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Differential effects of various fish proteins in altering body weight, adiposity, inflammatory status, and insulin sensitivity in high-fat–fed rats

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ABSTRACT

Mounting evidence suggests that the benefits of fish consumption are not limited to the well-appreciated effects of omega-3 fatty acids. We previously demonstrated that cod protein protects against the development of diet-induced insulin resistance. The goal of this study was to determine whether other fish protein sources present similar beneficial effects. Rats were fed a high-fat, high-sucrose diet containing protein from casein or fish proteins from bonito, herring, mackerel, or salmon. After 28 days, oral glucose tolerance tests or hyperinsulinemic-euglycemic clamps were performed; and tissues and plasma were harvested for biochemical analyses. Despite equal energy intake among all groups, the salmon-protein–fed group presented significantly lower weight gain that was associated with reduced fat accrual in epididymal white adipose tissue. Although this reduction in visceral adiposity was not associated with improved glucose tolerance, we found that whole-body insulin sensitivity for glucose metabolism was improved using the very sensitive hyperinsulinemic-euglycemic clamp technique. Importantly, expression of both tumor necrosis factor–α and interleukin-6 was reduced in visceral adipose tissue of all fish-protein–fed groups when compared with the casein-fed control group, suggesting that fish proteins carry anti-inflammatory properties that

GP analyzed the data and wrote the manuscript. JR conducted the clamp studies. LER performed the in vitro analysis and wrote parts of the manuscript. CL supervised the in vivo experiments and statistical analysis. PJW contributed to the manuscript writing. LF, HJ, and LB contributed to the design of the study and revised the manuscript. PB prepared fish protein extracts. AM designed and supervised the study and data interpretation and wrote the manuscript.

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may protect against obesity-linked metabolic complications. Interestingly, consumption of the salmon protein diet was also found to raise circulating salmon calcitonin levels, which may underlie the reduction of weight gain in these rats. These data suggest that not all fish protein sources exert the same beneficial properties on the metabolic syndrome, although anti-inflammatory actions appear to be common.

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1. Introduction

In recent decades, obesity has emerged as an ominous global health issue. Obesity is characterized by chronic low-grade inflammation and is associated with numerous pathological states including heart disease, high blood pressure, cancer, gallbladder disease, dyslipidemia, and diabetes [1-5]. As a result, antiobesity interventions have become the common form of therapy. Nutritional intervention remains a safe and effective means for prevention and mitigation of these serious conditions. In recent years, much focus has been placed on the beneficial effects of fish consumption [6-10]. Many of the positive effects of fish consumption on dyslipidemia and heart diseases have been attributed to omega-3 polyunsaturated fatty acids (n-3PUFA) contained in fish oils. However, we have previously demonstrated that the protein component may also play an important role in the beneficial effects of fish consumption [11-15]. In fact, we have found that the sole inclusion of cod protein into a high-fat, high-sucrose (HFHS) diet effectively protects against the development of obesity-linked insulin resistance and glucose intolerance in rats without influencing body weight gain [12]. Importantly, this beneficial effect was also observed in human trials. Indeed, during a test meal, cod protein lowered the insulin-glucose ratio in the subjects receiving cod protein, presenting a smaller insulin excursion as compared with those fed milk protein [16]. We also showed that consumption of a cod protein diet compared with diets containing other animal proteins improves insulin sensitivity in insulin-resistant individuals, and this was associated with a reduction in plasma C-reactive protein (CRP) [13,14], an inflammatory marker that is elevated in the insulin-resistant state [17].

Although there is mounting evidence that fish protein underlies at least part of the beneficial actions of fish consumption on glucose metabolism and inflammation, the mechanisms behind these effects remain unknown. The specific combination of high proportions of certain amino acids such as arginine and taurine with low levels of branched-chain amino acids found in fish meat could be one explanation for the beneficial properties of fish protein. We have previously observed that L6 myocytes exposed to cod-protein–derived amino acids showed greater rates of insulin-stimulated glucose uptake compared with cells incubated with casein- or soy-protein–derived amino acids [11]. It is also conceivable that specific peptides found in fish protein extracts could contribute to these effects. One example of a bioactive component of fish protein is salmon calcitonin (sCT). Salmon calcitonin is currently clinically used to inhibit osteoclast activity and protect against osteoporosis, Paget disease, and bone metastases [18]. Interestingly, sCT is a homolog of amylin, a pancreatic β-cell hormone involved in the regulation of satiation. Amylin has recently been shown to increase energy expenditure, suggesting that sCT may promote weight loss [19].

