Effect of eicosapentaenoic acid ethyl ester v. oleic acid-rich safflower oil on insulin resistance in type 2 diabetic model rats with hypertriacylglycerolaemia

Asako Minami, Noriko Ishimura, Sadaichi Sakamoto, Eiko Takishita, Kazuaki Mawatari, Kazuko Okada and Yutaka Nakaya*

Department of Nutrition, School of Medicine, The University of Tokushima, Tokushima, Japan

(Received 12 March 2001 – Revised 10 September 2001 – Accepted 3 October 2001)

The purpose of the present study was to test whether hyperlipidaemia and insulin resistance in type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats can be improved by dietary supplementation with purified eicosapentaenoic acid (EPA) or oleic acid (OA). Male OLETF rats were fed powdered chow (510 g fat/kg) alone (n 8) or chow supplemented with 1·0 g EPA- (n 8) or OA- (n 8) rich oil/kg per d from 5 weeks until 30 weeks of age. An oral glucose tolerance test and hyperinsulinaemic euglycaemic clamp was performed at 25 and 30 weeks of age. EPA supplementation resulted in significantly reduced plasma lipids, hepatic triacylglycerols, and abdominal fat deposits, and more efficient in vivo glucose disposal compared with OA supplementation and no supplementation. OA supplementation was associated with significantly increased insulin response to oral glucose compared with EPA supplementation and no supplementation. Inverse correlation was noted between glucose uptake and plasma triacylglycerol levels (r 2 0·86, P<0·001) and abdominal fat volume (r 2 0·80, P<0·001). The result of oral glucose tolerance test study showed that the rats fed EPA tended to improve glucose intolerance, although this was not statistically significant. Levels of plasma insulin at 60 min after glucose was significantly increased in rats fed OA compared with the other two groups. The results indicate that long-term feeding of EPA might be effective in preventing insulin resistance in diabetes-prone rats, at least in part, due to improving hypertriacylglycerolaemia.

Insulin resistance constitutes an important risk factor for arteriosclerosis and is commonly found among patients with glucose intolerance, obesity, diabetes, and hypertension, and dyslipidaemia. Piatti et al. (1995) showed that acute hypertriacylglycerolaemia, as induced by intralipid infusion, led to an inhibition of tissue glucose uptake, glucose oxidation, and insulin-induced suppressibility of hepatic gluconeogenesis. Widen et al. (1992) reported that type 2 diabetic patients with hypertriacylglycerolaemia are more insulin resistant than matched non-hypertriacylglycerolaemic patients. A number of studies have also shown an inverse correlation between insulin sensitivity and fasting triacylglycerol levels (Abbott et al. 1988; Widen et al. 1992). These results suggest that a correction in plasma triacylglycerol level can be an important factor in patients with insulin resistance.

Dietary fatty acids are among the most important nutrients determining plasma lipid concentration. Dietary intake of n-3 fatty acids is effective in lowering plasma concentration of triacylglycerol (Harris, 1993; Rustan et al. 1993; Ikemoto et al. 1996). Recently, purified n-3 fatty acids derived from fish oils were examined in terms of lipid metabolism in human subjects (Rambjor et al. 1996) and in rats (Ikeda et al. 1994). Storlien et al. (1987, 1991) have demonstrated that the insulin resistance produced by feeding a high-fat diet is prevented by fish-oil supplements in rats. Monounsaturated fatty acids (MUFA) have also been reported to be metabolically better in patients with non-insulin-dependent diabetes (Campbell et al. 1994; Gumbiner et al. 1998). However, only a few studies on the effects of eicosapentaenoic acid (EPA) and oleic acid (OA, a MUFA) on insulin sensitivity and lipid metabolism in

Abbreviations: EPA, eicosapentaenoic acid; GIR, glucose infusion rate; MUFA, monounsaturated fatty acid; OA, oleic acid; OLETF, Otsuka Long-Evans Tokushima Fatty.

