Dietary Cod Protein Improves Insulin Sensitivity in Insulin-Resistant Men and Women

A randomized controlled trial

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OBJECTIVE — The purpose of this article was to compare the effects of cod protein to those of other animal proteins on insulin sensitivity in insulin-resistant human subjects.

RESEARCH DESIGN AND METHODS — Insulin sensitivity (*M/I*) was assessed using a hyperinsulinemic-euglycemic clamp in 19 insulin-resistant subjects fed a cod protein diet and a similar diet containing lean beef, pork, veal, eggs, milk, and milk products (BPVEM) for 4 weeks in a crossover design study. Both diets were formulated to differ only in protein source, thus providing equivalent amounts of dietary fibers and monounsaturated, polyunsaturated (including n-3), and saturated fatty acids (1.1:1.8:1.0). β-Cell function, estimated by oral glucose tolerance test–derived parameters, was also assessed.

RESULTS — There was a significant improvement in insulin sensitivity ($P = 0.027$) and a strong tendency for a better disposition index (β-cell function × *M/I*) ($P = 0.055$) in subjects consuming the cod protein diet compared with those consuming the BPVEM diet. When median baseline *M/I* (4.8×10^{-3} mg · kg^{-1} · min^{-1} · pmol^{-1}) was taken into account, an interaction on the 30-min C-peptide–to–30-min glucose ratio, used as an index of β-cell function, was observed between diet and *M/I* status ($P = 0.022$). Indeed, this ratio strongly tended to increase in subjects with low *M/I* consuming the cod protein diet compared with those consuming the BPVEM diet ($P = 0.065$).

CONCLUSIONS — Dietary cod protein improves insulin sensitivity in insulin-resistant individuals and thus could contribute to prevention of type 2 diabetes by reducing the metabolic complications related to insulin resistance.

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Insulin resistance contributes to the pathophysiology of type 2 diabetes (1). Studies to reduce insulin resistance using insulin-sensitizing agents, such as thiazolidinediones, suggest that such therapies may delay or prevent progression from insulin resistance to type 2 diabetes (2,3) and preserve β-cell function (4). Among other therapeutic approaches, dietary interventions are also being studied.

An increasing number of studies have been performed to investigate the metabolic effects of dietary proteins on insulin and glucose homeostasis. According to von Post-Skagegard et al. (5), a cod protein meal, compared with a milk protein meal, lowered insulin levels and reduced insulin–to–C-peptide and insulin-to-glucose ratios in healthy women. In a previous study, both cod and soy proteins reduced fasting and postprandial glucose and insulin responses and increased peripheral insulin sensitivity compared with casein in rats fed a high-sucrose diet (6). Furthermore, dietary cod protein, compared with soy protein and casein, prevented the development of skeletal muscle insulin resistance (7) by normalizing insulin activation of the phosphatidylinositol 3-kinase/Akt-protein kinase B pathway and by improving GLUT4 translocation in skeletal muscle of high-fat–fed obese rats (8). Studies in humans also showed that including lean fish, whose major component is fish protein, as opposed to other animal proteins, in a prudent-type hypolipidemic diet increased sex hormone–binding globulin (9) and HDL_2 cholesterol concentrations (9,10), well-recognized parameters associated with insulin sensitivity (11).

Thus, the objective of this study was to compare the effects of dietary cod protein with those of other animal proteins (beef, pork, veal, and milk) on insulin sensitivity in insulin-resistant men and women. Based on previous studies in animals (7,8) and humans (9,10), we hypothesized that cod protein would improve insulin sensitivity in insulin-resistant subjects, compared with other animal proteins. Insulin secretion was also explored because both insulin sensitivity and secretion interact in determining diabetes risk (1).