It is conceivable that fish proteins carry diverse bioactive properties. Indeed, whereas bonito fish protein has not been shown to influence osteoclast function, it does lower blood pressure by acting on angiotensin-converting enzyme activity [20]. Other bioactive properties associated with fish protein sources include the control of plasma cholesterol, lipoproteins, and triglycerides [21]. In this study, we wanted to determine whether fish protein sources other than cod could effectively counteract the harmful effects of HFHS feeding in rats. We thus investigated the impact of salmon, bonito, herring, or mackerel protein sources on the physiological response to an HFHS diet. We found that inclusion of salmon protein uniquely protects against body weight gain and improves insulin sensitivity, whereas protein from salmon and all other fish sources display common anti-inflammatory properties that may protect against the metabolic consequences of obesity.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, St Constant, Quebec, Canada) weighing approximately 240 g were individually housed in wire-mesh cages in a temperature- and humidity-controlled room with a daily 12:12-hour light-dark cycle. Upon arrival, all rats were fed a grounded nonpurified commercial diet (Purina rat chow; Ralston Purina, Lasalle, Quebec, Canada) for at least 5 days. Following this period, rats were divided into 5 groups: HFHS-casein, HFHS-salmon, HFHS-bonito, HFHS-herring, and HFHS-mackerel. Purified diets and tap water were provided ad libitum for 28 days. Food intake and body weight were measured daily. The animal protocol was approved by the Animal Care Committee of Laval University.

2.2. Diets

The HFHS diet contained 20% (wt/wt) protein (casein, bonito, herring, mackerel, or salmon), 19.8% lard, 19.8% corn oil, 22% sucrose, and 5% cellulose and were supplemented with 1.4% vitamin mixture, 6.7% AIN-76 mineral mix, 0.2% choline bitartrate, and 0.02% butylhydroxyanisol and butylated hydroxytoluene. All HFHS diets were isoenergetic. All diet ingredients, except vitamin mix (Teklad, Madison, WI) and fish proteins, were purchased from MP Biochemicals (Cleveland, OH). Bonito protein hydrolysate was provided by Ocean Nutrition Canada (Halifax, Nova Scotia, Canada). Herring and

mackerel protein hydrolysates were provided by the Aquatic Products Technology Centre (CTPA, MAPAQ, Gaspé, Quebec, Canada). Salmon protein hydrolysate was provided by Marine Harvest Ingredients (Hjelmeland, Norway). The level of protein in the purified diets was adjusted to an isonitrogenous basis at the expense of carbohydrates. The fish protein used contained no 18:3 or 22:5 n-3PUFA; and the amounts of 20:5 and 22:6 n-3PUFA were less than 0.8 and 1.2 mg/100 g of diet, respectively.

2.3. *Experimental protocols*

At day 25, rats were cannulated via the carotid artery (oral glucose tolerance test [OGTT]) and the jugular vein (hyperinsulinemic-euglycemic clamp) under isoflurane anesthesia. All rats were within 4% of surgery weight on the day of the study. The OGTT and the hyperinsulinemic-euglycemic clamp protocol were performed in 2 separate groups of animals.

2.3.1. *Oral glucose tolerance test*

At day 28, after a 12-hour fast, rats received a 50% glucose solution orally (2 g/kg body weight). Blood samples (300 μL) were taken before (t = 0) and 15, 30, 60, 90, and 120 minutes after the glucose load. All erythrocytes were pooled, resuspended in saline, and injected back into the animals after the 30-, 60-, and 90-minute samples. Three days later (day 31), the soleus, extensor digitorum longus, gastrocnemius muscles, and epididymal, retroperitoneal, and perirenal white adipose tissue (WAT), as well as the liver, heart, and brown adipose tissue, were harvested, weighed, and snap frozen in liquid nitrogen.

2.3.2. *Hyperinsulinemic-euglycemic clamp*

Whole-body insulin sensitivity in unrestrained conscious casein- and salmon-protein–fed animals was determined by hyperinsulinemic-euglycemic clamp. Rats were fasted overnight and weighed then allowed to rest for 40 minutes before the first blood sample (300 μL). A continuous intravenous infusion of purified human insulin (Humulin R; Eli Lilly, Indianapolis, IN) was then started at 4.0 mU kg^{-1} min^{-1} and was continued for 140 minutes. Dextrose solution (25%) was infused at a variable rate to maintain blood glucose between 6 and 7 mmol/L. Arterial blood samples (40 μL) were taken at 5-minute intervals to monitor plasma glucose concentrations. Every 20 minutes, 300 μL of blood was withdrawn for determination of plasma insulin levels. Erythrocytes were suspended in saline and reinjected into the animals to prevent a fall in hematocrit and to minimize stress.

2.3.3. *sCT determination*

Circulating sCT was measured using the sCT enzyme-linked immunosorbent assay (ELISA) kit (Phoenix Peptide, Burlingame, CA). Overnight-fasted rats were refed the salmon diet for 30, 60, and 90 minutes and then killed. Plasma was harvested for analysis. The coefficient of variation for the sCT ELISA kit was less than 5%.