* Corresponding author: Dr Yutaka Nakaya, fax +81 88 633 7113, email nakaya@nutr.med.tokushima-u.ac.jp
Materials and methods

Animals and experimental design

Male OLETF rats aged 5 weeks were kindly provided by Tokushima Research Institute, Otsuka Pharmaceutical Co., Tokushima, Japan, and were maintained in our animal facilities under specific pathogen-free conditions (Institute of Animal Experimentation, Tokushima University). The animals were individually housed in an air-conditioned room (23 ± 1°C, lights on 08.00–20.00 hours). OLETF rats were randomly assigned into three groups of eight rats each. Two groups were maintained on one of two supplemented diets: chow + 1·0 g EPA ethyl ester/kg per d (EPA ethyl ester contained (g/kg): EPA 943, α-tocopherol 2; Mochida Pharmaceutical, Tokyo, Japan; EPA group), or chow + 1·0 g high-oleic-acid safflower oil/kg per d (safflower oil contained (g/kg): oleic acid 778, linolenic acid 1, palmitic acid 5, stearic acid 21, arachidic acid 2; Ajinomoto, Tokyo, Japan; OA group) for 25 weeks. The third group (control group) was maintained on standard rat chow alone (containing 51 g fat, 234 g protein and 572 g carbohydrate/kg; Oriental Yeast, Tokyo, Japan) with rat insulin as the standard.

Following the completion of the euglycaemic clamp test, rats were killed and liver and the intra-abdominal fat (mesenteric, epididymal, and retroperitoneal fat) were surgically removed and weighed separately. The extraction of lipids from liver tissue was performed as described by Folch et al. (1957). Plasma and liver triacylglycerol, cholesterol, and phospholipids levels were determined by enzymatic methods (Wako, Osaka, Japan). The levels of free fatty acids were determined enzymatically (Wako).

Table 1. Effect of dietary supplementation with eicosapentaenoic acid (EPA) and oleic acid (OA) for 25 weeks on fasting plasma lipids of diabetic rats†

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>EPA</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean SD</td>
<td>Mean SD</td>
<td>Mean SD</td>
</tr>
<tr>
<td>Triacylglycerol (mmol/l)</td>
<td>1·75 0·28</td>
<td>0·88* 0·27</td>
<td>1·72 0·29</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>1·70 0·18</td>
<td>1·23* 0·16</td>
<td>1·78 0·16</td>
</tr>
<tr>
<td>Phospholipids (mmol/l)</td>
<td>2·08 0·32</td>
<td>1·50* 0·24</td>
<td>2·20 0·13</td>
</tr>
<tr>
<td>Free fatty acids (mmol/l)</td>
<td>0·80 0·15</td>
<td>0·75 0·17</td>
<td>0·98 0·02</td>
</tr>
</tbody>
</table>

Mean values were significantly different from those of the control and of the OA groups: *P < 0·05.† For details of diets and procedures, see p. 158.

Statistical analysis

Results in the text are expressed as mean values and

Oral glucose tolerance tests

At 25 weeks of age, the rats underwent an oral glucose tolerance test after an overnight fast. Glucose (500 g/l water), 2 g/kg body weight, was administered orally. Blood was drawn from a tail vein at 0, 30, 60, and 120 min for measurement of the plasma glucose level. Blood samples were also taken from a tail vein at 0, and 60 min for measurement of plasma insulin levels.

Measurement of in vivo glucose disposal by euglycaemic clamp studies

Insulin-mediated whole-body glucose uptake was determined in 30-week-old anaesthetized rats using an euglycaemic insulin clamp. After an overnight fast, rats were anaesthetized by an intraperitoneal injection of pentobarbital sodium (50 mg/kg), and catheters were inserted in the jugular vein and carotid artery. Rats received a 1 h infusion of insulin (Novorin R; NOVO Nordisk, Bagsvaerd, Denmark; 60 pmol/kg per min). A glucose solution (100 g/l) was initiated at t = 0. The rate of infusion was adjusted to maintain the plasma concentration of glucose at 6·1 mmol/l. The whole-body glucose uptake during the final 20 min represents the glucose infusion rate (GIR).

Plasma glucose levels were determined by the glucose oxidase method (Tido-Tidex: Sankyo, Tokyo, Japan). Insulin levels were determined by an enzyme-linked immunosorbent assay (Levis insulin rat; Shibayagi, Gunma, Japan) with rat insulin as the standard.

