British Journal of Nutrition (2002), **87**, 157–162
DOI: 10.1079/BJN2001496

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

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(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 ($P<0.05$) 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 −0·86, $P<0·001$) and abdominal fat volume (r −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.

Otsuka Long-Evans Tokushima Fatty rats: Eicosapentaenoic acid: Hypertriacylglycerolaemia: Insulin resistance

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.

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diabetic model animals have been reported (Ikemoto *et al.* 1996; Mori *et al.* 1997).

Otsuka Long-Evans Tokushima Fatty (OLETF) rats, a genetic model of the spontaneous development of type 2 diabetes mellitus, show innate polyphagia, which causes rapid body-weight gain resulting in hypertriacylglycerolaemia hyperinsulinaemia, and hyperglycaemia (Kawano *et al.* 1992). In this model, insulin resistance is closely related to lipid profiles, especially in tissue levels of triacylglycerol. Thus, reduced insulin resistance would be expected at lowered plasma and tissue triacylglycerol levels. To test this possibility, we examined the effect of OA and purified EPA, starting at an early age, on the progression of insulin resistance in type 2 diabetic model rats.

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). Experimental diets were freshly prepared at 2 d intervals using oils that had been stored at −20°C. The food intake of rats treated with EPA or OA oils was adjusted (g) to those of the control group in the previous week. Control rats had free access to food. Body weight and food consumption were measured at weekly intervals throughout the experimental period.

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

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

(Mean values and standard deviations for eight rats per group)

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

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.

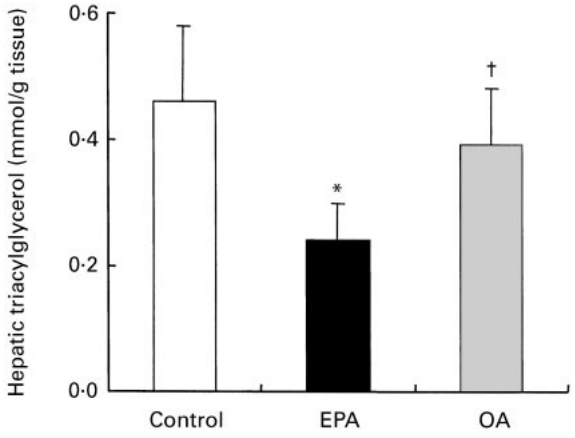

Fig. 1. Effect of dietary oil supplementation on the triacylglycerol content in liver of diabetic rats. EPA, eicosapentaenoic acid; OA, oleic acid. For details of diets and procedures, see p. 158. Values are means for eight rats per group with standard deviations shown by vertical bars. Mean value was significantly different from that of the control group: *$P<0{\cdot}05$. Mean value was significantly different from that of the EPA-fed group: †$P<0{\cdot}05$.

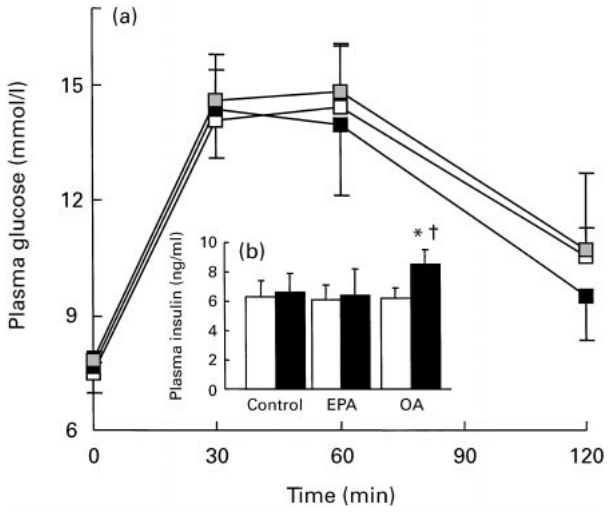

Fig. 3. Oral glucose tolerance test (a) in diabetic rats fed eicosapentaenoic acid- (EPA) or oleic acid- (OA) rich diets. □, control; ■, EPA; ■, OA. Plasma insulin concentration (b) measured at 60 min after loading. □, 0 min; ■, 60 min. For details of diets and procedures, see p. 158. Values are means 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: *$P<0{\cdot}05$. Mean value was significantly different from that of the EPA-fed group: †$P<0{\cdot}05$.

standard deviations. Data were analysed by ANOVA plus Bonferroni multiple comparison tests. A level of $P<0{\cdot}05$ 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 ($P<0{\cdot}05$) 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, $P<0{\cdot}01$), although OA treatment failed to prevent the accumulation of triacylglycerol (0·39 (SD 0·09) mmol/g tissue, NS).