2.3.4. *Cell culture and nitrite production*

J774A.1 macrophages were cultured in Dulbecco's Modified Eagle's Medium high glucose containing 10% fetal bovine serum. Macrophages were stimulated with or without 2.5 ng/mL lipopolysaccharide (LPS) in the presence of 1 mg/mL protein from casein or different fish sources as described in the figure legends. After 16 hours, the medium was harvested. Accumulation of nitrite was used as an index of inducible nitric oxide synthase activity. Nitrite was determined using the Griess method previously described [22].

2.4. *Biochemical determinations*

Glucose was measured using a handheld glucometer (Precision PCX; Abbott Diagnostics, Mississauga, Ontario, Canada). Plasma insulin before and during the OGTT was determined using a rat radioimmunoassay kit (Linco Research, St Charles, MO). Tumor necrosis factor (TNF)–α and interleukin (IL)-6 in retroperitoneal WAT were quantified using ELISA kits (BD optEIA, Oakville, Ontario, Canada). The coefficient of variations for the rat radioimmunoassay kit and the TNF-α kit were less than 4.6% and less than 4.3%, respectively.

2.5. *Statistical analyses*

All data are presented as means ± SEM. Randomized complete blocks experimental design was used and was analyzed using the repeated-measure procedure for OGTT of the Statistical Analysis System (version 9.1; SAS Institute, Cary, NC). To determine the effect of treatments on the various parameters, variations in initial body weight from the different arrivals of animals were added to the statistical model as a covariate in the mixed model analysis. Because WAT weight data were skewed, we used logarithmic transformations to normalize the distribution of this outcome variable. Differences were considered to be statistically significant at $P < .05$.

3. Results

3.1. *Effect of fish proteins on energy intake, body weight gain, and adiposity*

It was important to determine whether the fish protein source affected food intake in HFHS-fed rats. As shown in Fig. 1A, daily energy intake was similar for all groups irrespective of the protein source. Despite equivalent energy intake between all groups, body weight gain was significantly lower in salmon-protein–fed rats compared with the casein-fed group ($P = .0046$) (Fig. 1B). This was reflected by a reduction in the gross weight gain efficiency ratio, which represents the amount of weight gain in grams per calorie consumed ($P < .0001$) (Fig. 1C). Because caloric intake was not different in the salmon-protein–fed animals, these data suggest that energy expenditure was elevated in this group compared with the other fish- or casein-fed counterparts.

The protection against body weight gain in the salmon-protein–fed group was not explained by a reduction in muscle mass because the weights of gastrocnemius, soleus, extensor digitorum longus, and heart were similar between all experimental groups (data not shown). Interestingly, we found that feeding salmon protein prevents dietary-fat–mediated accretion of epididymal WAT compared with the casein-fed group ($P = .0154$) (Fig. 2A) This effect was specific to visceral fat

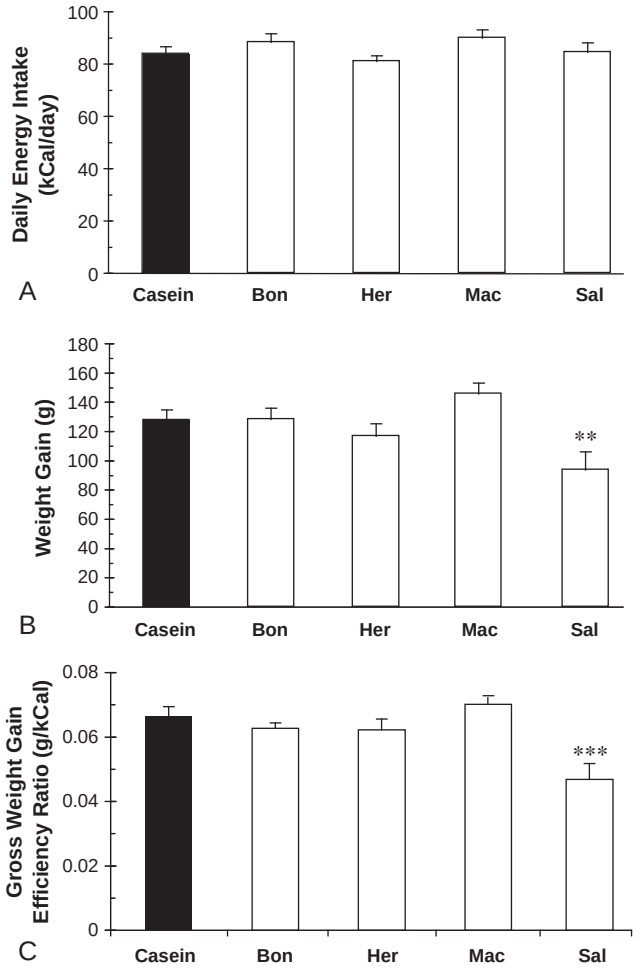

Fig. 1 – Effects of fish proteins on energy intake and body weight gain. Rats were fed for 28 days with an HFHS diet containing proteins from casein, bonito (Bon), herring (Her), mackerel (Mac), or salmon (Sal). Daily energy intake (kilocalories per day), body weight gain (grams), and the gross weight gain efficiency ratio (grams per kilocalorie) are shown in panels A, B, and C, respectively. **P < .01 and ***P < .001 vs casein group. Data are presented as mean ± SEM (n = 8-10).

because no modulation of the subcutaneous inguinal or brown adipose tissue depots was observed (Fig. 2B, C). Furthermore, no significant effect on liver weights or fasting plasma glucose, insulin, or triglyceride concentrations were observed (Table 1).

