Special Article

Fish–derived proteins and their potential to improve human health

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Emerging evidence from studies evaluating the effect of lean fish consumption in humans suggests that proteins from fish have several beneficial metabolic effects. Rest, or waste, material from the fishing industry contains high-quality proteins, and utilization of this material offers novel possibilities for the development of protein-containing products that might be beneficial for human consumption. Fish-derived peptides containing bioactive amino acid sequences suggested to beneficially influence pathways involved in body composition, hypertension, lipid profile, and regulation of glucose metabolism are of particular interest, although the results of published studies are conflicting. This review aims to summarize current knowledge from animal studies and clinical interventions in humans evaluating the effects of lean fish, fish proteins, and fish-derived peptides on outcomes related to metabolic health. Fish proteins have a high content of taurine, and animal trials suggest that taurine mediates some of the beneficial effects observed thus far, although the mechanisms by which fish peptides exert their action are not yet elucidated. At this time, the literature is inconsistent, and there is insufficient mechanistic evidence to support a beneficial effect of fish-derived peptides on metabolic health.

INTRODUCTION

The beneficial effects of fish consumption have traditionally been attributed to the marine-derived omega-3 (n-3) polyunsaturated fatty acids (PUFAs) present in fish, but emerging evidence from studies evaluating the effects of lean fish consumption suggests that the proteins from fish may also have several beneficial metabolic effects.[1–4] It is generally assumed that the consumption of fish has a protective effect against different diseases related to lifestyle. In particular, the relationship between cardiovascular disease and fish consumption has received much attention. The positive association between fish intake and decreased risk of stroke and coronary heart disease is well documented by meta-analyses of observational studies.[5,6] The results of studies investigating the possible associations between fish consumption and development of metabolic syndrome and type 2 diabetes mellitus (T2DM) are, however, inconsistent.[7–12] The beneficial effects of diets rich in fish have, to a large extent, been attributed to the marine n-3 PUFAs.[13,14] However, studies reporting beneficial health effects of lean fish in both animals and humans suggest that substances other than the marine n-3 PUFAs, such as fish protein, may beneficially influence metabolic health by improving insulin sensitivity, glucose metabolism, lipid status, and body composition.[12,15,16]

While the beneficial health effect of an adequate amount of protein in the diet is well established, the

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specific effect of bioactive peptides—beyond the supply of nutrients—has recently drawn increasing interest. Preliminary data suggest that bioactive peptides derived from marine resources may have a beneficial effect on several health outcomes related to metabolic health.[17–20] This increased interest and early research have provided a platform from which to examine the potential for better utilization of rest material from the fishing industry. Waste material with a high content of high-quality protein may be valuable for human consumption, and fish-derived protein hydrolysates with bioactive peptides are hypothesized to be particularly beneficial.[21]

This review aims to summarize current knowledge from animal studies and intervention studies in humans evaluating the effects of lean fish, fish proteins, and fish-derived peptides on metabolic pathways involved in outcomes related to metabolic health, including glucose metabolism, lipid status, hypertension, and body weight and composition.

FISH PROTEINS AND BIOACTIVE PEPTIDES

Fish and marine resources, including fish processing by-products, are excellent sources of high-quality protein, and an increasing amount of evidence points toward beneficial effects of these marine proteins on metabolic health.[1,4,22] The nutritional value of a food protein depends on several factors, including primary structure of the protein, susceptibility of the protein to enzymatic digestion, chemical changes of the protein during processing, amino acid composition of the protein, and content of essential amino acids in the protein. A high-quality protein source has a high relative content of essential amino acids.[23] In general, proteins from fish contain all the essential amino acids and have a particularly high content of the essential amino acids lysine and leucine. Of the nonessential amino acids, aspartic acid, glutamic acid, and alanine are usually present in very high amounts in marine protein sources, along with the amino acid–derived organic acid taurine.[24] The digestibility of most seafood proteins exceeds 90%, and thus the essential amino acids in marine proteins are highly exploitable.[23]

The fishing industries around the world generate a tremendous amount of protein-containing rest material, which is normally discarded but, when properly processed, can be used for human consumption. The utilization of marine by-products, such as waste and cuttings from which protein hydrolysates can be extracted, is both environmentally friendly and cost effective, with positive consequences for both the industry and human health.[17] Following ingestion of whole protein, bioactive peptides are formed naturally in the gut, either by enzymatic degradation (hydrolysis) or microbial fermentation. They can also be consumed as a nutritional supplement that contains already-hydrolyzed protein. The biological activity of a peptide derived from food is highly dependent on the structural properties of the peptide, including the molecular mass and characteristics of the amino acids present in the peptide, whereas the biological activity of a peptide derived from a protein hydrolysate is dependent on several factors, including the enzymes utilized in the hydrolysis process, the pH and temperature during hydrolysis, the duration of hydrolysis, and the enzyme-to-substrate ratio applied.[25] Bioactive peptides tend to be present as di- and tripeptides with low molecular mass and contain 2 to 20 amino acid residues. The bioactivity of peptides is linked to the presence of different amino acid sequences. A bioactive peptide may produce a local effect in the gastrointestinal tract or a systemic effect after being absorbed in the gut and entering the circulation.[26] Some of the amino acid sequences present in fish proteins are proposed to have a unique potential to beneficially modulate different metabolic pathways and thereby contribute to the prevention of disease (Figure 1). Different amino acid sequences present in fish-derived peptides are suggested to be capable of reducing hypertension by inhibiting angiotensin-1 converting enzyme, beneficially altering blood glucose metabolism through mechanisms such as inhibition of dipeptidylpeptidase-4, and altering the gut microbiota by contributing to increased conjugation of bile acids.[21]

ANIMAL STUDIES INVESTIGATING THE EFFECTS OF FISH PROTEIN

Studies with intact fish protein

Several studies in rats and mice have investigated the metabolic effect of intact fish protein in the diet, comparing the effect of proteins from fish with that of casein or proteins from terrestrial animals.

