A Comparative Study of High-Fat Diet Containing Fish Oil or Lard on Blood Glucose in Genetically Diabetic (db/db) Mice

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Summary The effects of high-fat diets containing fish oil or lard on blood glucose and plasma insulin after oral glucose loading were compared in genetically diabetic (db/db) mice, one of the animal models of non-insulin-dependent diabetes mellitus (NIDDM) with hyperinsulinemia. The blood glucose levels were significantly decreased in 27% of the mice fed high-fat diets containing 20% fish oil 30 and 60 min after the oral administration of glucose (both; \(p<0.05\)). Conversely, the plasma insulin levels were significantly increased 30 min after the glucose loading as compared to 27% of the mice fed high-fat diets containing 20% lard (\(p<0.01\)). In addition, a significant hypoglycemic effect was observed 60 min after the subcutaneous administration of insulin to mice on the fish oil diet (\(p<0.05\)), whereas no effect was demonstrated in the case of those on the lard diet. The average body weight of the fish oil-treated mice was not significantly different from that of the lard-treated mice. The fish oil diet has a beneficial effect on glucose tolerance by increasing the insulin secretory capacity from pancreatic \(\beta\) cells and also ameliorating insulin resistance.

Key Words fish oil, lard, db/db mice, insulin secretion, insulin sensitivity

The ingestion of \(n\)-3 polyunsaturated fatty acids (\(n\)-3 PUFA), particularly eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA), found in cold-water fish seems to lower mortality from coronary artery disease (1). Such reduced

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incidence of ischemic cardiovascular diseases (2) has been reported to be due to reduced triglyceride concentrations (3). Moreover, n-3 PUFA seem to beneficially interfere with platelet aggregation (4), blood rheology (5), fibrinolysis (6) and blood-pressure regulation (7,8), thus counteracting possible risk factors of arteriosclerosis.

Furthermore, despite a fat-enriched diet (9), the incidence of diabetes mellitus among Eskimos is low (2,10), which may hint either at a genetic trait or at a preventive effect by the daily consumption of 6–10 g of n-3 PUFA (9). A potentially beneficial effect of dietary fish oil supplementation on peripheral insulin resistance, probably subsequent to a change in cell membrane composition (11,12), is supported by studies in animals (13) and patients with non-insulin-dependent diabetes mellitus (NIDDM) (14). Concerning insulin secretion, both augmentation (15) and reduction (16,17) by n-3 PUFA have been reported. Although diabetic patients carry a high risk for cardiovascular disease (18), dietary intervention by fish oil, although potentially helpful in reducing such risks, has yielded controversial results in both insulin-dependent diabetic (19,20) and NIDDM (21–24) patients, because improved lipid metabolism may be outweighed by deterioration in blood glucose control. Conversely, improvements in insulin sensitivity and glucose tolerance by fish oil have been shown in nondiabetic subjects (25).

Therefore, the therapeutic potential of n-3 PUFA in disorders of glucose metabolism is still unclear (26). The observed variability in metabolic responses to fish oil supplementation in NIDDM may, however, result from the disease's undefinable duration and its pathophysiological heterogeneity. Therefore, to better determine the impact of dietary control and the effect of n-3 PUFA on glycemic control and insulin action, we chose a genetical NIDDM mouse model, db/db mice (27).

MATERIALS AND METHODS

Fish oil (EPA 17.9%, DHA 10.7%, d-α-tocopherol 2.1 IU/g, peroxide value 4.3 meq/kg), generously donated by Johnson & Johnson K.K. (Tokyo, Japan), and lard (Miyoshi-Yushi, Japan) were used in this experiment.

Animals. db/db mice (Clea, Tokyo, Japan), 12 weeks of age, were used. The db/db mice with blood glucose levels above 300 mg/100 mL were considered to be diabetic and used in this study. The mice were housed in an air-conditioned room at 22±2°C with a 12 h light–12 h dark cycle. The animals were kept in the experimental animal room for 6 weeks with free access to food and water. For the determination of blood glucose levels, blood samples were withdrawn from the cavernous sinus with a capillary under unanesthesia. The composition of the experimental diets is indicated in Table 1. The energy and chemical compositions of the experimental diets were metabolic energy 504 kcal/100 g, moisture 2.8%, crude protein 20.4%, crude fat 27%, crude fiber 0.1%, ash 4.7% and soluble non-nitrogenous materials 45% (high-fat diet containing fish oil is the same
Comparison of Fish Oil or Lard on Blood Glucose in db/db Mice

Table 1. Composition of the experimental diets (%).

| Ingredients         | Lard | Fish oil |
|---------------------|------|----------|
| Casein              | 18   | 18       |
| Sucrose             | 41   | 41       |
| Yeast, beer         | 2    | 2        |
| Beef fat            | 7    | 7        |
| Lard                | 20   | —        |
| Fish oil            | —    | 20       |
| Soybean oil         | 1    | 1        |
| White fish meal     | 5    | 5        |
| Skim milk powder    | 1.5  | 1.5      |
| CaHPO₄·2H₂O          | 1.5  | 1.5      |
| USP*-Vitamin        | 1    | 1        |
| USP*-Mineral        | 2    | 2        |

*USP: United States Pharmacopeia.

composition as that containing lard).

