Dietary Fish Oil Enhances Insulin Sensitivity in Miniature Pigs

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ABSTRACT The effects of dietary fish oil, MaxEPA, and corn oil on insulin sensitivity were examined in male miniature pigs. The pigs (20-35 kg) received 750 g of nonpurified diet per day (160 g/kg protein, 50 g/kg fat) with the addition of either 30 g corn oil or 30 g MaxEPA, resulting in 90 g total fat per kg diet for 4-5 wk. The MaxEPA diet provided 12.6 g (n-3) polyunsaturated fatty acids per kg diet (6.7 g eicosapentaenoic acid, 4.8 g docosahexaenoic acid), 4.7 g (n-3) polyunsaturated fatty acids and 147 mg cholesterol. The corn oil diet provided 22.7 g (n-6) polyunsaturated fatty acids per kg diet and no (n-3) polyunsaturated fatty acids; cholesterol was added to equal the amount in the MaxEPA. After overnight withdrawal of food, intravenous glucose tolerance tests were conducted in conscious pigs by using previously placed jugular vein catheters. Plasma glucose responses and the areas under the plasma glucose curves were similar in seven MaxEPA- and five corn oil-fed pigs. However, the incremental areas under the insulin curves were significantly lower for the pigs fed MaxEPA. Thus values for insulin sensitivity (SI), determined with Bergman’s minimal model, were significantly higher for MaxEPA than for corn oil-fed pigs, whereas the rate of glucose disappearance (Kg), did not differ between the two groups. Therefore, substitution of (n-3) for (n-6) polyunsaturated fatty acids in dietary lipids is associated with enhanced insulin sensitivity in male pigs. J. Nutr. 126: 1549–1553, 1996.

INDEXING KEY WORDS:

- miniature pigs
- fish oil
- (n-3) fatty acids
- insulin sensitivity

Interest in the effects of (n-3) polyunsaturated fatty acids on glucose metabolism stem from the observation that the incidence of diabetes mellitus may be lowered by dietary fish oil (Mouratoff et al. 1967). Hypothetically, the enrichment of tissue phospholipids with (n-3) polyunsaturated fatty acids may enhance both insulin sensitivity and secretion [Lardinois 1987]. With respect to insulin action, both insulin binding and insulin responsiveness are influenced by dietary fat [Clandinin et al. 1993]. Changes in insulin secretion have been attributed to altered products of cyclooxygenase, lipoxygenase and phospholipases, which act as second messengers for insulin secretion [Robertson 1986]. Thus, dietary fish oils may alter both the action and secretion of insulin, resulting in changes in glucose tolerance and sensitivity to insulin.

In humans with Type 2 diabetes mellitus, deleterious effects of fish oils on glucose tolerance have been noted [Malasanos and Stacpoole, 1991]. Kasim (1993) suggested that (n-3) polyunsaturated fatty acids inhibit insulin secretion and increase hepatic glucose output without affecting peripheral insulin sensitivity. In contrast, beneficial effects have also been reported [Fasching et al. 1991, Feskens et al. 1991, Popp-Snijders et al. 1987, Zak et al. 1989]. In a recent study of Alaska natives, daily consumption of seal oil or salmon was associated with a lower prevalence of impaired glucose tolerance and diabetes [Adler et al. 1994]. In another study, (n-3) polyunsaturated fatty acids had no effect on glucose or insulin levels, whereas significant reduction in blood pressure occurred in humans with combined hyperlipidemia [Grundt et al. 1995]. Similarly, in mildly obese men with noninsulin dependent diabetes mellitus, (n-3) polyunsaturated fatty acids did not alter insulin sensitivity [Pelikanova et al. 1993]. These studies are not readily comparable because they examined variable dietary practices for different types of subjects. Furthermore, the degree of unsaturation of membrane

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phospholipids may be as important as the type of polyunsaturated fatty acids present. Borkman et al. (1993) demonstrated that insulin sensitivity is positively correlated with the degree of unsaturation in skeletal muscle phospholipids in humans; notably, insulin sensitivity was positively correlated with the percentage of 20:4n-6 and the total amount of C20-C22 polyunsaturated fatty acids in muscle. In studies on rats, positive effects on insulin action were reported after enrichment of dietary [n-3] polyunsaturated fatty acids (Clandinin et al. 1993). We showed that MaxEPA in comparison with safflower oil does not alter intravenous glucose tolerance in conscious rats (Behme et al. 1993), although other investigators found efficacious results with [n-3] polyunsaturated fatty acids (Liu et al. 1994, Storlien et al. 1987).

Modern domestic pigs resemble humans in many ways and have been used extensively as models for aberrations in carbohydrate and lipid metabolism (Mersmann 1986). In both humans (Nestel et al. 1984) and pigs (Huff and Telford 1989), dietary fish oils reduce plasma triglycerides. Thus we undertook a pilot study in miniature pigs of insulin sensitivity determined by applying Bergman's minimal model to a frequently sampled intravenous glucose tolerance test (Bergman et al. 1979). We report that insulin sensitivity was enhanced in pigs fed supplementary [n-3] polyunsaturated fatty acids compared with pigs fed supplementary [n-6] polyunsaturated fatty acids.