Cod protein in combination with fish oil lowered the rate of hepatic triacylglycerol secretion in rats when compared with casein in a 28-day dietary intervention study, thereby beneficially altering lipid metabolism.[27] A study in rats that compared the metabolic effect of a 4-week high-fat diet that included protein from cod, soy, or casein found that cod and soy proteins, when compared with casein, improved fasting glucose tolerance and peripheral insulin sensitivity. Postprandial samples taken after test meals revealed that the rats fed cod or soy protein had lower plasma insulin concentrations, possibly due to decreased release of pancreatic insulin and increased removal of insulin by the liver.[28] Furthermore, the high-fat diets with soy and casein induced severe insulin resistance, while the high-fat diet

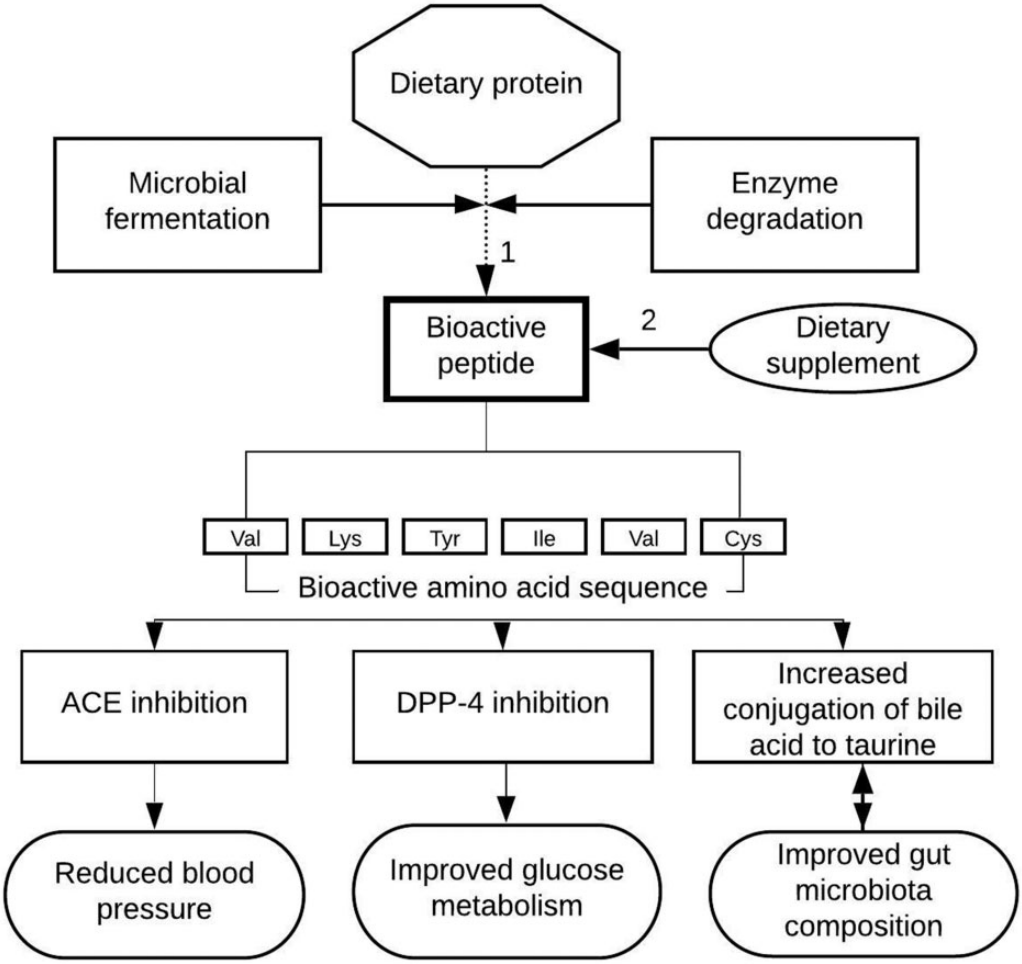

Figure 1 **Potential effects of bioactive fish-derived peptides.** Bioactive peptides can be formed naturally by ingestion of whole protein and subsequent microbial fermentation or degradation of enzymes in the gut, or they can be consumed as a nutritional supplement with already-hydrolyzed protein. They tend to have 2 to 20 amino acid residues, and their bioactive effect is linked to the presence of different amino acid sequences (simplified example shown in figure). Some of the peptide sequences present in fish proteins have been proposed to have a unique potential to beneficially modulate different metabolic pathways and thereby contribute to the prevention of disease. Different amino acid sequences present in fish-derived peptides have been proposed to be capable of reducing hypertension by inhibiting ACE, altering blood glucose metabolism beneficially through different mechanisms such as inhibition of DPP-4, and contributing to increased conjugation of bile acids. *Abbreviations*: ACE, angiotensin-converting enzyme; Cys, cysteine; DPP-4, dipeptidylpeptidase-4; Ile, isoleucine; Lys, lysine; Tyr, tyrosine; Val, valine.