3.2. *Effect of fish proteins on TNF-α and IL-6 protein expression in adipose tissue*

The HFHS-induced obesity is characterized by inflammation as revealed by elevated expression of the proinflammatory cytokines TNF-α and IL-6 in WAT of casein-fed animals [23]. Tumor necrosis factor–α was significantly reduced in HFHS animals fed bonito ($P = .0190$), herring ($P = .0157$), mackerel ($P = .0496$), or salmon protein ($P = .0328$) (Fig. 3A) compared with casein-fed controls. Interleukin-6 was also significantly reduced in HFHS animals fed bonito, herring, mackerel, or salmon proteins compared with casein-fed rats ($P = .0177$, $P = .0188$, $P = .0405$, and $P = .0276$, respectively) (Fig. 3B).

3.3. *Effect of fish proteins on inflammatory activity in LPS-treated macrophages*

To further understand the mechanism by which fish proteins exert their anti-inflammatory actions, we examined

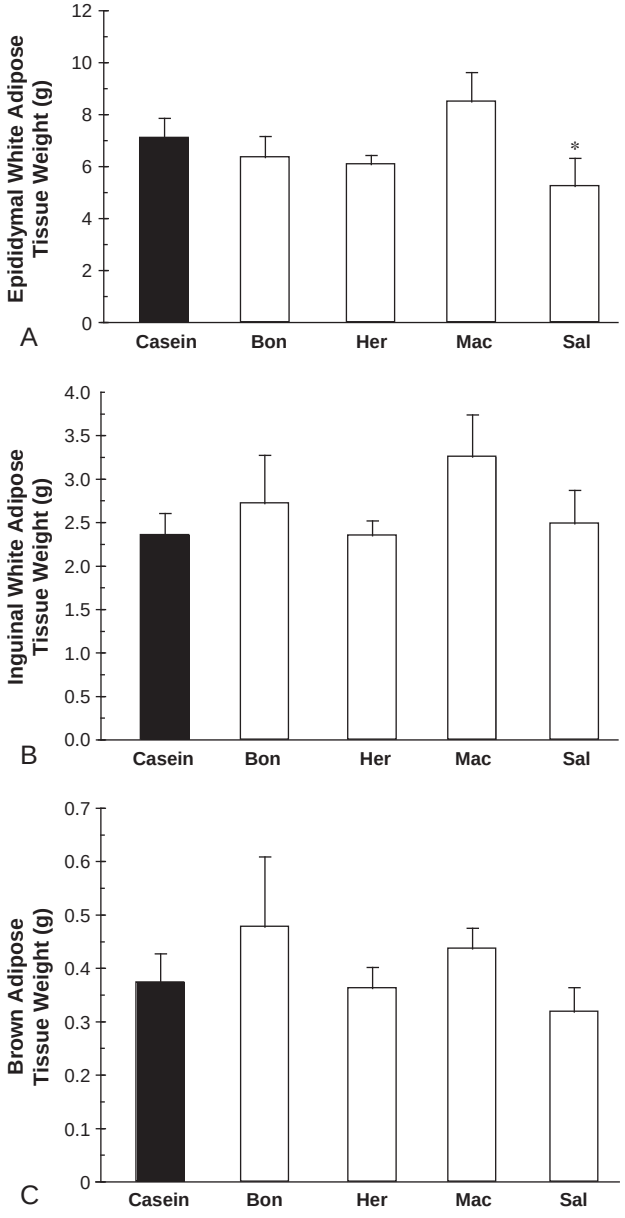

Fig. 2 – Dietary salmon protein reduces visceral adiposity in HFHS-fed rats. Rats were fed for 28 days with an HFHS diet containing protein from casein, bonito (Bon), herring (Her), mackerel (Mac), or salmon (Sal). Adipose tissue depots were harvested and weighed at the time the rats were killed. Weights (grams) of epididymal (visceral) WAT, inguinal (subcutaneous) adipose tissue, and brown adipose tissue are shown in panels A, B, and C, respectively. *P < 05 vs casein-fed group. Data are presented as mean ± SEM (n = 8-10).

