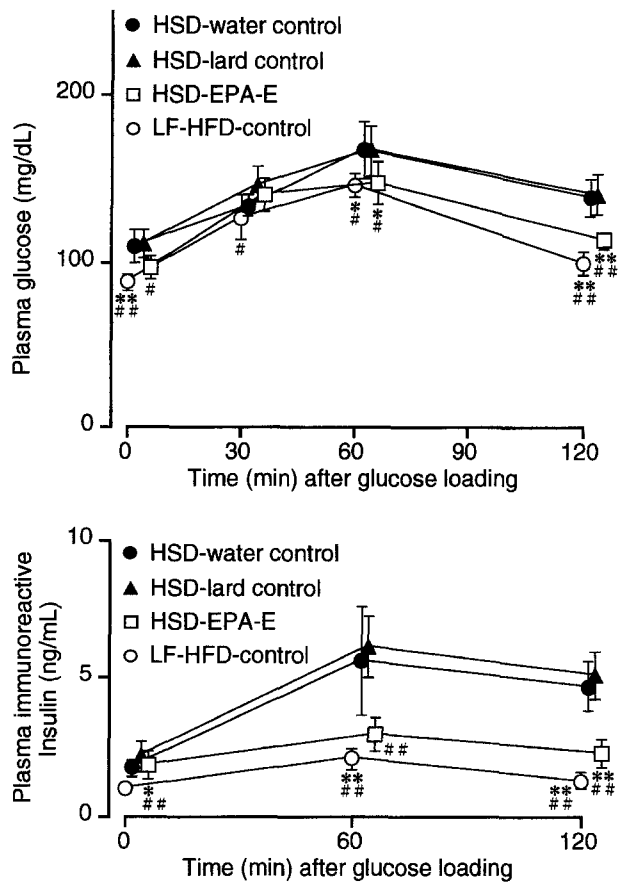

Fig 2. Plasma glucose and insulin levels after an oral glucose load in Dahl-S rats. Data are the mean ± SD (n = 6). *$P < .05$, **$P < .01$ *v* HSD-water control. #$P < .05$, ##$P < .01$ *v* HSD-lard control.

Hyperinsulinemic-Euglycemic Clamp

Both HSD-EPA-E and LF-HFD control groups showed significantly decreased basal blood glucose compared with the HSD-water or -lard control group, and the value for the HSD-lard control group was similar to that for the HSD-water control group. In addition, the steady-state GIR for the HSD-EPA-E group and LF-HFD control group was, respectively, about 2.8 and 4.0 times greater than the rate for the HSD-water or -lard control group, and these differences were significant.

Table 4. Changes in Urinary Output of Catecholamines in Dahl-S Rats Treated Orally With EPA-E for 16 Weeks

Parameter	Dosing Period (wk)	HSD-Water Control	HSD-Lard Control	HSD-EPA-E	LF-HFD Control
Urinary volume (mL/d)	8	25.6 ± 9.3	27.8 ± 10.2	22.8 ± 3.9	21.7 ± 4.9
	16	28.6 ± 12.6	33.6 ± 10.1	26.0 ± 5.6	21.8 ± 6.8
Epinephrine (ng/d)	8	187 ± 53	170 ± 29	175 ± 40	146 ± 26*‡
	16	331 ± 72	306 ± 83	302 ± 85	253 ± 83*
Norepinephrine (ng/d)	8	731 ± 75	772 ± 93	717 ± 115	726 ± 106
	16	833 ± 69	885 ± 126	820 ± 134	747 ± 123‡
Dopamine (ng/d)	8	3,732 ± 393	3,486 ± 505	3,334 ± 355	2,712 ± 435†§
	16	3,205 ± 341	2,946 ± 511	3,023 ± 293	2,639 ± 482

NOTE. Values are the mean ± SD (n = 12).
*$P < .05$, †$P < .01$ *v* HSD-water control.
‡$P < .05$, §$P < .01$ *v* HSD-lard control.