with cod protein fully prevented the development of insulin resistance. The effect of the cod protein was linked to a direct action of amino acids on insulin-stimulated glucose uptake in skeletal muscle cells.[15]

The same research group later investigated the cellular mechanisms behind this action and reported a beneficial effect of cod protein on insulin sensitivity, which was attributed to maintenance of the insulin receptor substrate-1-associated phosphatidylinositol 3-kinase (PI3K) pathway, which in turn led to improved translocation of glucose transporter 4 to the T-tubules in the cells.[29] This pathway was significantly downregulated, leading to reduced insulin sensitivity when the rats were fed high-fat diets with soy or casein. Together, these results indicate that dietary cod protein, or components of the cod protein, may act as a natural insulin-sensitizing agent that can possibly prevent insulin resistance linked to obesity by normalizing the insulin activation of the PI3K/Akt pathway and improving translocation of glucose transporter 4 present on the cell surface.[29] Importantly, it should be noted that both studies are based on values from a hyperinsulinemic-euglycemic clamp and tracer injection in rats; therefore, the proposed mechanisms might not be translatable to humans, and the results must be interpreted with caution.

A number of animal trials indicate that high-fat diets containing lean seafood are less obesogenic than high fat-diets containing meat from terrestrial animals. Mice fed a Western diet high in fat and sucrose and

containing a mixture of lean seafood (ling, rose fish, cod, wolf fish) and muscle from Canadian scallop were reported to gain less adipose tissue mass than mice fed a Western diet containing a mixture of skinless chicken breast, pork tenderloin, and beef sirloin.[30] A diet containing a mixture of protein from cod and scallop was reported to reduce fat mass and improve glucose tolerance when compared with isoenergetic diets containing either chicken or casein, respectively, in mice fed a high-fat diet for 6 weeks.[31] The feed efficiency values were lower in the mice fed cod/scallop and casein than in the mice fed chicken. In addition the mice fed cod/scallop and casein had reduced adipose tissue mass in compared with mice fed chicken. In addition, the mice fed chicken had both elevated concentrations of triacylglycerol in the liver and elevated fasting plasma cholesterol concentrations compared with the other groups. These findings show that protein from different food sources modulates the energy balance differently in mice. Compared with other protein sources, the cod protein mix was more likely to prevent diet-induced obesity and improve glucose tolerance.[31] It should be noted that this study investigated protein from a combination of cod and scallop, and not protein from cod in particular. The effect of other sources of fish proteins has been studied in rats fed a high-fat, high-sucrose diet.[32] Proteins were provided from casein, salmon, herring, mackerel, or bonito. Despite equal energy intake, the group fed salmon protein had a significantly lower weight gain, reduced visceral adiposity, and improved insulin sensitivity compared with the other groups.[32] Collectively, these data suggest that different sources of fish protein may exert different metabolic effects.

A recent study investigated whether changing the protein source from pork to cod in a regular Western diet would alter the endocannabinoid tone in mice and thereby reduce both the development of obesity and the accumulation of fat in the liver.[33] The results showed that the cod-fed mice had significantly lower concentrations of 2 major circulating endocannabinoids, a lower increase in adipose tissue, and a lower content of hepatic lipids than the pork-fed mice. Protein from seafood has a high content of taurine, and a negative correlation between adiposity and intake of taurine and glycine has been demonstrated in mice fed chicken, cod, crab, or scallop in high-fat, high-sucrose diets.[34] In line with this, both taurine[35] and glycine[36,37] are reported to attenuate obesity development in rodents, and increased intakes of glycine, taurine, arginine, and lysine, all found in fish protein, are associated with anti-inflammatory effects in rats.[38]

Together, these findings suggest an overall beneficial metabolic effect of protein sources from lean seafood.

Studies with fish protein hydrolysates

Several animal studies have investigated the specific effect of fish protein hydrolysates from different kinds of fish and marine protein sources. Most studies have compared the effect of a fish protein hydrolysate with casein and/or soy.

A fat-free protein hydrolysate from salmon, when compared with casein, has been reported to possibly have a cardioprotective effect in rats by reducing plasma total cholesterol, increasing high-density lipoprotein cholesterol (HDL-C), and lowering acyl-coenzyme A:cholesterol acyltransferase (ACAT) activity in the liver.[39] However, the same beneficial effects seen in the rats fed fish protein hydrolysate were observed in the rats fed soy protein, and thus the effect of the fish protein was not unique. Both the soy protein diet and the fish protein hydrolysate diet had low ratios of both methionine to glycine and lysine to arginine compared with the casein diet, and this was proposed to contribute to the reduction in cholesterol levels.

A study in rats that compared a salmon protein hydrolysate with casein found that the rats fed the salmon protein hydrolysate became resistant to high-fat-diet–induced obesity, had reduced postprandial plasma glucose and triacylglycerol levels, and lower triacylglycerol levels in the liver compared with the rats fed casein.[40] The plasma bile acid concentrations were elevated in the rats fed the salmon protein hydrolysate, a finding associated with the induction of genes involved in energy metabolism. These genes were found to stimulate an increase in energy expenditure and a decrease in body mass. Overall, the findings indicated the salmon protein hydrolysate diet, when compared with the casein diet, to have a beneficial effect on several metabolic markers.[40] A similar effect in rats has been reported for a fish protein hydrolysate from saithe when compared with proteins from soy and casein.[41] In this study, the fish hydrolysate, which had a high content of taurine and glycine, was shown to elevate fasting levels of bile acids and reduce visceral adipose tissue mass in rats. In both experiments, the casein or soy control diet was supplemented with 3% L-cysteine to avoid depletion of sulfur-containing amino acids. These findings on lipid metabolism are supported by a later study indicating that a fish protein hydrolysate from salmon, also rich in taurine and glycine, beneficially altered the fatty acid composition in liver and adipose tissue and increased plasma carnitine levels in a mouse model of chronic inflammation.[42] The mice were fed a high-fat diet containing either 20% casein (control) or 15% fish protein hydrolysate and 5% casein.