Plasma and liver lipid assay

Following the completion of the euglycaemic clamp test, rats were killed and liver and the intra-abdominal fat (mesenteric, epididymal, and retroperitoneal fat) were surgically removed and weighed separately. The extraction of lipids from liver tissue was performed as described by Folch et al. (1957). Plasma and liver triacylglycerol, cholesterol, and phospholipids levels were determined by enzymatic methods (Wako, Osaka, Japan). The levels of free fatty acids were determined enzymatically (Wako).

Statistical analysis

Results in the text are expressed as mean values and
standard deviations. Data were analysed by ANOVA plus Bonferroni multiple comparison tests. A level of $P < 0.05$ was accepted as statistically significant.

**Results**

**Body weight and food intake**

The average food intakes during the experimental period in the control, EPA- and OA-fed rats were 24.4 (SD 0.5), 24.0 (SD 1.1), and 24.9 (SD 0.4) g/d respectively. There were no significant differences among rats with and without treatment. The final body weight of the control rats was not significantly different from the EPA- or OA-treated rats (629 (SD 8), 634 (SD 13) and 661 (SD 8) g respectively).

**Plasma and liver lipids**

The concentrations of fasting plasma lipids in each group are shown in Table 1. Fasting plasma levels of triacylglycerol, cholesterol, and phospholipids were significantly ($P < 0.05$) decreased by the EPA treatment, compared with those of control rats, but not by OA treatment. The concentration of plasma free fatty acids showed no significant effect by EPA or OA treatment.

Fig. 1 shows the concentrations of triacylglycerol in the liver. The control group showed high concentration of triacylglycerol (0.46 (SD 0.12) mmol/g tissue). EPA treatment improved these values (0.24 (SD 0.06) mmol/g tissue, $P < 0.01$), although OA treatment failed to prevent the accumulation of triacylglycerol (0.39 (SD 0.09) mmol/g tissue, NS).

**Abdominal fat accumulation**

Fig. 2 shows intra-abdominal fat accumulation for each group. The relative intra-abdominal fat pad weight of control rats was 112.0 (SD 7.6) g/kg body weight and significantly decreased by treatment with EPA (94.0 (SD 6.7) g/kg body weight, $P < 0.05$) but not by treatment with OA (118.6 (SD 9.6) g/kg body weight, NS).

**Glucose tolerance**

Fig. 3 summarizes the oral glucose tolerance test values at 25 weeks of age for each group. A significant increase in concentrations of basal plasma glucose as the result of treatment with OA (7.83 (SD 0.22) v. 6.94 (SD 0.50) mmol/l, $P < 0.05$) compared with control rats was detected, but treatment with EPA (7.67 (SD 0.44) mmol/l, NS) had no effect. The rats that had been fed EPA showed the lowest value among groups at 60 and 120 min after glucose loading, although these values did not reach the level of

![Graph](https://www.cambridge.org/core/journals/hypertriacylglycerolaemia-in-diabetic-rats/figs/1eff756527.jpg)

**Fig. 1.** Effect of dietary oil supplementation on the triacylglycerol content in liver of diabetic rats. EPA, eicosapentaenoic acid; OA, oleic acid. For details of diets and procedures, see p. 158. Values are means for eight rats per group with standard deviations shown by vertical bars. Mean value was significantly different from that of the control group: * $P < 0.05$. Mean value was significantly different from that of the EPA-fed group: † $P < 0.05$.

![Graph](https://www.cambridge.org/core/journals/hypertriacylglycerolaemia-in-diabetic-rats/figs/248c347265.jpg)

**Fig. 2.** Effect of dietary oil supplementation on intra-abdominal fat accumulation in diabetic rats. EPA, eicosapentaenoic acid; OA, oleic acid. For details of diets and procedures, see p. 158. Values are means for eight rats per group with standard deviations shown by vertical bars. Mean value was significantly different from that of the control group: * $P < 0.05$. Mean value was significantly different from that of the EPA-fed group: † $P < 0.05$.