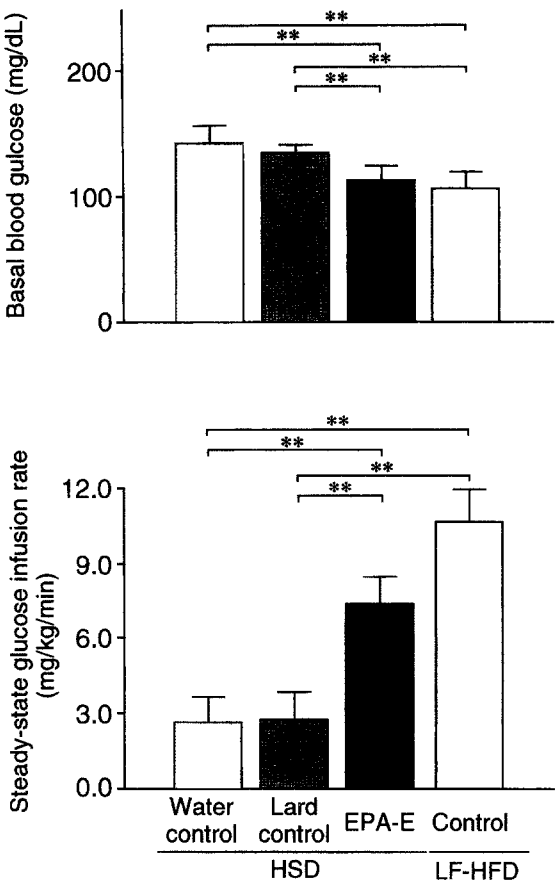

Fig 3. Insulin resistance in the euglycemic insulin-glucose clamp test in Dahl-S rats. Each column represents the mean ± SD (n = 6). **$P < .01$ for the indicated groups.

The HSD-lard control group retained a rate similar to that of the HSD-water control group (Fig 3).

Plasma Lipid Levels

The HSD-EPA-E group showed significantly decreased triglycerides, phospholipids, and free fatty acids (only *v* HSD-water control) compared with the HSD-water or -lard control group, and the HSD-lard control group was similar to the HSD-water control group for these parameters. Values for the LF-HFD control group were similar to values for the HSD-EPA-E group, but the triglyceride level in the former was about half that in the latter. On the other hand, total cholesterol was essentially the same in all groups (Table 5).

Body Fat Distribution

The HSD-EPA-E group showed a significantly decreased brown fat content compared with the HSD-water control group, and a significant decrease in the content of mesenteric, subcutaneous, and brown fat compared with the HSD-lard control group. The HSD-lard control group showed a significantly increased amount of subcutaneous fat compared with the HSD-water control group. The LF-HFD control group had significantly less mesenteric, subcutaneous, and brown fat than the HSD-water and -lard control groups (Fig 4).

Table 5. Effects of 16 Weeks of EPA-E Treatment on Plasma Lipid Levels in Dahl-S Rats

Plasma Lipid	HSD-Water Control	HSD-Lard Control	HSD-EPA-E	LF-HFD Control
Triglycerides (mg/dL)	265 ± 78	271 ± 42	136 ± 52†§	66 ± 27†§
Total cholesterol (mg/dL)	172 ± 21	153 ± 7	158 ± 26	149 ± 24
Phospholipids (mg/dL)	256 ± 31	236 ± 17	210 ± 29†‡	197 ± 28†§
Free fatty acids (mEq/L)	1.38 ± 0.32	1.31 ± 0.44	1.06 ± 0.29*	1.02 ± 0.22*

NOTE. Values are the mean ± SD (n = 12).
*$P < .05$, †$P < .01$ *v* HSD-water control.
‡$P < .05$, §$P < .01$ *v* HSD-lard control.

UCP Levels in Brown Adipose Tissue

The HSD-EPA-E group showed a significant increase in UCP in brown adipose tissue compared with the HSD-water or -lard control group. The HSD-lard control group had a significant decrease in UCP compared with the HSD-water control (Fig 5).

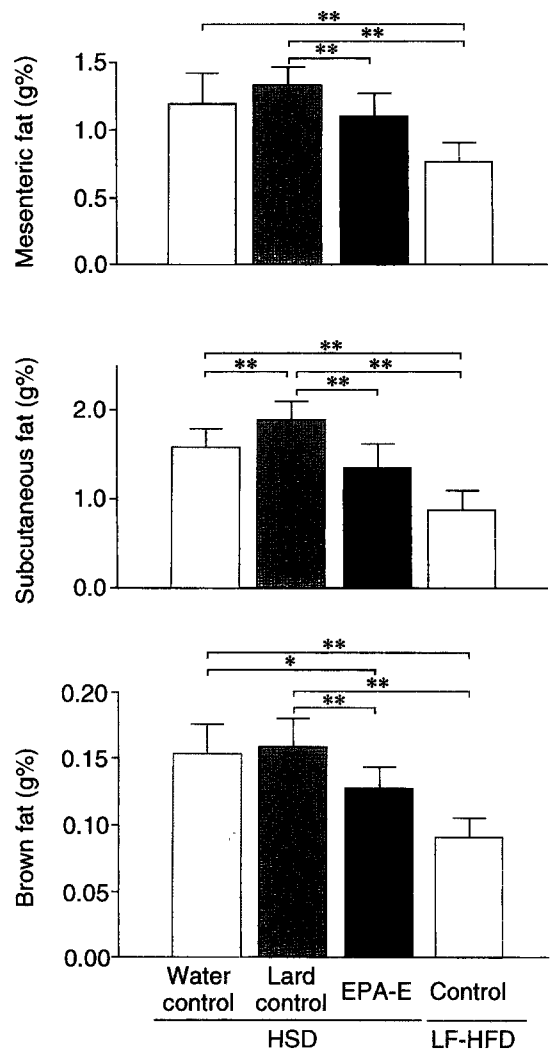

Fig 4. Body fat distribution in Dahl-S rats. Each column represents the mean ± SD (n = 6). *$P < .05$, **$P < .01$ for the indicated groups.