A recent study investigated the effect of a diet comprised of 75% protein from casein/whey and 25% protein from fish protein hydrolysates derived from either

herring or salmon.[16] The rats fed the herring-containing diet presented lower levels of serum HDL-C and low-density lipoprotein cholesterol (LDL-C) as well as higher levels of serum triacylglycerol than the rats fed the casein/whey diet, whereas the rats fed the salmon-containing diet gained more weight and had better postprandial blood glucose regulation than the rats fed the casein/whey diet. These findings imply a negative effect of herring on lipid status. The ratios of lysine to arginine and methionine to glycine were lower in the 2 fish diets than in the casein/whey diet, whereas taurine was present only in the fish diets. The authors linked the observed effects to the bioactive motives present in the different fish protein hydrolysates.[16] The same 2 fish protein hydrolysates were later reported to contain several peptide sequences with possible angiotensin-1 converting enzyme (ACE)-inhibiting activities and to beneficially alter the urine concentrations of glucose, protein, and cystatin C.[43]

Discussion of results from animal studies

Overall, the findings from current animal studies are not clear regarding the specific metabolic effect of either fish protein or fish protein hydrolysates. Several studies report beneficial metabolic effects of diets containing protein from fish, but results about whether fish proteins are more beneficial than other sources of protein are inconsistent. The use of different control diets makes the comparison of results challenging.

Likewise, the results of studies investigating the effect of different types of fish protein hydrolysates are highly inconsistent. As in the studies that examined fish protein, the use of different protein sources in control diets makes a valid comparison of the effects of fish protein hydrolysates difficult. Several studies have suggested that fish protein hydrolysates contain bioactive peptides that may have beneficial metabolic effects on health outcomes related to lifestyle diseases, but the lack of identified mechanisms weakens these findings. Although several different peptide sequences have been identified in marine-derived proteins and are suggested to beneficially modulate metabolic pathways, this is a new area of research that requires further investigation in humans before the potential of these peptides as an ingredient in nutritional supplements or nutraceutical products can be determined.[21]

In many of the animal studies in this review, casein in the form of whole protein was used in the control diet. Comparing the effect of fish protein with casein may not be straightforward, in part because of the low content of sulfur-containing fatty acids in casein. To avoid depletion of sulfur amino acids that would also lead to taurine deficiency, commercially available diets comprising 20% protein in form of casein are supplemented with 0.3% methionine or cysteine to meet the requirements of the American Institute of Nutrition.[44] However, dairy proteins, such as casein and whey, have a high content of branched-chain amino acids that may possess antiobesogenic properties[45,46] and reduce insulin signaling.[47] In addition, the metabolic response to casein is well investigated, and casein is known to be a slowly digested protein capable of lowering the rate of gastric emptying.[48,49] This could arguably cause problems related to the rate of digestion when casein is compared with other sources of protein. In particular, when the effects of high-protein diets are examined, casein as a protein source does not seem to be representative.[33,50] The pattern of digestion of casein is unique, and casein has been shown to affect gut hormones involved in glucose metabolism by generating an insulinotropic effect and affecting the absorption rate of different amino acids.[51] Thus, the use of casein as a control protein can arguably be a limitation. It can be questioned whether the observed beneficial metabolic effects of fish proteins in the studies using casein as control are attributable to a truly beneficial effect of fish proteins or simply to a correction of a negative metabolic effect of casein. Rodent studies in which different protein sources are compared with casein should therefore be interpreted with caution.

Furthermore, the form of the casein protein used, ie, intact protein vs hydrolyzed peptides, has been shown to be decisive for the metabolic effect when casein is investigated in animal models.[52,53] Hydrolyzed casein, when compared with intact casein, has been found to induce physiological changes that result in decreases in body mass, adipose tissue mass, and plasma insulin concentrations.[53] Hydrolyzed casein has also been shown to facilitate beneficial changes in carbohydrate and amino acid metabolism associated with reduced glucose concentrations and lipid levels in mice.[52]

Overall, these findings suggest that hydrolyzed proteins in general, and not just a fish protein per se, may be particularly effective in beneficially altering metabolism, and thus studies comparing hydrolyzed fish protein with whole casein must acknowledge this when results are interpreted. In addition, in the animal studies included in the current review, the different fish protein hydrolysates investigated contain crude protein extracts with different compositions of amino acids as well as different amounts of ash, maltodextrin, and moisture. Thus, it is possible that an effect could be caused by the composition of other nutrient or non-nutrient components of the test material. Overall, the current animal studies have several limiting issues, and thus the results should be interpreted with caution.

INTERVENTION STUDIES INVESTIGATING THE EFFECTS OF FISH PROTEIN IN HUMANS

Several studies in humans have investigated the health effect of an intervention with lean fish, but the type of intervention, the study participants, and the outcome measures differ greatly between studies (Table 1)[1–4,54–62]. Most intervention studies have compared lean fish with fatty fish or a non-seafood diet containing equal amounts of protein from lean meat, eggs, chicken, and dairy products.