Table 1 – Effects of dietary treatment on basal plasmatic values of glucose, insulin, and triglycerides concentrations in fasted animals

	Protein source				
	Casein	Herring	Salmon	Bonito	Mackerel
Glycemia, mmol/L	6.5 ± 0.2	6.7 ± 0.2	6.7 ± 0.2	6.2 ± 0.3	6.1 ± 0.3
Insulinemia, pmol/L	0.248 ± 0.063	0.174 ± 0.038	0.291 ± 0.070	0.164 ± 0.050	0.334 ± 0.076
Triglycerides, mmol/L	0.868 ± 0.110	0.679 ± 0.107	0.577 ± 0.074	0.615 ± 0.073	0.787 ± 0.113

Values are mean ± SEM; n = 7 to 9. All statistical analyses were performed in SAS 9.2 using a mixed procedure with contrasts between casein and every single fish protein. No significant differences were found between groups.

whether fish proteins could inhibit LPS-induced inflammation in macrophages. It is well documented that infiltrating macrophages play a major role in adipose tissue inflammation in obesity, and it was therefore important to determine whether fish proteins could act directly on these inflammatory cells. We used nitrite production in the culture medium as a readout of inducible nitric oxide synthase activity and an index of macrophage inflammatory status. Interestingly, whereas salmon protein effectively blunted LPS-induced nitrite production in macrophages ($P < .05$), this was not the case for the other fish protein sources (Fig. 4). These data suggest that although all fish protein sources possess anti-inflammatory activity in adipose tissue in vivo, only salmon protein can directly blunt inflammation in macrophages.

3.4. *Effect of fish proteins on glucose tolerance and insulin sensitivity*

The response of the rats to a physiological glucose challenge was first tested using an OGTT. No significant improvement in the glycemic excursions, area under the glucose curve, or area under the insulin curve was observed in any of the fish-protein–fed groups compared with the casein-fed group (Fig. 5A, B, and C, respectively). Because salmon-protein–fed rats presented less visceral adiposity in addition to reduced expression of inflammatory cytokines in adipose tissue, the lack of improvement in glucose tolerance in this group was particularly surprising. The more sensitive euglycemic-hyperinsulinemic clamp technique was thus carried out to determine whether a more discrete improvement in insulin sensitivity may have passed undetected in the HFHS salmon-protein–fed group. Using this criterion standard test for the determination of insulin sensitivity, we found that the glucose infusion rate required for maintaining glycemia between 6 and 7 mmol/L was higher in the salmon-protein–fed group compared with HFHS casein-fed animals (Fig. 5D).

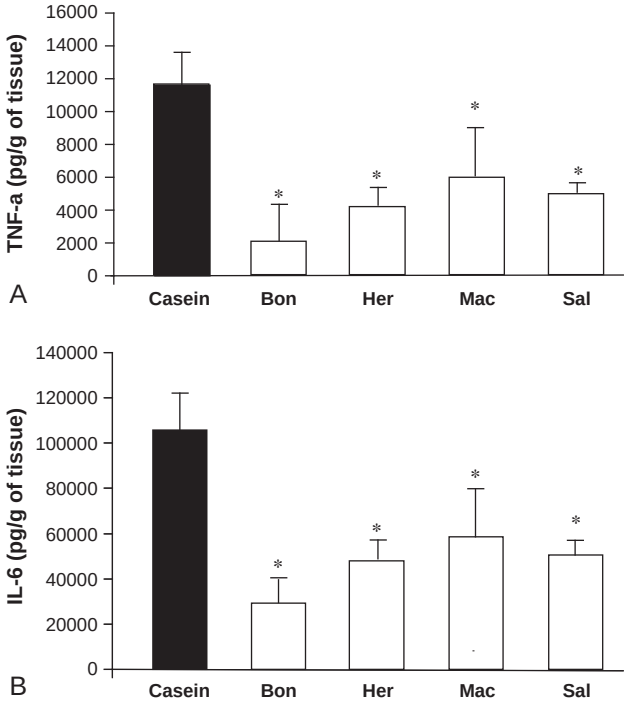

Fig. 3 – Effect of fish protein sources on adipose cytokine expression. Tumor necrosis factor–*α* (picograms per gram of tissue) (A) and IL-6 (picograms per gram of tissue) (B) were measured from the retroperitoneal fat depot that was harvested from rats fed an HFHS diet containing protein from casein, bonito (Bon), herring (Her), mackerel (Mac), or salmon (Sal). *P < 05 vs casein-fed group. Data are presented as mean ± SD (n = 2) or SEM (n = 3-4).

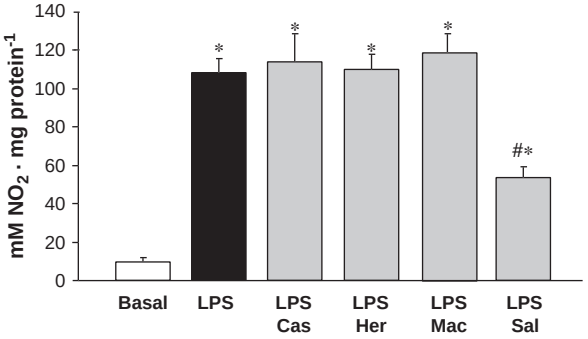

Fig. 4 – Effect of fish protein sources on macrophage activity following LPS treatment. Measurement of nitrite accumulation in the incubation medium after macrophages were treated for 16 hours with or without LPS (2.5 ng/ml) in the presence of casein (Cas), herring (Her), mackerel (Mac), or salmon (Sal) protein (1 mg/ml). *P < 05 vs basal; #P < .05 vs LPS. Data are presented as mean ± SEM from 4 to 7 independent experiments and are expressed as micromoles per liter of NO_2 per milligram of protein.