RESEARCH DESIGN AND METHODS

— After a medical examination and evaluation, including routine

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Abbreviations: BPVEM, lean beef, pork, veal, eggs, milk, and milk products; FPG, fasting plasma glucose; IAUC, incremental area under the curve; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PUFA, polyunsaturated fatty acid.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

laboratory testing and a 75-g oral glucose tolerance test (OGTT), 10 men and 9 women, recruited through media advertising in the Quebec City metropolitan area, aged 40–65 years, who were overweight or obese (BMI >25 kg/m^2) were selected. Exclusion criteria included smoking; chronic, metabolic, or acute disease; use of medication known to affect lipid or glucose metabolism; major surgery in the 3 months before onset of the study; significant weight change (±10%) within the 6 months that preceded the study; and incompatibility with fish consumption (allergy, intolerance, or dislike) and/or calcium supplementation. Inclusion criteria were fasting plasma insulin >90 pmol/l (12), which is >75th percentile for fasting insulin levels in a sample from the adult Quebec population (13), with fasting plasma glucose (FPG) <7.0 mmol/l and 2-h plasma glucose <11.1 mmol/l. Because insulin sensitivity reductions in the luteal phase have been reported (14), the effect of menstrual phase was controlled by performing all tests during the follicular phase (days 4–11 of the menstrual cycle) for the two premenopausal women. Participants provided written informed consent after the experimental protocol was carefully explained. This study was approved by the Clinical Research Ethical Committee of Laval University Hospital Center.

The study included two experimental periods in a crossover design. After a first 2-week run-in period, subjects were randomly assigned to either a cod protein diet or a diet containing lean beef, pork, veal, eggs, milk, and milk products (BPVEM) for 4 weeks. At the end of the first experimental period, participants returned to their usual diet for a washout period of 4 weeks including a second 2-week run-in period. They were subsequently assigned to the other experimental diet for an additional 4 weeks. Subjects were asked to maintain their physical activity level and to refrain from alcohol consumption for the entire study period.

Diets

Experimental diets, given as 7-day rotating menus, were formulated to meet the National Cholesterol Education Program Adult Treatment Panel III (15), American Diabetes Association (16), and Dietary Reference Intakes (17) recommendations, thus providing 51–52% of total energy as carbohydrate, 18–19% as protein, 32% as fat (with <10% as saturated fat), 225 mg cholesterol/day, and 30 g dietary fibers/day. Nutritional composition of the experimental diets was calculated using a computer-assisted analysis of the Canadian Nutrient File database (18).

Table 1—*Nutrient composition of the experimental diets

	Diet	
	BPVEM	Cod protein
Energy (kJ)	10,984	10,920
Carbohydrates (% of energy)	51	52
Lipids (% of energy)	32	32
Protein (% of energy)	19	18
Alanine (g)	5.53	6.02
Arginine (g)	6.26	7.04
Aspartic acid (g)	10.05	11.59
Cysteine (g)	1.56	1.45
Glutamic acid (g)	23.35	21.11
Glycine (g)	4.99	5.15
Histidine (g)	3.38	3.05
Isoleucine (g)	5.38	4.96
Leucine (g)	9.28	8.64
Methionine (g)	2.52	2.73
Lysine (g)	7.83	8.21
Phenylalanine (g)	5.09	4.79
Proline (g)	7.91	5.55
Serine (g)	5.33	4.96
Threonine (g)	4.60	4.53
Tryptophan (g)	1.44	1.30
Tyrosine (g)	4.18	3.71
Valine (g)	6.21	5.68
BCAA (g)	20.87	19.28
EAA (g)	45.73	43.89
PUFA (g)	25	26
MUFA (g)	39	39
SFA (g)	22	23
PUFA-to-MUFA-to-SFA ratio	1.1:1.8:1.0	1.1:1.7:1.0
ω-3 (g)	3.5	3.3
ω-6 (g)	20.9	21.6
Cholesterol (mg)	228	220
Total fiber (g)	28.0	29.7
Calcium (mg)	1595	1487
Vitamin D (μg)†	15.3	12.8

*Average of the 7-day menu cycle for the 11,000-kJ diets, as determined by using the Canadian Nutrient File database (18). †Values include the vitamin D provided by cod liver oil in the BPVEM diet and by calcium and vitamin D supplements in the cod protein diet. BCAA, sum of branched-chain amino acids (isoleucine, leucine, and valine); EAA, sum of essential amino acids (histidine, isoleucine, leucine, methionine, lysine, phenylalanine, threonine, tryptophan, and valine); MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

