Molecular Nutrition & Food Research
www.mnf-journal.com

A Narrative Review of the Anti-Hyperglycemic and Satiating Effects of Fish Protein Hydrolysates and Their Bioactive Peptides

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Prevalence of type 2 diabetes and overweight/obesity are increasing globally. Food supplementation as a preventative option has become an attractive option in comparison to increased pharmacotherapy dependency. Hydrolysates of fish processing waste and by-products have become particularly interesting in a climate of increased food wastage awareness and are rapidly gaining traction in food research. This review summarizes the available research so far on the potential effect of these hydrolysates on diabetes and appetite suppression. Scopus and Web of Science are searched using eight keywords (fish, hydrolysate, peptides, satiating, insulinotropic, incretin, anti-obesity, DPP-4 [dipeptidylpeptidase-4/IV]) returning a total of 2549 results. Following exclusion criteria (repeated appearances, non-fish marine sources [e.g., macroalgae], and irrelevant bioactivities [e.g., immunomodulatory, anti-thrombotic]), 44 relevant publications are included in this review. Stimulation of hormone secretion, regulation of glucose uptake, anorexigenic potential, identified mechanisms of action, and research conducted on the most potent bioactive peptides identified within these hydrolysates are all specifically addressed. Results of this review conclude that despite wide methodological variation between studies, there is significant potential for the application of fish protein hydrolysates in the management of bodyweight and hyperglycemia.

1. Introduction

Protein is the primary source of amino acids and an important source of energy within the diet. In addition to this basic nutrition, proteins contain motifs that may possess bioactivity. A large focus of recent food science research has been the identification of various bioactivities in otherwise low-value protein sources for potential application as a functional food ingredient.[1] Thus, there has been a significant amount of research undertaken to mine for bioactive peptides from hydrolysates of various sources of protein. These protein sources include milk whey and casein, shellfish, egg, wheat, fish, and soybean amongst others.[2–5] From these various sources, there has been a wide range of bioactivities reported including anti-cancer, mineral-binding, immunomodulatory, osteoprotective, antimicrobial, antihypertensive, anti-inflammatory, anti-diabetic, and anorexigenic capabilities. When combined with the promising techno-functional properties which some proteins exhibit, such as high solubility, good emulsifying capacity and stability, oil binding, and water holding capacity,[6,7] these have become an extremely promising source of ingredients for functional food applications.

Of all the sources which have been investigated so far, dairy proteins such as whey and casein have likely been the most heavily studied.[8–10] Fish/marine products have also become

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The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/mnfr.202000403

DOI: 10.1002/mnfr.202000403

promising protein sources, as the application of fish protein sources as bioactive ingredients would simultaneously improve the quality of dietary protein, whilst also alleviating some of the excess wastage associated with the fishing industry and increasing the value of low-value fish now landed due to EU regulations.[11,12] This is reflected in the increase in availability of fish hydrolysate products commercially (e.g., Nutripeptin by Copalis). Despite a growing body of research on the anti-diabetic and anti-obesogenic effect of fish proteins, to the best of our knowledge there has been little consolidation of this data. This prompted the current analysis which aims to specifically review the current available literature on the effectiveness of fish peptides for alleviating hyperglycemia and obesity.

1.1. The Production of Fish Protein Hydrolysates (FPH)

As mentioned above, much of the bioactivity identification has been carried out in fish proteins which have been modified through hydrolysis. The production of peptides with anti-diabetic and anorexigenic properties has primarily been through either enzymatic or acid hydrolysis, with research often focusing on peptides <3 kDa. This is partially due to the improved gastrointestinal stability of these peptides compared to the native proteins which are commonly degraded by acid hydrolysis and enzymes present in the gastrointestinal tract.[13,14] Furthermore, previously identified fish hydrolysate-derived peptides with anti-diabetic activity have been <1.7 kDa.[15–17] By producing hydrolysates using enzymes under controlled conditions, the possibility of variable bioactivity is reduced; however, this does not completely eliminate variation as there may still be some variation between hydrolysate batches. The advantage of enzymatic hydrolysis over, for example, acid or microbial hydrolysis, is the relatively short time required to produce a high degree of hydrolysis. The resulting short peptides are believed to be more resistant to gastrointestinal digestion than intact proteins.[17–19] This makes them of much greater interest for application in functional foods as well as for potential pharmacotherapeutic uses.

The peptides generated during hydrolysis depend to a large extent on the specificity of the proteolytic enzyme(s) used, hydrolysis temperature, pH, and enzyme/substrate ratio.[20] For example, many hydrolysates are the result of sequential hydrolysis in which an endopeptidase (a peptidase which will not cleave peptides to singular amino acids) is used first followed by later addition of an exopeptidase (a peptidase which will create smaller peptides and cleave single amino acids from the end of peptide chains).[21] Given the wide variety of enzyme choices to be considered depending on the primary desired outcome (small peptides, mid-length peptides, etc.) and the use of enzymes which can also de-bitter proteins and create better flavors (e.g., Flavourzyme), there are abundant possible permutations of peptides which can be produced as a result of the hydrolysis process and this is reflected in the variability of the data presented herein and differences in dosages used.