**MATERIALS AND METHODS**

Miniature pigs (20–35 kg) were obtained from Hyde Park Farms (Hyde Park, Ontario, Canada) and individually housed in metabolic cages in a room with a 12-h light and dark cycle. Each pig received 750 g/d of nonpurified diet (160 g protein, 50 g fat, 50 g fiber per kg, pig chow Ralston Purina, Woodstock, Ontario, Canada). Just before feeding, 30 g corn oil (Mazola, Best Foods, Toronto, Ontario, Canada) or 30 g MaxEPA (kindly provided by R. P. Scherer Canada, Windsor, Ontario, Canada) were added to the 750 g nonpurified diet, resulting in 90 g total fat per kg diet. The MaxEPA diet provided 12.6 g [n-3] polyunsaturated fatty acids per kg diet (6.7 g eicosapentaenoic acid, 4.8 g docosahexaenoic acid), 4.7 g/kg [n-6] fatty acids and 147 mg/kg cholesterol. The corn oil diet provided 22.7 g [n-6] polyunsaturated fatty acids per kg diet and no [n-3] polyunsaturated fatty acids; cholesterol was dissolved in the corn oil to equal the amount in the MaxEPA. The MaxEPA oil was stored in daily doses under N2 in sealed vials at 4°C until just before feeding; the oils were mixed with the feed and consumed within 1 h. All animal protocols were approved by the University Council on Animal Care, University of Western Ontario.

**Insulin sensitivity.** Insulin sensitivity [SI] was determined by applying Bergman's minimal model to a frequently sampled intravenous glucose tolerance test (Bergman et al. 1979). An indwelling Silastic catheter (0.2 cm ID) was surgically implanted in one external jugular vein of pigs under halothane anesthesia. The catheters were tunneled under the skin and externalized in the middle of the back. Three-way stopcocks were attached and held in place with a bandage and elastic netting. The catheters were kept patent with 7% EDTA. Tests were carried out 4–5 wk after institution of the diets and 2–4 wk after surgery to place indwelling catheters; the pigs were growing and feeding normally. Food was withheld overnight and insulin sensitivity tests were conducted in unanesthetized animals. Glucose (0.4 g/kg body weight) was injected through the catheter as a 25 g/L solution over 3 min followed by 20 mL saline. When half the glucose had been injected, the timer was set at 0 min. Tolbutamide (Orinase, Upjohn, Kalamazoo, MI), 250 mg in 5 mL saline, was injected at 10 min. Blood samples of 4 mL were obtained at −15, −10, −5, −1, 3, 4, 5, 6, 7, 9, 11, 12, 13, 14, 16, 18, 20, 25, 30, 35, 40, 50, 60, 70, 80, 100, 120, 140, 160 and 180 min. Blood samples were placed in test tubes containing sodium fluoride and trasylosil (350 KIU Aprotinin, Novo, Copenhagen, Denmark). Plasma was collected and stored at −20°C for glucose and insulin analyses. Area under the curve for plasma insulin was calculated geometrically for the insulin values after tolbutamide injection. Values for SI insulin sensitivity and glucose effectiveness (Sgi), were determined with the minimal model (Bergman et al. 1979). In this model, the dynamic relationship between plasma glucose and insulin after an intravenous glucose tolerance test is evaluated. Briefly, the model assumes the existence of two compartments: the insulin compartment, I, remote from plasma, and the glucose space, G, represented by plasma glucose. The rate of change of glucose is defined as the difference between net hepatic glucose balance and glucose uptake by peripheral tissues. The equations for the model are dG/dt = [P1]XG + P4 and dX/dt = P2[X + P3J[ti]. The parameters, P1–P4, define the rate of change of plasma glucose; X represents the effect of insulin in the remote compartment on glucose disappearance and I[ti], the time course of plasma insulin concentrations. The parameters are determined from the insulin and glucose responses for the modified intravenous glucose tolerance test. The effect of glucose to enhance its own disappearance from the plasma compartment, the glucose effectiveness, Sgi = P1. Insulin sensitivity, Sgi, is defined as the effect of insulin to augment glucose effectiveness, Sgi = −P3/P2, the fractional glucose disappearance per

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3 Abbreviations used: Kgc, glucose disappearance rate; P/S, polyunsaturated fatty acids/saturated fatty acids; Sc, glucose effectiveness; Sgi, Insulin sensitivity index.
unit insulin concentration. $K_G$ is the rate of disappearance of plasma glucose after tolbutamid injection, expressed as a percent of the initial peak concentration per minute.