Some studies have reported a beneficial effect of lean fish consumption on lipid status,[1,2] whereas others have reported no effect or a negative effect on lipid concentrations.[55–57,59] Two studies have reported a beneficial effect of lean fish on glucose metabolism,[3,4] whereas 1 study reported no effect.[61] One study has reported lean fish to reduce blood pressure in patients with cardiovascular disease,[62] but the same effect was not seen in a group of overweight or obese individuals.[54]

Body weight

Only 1 study has evaluated the effect of cod protein, consumed as part of a weight loss diet, on weight management.[54] The study included 126 overweight or obese individuals and evaluated the effects of different amounts of cod (150 g, 3 or 5 times per week, compared with a non-seafood diet) in an energy-restricted weight loss diet. Total energy expenditure and a 30% reduction in caloric intake were calculated individually for each participant. All 3 diets had an identical macronutrient composition and a similar distribution of energy (30%E from lipids, 50%E from carbohydrates, and 20%E from protein). The authors found a dose-dependent relationship between cod consumption and weight loss, with the diet containing the most cod showing the most favorable effect. Change in cardiovascular risk factors were similar between the 3 groups, but when all groups were merged, the prevalence of metabolic syndrome dropped from 29% to 21%.[54] Notably, the study implemented a 2-day weighed food record only at baseline and during the last week of the intervention, and the lack of controlling for diet and caloric intake during the intervention can arguably be considered a limitation of the design. Controlling for diet could possibly have revealed differences in energy intake that may have explained the weight loss beyond the effect of cod protein.

Lipid status

Several studies of the effect of lean fish protein on lipid metabolism have been conducted, but results have been inconsistent. A large proportion of these compared the effect of lean fish vs fatty fish. Both salmon and cod were found to lower triacylglycerol levels equally when compared with an isocaloric potato diet in a 15-day parallel-group intervention performed in 30 healthy individuals.[2] The 3 study diets differed in the amount of n-3 fatty acids provided, with the potato diet containing 144 mg/d, the cod diet 154.3 mg/d, and the salmon diet 5412.1 mg/d. The study found both lean and fatty fish to improve lipid status by reducing the ratio of 18:1n-9 to 18:0 in plasma.[2] This finding is not consistent with the results of a 4-week parallel-group intervention conducted in 38 healthy individuals who were given 750 g of cod, salmon, or lean meat per week. Importantly, no differences in energy or macronutrient intake were observed between the groups, either at baseline or after the 4-week intervention. In this study, salmon, but not cod, significantly reduced triacylglycerol concentrations and increased fasting levels of HDL-C in serum when compared with lean meat. The salmon intervention, compared with the cod intervention, increased HDL-C.[59] This finding is supported by a study in 33 patients with coronary heart disease.[58] Consumption of lean fish or fatty fish was compared with consumption of lean meat as a control in an 8-week intervention. Fatty fish, but not lean fish, increased the particle size of HDL-C, which might be beneficial in patients with coronary heart disease.[58] The energy intake during the intervention did not change from the baseline measures in any of the groups, but, as expected, the intake of n-3 fatty acids increased in the group that ate fatty fish.

Studies that do not compare lean and fatty fish but only investigate the effects of lean fish vs a non-seafood diet report inconsistent findings. Several intervention studies report either a negative effect or no effect of a lean fish intervention. Two 4-week crossover trials in post- and premenopausal women evaluated the effect of a diet containing proteins from lean white fish compared with the effect of an isocaloric diet containing proteins from milk, eggs, pork, veal, and beef.[55,56] The trial performed in 15 postmenopausal women reported the diet containing lean white fish to induce higher concentrations of total plasma cholesterol and HDL-C than the non-seafood diet, thereby negatively affecting the lipid profile.[55] The 2 test diets contained similar amounts of energy and an equal distribution of energy provided from protein (19%E), lipids (29%E), and carbohydrates (52%E). Cholesterol content was similar in both diets. The weight of the participants did not change during the intervention.

The crossover trial in 14 premenopausal women performed some years later supported this finding and reported no significant improvements in lipid profiles assessed after the lean fish diet.[56] The lean fish diet and

Table 1 **Overview of controlled, clinical intervention studies evaluating the effect of lean fish intake on metabolic markers in humans**