2. Search Procedure

Two databases were employed for construction of this review: Scopus (https://www.scopus.com) and Web of Science (http://apps.webofknowledge.com). All searches were carried out before August 12, 2020. Eight keywords were chosen for searching after consideration of terms most relevant to the area of interest; fish, hydrolysates, peptides, satiating, insulinotropic, incretin, anti-obesity, DPP-4 (dipeptidylpeptidase-4/IV). When searching, the words fish AND hydrolysates were always used, with a third interchangeable word chosen from one of the remaining six. In total, this resulted in 2549 results, which were refined to 113 possibly relevant citations. Publications which used other marine material (e.g., macroalgae) or did not specifically deal with anti-diabetic/satiating activity were excluded. Furthermore, many publications appeared more than once throughout the searches and numbers were reduced significantly when duplicates were removed. Following this exclusion process, 44 manuscripts were deemed sufficiently relevant to the theme of this review. This search process is summarized in **Figure 1**.

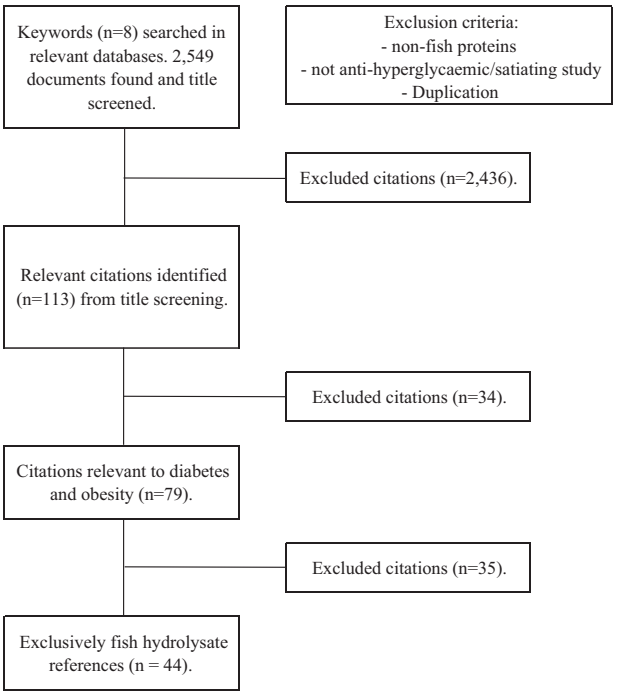

Figure 1. Summary of search procedure used for identification of relevant literature.

3. Anti-Hyperglycaemic Potential of Fish Protein Hydrolysates

Diabetes is a severe metabolic disorder which is estimated to affect 451 million people worldwide.[22] Type 2 insulin-resistant diabetes mellitus (T2DM) accounts for ≈90% of all cases, and is characterized by impaired insulin secretion, increased hepatic glucose production, and decreased insulin action. As direct use of insulin/stimulation of insulin secretion may cause hypoglycemia and further decrease in insulin sensitivity, many pharmacological treatments have recently focused on the development of incretin mimetics and prolonging the action of endogenous incretin hormones.[23–25] The prevalence of diabetes is rapidly increasing worldwide, and despite the increase in

Table 1. Summary of research on bioactivities from fish hydrolysates relevant to hyperglycaemia.