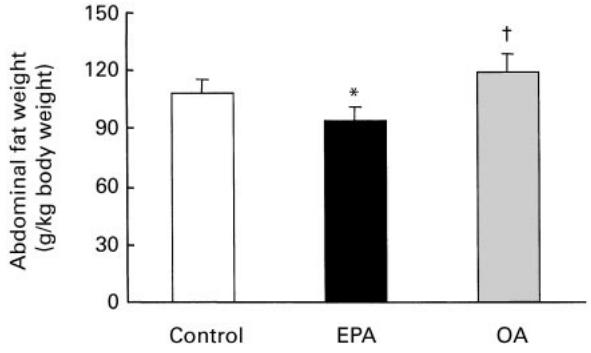

Fig. 2. Effect of dietary oil supplementation on intra-abdominal fat accumulation in diabetic rats. EPA, eicosapentaenoic acid; OA, oleic acid. For details of diets and procedures, see p. 158. Values are means for eight rats per group with standard deviations shown by vertical bars. Mean value was significantly different from that of the control group: *$P<0{\cdot}05$. Mean value was significantly different from that of the EPA-fed group: †$P<0{\cdot}05$.

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, $P<0{\cdot}05$) 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, $P<0{\cdot}05$) 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

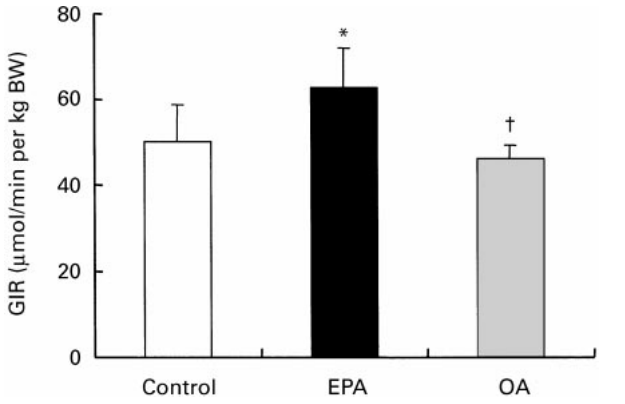

Fig. 4. Effects of dietary oil supplementation on insulin-stimulation glucose disposal *in vivo* in diabetic rats. GIR, glucose infusion rate; BW, body weight; EPA, eicosapentaenoic acid; OA, oleic aid. For details of diets and procedures, see p. 158. Values are means for seven or eight rats per group with standard deviations shown by vertical bars. Mean value was significantly different from that of the control group: *$P<0{\cdot}05$. Mean value was significantly different from that of the EPA-fed group: †$P<0{\cdot}05$.

statistical significance. 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) *v.* 50·1 (SD 8·98) μmol/min per kg body weight, $P<0{\cdot}05$), 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, $P<0{\cdot}001$) and plasma free fatty acid levels (r −0·67, $P<0{\cdot}01$). GIR values also show a significant inverse correlation with the relative weight of intra-abdominal fat (r −0·80, $P<0{\cdot}001$).