The nutrient composition of the formulated experimental diets is presented in Table 1. The cod protein diet consisted of cod fillets and the BPVEM diet consisted of lean beef, pork, and veal, eggs and egg substitutes, and skimmed milk and milk products as the main protein sources. A proportion of 58–68% of daily dietary proteins came from cod or BPVEM proteins, whereas the remaining proteins were of vegetable origin. The cod protein diet provided more alanine, arginine, aspartic acid, glycine, methionine, and lysine and less cysteine, glutamic acid, histidine, isoleucine, leucine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, and branched-chain amino acids (isoleucine, leucine, and valine) than the BPVEM diet. Both experimental diets were adjusted to provide equivalent amounts of monounsaturated, polyunsaturated (polyunsaturated fatty acids [PUFAs]), and saturated fats (1.1:1.8:1.0), as well as dietary fibers. Cod liver oil was added to the BPVEM diet to provide amounts of n-3 PUFAs similar to those in the cod protein diet. α-Tocopherol (5 mg/g eicosapentaenoic acid

Table 2—*Physical and clinical characteristics of the subjects at baseline*

	Men	Women
n	10	9
Age (years)	53.8 ± 2.6	55.4 ± 2.9
Body weight (kg)	92.7 ± 4.9	89.4 ± 4.4
BMI (kg/m^2)	30.9 ± 1.3	33.8 ± 1.6
Waist circumference (cm)	107.2 ± 4.0	107.0 ± 2.9
Hip circumference (cm)	110.1 ± 2.8	121.4 ± 3.4
Cholesterol (mmol/l)		
Total	5.64 ± 0.39	5.27 ± 0.29
LDL	3.85 ± 0.32	3.22 ± 0.34
HDL	1.05 ± 0.05	1.45 ± 0.13
Total–to–HDL cholesterol ratio	5.41 ± 0.41	3.89 ± 0.42
Total triacylglycerols (mmol/l)	1.87 ± 0.20	1.69 ± 0.23
Fasting plasma glucose (mmol/l)	6.0 ± 0.1	6.1 ± 0.2
2-h plasma glucose (mmol/l)	8.0 ± 0.6	8.2 ± 0.7
Fasting insulin (pmol/l)	130 ± 22	146 ± 30

Data are means ± SEM.

+ docosahexaenoic acid) was added to the cod liver oil to protect the PUFAs against oxidation. The cod liver oil was divided into aliquots, carefully put under a nitrogen atmosphere, and frozen immediately. Each aliquot was thawed just before being added to the meal and served to the participants. Subjects were asked to take daily calcium (600 mg) and vitamin D (125 IU) supplements during the cod protein diet because no milk or milk products were included. They received one or two tablets to reach adequate intakes (19) for both nutrients.

Subjects were asked to complete a 5-day food diary (4 weekdays and 1 weekend day) before the onset of the study to determine their usual energy intake. Participants were provided with kitchen scales and asked to weigh or measure with household measurement tools (e.g., cups and tablespoons) all foods and beverages consumed. Eight different energy levels were established for each diet (from 7,500 to 16,500 kJ). Subjects began the study at the energy level nearest to their usual intake. Subjects were weighed every day before lunch and were moved from one level to another when their body weight fluctuated. A maximum body weight variation of 2 kg was allowed within each experimental period. Waist circumference was also determined before and after each diet, as described previously (20).

On weekdays, lunches and dinners were prepared and eaten at the metabolic kitchen of the Institute of Nutraceuticals and Functional Foods of Laval University. All food was precisely weighed (to the nearest 0.1 g). Weekend lunches and dinners were distributed on Friday. Breakfast and snacks were purchased and prepared by the participants from a list of foods indicating the type and quantity of food to be consumed. Participants were provided with kitchen scales to weigh food. Subjects' compliance was assessed by an oral questionnaire every day, and any deviation from the diets was noted.