Reference	Outcome measure	Participants	Design	Duration of intervention	Amount and type of lean fish	Control	Result
Ramel et al (2009)[54]	Body weight	100 overweight or obese individuals (3 groups)	RCT, parallel group	8 wk	150 g of cod 3×/wk or 150 g of cod x 5×/wk	Seafood-free diet	Dose–response relationship observed between weight loss and cod consumption
Jaques et al (1992)[55]	Lipid metabolism	15 healthy premenopausal women	RCT, crossover	4 wk	70%–75% of daily protein from cod, sole, haddock, halibut, and pollock	Beef, pork, veal, egg, and dairy products	Lean fish diet caused higher concentrations of total cholesterol and LDL-C
Gascon et al (1996)[56]	Lipid metabolism	14 healthy premenopausal women	RCT, crossover	4 wk	20% of energy intake as lean fish protein	Beef, port, weal, eggs, and dairy products	No significant effect of lean fish on lipid profile
Lacaille et al (2000)[57]	Lipid metabolism	11 normolipidemic men	RCT, crossover	NA	NA	Beef, pork, veal, eggs, and dairy products	Lean fish diet induced higher levels of plasma total cholesterol and LDL-C
Telle-Hansen et al (2012)[2]	Lipid metabolism	20 healthy individuals (3 groups)	RCT, parallel group	15 d	150 g of cod per day	Salmon or potato	Both salmon and cod improved lipid status
Erkkila et al (2014)[58]	Lipid metabolism	33 patients with CHD (3 groups)	RCT, parallel group	8 wk	4 meals of lean fish per week	Fatty fish or lean meat	Fatty fish, but not lean fish, increased HDL particle size
Aadland et al (2015),[1] (2016)[3]	Lipid metabolism	20 healthy individuals	RCT, crossover	4 wk	60% of total protein per day from lean fish	Non-seafood protein sources	Lean seafood improved lipid status
Hagen et al (2016)[59]	Lipid metabolism	38 healthy individuals (3 groups)	RCT, parallel group	4 wk	750 g of cod per week	Salmon or lean meat	Salmon, but not cod, improved lipid profile
Ouellet et al (2007),[4] (2008)[60]	Glucose metabolism	19 overweight or obese insulin-resistant individuals	RCT, crossover	4 wk	58%–68% of total daily protein intake from cod	Beef, pork, veal, egg, and dairy products	Cod group showed improved insulin sensitivity and lower CRP
Aadland et al (2016)[3]	Glucose metabolism	20 healthy individuals	RCT, crossover	4 wk	60% of total protein per day from lean fish	Non-seafood protein sources	Lean seafood reduced postprandial C-peptide concentrations
Helland et al (2017)[61]	Glucose metabolism	68 overweight or obese individuals (3 groups)	RCT, parallel group	8 wk	750 g of cod per week	Salmon or non-seafood diet	Salmon, but not cod, beneficially altered glucose metabolism
Erkkila et al (2008)[62]	Hypertension	33 patients with CHD (3 groups)	RCT, parallel group	8 wk	4 meals lean fish per week	Fatty fish or lean meat	Lean fish reduced blood pressure
Ramel et al (2009)[54]	Hypertension	100 overweight or obese individuals (3 groups)	RCT, parallel group	8 wk	150 g of cod 3×/wk or 150 g of cod 5×/wk	Seafood-free diet	Lean fish had no effect on blood pressure

Abbreviations: CHD, coronary heart disease; CRP, C-reactive protein; LDL, low-density lipoprotein; NA, not available; RCT, randomized controlled trial.

the non-seafood diet contained equal amounts of energy as well as an equal distribution of energy from protein (20%E), lipids (30%E), and carbohydrates (50%E). The lean fish group was given a daily calcium and vitamin D supplement because dairy products had been eliminated from the diet, and all participants maintained a stable weight throughout the intervention. Another study comparing a non-seafood diet with a lean fish diet in 11 normolipidemic men reported the lean fish diet to induce higher concentrations of plasma cholesterol and HDL-C. This effect was associated with variations in plasma sex hormone status and lipoprotein lipase activity.[57] The 2 test diets were isocaloric and had a similar nutrient distribution (18%E from protein, 52%E from carbohydrates, 32%E from lipids) and a similar ratio of polyunsaturated to saturated fatty acids.

In contrast, a randomized crossover trial in 20 healthy individuals that compared the effect of protein from lean seafood with that of non-seafood protein reported lean seafood protein to significantly reduce fasting and postprandial serum triacylglycerol and prevent an elevated ratio of total cholesterol to HDL-C in fasting and postprandial serum samples.[1] The lean seafood diet consisted of cod, pollock, saithe, and scallops, whereas the non-seafood diet included lean beef, chicken filet, turkey filet, pork, eggs, milk, and dairy products. The experimental diets were isocaloric, with a similar distribution of nutrients (19%E from protein, 29%E from lipids, and 52%E from carbohydrates). Participants who consumed the non-seafood diet were supplemented with cod liver oil to ensure both groups had a similar intake of eicosapentaenoic acid and docosahexaenoic acid. Each group was followed for 4 weeks, separated by a 5-week washout period. The same authors later reported that the lean seafood diet beneficially altered the fasting and postprandial ratio of triacylglycerol to HDL-C.[3]

Glucose metabolism

A 4-week crossover study in 19 overweight or obese individuals compared the effect of a cod protein diet with the effect of a diet containing similar amounts of protein from lean beef, pork, veal, eggs, milk, and dairy products. The diets differed only in the source of protein and provided equal amounts of carbohydrates and lipids, including n-3 PUFAs. The cod protein diet significantly improved insulin sensitivity and reduced the levels of circulating C-reactive protein compared with the mixed-protein diet.[4,60] This is consistent with the results of a 4-week crossover study in 20 healthy individuals in whom a lean seafood diet did not alter serum glucose or insulin concentrations but reduced postprandial C-peptide and lactate concentrations when compared with a non-seafood diet.[3] The 2 experimental diets contained equal amounts of energy (29%E lipids, 52%E carbohydrates, 19%E protein), with lean seafood or non-seafood sources contributing 60%E of total protein intake. They concluded that the lean seafood diet beneficially altered lipid status and influenced glucose metabolism in a manner that may beneficially affect the long-term development of insulin resistance, T2DM, and cardiovascular disease.[1,3]

The same group that reported a beneficial effect of salmon intake on lipid status also investigated the effect of 750 g of cod or salmon per week compared with non-seafood proteins on glucose metabolism in 68 overweight or obese individuals for 8 weeks.[61] Intake of energy and macronutrients did not change within the groups during the intervention. Salmon, but not cod, significantly improved postprandial glucose regulation and increased insulin C-peptide concentrations to a lesser degree than cod or control.[61] These findings suggest that intake of fatty fish, but not lean fish, beneficially alters glucose and lipid metabolism and, hence, reduces the risk for insulin resistance, T2DM, and cardiovascular disease.