Oral glucose tolerance test. After overnight fasting (18 h), the glucose (2 g/kg body weight) solution was administered orally. Blood samples were collected before administration of the glucose and 30, 60 and 120 min thereafter. Blood samples for plasma insulin determination were also taken before the administration of glucose and 30 min thereafter.

Insulin tolerance test. After overnight fasting (18 h), the insulin (0.5 U/kg body weight) solution was administered subcutaneously. Blood samples were collected before administration of the insulin and at 30, 60 and 120 min thereafter. Oral glucose tolerance and insulin tolerance tests were two separate experiments.

Determination of blood glucose and insulin. The blood glucose levels in mice were determined by the glucose oxidase method (28), and plasma insulin was measured by the double-antibody method (29). All the data were expressed as M±SE, and Student’s paired or unpaired t-tests were used for the statistical analysis. The values were considered to be statistically different when the p value was less than 0.05.

RESULTS

Effects of fish oil and lard on body weight and blood glucose in genetically diabetic (db/db) mice

The average body weight of the fish oil-treated mice was not significantly different from that of the lard-treated mice (Fig. 1). The effect of fish oil on the blood glucose of db/db mice is shown in Fig. 2. The blood glucose levels in db/db mice fed high-fat diets containing fish oil tended to decrease 6 weeks after administration as compared to the mice fed high-fat diets containing lard, but there was no statistical difference found between them.
Fig. 1. Change in body weight. Each value represents the M±SE from 5 mice. ○, Lard; ●, fish oil.

Fig. 2. Effects of fish oil and lard on blood glucose in db/db mice. Each value represents the M±SE from 5 mice. ○, Lard; ●, fish oil.

Fig. 3. Effects of fish oil and lard on blood glucose by glucose tolerance test in db/db mice. Each value represents the M±SE from 5 mice. Significantly different from lard, *p<0.05. ○, Lard; ●, fish oil.

Oral glucose tolerance test
At 6 weeks, fish oil-treated mice showed a significant decrease in blood glucose levels 30 and 60 min after the loading of glucose (both p<0.05) (Fig. 3). The plasma insulin levels before and 30 min after loading are shown in Fig. 4. The plasma insulin level was found to be significantly increased 30 min after loading in
Comparison of Fish Oil or Lard on Blood Glucose in db/db Mice

Fig. 4. Effects of fish oil and lard on insulin by glucose tolerance test in db/db mice. Each value represents the M±SE from 5 mice. Significantly different from control, **p<0.01. ○, Lard; ●, fish oil.

Fig. 5. Effects of fish oil and lard by insulin tolerance test in db/db mice. Each value represents the M±SE from 5–6 mice. Significantly different from lard, *p<0.05. ○, Lard; ●, fish oil.

Insulin tolerance test
A significant decrease in blood glucose was observed in fish oil-treated mice 60 min after insulin administration as compared to the corresponding fasting blood glucose (p<0.05) (Fig. 5). In contrast, no differences in blood glucose were observed at any point after the administration of insulin in the case of lard-treated mice.

DISCUSSION
This study shows that a fish oil diet improved glucose tolerance after oral glucose loading as compared to a lard diet in db/db mice, one of the animal models of NIDDM. The improvement by the fish oil diet was accompanied by increased...
plasma insulin levels, supposedly indicating increased insulin secretory capacity from pancreatic β cells, as has been reported previously (15). These findings suggest that a fish oil diet has a beneficial effect on diet therapy in NIDDM. In addition, insulin administration resulted in a significant decrease in blood glucose levels only in mice on high-fat diets containing fish oil, and not in those on high-fat diets containing lard. This fact shows that fish oil can lessen the insulin resistance in NIDDM as compared to lard. These results support a previous report that fish oil improves insulin action in adipocyte and also the glucose metabolism in insulin-resistant mice (30).

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*J Nutr Sci Vitaminol*
Comparison of Fish Oil or Lard on Blood Glucose in db/db Mice

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Vol 43, No 2, 1997