OGTT

A 75-g OGTT was conducted before and after each experimental period after a 12-h overnight fast as described previously (20). Blood samples were drawn at −15, 0, 15, 30, 60, and 120 min for the determination of plasma glucose, insulin, and C-peptide concentrations. The total glucose, insulin, and C-peptide incremental areas under the curve (IAUCs) during the OGTT were determined with the trapezoid method. The 30-min C-peptide–to–30-min glucose ratio was calculated as an index of β-cell function (21). OGTTs were performed at the Diabetes Research Unit of Laval University Hospital Center.

Hyperinsulinemic-euglycemic clamp

A 120-min hyperinsulinemic-euglycemic clamp was performed to determine insulin sensitivity before and after each experimental diet as described previously (20). The insulin-stimulated glucose disposal rate (*M*) was calculated from the glucose infusion rate during the last 30 min of the clamp divided by body weight (kilograms). Insulin sensitivity (*M*/*I*) was then determined as the *M* value divided by the mean insulin concentration during the last 30 min of the clamp (*I*). The disposition index, which is a measure of the ability of β-cells to compensate for insulin resistance (22), was calculated as follows: β-cell function (30-min C-peptide–to–30-min glucose ratio) × insulin sensitivity (*M*/*I*). The clamps were performed at the Diabetes Research Unit of Laval University Hospital Center.

Statistical analyses

Based on previous published data (7,9), a minimum of 16 subjects were needed to provide 90% power to detect a treatment difference of 30% in insulin sensitivity at $P < 0.05$. Statistical analyses were performed using the Statistical Analysis System (version 9.0; SAS Institute, Cary, NC). The PROC MIXED procedure for an ANOVA for crossover design with two periods as described by Hills and Armitage (23) was used to compare the effects of the two dietary treatments. As no effect of experimental period or diet sequence and no residual effect of the first experimental period over the second period were observed for any measured variables, the data for experimental period, diet sequence, and dietary treatment were pooled. The potential interactions of the diets with sex, body weight change, and baseline insulin sensitivity status on the measured variables were tested by individual entry of terms and interaction terms into the ANOVA model. When an interaction was significant, the least significant difference test was then used for multiple comparisons. Baseline insulin sensitivity status was stratified according to the baseline median value (4.8×10^{-3} mg · kg^{-1} · min^{-1} · pmol^{-1}). Data from two subjects for the hyperinsulinemic-euglycemic clamp variables were excluded because of technical problems during one of their clamps. Data for glucose, insulin, and C-peptide from one subject were missing, so she was excluded from all analysis needing these data (IAUC, β-cell function, and disposition index). The significance level was set to $P < 0.05$. Results are expressed as means ± SEM.

RESULTS

Subject baseline characteristics

All subjects completed the study. Subjects' physical and clinical characteristics at baseline are presented in Table 2. All of our subjects were overweight or obese (BMI 27–41 kg/m^2) (15) with increased

abdominal adiposity (waist circumferences >100 cm for men and >96 cm for women) (15) and hyperinsulinemia (13). According to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (24), 21% of the subjects could be classified as having normal glucose tolerance (NGT), 11% as having isolated impaired fasting glucose (FPG between 6.1 and 6.9 mmol/l and 2-h plasma glucose <7.8 mmol/l), and 32% as having isolated impaired glucose tolerance (IGT) (FPG <6.1 mmol/l and 2-h plasma glucose between 7.8 and 11.0 mmol/l), whereas 37% had both impaired fasting glucose and IGT (FPG between 6.1 and 6.9 mmol/l and 2-h plasma glucose between 7.8 and 11.0 mmol/l).

Anthropometric measures

Before the 4-week intervention, there were no significant differences in mean initial body weight and waist circumference between the BPVEM (91.0 ± 3.3 kg and 106.6 ± 2.5 cm, respectively) and the cod protein (90.7 ± 3.2 kg and 106.4 ± 2.5 cm, respectively) diet groups. After the 4-week intervention, no significant differences in body weight and waist circumference were observed between the BPVEM (90.0 ± 3.2 kg and 106.4 ± 2.6 cm, respectively) and the cod protein (89.7 ± 3.2 kg and 106.3 ± 2.6 cm, respectively) diet groups.