![Graph](https://www.cambridge.org/core/journals/hypertriacylglycerolaemia-in-diabetic-rats/figs/3e9e4e05b.jpg)

**Fig. 3.** Oral glucose tolerance test (a) in diabetic rats fed eicosapentaenoic acid- (EPA) or oleic acid- (OA) rich diets. □, control; ■, EPA; ▲, OA. Plasma insulin concentration (b) measured at 60 min after loading. □, 0 min; ■, 60 min. For details of diets and procedures, see p. 158. Values are means for seven or eight rats per group with standard deviations shown by vertical bars. Mean value was significantly different from that of the control group: * $P < 0.05$. Mean value was significantly different from that of the EPA-fed group: † $P < 0.05$. 

![Graph](https://www.cambridge.org/core/journals/hypertriacylglycerolaemia-in-diabetic-rats/figs/b4e1e4af5.jpg)

Fig. 3. Oral glucose tolerance test (a) in diabetic rats fed eicosapentaenoic acid- (EPA) or oleic acid- (OA) rich diets. □, control; ■, EPA; ▲, OA. Plasma insulin concentration (b) measured at 60 min after loading. □, 0 min; ■, 60 min. For details of diets and procedures, see p. 158. Values are means for seven or eight rats per group with standard deviations shown by vertical bars. Mean value was significantly different from that of the control group: * $P < 0.05$. Mean value was significantly different from that of the EPA-fed group: † $P < 0.05$.
There was no significant difference in the levels of fasting plasma insulin among the three groups. Plasma insulin response to oral glucose was significantly increased by OA treatment at 60 min after loading, but was not altered by EPA treatment (Fig. 3).

**In vivo glucose disposal**

Fig. 4 shows GIR values for each group. Supplementation of EPA significantly improved the decreased GIR compared with the control groups (62.7 (SD 9.4) μmol/min per kg body weight, $P<0.05$), but that of OA did not (45.8 (SD 3.2) μmol/min per kg body weight). As shown in Fig. 5, GIR values were inversely correlated with the concentration of plasma triacylglycerols ($r = -0.86$, $P<0.001$) and plasma free fatty acid levels ($r = -0.67$, $P<0.01$). GIR values also show a significant inverse correlation with the relative weight of intra-abdominal fat ($r = -0.80$, $P<0.001$).

**Discussion**

The results herein show that EPA supplementation lowers plasma triacylglycerol levels and abdominal fat accumulation and improves insulin resistance in type 2 diabetic model OLETF rats, suggesting that EPA might be useful for the treatment of type 2 diabetes. However, OA did not improve hypertriacylglycerolaemia or insulin resistance in OLETF rats.

Fish oil consistently reduces plasma triacylglycerol levels. In an animal study, the concentration of plasma triacylglycerol was significantly lower in rats that had been fed EPA than in those fed docosahexaenoic acid (Ikeda et al. 1994). Zhang et al. (1993), using perfused rat livers, observed that EPA, but not docosahexaenoic acid, resulted in reduced hepatic VLDL synthesis and secretion or an accelerated rate of removal. In accordance with these animal observations, Rambjor et al. (1996) concluded that EPA is primarily responsible for the hypotriacylglycerolaemic effect of fish oil in man. In the present study, plasma triacylglycerol was reduced by 50% by EPA supplementation compared with OA supplementation and no supplementation, and concomitant significant reductions...
in hepatic triacylglycerol content and abdominal fat accumulation were observed.

A previous study showed that hypertriacylglycerolaemia was associated with a reduction in peripheral glucose uptake, whereas the suppressive effect of insulin on hepatic glucose production was unaffected (Mckane et al. 1990). It is conceivable that triacylglycerol-rich particles might influence the binding of insulin to its receptor or interfere with early post-binding steps (Bieger et al. 1984). Moreover, hypertriacylglycerolaemic patients typically show a resistance to the antilipolytic effect of insulin (Yki-Jarvinen & Taskinen, 1988). Thus, hyperinsulinaemia in insulin-resistant subjects can be associated with increased levels of plasma triacylglycerols.