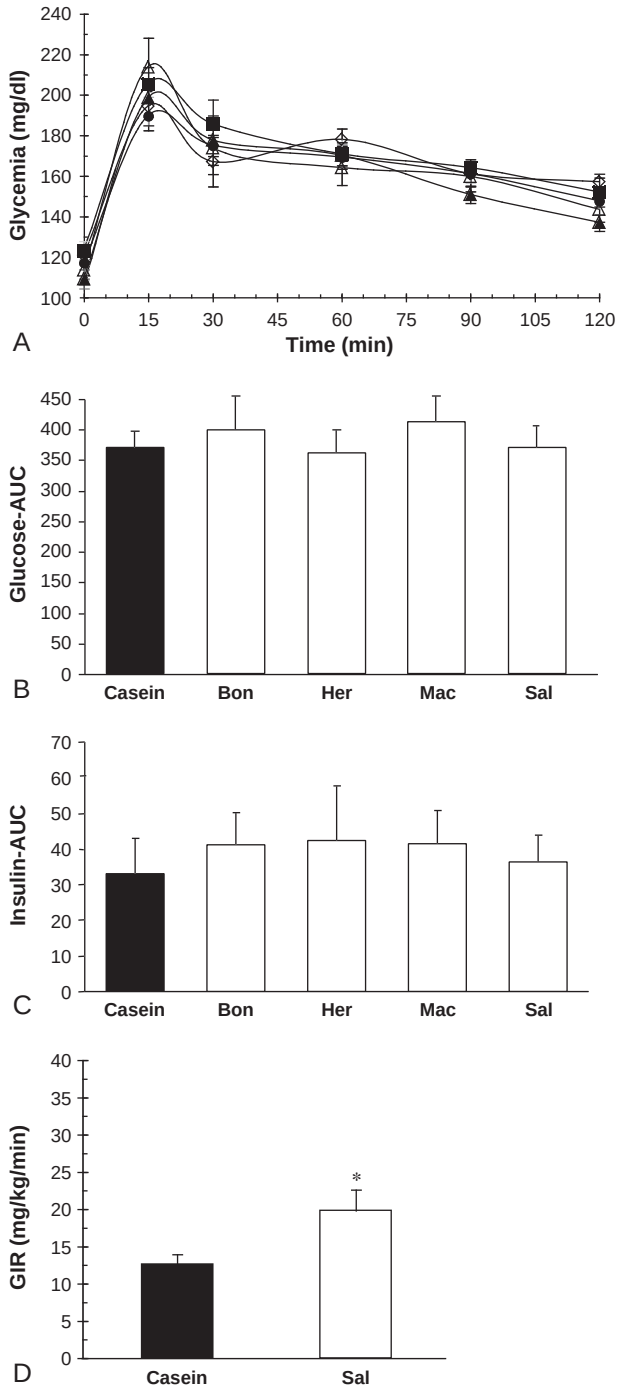

Fig. 5 – Effect of fish protein sources on glucose tolerance and insulin sensitivity. A, The glycemic excursion curves during the OGTT for HFHS rats fed casein (filled circle), bonito (open triangle), herring (open diamond), mackerel (filled triangle), or salmon (filled square) proteins. B and C, The area under the glucose curve (glucose-AUC) and area under the insulin curve (insulin-AUC) during the glucose tolerance test. D, The glucose infusion rate (GIR) during the hyperinsulinemic euglycemic clamp. The casein group is represented by the gray bar, and the salmon group is represented by the white bar. *P < .05 salmon- 05 salmon- vs casein-fed groups. Data are presented as mean ± SEM (n = 6-8).

3.5. *Circulating sCT is elevated postprandially in salmon-fed rats*

Because feeding salmon protein to HFHS-fed rats reduced weight gain and visceral adiposity, we hypothesized that sCT contained within the salmon protein source might account for this effect. Indeed, sCT binds with high affinity to the amylin receptor, a pathway known to activate energy expenditure. We predicted that if sCT was present in the salmon protein, it could potentially be detected in the plasma following a meal. Therefore, we measured plasma sCT in the HFHS salmon-fed group. We found that sCT was significantly elevated in the circulation of these rats 60 and 90 minutes after a meal (Fig. 6).