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

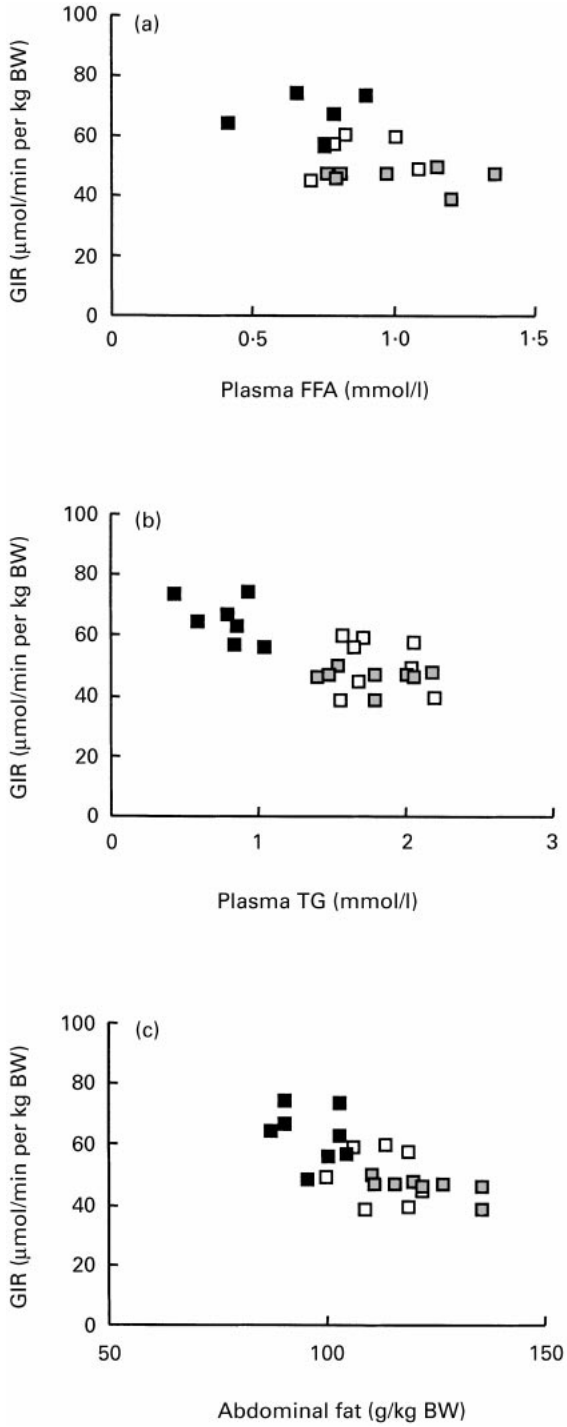

Fig. 5. Correlation between insulin-stimulated glucose disposal and (a) plasma free fatty acids (r −0·67, $P<0{\cdot}01$), (b) plasma triacylglycerol (r −0·86, $P<0{\cdot}001$), and (c) relative intra-abdominal fat weight (r −0·80, $P<0{\cdot}001$) in diabetic rats fed eicosapentaenoic acid- (EPA) or oleic acid- (OA) rich diets. GIR, glucose infusion rate; BW, body weight; FFA, free fatty acids; TG, triacylglycerol. □, Control; ■, EPA; ■, OA. For details of diets and procedures, see p. 158.

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 ($P<0{\cdot}05$) 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 inhibited the increment of abdominal fat depots 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 (Schectman *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 ($P<0{\cdot}001$) 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

Abbott WGH, Lillioja S, Young AA, Zawadzki JK, Yki-Jarvinen H, Christin L & Howard BV (1988) Relationships between plasma lipoprotein concentrations and insulin action in an obese hyperinsulinemic population. *Diabetes* **36**, 897–904.

Bieger WP, Michel G, Barwich D, Bhiel K & Wirth A (1984) Diminished insulin receptors on monocytes and erythrocytes in hypertriglyceridemia. *Metabolism* **33**, 982–987.

Campbell L, Marmot PE, Dyer J, Borkman M & Storlien LH (1994) The high-monounsaturated fat diet as a practical alternative for NIDDM. *Diabetes Care* **17**, 177–182.

Folch J, Lees M & Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–506.

Friedberg CE, Janssen MJEM, Heine RJ & Grobbee DE (1998) Fish oil and glycemic control in diabetes. *Diabetes Care* **21**, 494–500.

Gumbiner B, Low CC & Reaven PD (1998) Effects of a monounsaturated fatty acid-enriched hypocaloric diet on cardiovascular risk factors in obese patients with type 2 diabetes. *Diabetes Care* **21**, 9–15.

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

removal rates. *American Journal of Clinical Nutrition* **58**, 68–74.

Hill JO, Peters JC, Lin D, Yakubu F, Greene H & Swift L (1993) Lipid accumulation and body fat distribution is influenced by type of dietary fat fed to rats. *International Journal of Obesity* **17**, 223–236.

Ikeda I, Wakamatsu K, Inayoshi A, Imaizumi K, Sugano M & Yazawa K (1994) α-Linolenic, eicosapentaenoic and docosahexaenoic acids affect lipid metabolism differently in rats. *Journal of Nutrition* **24**, 1899–1906.

Ikemoto S, Takahashi M, Tsunoda N, Maruyama K, Itakura H & Ezaki O (1996) High-fat diet-induced hyperglycemia and obesity in mice: Differential effects of dietary oils. *Metabolism* **45**, 1539–1546.