Glucose-insulin homeostasis

From the OGTT, no significant differences were observed between the effects of the experimental diets on FPG and glucose IAUC, indicating that glucose tolerance was not modified, and on fasting plasma insulin, insulin IAUC, C-peptide, and C-peptide IAUC (Table 1 of the online appendix [available at http://dx.doi.org/10.2337/dc07-0273]). As shown in Fig. 1*A*, dietary cod protein increased *M/I* by 29% (from 5.6 ± 0.6 to 7.2 ± 0.8 × 10^{-3} mg · kg^{-1} · min^{-1} · $pmol^{-1}$), whereas dietary BPVEM decreased it by 3% (from 6.4 ± 0.8 to 6.2 ± 0.7 ×10^{-3} mg · kg^{-1} · min^{-1} · $pmol^{-1}$), showing a significant improvement in insulin sensitivity in subjects consuming the cod protein diet compared with those consuming the BPVEM diet ($P = 0.027$). No correlation between weight change and changes in insulin sensitivity ($n = 34$; $r = 0.002$, $P = 0.990$) was observed. Also of interest, there was a strong tendency for a better disposition index after the cod protein diet compared with the BPVEM diet ($P = 0.055$), suggesting improved overall glucose homeostasis.

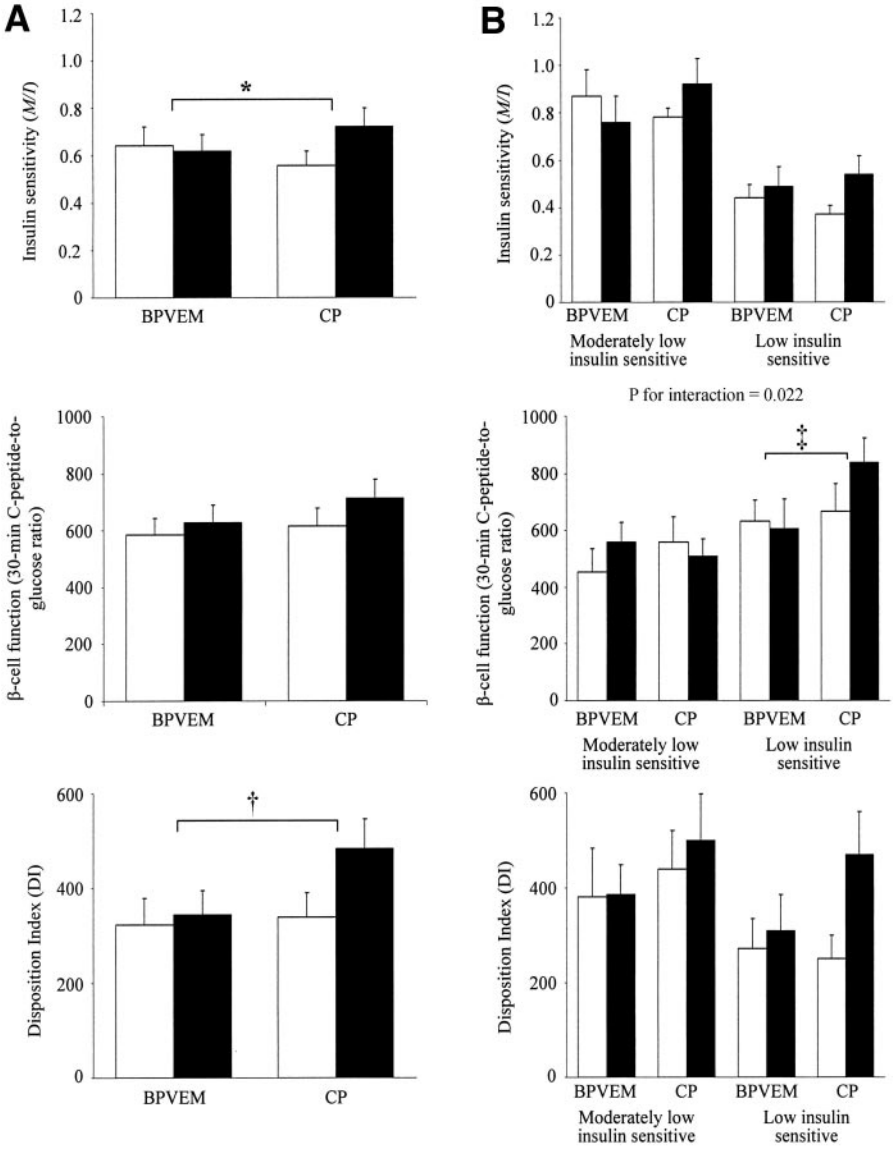

Figure 1— *Effects of cod protein (CP) compared with other animal proteins (BPVEM) on insulin sensitivity (top), β-cell function (middle), and disposition index (bottom) before (□) and after (■) 4 weeks of feeding of insulin-resistant subjects* (A) *and the interaction between diet and baseline insulin sensitivity (median 4.8 × 10^{-3} mg · kg^{-1} · min^{-1} · $pmol^{-1}$)* (B). *Values are means ± SEM.* *P = *0.027*, †P = *0.055, and* ‡P = *0.065 (ANOVA for crossover design). Insulin sensitivity and β-cell function data were available for 17 subjects. Disposition index data were available for 15 subjects. Data were available for 9 subjects in the low insulin-sensitivity subgroup and for 8 subjects in the moderately low insulin-sensitivity subgroup for insulin sensitivity and β-cell function. Data were available for 8 subjects in the low insulin-sensitivity subgroup and for 7 subjects in the moderately low insulin-sensitivity subgroup for the disposition index.*