4. Discussion

The main goal of this study was to determine whether fish protein sources other than cod possessed beneficial properties that could be useful for the prevention of obesity-related metabolic defects. We thus tested several proteins isolated from salmon, bonito, herring, and mackerel in HFHS-fed obese rats that are prone to developing insulin resistance. We found that salmon protein prevents body weight gain caused by HFHS feeding without altering energy intake. As expected, the prevention of weight gain on the HFHS diet was associated with improved insulin sensitivity as measured by the hyperinsulinemic-euglycemic clamp technique.

In our previous study using cod protein, we observed protection against insulin resistance in HFHS-fed animals without any reduction in body weight gain. In the present case, however, one could presume that the insulin-sensitizing effect observed in the salmon-protein–fed group is, at least in

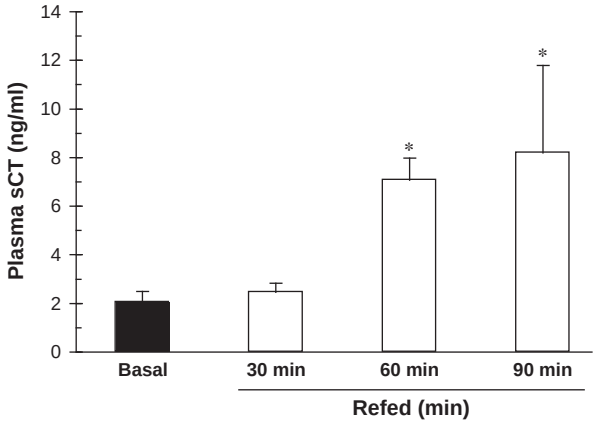

Fig. 6 – Salmon calcitonin can be detected in the plasma of salmon-protein–refed animals. Following an overnight fast, animals were refed for 30, 60, or 90 minutes with the HFHS diet containing salmon as the protein source. Salmon calcitonin concentration detected in the plasma is presented in nanograms per milliliter. Black bar represents basal calcitonin levels in fasted animals. White bars represent time points in the refed state. *P < 05 vs fasted state. Data are presented as mean ± SEM (n = 3-5).

part, related to the reduction of their visceral WAT. Indeed, the reduction of weight witnessed in this group was partially explained by a reduction in the size of visceral fat, which is known to be a major determining factor in the development of obesity-linked insulin resistance [24]. Furthermore, in the obese state, the infiltration of visceral adipose tissue by proinflammatory macrophages correlates highly with adipocyte size [25]. Therefore, a reduction of fat accumulation in this depot could explain both the insulin-sensitizing effect and the reduced cytokine expression observed in salmon-protein–fed animals.

Although we failed to detect a significant effect of fish proteins during the OGTT, we confirmed the beneficial action of salmon protein on whole-body insulin action when directly measuring insulin sensitivity using the hyperinsulinemic-euglycemic clamp technique. Because of the technically challenging and time-consuming nature of the clamp procedure, we did not perform this assessment for all the fish-protein–fed groups and rather elected to focus on the salmon-protein–fed group where modulation of body weight in addition to an anti-inflammatory effect was observed. Nevertheless, further clamp studies following a longer duration of treatment are warranted to test whether the ability of the other fish protein sources to blunt adipose cytokine expression will eventually improve insulin sensitivity even in the absence of changes in adiposity and body weight gain.

Fish consumption has been reported to be independently and inversely associated with circulating levels of several inflammatory markers [26,27]. However, the anti-inflammatory actions of fish are most often ascribed to its oils rather than proteins because long-chain n-3PUFA have been extensively studied for their ability to reduce inflammation [28]. In contrast, little is known about the potential impact of fish proteins on inflammation. Recently, it has been shown that dietary cod protein decreases the circulating levels of high-sensitivity CRP, a well-established biomarker of inflammation in obesity, when compared with an isocaloric diet composed of other animal proteins [14]. Our results suggest that fish proteins exert these anti-inflammatory effects by reducing TNF-α and IL-6 expression in visceral adipose tissue. This is further supported by the fact that cod protein also reduced TNF-α and IL-6 expression in visceral adipose tissue (unpublished data). Interestingly, further in vitro experiments in LPS-treated macrophages revealed that not all the fish protein sources tested in our study exert their anti-inflammatory actions via the same mechanism because only salmon protein was able to blunt macrophage inflammatory activity in this model. Tumor necrosis factor–α and IL-6 production was also assessed in LPS-treated macrophages in the presence of casein and fish proteins. However, we found no reduction of these cytokines after treatment with the fish proteins (unpublished data), indicating that the reduction of TNF-α and IL-6 in the retroperitoneal fat depot is likely not explained by inhibition of infiltrated macrophages. Further studies will be needed to clarify whether specific peptides or amino acids within fish protein underlie these inhibitory effects and by which mechanism they reduce inflammation in adipose tissue. It has been proposed that the anti-inflammatory action of fish protein could be a result of the particularly high level of taurine in fish meat [13,14]. In fact, taurine has been shown to suppress inflammatory cytokines including TNF-α and IL-6 in activated macrophages [29]. Furthermore, a recent study has shown that a diet containing a fish protein rich in taurine and glycine reduces visceral fat accumulation in rats [30].