**Analyses.** Plasma glucose concentrations were determined with a Beckman glucose analyzer (Fullerton, CA). Plasma immunoreactive insulin was measured by using charcoal-separated radioimmunoassay (Herbert et al. 1965) with porcine $^{125}$I-insulin from New England Nuclear (Du Pont Canada, Mississauga, Ontario, Canada), porcine monocomponent insulin (Novo) as standard and insulin antibody obtained from P. Wright, Cambridge, UK. Plasma glucose and insulin responses to intravenous glucose at each time point, $S_t$ and $S_G$, values, and $K_G$ values were compared with unpaired t-tests for the dietary groups. All values are reported as means $\pm$ SEM. Differences were considered significant if $P < 0.05$.

**RESULTS**

Mean values for body weight and basal plasma glucose at the time of the frequently sampled intravenous glucose tolerance test did not differ between the groups (Table 1). In conscious pigs, plasma glucose responses to intravenous glucose were similar in seven MaxEPA- and five corn oil-fed pigs (Fig. 1). Mean values for basal and maximal immunoreactive insulin were not significantly different. Mean values for both $S_t$ and $S_G$ were significantly higher for MaxEPA-fed pigs. These results are consistent with the observation that although $K_G$ values did not differ between the two groups, the mean incremental area under the insulin

![FIGURE 1 Intravenous glucose tolerance tests in conscious miniature pigs fed nonpurified diet with the addition of 40 g corn oil per kg diet ($n = 5$) or 40 g MaxEPA per kg diet ($n = 7$) for 4–5 wk. Values are means $\pm$ SEM. Plasma glucose and immunoreactive insulin responses were determined after bolus venous infusion of 0.4 g glucose per kg body weight after overnight withdrawal of food, with infusion of 250 mg tolbutamide at 10 min. Curve after tolbutamide injection (11–50 min) was significantly lower in the MaxEPA group than in the corn oil group (Table 1).

**DISCUSSION**

Sensitivity to the actions of insulin is a major determinant in the regulation of glucose balance along with the effects of counterregulatory hormones and neural influences. Many studies indicate that (n-3) polyunsaturated fatty acids enhance insulin sensitivity in rats but may have deleterious effects in humans. Our current results in pigs agree with the beneficial effects of (n-3) polyunsaturated fatty acids shown in rats.

Insulin action at its target tissues may be altered by the substitution of (n-3) for (n-6) fatty acids in liver, muscle and adipose tissue. Insulin binding to its receptors, insulin-stimulated glucose transport and lipogenesis in adipocytes were all enhanced by a diet with a high polyunsaturated fatty acid/saturated fatty acid (P/S) ratio; insulin-dependent and basal muscle metabolism were also enhanced in rats fed a diet high in (n-
3) polyunsaturated fatty acids (Clandinin 1993). They attribute these effects to changes in the physical characteristics of cellular membranes with increased P/S as well as to changes in eicosanoids caused by substitution of (n-3) for (n-6) polyunsaturated fatty acids in tissue lipids. Thus our results are in keeping with these effects and may be attributed to mechanisms similar to those demonstrated in rats.

This pilot study shows that insulin sensitivity in miniature pigs is enhanced by (n-3) fatty acids provided in a low fat diet. Many reports of improved insulin action with (n-3) fatty acids have been conducted with a high fat diet showing that the insulin resistance produced by a high fat diet is ameliorated by (n-3) fatty acids (Malasanos and Stacpoole 1991, Storlien et al. 1987). Although the latter may be more relevant to humans consuming diets with a much higher fat concentration than the 9 g/100 g used in this pig study, it is important to note that the current results demonstrate improved sensitivity to insulin without a background of relative insulin resistance.

In this study, insulin sensitivity was determined by analyzing a modified intravenous glucose tolerance test with Bergman’s minimal model with its inherent assumptions (Bergman et al. 1979). Using this method, whole body sensitivity is estimated, but hepatic glucose output and peripheral glucose uptake are not differentiated. Furthermore, the influence of the gastrointestinal tract on insulin secretion and action is not considered during intravenous glucose tests; a more physiological test of insulin action would include disposition of oral nutrient loads. These tests may be expected to vary with dietary fat because enterocytes in the intestinal brush border are rapidly altered in response to dietary fat (Clandinin et al. 1993). Besides the immediate effects that structural and functional lipids in the intestinal cells may have on nutrient absorption, undoubtedly there will be effects on secretion of gastrointestinal hormones as well. In turn, these hormones influence insulin secretion such as the stimulatory effect of glucagon-like peptide-1 on insulin secretion (Dupre et al. 1995). Thus hypothetically, the P/S and enrichment with (n-3) polyunsaturated fatty acids can modulate insulin action by a number of mechanisms.

In summary, this pilot study in conscious pigs demonstrates the application of Bergman’s minimal model to a modified intravenous glucose tolerance test in this species and supports the hypothesis that (n-3) polyunsaturated fatty acids enhance insulin sensitivity.

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LITERATURE CITED


