Docosahexanoic Acid (DHA) Improved Glucose and Lipid Metabolism in KK-A' Mice with Genetic Non-Insulin-Dependent Diabetes Mellitus (NIDDM)

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The hypoglycemic and hypolipidemic effect of docosahexaenoic acid (DHA; C22:6ω3) ethyl ester was examined in KK-A' mice and neonatal streptozotocin-induced diabetic (NSZ) which are respectively obese and lean animal models of non-insulin-dependent diabetes mellitus (NIDDM), and in ddY normal mice. Single administration of DHA (500 mg/kg body weight) to KK-A' mice significantly reduced (p < 0.05) the blood glucose levels (BG) (p < 0.05) and plasma free fatty acid levels (FFA) (p < 0.05) at 10 h after oral administration when compared with control group. DHA (500 mg/kg body weight)-treated NSZ and normal mice, however, showed no change in these parameters. In addition, repeated administration of DHA (100 mg/kg) to KK-A' mice significantly suppressed the increment of BG (p < 0.05) and plasma triglyceride levels (TG) (p < 0.01), and significantly decreased FFA (p < 0.05) at 30 d compared with control group. DHA also significantly decreased the blood glucose at 60 and 120 min on insulin tolerance test (ITT). From these findings, it seems likely that DHA exhibits its hypoglycemic effects by increasing insulin sensitivity. It is concluded that DHA would be useful for treatment of obese type NIDDM with insulin resistance.

Key words hypoglycemic effect; hypolipidemic effect; docosahexaenoic acid; KK-A' mouse; non-insulin-dependent diabetic mellitus

In the early 1970s, epidemiologic studies conducted in Greenland Eskimos by Bang and colleagues led to the hypothesis that fish oil rich in ω3 polyunsaturated fatty acid (ω3PUFA) and coronary heart disease (CHD). The effect of fish oil intake has, in turn, been attributed to ω3PUFA, especially eicosapentaenoic acid (EPA; C22:5ω3) and docosahexaenoic acid (DHA; C22:6ω3). DHA contains 22 carbon atoms with 6 double bonds, the first one at position 3 from the methyl terminal. Δ6-Linoleic acid belongs to the ω3 family, and is metabolized to EPA and DHA by desaturase and elongase. NIDDM has been reported to reduce omega-3 and omega-6 desaturase activity, and also EPA and DHA in both liver and muscle phospholipid.5)

The aim of this study was, accordingly, to examine the effect of DHA on glycemic and lipidemic metabolism in KK-A' mice, one of the animal models of obese type genetic NIDDM with hyperinsulinemia, and neonatal streptozotocin-induced diabetic (NSZ) mice, one of the animal models of lean type NIDDM with hypoinsulinemia.

MATERIALS AND METHODS

Materials DHA ethyl ester was purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan) and stored at −40℃ until use. Antioxidant used was DL-α-tocopherol (40 mg/kg body weight) with DHA in the repeated administration.

Animals Adult male ddY mice (5 weeks old, SLC, Shizuoka, Japan) weighing 22—25 g were used as a normal mice. They were housed in an air-conditioned room in plastic cages at 22 ± 2℃ with a 12 h light—12 h dark cycle.

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The animals were kept in the experimental animal room for 7 d with free access to food and water. NSZ mice were produced by subcutaneous injection with 90 mg/kg body weight of streptozotocin (STZ, Sigma, Tokyo, Japan) freshly dissolved in citrate buffer, pH 4.5 to neonatal mice (1 to 2 d old). Five weeks after injection of STZ, the blood glucose levels (BG) of all the mice were determined. KK-A' mice (CLEA, Tokyo, Japan) 12 weeks of age were also used. Both NSZ and KK-A' mice with BG above 300 mg/dl were considered to be diabetic and were used in this study. DHA was given orally by force. In repeated administration, DHA was administered at 10:00—11:00 am. Blood samples were taken before the administration.