It should be noted that lipid contaminants are unlikely to be implicated in the beneficial effects of fish proteins reported here because both 18:3 and 22:5 n-3PUFA were not detectable in any of the fish protein used and the amounts of 20:5 and 22:6 n-3PUFA present were found to be negligible in this experiment. Indeed, we performed hyperinsulinemic-euglycemic clamp studies that confirmed that the quantities of n-3PUFA contained in our fish protein fail to improve insulin sensitivity in HFHS casein-fed rats (unpublished data).

It has been shown that functional calcitonin and/or calcitonin-gene–related peptide is detectable in fish and shrimp protein hydrolysates [31]. Notably, calcitonin-gene–related peptide and calcitonin were found to prevent macrophage activation [32]. It is thus possible that fish calcitonin present in the fish protein sources used here could explain their anti-inflammatory properties. Interestingly, fish calcitonin is 40 times more potent than mammalian calcitonin when binding to the mammalian calcitonin receptor [33]. In particular, sCT has been extensively studied for its high biological potency and is used therapeutically for the treatment of hypercalcemia or osteoporosis [34,35]. Salmon calcitonin has also been studied for its capacity to irreversibly bind to and strongly activate the mammalian amylin receptor, which is known for its role in the negative regulation of body weight, through its capacity to increase energy expenditure [19,36,37]. Importantly, sCT treatment has been shown to directly increase energy expenditure, thus providing a potential explanation for the prevention of obesity in the HFHS rats consuming salmon protein [19,38]. Although calcitonin structure does not vary greatly among fish species, it is not known whether calcitonin derived from other fish sources also possess the same potent ability to activate the amylin receptor. In either case, because we could not measure the respective fish-derived calcitonin in the circulation of bonito-, herring-, and mackerel-protein–fed rats (only an sCT kit is commercially available), we cannot rule out the possibility that these forms of calcitonin might also influence energy metabolism and body weight. However, the lack of effect of the other fish protein sources on adiposity and body weight gain would suggest that calcitonin is unlikely to be active in these groups, at least during the time course of our studies.

A previous study has shown that intraperitoneal injection of a single dose of approximately 1.6 μg of sCT per rat is sufficient to stimulate energy expenditure [38]. Unfortunately, in this study, the authors did not measure the plasma concentration of sCT following injection; therefore, it is difficult to directly compare their findings with our own work. Nevertheless, here we saw an increase of 6 ng/mL of sCT in the circulation following a single meal. It is thus conceivable that an elevation of this magnitude following each meal for a period of 1 month could have a physiological impact on energy expenditure in our study; however, this remains to be demonstrated. Alternatively, because insulin is known to be a potent antinatriuretic hormone, the reduced body weight gain in the more insulin-sensitive salmon-protein–fed group

might also reflect reduced insulin-dependent water retention in these rats [39].

One limitation of this study is that our different fish protein sources came from multiple commercial suppliers. Thus, we cannot rule out that differences in farming and/or hydrolyzation processes underlie some of the differences observed between the effects of different fish protein sources on the prevention of body weight gain. On the other hand, this may also be considered a strength of our study because it would suggest that the common finding of anti-inflammatory effects observed in the adipose tissue of all fish-protein–fed groups is not dependent on fish farming conditions or hydrolyzation processes but is intrinsic to fish protein per se.

Another strength of our work is the potential for translation to dietary intervention studies in humans. Indeed, the finding of intrinsic anti-inflammatory activity in fish proteins is in line with the outcome of a recent clinical trial that showed that consumption of lean fish was an effective means of reducing circulating CRP in human plasma [40]. Furthermore, our data might also explain some of the health benefits of the "prudent diet" in which fish represents an important dietary protein source. Reminiscent of our finding in salmon-protein–fed rats, the "prudent diet" has been associated with reduced indices of obesity in populations with and without a family history of obesity [41]. Moreover, adherence to the "prudent diet" was also linked to reduced risk of cardiovascular-disease–associated mortality, for which both inflammation and obesity are independent risk factors [42]. Importantly, similar associations with obesity, inflammation, and cardiovascular disease mortality have also been reported for the "Mediterranean diet," which also contains elevated levels of fish [43-45]. In light of these promising findings, further human studies with fish proteins are certainly warranted.

Taken together, these data suggest that anti-inflammatory actions appear to be common to all fish protein sources despite varying effects on glucose homeostasis. The broad implication of these results lies in the fact that inflammation is central to all major metabolic complications of obesity such as atherosclerosis, type 2 diabetes mellitus, and cardiovascular disease. We also show that salmon protein might have an additional benefit by protecting against visceral obesity possibly through its calcitonin content that could be a useful resource in the fight against the obesity epidemic. Finally, future studies examining the molecular basis of the anti-inflammatory effects of fish protein are highly warranted to develop novel means to prevent the metabolic aberrations that accompany the obese state.

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