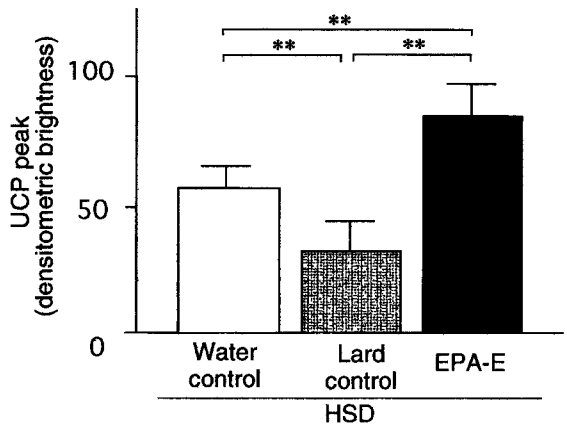

Fig 5. Western blot analysis of UCP in scapular brown adipose tissue in Dahl-S rats. Each column represents the mean ± SD (n = 6). **$P < .01$ for the indicated groups.

Fatty Acid Composition of Skeletal Muscle Phospholipids

The HSD-EPA-E group showed significant increases in C18:2 (linoleic acid; only *v* HSD-water control), C20:5 (eicosapentaenoic acid), and C22:5 (docosapentaenoic acid) and, conversely, decreases in C16:0 (palmitic acid; only *v* HSD-water control), C20:4 (arachidonic acid), and C22:6 (docosahexaenoic acid; only *v* HSD-water control) compared with the HSD-water or -lard control group. The HSD-lard control group was similar to the HSD-water control group with respect to these fatty acids. The LF-HFD control group showed a significant decrease in C16:0, C18:1 (oleic acid; only *v* HSD-water control), C22:5, and C22:6 compared with the HSD-water or -lard control group (Table 6).

Histopathological Findings

The kidney showed glomerular, tubular, and arterial lesions of moderate to severe degree in the HSD-water control, -lard control, and -EPA-E groups, but the pancreas showed little histopathological change. In addition, there was a thickening of the arterial wall, inflammatory cell infiltration around interlobular arteries in the liver, and hypertrophy of the aortic intima of moderate to severe degree in all four groups, including the LF-HFD control group (data not shown).

Table 6. Effects of 16 Weeks of EPA-E Treatment on Fatty Acid Composition of Skeletal Muscle Phospholipids in Dahl-S Rats

Fatty Acid (μg/g)	HSD-Water Control	HSD-Lard Control	HSD-EPA-E	LF-HFD Control
Saturated				
C16:0	1,597 ± 75	1,599 ± 101	1,516 ± 79*	1,457 ± 84†§
C18:0	877 ± 87	881 ± 109	841 ± 8	808 ± 68
Monounsaturated				
C18:1	264 ± 34	271 ± 31	256 ± 30	243 ± 17‡
n-6 polyunsaturated				
C18:2	929 ± 77	956 ± 155	1,074 ± 127†	914 ± 94
C20:4	1,229 ± 76	1,217 ± 115	853 ± 66†§	1,224 ± 106
n-3 polyunsaturated				
C20:5	1 ± 3	0 ± 0	71 ± 7†§	3 ± 5
C22:5	138 ± 13	143 ± 19	358 ± 46†§	115 ± 11†§
C22:6	1,115 ± 143	1,081 ± 111	987 ± 82*	974 ± 89*‡

NOTE. Values are the mean ± SD (n = 12).
*$P < .05$, †$P < .01$ *v* HSD-water control.
‡$P < .05$, §$P < .01$ *v* HSD-lard control.