Kawano K, Hirashima T, Mori S, Saitho Y, Kurosumi M & Natori T (1992) Spontaneous long-term hyperglycemic rat with diabetic complications: Otsuka Long-Evans Tokushima Fatty (OLETF) strain. *Diabetes* **41**, 1422–1428.

Keys A, Menotti A, Karvonen MJ, Aravanis C, Blackburn H, Buzina R, Djordjevic B, Dontas AS, Fidanza F, Kyes MH, Kromhout D, Nedeljkovic S, Punsar S, Seccareccia F & Toshima H (1986) The diet and 15 year death rate in Seven Countries Study. *American Journal of Epidemiology* **124**, 903–915.

Mckane WR, Stevens AB, Woods R, Andrews WJ, Henry RW & Bell PM (1990) The assessment of hepatic and peripheral insulin sensitivity in hypertriglyceridemia. *Metabolism* **39**, 1240–1245.

Mori Y, Murakawa Y, Hata S, Yokoyama J, Tajima N, Ikeda Y, Nobukata H, Ishikawa T & Shibutani Y (1997) 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. *Metabolism* **46**, 1458–1464.

Ney DM, Lasekan JB & Kim J (1989) Relative effects of dietary oleic- and linoleic-rich oils on plasma lipoprotein composition in rats. *Journal of Nutrition* **119**, 857–863.

Otto DA, Baltzell JK & Wooten JT (1992) Reduction in triacylglycerol levels by fish oil correlates with free fatty acid levels in ad libitum fed rats. *Lipids* **27**, 1013–1017.

Parillo M, Rivellese AA, Ciadullo AV, Capaldo B, Giacco A, Genovese S & Riccardi G (1992) A high-monounsaturated-fat/low-carbohydrate diet improves peripheral insulin sensitivity in non-insulin-dependent diabetic patients. *Metabolism* **41**, 1373–1378.

Piatti PM, Monti LD, Baruffaldi L, Magni F, Paroni R, Fermo I, Costa S, Santambrogio G, Nasser R, Marchi M, Galli-Kienle M, Pontiroli AE & Pozza G (1995) Effects of an acute increase in plasma triglyceride levels on glucose metabolism in man. *Metabolism* **44**, 883–889.

Rambjor GS, Walen AI, Windsor SL & Harris WS (1996) Eicosapentaenoic acid is primarily responsible for hypotriglyceridemic effect of fish oil in humans. *Lipids* **31**, S45–S49.

Riccardi G, Rivellese AA & Mancini M (1987) The use of diet to lower plasma cholesterol levels. *European Heart Journal* **8**, Suppl. E, 79–85.

Rivellese AA, Giacco R & Genovese S (1990) Effects of changing amount of carbohydrate in diet on plasma lipoproteins and apolipoproteins in type II diabetic patients. *Diabetes Care* **13**, 446–448.

Rustan AC, Hustvedt BE & Drevon CA (1993) Dietary supplementation of very long-chain *n*-3 fatty acids decreases whole body lipid utilization in the rat. *Lipid Research* **34**, 1299–1309.

Schectman G, Kaul S & Kissebah AH (1988) Effect of fish oil concentrate on lipoprotein composition in NIDDM. *Diabetes* **37**, 1567–1573.

Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S & Kraegen EW (1991) Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. *Diabetes* **40**, 280–289.

Storlien LH, Kraegen EW, Chisholm DJ, Ford GL, Bruce DG & Pascoe WS (1987) Fish oil prevents insulin resistance induced by high-fat feeding in rats. *Science* **21**, 885–888.

Widen E, Ekstrand A, Saloranta C, Franssila-Kallunki A, Eriksson J, Schalin-Jantti C & Groop L (1992) Insulin resistance in type 2 (non-insulin-dependent) diabetic patients with hypertriglyceridemia. *Diabetologia* **35**, 1140–1145.

Yki-Jarvinen H & Taskinen MR (1988) Interrelationships among insulins antilipolytic and diabetic patients with endogenous hypertriglyceridemia. *Diabetes* **37**, 1271–1278.

Zhang ZJ, Wilcox HG, Castellani L, Fungwe TV, Elam MB & Heimberg M (1993) Concentration-dependent effects of eicosapentaenoic acid on very low density lipoprotein secretion by the isolated perfused rat liver. *Lipids* **28**, 419–425.