In conclusion, beneficial effects of cod protein on glucose metabolism and insulin sensitivity have been reported in several studies, but the findings are inconsistent.

Hypertension

A few studies have investigated the specific effect of cod protein consumption on hypertension. The previously described study investigating the effect of cod consumption in a weight loss diet reported no effect of different amounts of cod consumption on blood pressure.[54] A study examining the effect of lean fish or fatty fish compared with lean meat as control in patients with coronary heart disease found lean fish (consumed 4 times per week) to reduce blood pressure and, hence, to be beneficial in this group.[62] The participants were all given similar instructions by a dietitian about following a diet recommended for patients with coronary heart disease, with group-specific instructions to include 100 to 150 g of fish (fatty or lean) in 4 meals per week. The control group was instructed to consume lean meat and less than 1 fish meal per week.

INTERVENTION STUDIES WITH FISH PEPTIDE SUPPLEMENTS IN HUMANS

Several recent studies have investigated the effect of supplements with marine peptides (protein hydrolysates) on metabolic health in humans, with overall promising findings.

Two recent studies, both of which used an 8-week intervention protocol, have reported on the metabolic effect of a cod protein hydrolysate in overweight or obese individuals.[19,22] Vikøren et al[19] conducted a study in 34 individuals and were the first to investigate the specific effect of a cod protein hydrolysate on metabolic markers. Participants were divided in 2 groups who received either placebo tablets or fish-derived peptides. The fish peptide group was given 3 g of fish protein supplement per day for the first 4 weeks and 6 g per day for the last 4 weeks. Eight weeks of supplementation led to lower fasting and postprandial glucose levels, a beneficially altered postprandial insulin C-peptide concentration, reduced body fat, increased lean body mass, and reduced LDL-C. No effect on blood pressure was found, but overall, a beneficial effect on several metabolic markers was observed.[19] A study by the same research group did not observe the same significant beneficial effect on insulin regulation but found that 6 g of cod peptide supplementation per day significantly lowered serum concentrations of nonesterified fatty acids.[22]

One study has investigated the effect of a peptide from a sardine muscle hydrolysate on blood pressure in a randomized controlled trial of 29 individuals. The authors reported the specific valyl-tyrosine peptide from sardine to have a significant antihypertensive effect in individuals with mild hypertension by inhibiting ACE.[63]

A study investigating the effect of 2 different doses of supplementation with a fish protein hydrolysate from blue whiting in 120 overweight individuals reported both doses to improve body composition and decrease body weight as well as increase levels of cholecystokinin and glucagon-like peptide 1 in serum.[18] The trial lasted for 90 days, and participants were allocated into 3 arms, receiving either 1.4 g or 2.8 g of fish protein hydrolysate per day or 1.4 g of whey protein isolate as placebo, in addition to receiving an individually adjusted mildly hypocaloric diet (−300 kcal/d). Both doses of fish protein hydrolysate were found to significantly reduce body weight, body mass index, and fat mass as well as waist, thigh, and hip circumference when compared with placebo. The effect was equal for both doses, indicating a plateau effect starting at 1.4 g.

Recently a randomized, double-blind crossover trial investigating the effect of a cod protein hydrolysate on postprandial glucose metabolism was performed in 41 healthy, middle-aged individuals.[20] The results support the findings of Vikøren et al,[19] who reported beneficial postprandial alterations in glucose values after supplementation with a fish protein hydrolysate. A single dose of 20 mg of cod protein hydrolysate per kilogram of body weight, when compared with casein as control, was found to significantly reduce postprandial insulin concentrations without affecting blood glucose levels in healthy individuals.[20] The effect observed in this study should be further investigated in patients with impaired glucose metabolism.

Discussion of results from human studies

Overall, the results from studies evaluating the metabolic effect of fish proteins show highly inconsistent results. It must be noted that the studies performed in obese and overweight individuals, in contrast to studies performed in healthy individuals, pose a highly different starting point with regard to metabolic health, making comparison of the results difficult. Furthermore, both the type of intervention and the amount of lean fish provided in the different studies vary greatly. The studies evaluating the effect of lean fish in comparison with fatty fish may have been influenced by n-3 fatty acids, which are present in much higher concentrations in salmon than in cod. A major consideration when evaluating the effect of a dietary intervention based on fish consumption is to definitively determine whether the observed effect is due to the intervention or simply to the removal of other foods with a nonbeneficial health effect, such as red meat. Studies investigating the effect of different fish protein hydrolysates are more consistent in their findings and report an overall promising metabolic effect of supplements containing low concentrations of peptides from fish. The fact that these studies do not alter the nutritional composition of the participants' diets, but instead simply add a peptide on top of the normal diet, is arguably a strength compared with the intervention studies that used whole fish. Although current results are inconsistent overall, most of the research reveals several beneficial health effects of lean fish consumption.