DISCUSSION

We previously reported that Dahl-S rats, a model of salt-sensitive hypertension, fed a HSD showed an elevation in systolic blood pressure and increases in the urinary output of catecholamines, urinary sodium excretion, plasma insulin, and body weight in comparison to Dahl-S rats fed a LF-HFD.[10] Furthermore, we recently reported that long-term administration of highly purified EPA-E prevents the development of insulin resistance in OLETF rats, a model of spontaneous NIDDM with obesity, but lard was detrimental.[8]

In this study, we examined the effect of EPA-E on the development of hypertension and insulin resistance in Dahl-S rats fed a HSD. Lard and a LF-HFD served as a reference control and a calorically deprived reference, respectively. In the 16-week oral administration study, the HSD-EPA-E group was roughly similar to the HSD-water control group in terms of systolic blood pressure, urinary output of catecholamines, and body weight gain. Namely, EPA-E had no effect on the development of hypertension and obesity in HSD-fed Dahl-S rats. Also, lard had no effect on either parameter. In contrast, the LF-HFD control group showed significant decreases in body weight gain, food efficiency, body fat content, and urinary output of catecholamines. The decrease in catecholamines in the LF-HFD control group may have been due to the lower body weight gain.[18]

However, the HSD-EPA-E group 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 was remarkably improved in the HSD-EPA-E group, being approximately three times higher than the GIR in the HSD-water or -lard control group. Also, the basal blood glucose level in the HSD-EPA-E group was comparable to that in the LF-HFD control group, and the GIR for the former was about 70% of the GIR for the latter. These findings suggest that EPA-E prevents the development of dietary insulin resistance in Dahl-S rats. These results obtained for the HSD-EPA-E group support our previous data on EPA-E–treated OLETF rats.[8] In contrast, the HSD-lard control group received no beneficial effect in terms of the above-mentioned parameters.

In the OGTT, the HSD-EPA-E group showed decreased plasma glucose at 60 and 120 minutes and tended to show the same effect in terms of plasma insulin. Therefore, we could clearly demonstrate by the OGTT a preventive effect of EPA-E on the development of dietary insulin resistance in Dahl-S rats with respect to plasma glucose and insulin levels.

In the previous study using OLETF rats, we already proposed that the beneficial effect of EPA-E on insulin resistance is likely dependent on the modification of phospholipid components of the skeletal muscle membrane.[8] Thus, we also analyzed the skeletal muscle of treated Dahl-S rats. The HSD-EPA-E group showed prominent increases in C18:2, C20:5, and C22:5 and

decreases in C16:0, C20:4, and C22:6. Such a modification of phospholipid components of the skeletal muscle membrane is considered to occur as follows. The formation of interconversion products from C18:2, namely, C18:2 → delta-6-desaturase → C18:3 → elongase → C20:3 → delta-5-desaturase → C20:4, was significantly inhibited by the treatment with EPA-E (C20:5). As a result, C18:2 significantly accumulated and, conversely, C20:4 significantly decreased in the muscle membrane. EPA-E probably inhibits the metabolism of C18:2 by two processes. One explanation may be an inhibitory effect of EPA-E on the elongation/desaturation of n-6 fatty acids. The other is the competition for acylation with the unsuccessful fatty acid being catabolized at an increased rate. In addition, the C20:5 content was remarkably increased by treatment with EPA-E, and C20:5 was converted to C22:5 by elongase. Consequently, C20:5 and C22:5 significantly accumulated, and, conversely, C22:6 significantly decreased. In addition, the decrease in C22:6 in muscle phospholipids may be related to the decrease in the plasma phospholipid level in the HSD-EPA-E group, but the details of this relationship remain unclear. In a comparison of these results obtained in EPA-E–treated Dahl-S rats fed a HSD to results for EPA-E–treated OLETF rats,[8] although the former showed decreased C16:0 and C22:6 and the latter showed increases, the remaining fatty acids showed similar changes in both animals.

Regarding the effect of EPA-E on lipid metabolism in Dahl-S rats fed the HSD, EPA-E–treated rats showed significant decreases in plasma triglycerides, phospholipids, and free fatty acids. These findings suggest that EPA-E has a desirable effect on lipid metabolism in Dahl-S rats fed a HSD. In terms of body fat distribution, EPA-E–treated rats showed a significant decrease in brown adipose tissue available for thermogenesis but, conversely, a significant increase in its UCP content, which exists especially in brown adipose tissue and plays an important role in the control of energy metabolism. These results suggest that the function of brown adipose tissue may be maintained with the consequence that EPA-E has no effect on body weight gain in Dahl-S rats fed a HSD. Lard-treated control rats had no change in brown adipose tissue content but showed a significant decrease in its UCP content compared with water-treated controls. These findings suggest that lard may influence the biosynthesis of UCP in adipose tissue without affecting the weight of the tissue.

In conclusion, this study provides evidence that EPA-E prevents the development of insulin resistance in Dahl-S rats with dietary obesity but does not reduce the blood pressure. The prevention of insulin resistance by treatment with EPA-E is likely attributable to the modification of phospholipid components of the skeletal muscle membrane.

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