Insulin Tolerance Test Insulin tolerance tests were performed at the end of the repeated study. Twenty-four hours before the test, food was removed from the cages. Insulin (0.5 U/kg body weight) solution was administered subcutaneously. Blood samples were collected before the administration of the insulin and at 30, 60 and 120 min later.

Determination of Blood Samples For the determination of blood factors, the blood samples were withdrawn from the cavernous sinus via a capillary. After centrifugation, BG, plasma triglyceride levels (TG), plasma cholesterol levels and plasma free fatty acid levels (FFA) in these animals were determined using commercial reagents (Glucose C-Test Wako, Triglyceride G-Test Wako, Cholesterol E-Test Wako, NEFA C-Test Wako, Wako Pure Chemical Industries, Ltd.). Plasma insulin levels were measured by the double antibody method.

Statistical Analysis All the data were expressed as means ± S.E. Analysis of variance (ANOVA) and Student’s t-test were used for the statistical analysis. Values were considered to be significantly different when p value was
RESULTS

Single Administration of DHA The mean levels of the blood samples in mice at 10 h after oral administration of DHA are shown in Fig. 1. DHA-treated KK-A' mice showed significantly decreased BG at 10 h after the oral administration ($p < 0.01$). Mice treated with tolbutamide (a known sulfonylurea hypoglycemic agent) (50 mg/kg) showed lower BG 10 h after the administration ($p < 0.05$). DHA (500 mg/kg)-treated mice also had decreased FFA when compared with control group (Fig. 2). However no changes in TG or total cholesterol (TC) were observed at any time between control and DHA groups (data not shown). NSZ mice and normal mice, on the other hand, had unchanged blood glucose (NSZ mice: control 795 ± 58, DHA 769 ± 46 mg/dl, normal mice: control 194 ± 11, DHA 206 ± 12 mg/dl).

Repeated Administration of DHA No changes in body weight were observed between the control and DHA groups. DHA-(100 mg/kg) treated KK-A' mice showed decreased BG (Fig. 3), TG (Fig. 4) and FFA (Fig. 5) after 30 d, however, TC did not change. We measured plasma insulin concentration after the repeated administration study. The DHA-treated group tended to show a decrease in plasma insulin after 30 d (Fig. 6).

Insulin Tolerance Test The fasting blood glucose level in KK-A' mice was higher than that in normal mice (KK-A' mice 173 ± 18, normal mice 89 ± 7 mg/dl, $p < 0.01$). Blood glucose concentrations during the insulin tolerance test are shown in Fig. 7. DHA (100 mg/kg) was administered orally to KK-A' mice once a day. Blood samples were taken for glucose determinations. Each value represents the mean ± S.E. of 4-6 mice. Significantly different from control, *$p < 0.05$, **$p < 0.01$ (by ANOVA).
Fig. 5. Effect of DHA on Free Fatty Acid in KK-A' Mice (Repeated Administration)
DHA (100 mg/kg) was administered orally to KK-A' mice once a day. Blood samples were taken for free fatty acid determinations. Each value represents the mean ± S.E. of 7 mice. Significantly different from control, *p<0.05.

Fig. 6. Effect of DHA on Insulin Concentration in KK-A' Mice
DHA (100 mg/kg) was administered orally to KK-A' mice once a day. Blood samples were taken for insulin determinations. Each value represents the mean ± S.E. of 5–7 mice.

duced insulin secretion by polyunsaturated fatty acids are reported. In addition, DHA-treated mice showed a decrease in BG in insulin tolerance test. Insulin (0.5 U/kg)-treated KK-A' mice did not show a decrease in BG because of insulin resistance in peripheral tissues. Improvement by fish oil of insulin sensitivity and intravenous glucose tolerance has reportedly been shown in nondiabetic subjects. From these findings, it seemed likely that DHA exhibited its hypoglycemic effects by increasing insulin sensitivity. DHA also improved lipid metabolism, indicating that it is useful for diabetic complications, because diabetic patients have elevated BG and TG by metabolic derangement. Further study will indicate how DHA could become a useful drug in the treatment of NIDDM.

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REFERENCES