To determine the effects of both diets on insulin secretion in relation to insulin resistance status, subjects were divided into two subgroups, the low insulin-sensitivity subjects and the moderately low insulin-sensitivity subjects, according to their baseline *M/I* (median 4.8 × 10^{-3} mg · kg^{-1} · min^{-1} · $pmol^{-1}$). Interestingly, a significant interaction on the 30-min C-peptide–to–30-min glucose ratio ($P = 0.022$), used as an index of β-cell function, was observed between the subgroups in their response to the cod protein and BPVEM diets (Fig. 1*B*). Although there was no difference between the cod protein and BPVEM diets ($P = 0.113$) in the moderately low insulin-sensitivity subjects, we observed a 25% increase in the 30-min C-peptide–to–30-min glucose ratio with the cod protein diet (from

668 ± 97 to 836 ± 86) and a slight 4% decrease (from 632 ± 73 to 605 ± 104) with the BPVEM diet ($P = 0.065$) in the low insulin-sensitivity subjects, suggesting a strong tendency for improved insulin secretion with cod protein in these subjects.

CONCLUSIONS — The present results provide the first evidence that cod protein improves insulin sensitivity compared with other animal proteins in insulin-resistant men and women. In our study, the consumption of both controlled-feeding diets resulted in an average weight loss of 1 kg (1%). A 5–10% body weight loss (25), particularly from visceral adipose tissue (26), has been reported to improve insulin sensitivity in overweight subjects with insulin resistance. In this study, waist circumference, which is a good marker for visceral fat (27), remained unchanged during both diets. Moreover, no significant interactions of diet by weight change were seen for any of the measured variables. Therefore, the observed changes in *M/I* and β-cell function are unlikely to be due to this slight body weight loss.