Type 2 diabetic patients with hypertriacylglycerolaemia are more insulin resistant than type 2 diabetic patients without hypertriacylglycerolaemia (Yki-Jarvinen & Taskinen, 1988; Widen et al. 1992). Therefore, a reduction in plasma triacylglycerol levels might improve insulin sensitivity. These results indicate that EPA might be particularly beneficial in the treatment of type 2 diabetic subjects in whom hypertriacylglycerolaemia is present.

In the study of oral glucose tolerance, the rats that had been fed EPA tended to improve glucose intolerance, but it did not reach the level of statistical significance. Plasma insulin response to oral glucose was significantly (P<0·05) increased by OA treatment at 60 min after loading, but was not altered by EPA treatment.

The results of the GIR study, an index of insulin sensitivity in the whole body, demonstrated that an improvement of the GIR was inversely correlated with concentrations of fasting plasma triacylglycerol levels and free fatty acid levels. This is consistent with previous studies (Abbott et al. 1988; Widen et al. 1992) which reported an inverse correlation between rates of insulin-mediated glucose disposal and levels of serum triacylglycerol.

Previous studies have shown that diets which contain fish oil may induce less abdominal fat deposits than diets with other types of fat in mice (Ikemoto et al. 1996) and rats (Hill et al. 1993). The present study showed that dietary supplementation of EPA increased the inhibition of abdominal fat deposits in OLETF rats. EPA and OA groups received a more dietary-energy-dense diet (about chow + 5% energy) due to the oils. There were no significant differences in energy intake between oil-treated rats and control rats, although OA group tended to show greater body weight. On the other hand, EPA group showed less accumulation of abdominal fat. A decrease in abdominal fat mass in fish oil-fed rats has been demonstrated to be linked to the reduction in triacylglycerol and free fatty acids in plasma (Otto et al. 1992). Our results are in good agreement with these studies. However, Mori et al. (1997) observed that the mesenteric fat mass in OLETF rats is significantly increased by an EPA-supplemented diet compared with those without supplementation. The reason for this difference might involve the fact that different doses of EPA were used, namely, 0·3 g/kg. It is well known that the triacylglycerol lowering effects of n-3 fatty acids are dosage dependent (Scheetman et al. 1988; Friedberg et al. 1998).

MUFA are reported to have a beneficial effect on the cardiovascular risk factor profile in diabetic (Rivellese et al. 1990) and non-diabetic populations (Riccardi et al. 1987). MUFA-enriched hypoenergetic diets potentiate the beneficial effects of weight loss and thus, ameliorate cardiovascular risk factors in obese patients with type 2 diabetes (Gumbiner et al. 1998). Low mortality rates from cardiovascular disease in Mediterranean countries can be explained, in part, by the use of olive oil, which is rich in MUFA (Keys et al. 1986). Parillo et al. (1992) reported that diets, which are high in MUFA and low in carbohydrates, decrease both postprandial glucose and insulin levels, and improve peripheral insulin sensitivity in patients with non-insulin-dependent diabetes. They also found a decrease in plasma triacylglycerol levels. In contrast to these reports, a MUFA-rich diet, i.e. an oleic acid-supplemented diet, had no effect on insulin sensitivity in diabetic rats in the present study. This discrepancy could be due to species differences between rat and human subjects, or differences in the diet used in the studies. In agreement with our study, Ney et al. (1989) reported that high oleic acid levels increased plasma cholesterol and triacylglycerol levels in rats. In addition, we simply added MUFA, but did not use a low-carbohydrate diet.

Obesity, especially intra-abdominal fat obesity, is frequently accompanied by hyperlipidaemia, glucose intolerance, and insulin resistance. The decrease in abdominal fat accumulation by EPA might also contribute to an increase in the action of insulin. In the present study, EPA supplementation lowered plasma triacylglycerol levels and abdominal fat accumulation and improved insulin resistance. We found a significant (P<0·001) correlation existed between the GIR values obtained by euglycaemic clamp test and relative weight of abdominal fat in type 2 diabetic model OLETF rats. This suggests that the effect of EPA on insulin sensitivity is also related to a decreased abdominal fat accumulation and EPA is expected to have a beneficial effect on insulin sensitivity in type 2 diabetes.

References


Harris WS & Muzio F (1993) Fish oil reduces postprandial triglyceride concentrations without accelerating lipid-emulsion