POSSIBLE LINKS BETWEEN FISH PROTEINS AND GUT MICROBIOTA

Obesity and metabolic diseases such as T2DM are associated with a dysbiotic gut microbiota, seen as a deviation of the organization of microbes that would promote optimal metabolic homeostasis.[64] So far, little is known about the ability of different protein sources to modulate the gut microbiota. Some recent animal studies have found that a diet containing proteins from lean seafood tends to be less obesogenic than a regular Western diet containing proteins from meat such as chicken, pork, or beef.[30,65] Comparison of the gut microbiome of mice fed 2 different Western diets (with lean seafood vs lean meat) revealed significant differences in the abundance of microbial genes.[30]

Marine protein sources tend to have moderate to high content of the branched-chain amino acids valine, leucine, and isoleucine. These amino acids participate in several metabolic pathways and are present in high concentrations shown to counteract obesity development in mice fed a high-fat diet.[66] Furthermore, supplementation with branched-chain amino acids is demonstrated to beneficially alter the composition of the gut microbiota by increasing the abundance of species associated with protection against obesity development.[67] However, compared with proteins from fish, the milk proteins casein and whey have an even higher content of branched-chain amino acids, and casein has been shown to be more efficient than proteins from cod in preventing weight gain and an increase in adipose tissue in mice.[65]

Fish proteins, the gut microbiota, and lipid status

A randomized controlled crossover trial previously reporting on glucose and lipid metabolism in response to lean seafood consumption in 20 healthy human individuals[1,3] recently also reported on the effect of lean seafood and non-seafood consumption on fecal metabolites and the gut microbiome.[68] The authors observed a twofold increase in fecal trimethylamine excretion after the lean seafood intervention. Moreover, the ratio between total cholesterol and HDL-C and the circulating levels of triacylglycerol and trimethylamine *N*-oxide (a metabolite associated with increased risk of cardiovascular disease) were each associated with specific gut bacteria. The non-seafood diet caused a decreased abundance of *Clostridium* cluster IV as well as an increased ratio of *Firmicutes* to *Bacteroides*. Overall, the authors concluded that the presence of seafood in the diet affects the gut microbiome composition and activity, which in turn seems to affect circulating concentrations of trimethylamine *N*-oxide and, hence, the risk for cardiovascular disease.[68]

Taurine, bile acid metabolism, and the gut microbiota

Marine protein sources tend to have a higher level of taurine, an amino acid–derived organic compound shown to prevent diet-induced weight gain and improve insulin sensitivity in rats, than other animal protein sources.[69,70] A fish protein hydrolysate diet with a high content of taurine was found to elevate plasma bile acids and reduce visceral adipose tissue mass in rats.[41] Interestingly, the same research group later reported that the nutritional regulation of bile acid metabolism was associated with beneficial alterations in several markers involved in the development of metabolic syndrome.[40] Primary bile acids are synthesized from cholesterol in the liver before they are conjugated with taurine or glycine and further metabolized into secondary bile acids by the gut microbiota. These bile acids serve as ligands for nuclear receptors involved in the development of obesity and metabolic disorders.[71,72] For instance, it has been reported that patients with T2DM have elevated levels of taurine-conjugated bile acids,[73] and a high ratio of 12α-hydroxy to non-12α-hydroxy bile acid has been associated with lower insulin sensitivity.[74]

Treatment of naive T2DM patients with Acarbose, an alpha-glycosidase inhibitor used as an alternative to metformin in several Asian countries, increases the ratio between primary and secondary bile acids and plasma levels of unconjugated bile acids, possibly by changing the relative abundance of microbial genes involved in bile acid metabolism.[75] In that study, multiple correlations between changes in plasma bile acids and clinical parameters, including body weight, homeostasis model assessment–insulin resistance, and lipid profile were found. Of note, patients with a higher baseline abundance of *Bacteroides* organisms in their gut microbiota and lower levels of secondary bile acids exhibited better therapeutic responses to Acarbose treatment, including reduced body mass index, improved insulin resistance status, and improved lipid profile, suggesting that baseline metagenome signatures may be used to stratify patients with T2DM prior to treatment. The role of the gut microbiota in regulating the metabolism of secondary bile acids poses novel questions about whether different marine proteins, which have different amino acid compositions, may be able to prevent or treat obesity and impaired glucose metabolism.

FUTURE RESEARCH DIRECTIONS

Although the overall beneficial health effects of fish consumption are well documented, more knowledge is needed on the specific effects of proteins from fish, particularly how fish proteins affect the metabolic pathways involved in the development of disease and impaired metabolic health. Increasing interest in the relationship between metabolic health and the gut microbiota may lead future research in exciting novel directions. Few studies have investigated the specific relationship between consumption of fish proteins and alterations in the gut microbiota. Although some single studies suggest that proteins from fish may benefit the gut microbiota composition and, hence, influence body weight, lipid status, and bile acid metabolism, no conclusions can be drawn from the existing literature. Future research should investigate whether fish proteins can influence the gut microbiota composition and, hence, affect the development of disease, and should determine

whether dietary supplements with bioactive marine peptides affect the gut microbiota and improve health. Taurine contained within marine compounds has been highlighted as a possible modulator of bile acid metabolism and, thus, improved metabolic health. As bile acid metabolism is dependent on the gut microbiota composition, the relationship between bile acid metabolism and specific taurine-containing proteins or supplements, along with implications for the gut microbiota and metabolic health, should be investigated in more detail in future human studies. In conclusion, future research should investigate the specific metabolic health effects of nutritional supplements containing marine peptides with possibly bioactive sequences.

CONCLUSION

Increasing evidence suggests that components other than the long-chain n-3 fatty acids from fish may have a beneficial health effect, and recent literature points toward proteins and peptides from fish as possibly being able to influence metabolic health. However, results from both experimental studies in animals and clinical studies in humans evaluating the effect of fish proteins and peptides are highly inconsistent, and no clear mechanistic effects are known thus far. Although novel results indicate a beneficial effect of marine peptides and dietary supplements containing fish protein hydrolysates on pathways involved in metabolic health, there is a lack of knowledge on the specific mechanisms for these actions.

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