The 29% increase in *M/I* obtained with the consumption of cod protein is in good agreement with the beneficial effects of cod protein already observed in animal studies. Indeed, Lavigne et al. (6,7) reported a better insulin sensitivity in cod protein–fed rats compared with rats fed casein. The mechanism underlying the beneficial effect of cod protein on insulin sensitivity could be attributed to its specific amino acid composition. A previous cell culture study in our laboratory (7) indeed showed that L6 myocytes incubated in an amino acid mixture corresponding to the concentrations of plasma amino acids in rats fed cod protein were more insulin sensitive than those incubated in amino acid mixtures representing plasma amino acids of rats fed casein or soy protein. Interestingly, these effects were observed in the total absence of n-3 PUFAs and therefore support the concept that the protein in fish per se can influence insulin sensitivity. Specific amino acids or mixtures of amino acids have been suggested to be responsible for the observed effects on insulin action. Lower branched-chain amino acids (valine, leucine, and isoleucine) and the higher arginine content of the cod protein diet compared with the BPVEM diet are of particular interest. Infusion of branched-chain amino acids was shown to inhibit insulin-stimulated glucose uptake in the forearm muscle (28). This inhibition is probably linked to activation of the mammalian target of the rapamycin/S6K1 pathway, which has recently been found to be responsible for nutrient-induced insulin resistance in humans (29). As for arginine, a study in patients with type 2 diabetes reported that the administration of L-arginine for 1 month significantly increased peripheral insulin sensitivity (30). Arginine is a substrate for nitric oxide production, which induces vasodilatation and thus contributes to better glucose disposal by increasing muscle perfusion and providing adequate insulin and glucose supply. Furthermore, it has been shown that taurine, whose content is about three to four times greater in white fish than in beef and pork (31), may improve insulin sensitivity in animal models of insulin resistance (32) and type 2 diabetes (33) through lowered protein tyrosine phosphatase and increased protein tyrosine kinase (32). Therefore, our data suggest that cod protein improves insulin sensitivity possibly through its amino acid composition acting on the insulin signaling pathway.

We added cod liver oil to the BPVEM diet to provide equivalent amounts of n-3 PUFAs in both diets. However, when we measured the n-3 PUFA content in plasma phospholipids, we observed a greater increase after consumption of the cod protein diet (from 5.74 ± 0.29 to 8.62 ± 0.21% of total fatty acids) than after consumption of the BPVEM diet (from 5.73 ± 0.26 to 7.20 ± 0.21% of total fatty acids) ($P < 0.001$), suggesting, as did Visioli et al. (34), that n-3 PUFAs consumed in the form of fish could be more available than those consumed in the form of added fish oil. Most intervention studies in diabetic humans have reported no change in insulin sensitivity after n-3 PUFA supplementation of 2 weeks to 6 months (35,36). However, some beneficial effects of n-3 PUFAs on insulin sensitivity have been observed in animal models (35) and on some parameters related to insulin sensitivity (e.g., triglycerides, hypertension, and inflammation) in human studies (36,37). Therefore, we cannot completely rule out a contribution of n-3 PUFAs to the improvement in insulin sensitivity after consumption of the cod protein diet, suggesting that cod protein could interact with n-3 PUFAs in improving insulin sensitivity, but further studies are needed to address this point.

It is well recognized that in insulin-resistant subjects, NGT is maintained by a compensatory increase in insulin secretion. However, insulin secretion progressively fails to adequately increase when moving from NGT to IGT to type 2 diabetes (1). In the present study, we observed a strong tendency ($P = 0.055$) for a greater increase in the disposition index with the cod protein diet, suggesting a better capacity of β-cells to adequately compensate for insulin resistance and, therefore, a potential to decrease the risk of progression from NGT/IGT to type 2 diabetes when a cod protein diet is consumed compared with a BPVEM diet. Because overall β-cell function was not different between the diets, the improvement in insulin sensitivity after consumption of the cod protein diet seems to account for the improvement in the disposition index. However, β-cell function strongly tended to increase in the subgroup of subjects with low insulin sensitivity after consumption of the cod protein diet compared with the BPVEM diet ($P = 0.065$), suggesting that subjects with the potentially greatest risk of progressing to type 2 diabetes benefit more from consuming cod protein. These results were observed in a small number of subjects and thus need to be further explored in a larger cohort.

In conclusion, the data from this study indicate that in the short-term, consumption of cod protein is effective as a dietary insulin sensitizer and, thus, could contribute to reducing the metabolic complications related to insulin resistance, which may prevent type 2 diabetes. In order to support dietary recommendations, further studies are required to determine the optimal number of servings of fish needed to obtain these health benefits. Additional studies will also be required to clarify the potential effects on insulin secretion and to elucidate the cellular mechanisms underlying the beneficial effects observed with cod protein.

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