Source	Study type	Effect	Reference
Alaska pollock[a)]	In vitro	$IC_{50} \geq 0.8$ mg mL^{-1}	[96]
Atlantic salmon[a,b)]	In vitro	↑ GLP-1 secretion, ↑ insulin secretion	[32]
Atlantic cod	Human	↓ Postprandial plasma insulin. ↔ plasma glucose and GLP-1	[35]
Atlantic salmon[a)]	In vivo	↓ Postprandial blood glucose and plasma DPP-4 activity. ↑ plasma insulin and insulin:glucagon ratio	[48]
Atlantic salmon[b)]	In vitro	$IC_{50} \geq 0.3$ mg mL^{-1}	[97]
Atlantic salmon[a)]	In vitro	$IC_{50} \geq 0.08$ mg mL^{-1}	[98]
Barbel[a)]	In vitro	IC_{50} - 2.21 mg mL^{-1}	[99]
Bester sturgeon[a,c)]	In vivo	↑ Glucose tolerance	[79]
Blue whiting[d)]	In vitro and in vivo	↑ GLP-1 secretion, ↑ insulin secretion. ↑ glucose tolerance after 90 min	[31]
Boarfish[e)]	In vitro and in vivo	↑ Insulin secretion and GLP-1 secretion. IC_{50}: 1.18 mg mL^{-1}. ↑glucose tolerance	[33]
Cod	Human	↔ Insulin, glucose, and GLP-1 after 8 weeks	[91]
Cod	Human	↔ insulin, glucose, and GLP-1	[90]
Green crab[e)]	In vitro	↑ GLP-1 release, and DPP-4/α-amylase inhibition	[100]
Halibut, hake, tilapia, and milkfish[a)]	In vitro and in vivo	↓ ≤55% in DPP-4 activity by peptides ≤1.5 kDa (derived from Tilapia skin). ↓plasma DPP-4 activity after 30 days, ↑ plasma insulin, active GLP-1, and postprandial glycemic control	[41]
Herring[d)] and salmon[c)]	In vivo	↑ Glucose tolerance after 22 days w/ salmon	[68]
Herring, salmon, and cod	Human	↑ Glucose tolerance after 90 min	[92]
Greater weever[d)]	In vitro	$IC_{50} \geq 0.38$ mg mL^{-1}	[101]
Marine collagen peptides[e)]	Human	↑ Fasting blood glucose and insulin, GHbA1c, and insulin sensitivity	[51]
Portuguese oyster[e)]	In vitro	45% DPP-4 inhibition at 2 mg mL^{-1}	[102]
Rainbow trout[a)]	In vitro	≤44% DPP-4 inhibition by peptides <3 kDa	[103]
Rainbow trout[c)]	In vitro	$IC_{50} \geq 1.23$ mg mL^{-1}	[104]
Salmon[c)]	In vitro	↑ Glucose uptake	[50]
Sardine[d)]	In vitro	$IC_{50} \geq 1.83$ mg mL^{-1}	[105]
Skate[a)]	In vivo	↓ Plasma glucose, insulin and insulin resistance	[87]
Tilapia[a)]	In vivo	↑ Glycemic control, when given orally or i.p. ↑ plasma insulin at 3 g kg^{-1}	[34]
Unicorn leatherjacket[a)]	In vitro	≤50% α-amylase inhibition	[106]

IC_{50}, concentration of condition required to inhibit DPP-4 activity by 50%; BW, bodyweight. Superscript letters indicate the source of the hydrolysate; [a)] Skin; [b)] Trimmings; [c)] Bone; [d)] Muscle; [e)] Meat. Lack of superscript letter indicates source was not clarified by the authors.

efficacy and longevity of pharmaceutical treatments, these do little to curb the rise in prevalence.[25,26] Dietary strategies are becoming increasingly relevant for management and prevention of these disorders. Previously, research has shown that acute co-ingestion of protein with a glucose/carbohydrate load improves postprandial glycemic parameters.[27–29]

3.1. Glycemic Control through Hormone Secretion

Some of the improvement in glycemic parameters with fish hydrolysates has been observed to happen through hormone secretion—promoting insulin secretion, inhibition of dipeptidylpeptidase-4 (DPP-4) enzyme activity (for extension of endogenous incretin hormone half-life), and secretion of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). For example, it has been well established from in vitro studies that many fish protein hydrolysates (FPH) contain potent DPP-4 inhibiting motifs (summarized in **Table 1**). The inhibition of DPP-4 and prolongation of GLP-1 half-life is of greater interest in the development of functional foods recently as a statement from EFSA concluded that a reduction in post-prandial glucose response "may be considered a beneficial physiological effect as long as insulin responses are not disproportionally increased".[30] FPH were found to cause an increase in insulin secretion in vitro by Harnedy et al.[31,32] and Parthsarathy et al.[33] Furthermore, Parthsarathy and colleagues and Harnedy et al. showed a significant increase in plasma insulin in healthy mice.[31,33]

An increase in plasma insulin was also shown by Iba et al.[34]; however, this involved a very high dose (3 g kg^{-1} bodyweight) which may be difficult to achieve when translating this to human studies. Despite these promising in vivo results, none of the human studies with fish hydrolysates have shown an increase in plasma insulin, while one study showed a decrease in plasma insulin.[35] Research in dairy hydrolysates has followed a similar focus of investigating the impact of insulin on glycemic control improvement.[36–38] If fish hydrolysates were to stimulate insulin secretion through secretion of endogenous incretin hormones or prolongation of half-life, there would be no risk of hypoglycemia as the insulinotropic action of incretin hormones is dependent on the prevailing blood glucose concentration.

Various studies have investigated GLP-1 secretion, both in vitro and in vivo,[32,39–41] with particular attention being drawn to the work of Wang and colleagues[41] which is the only study investigating the effects of chronic administration on plasma GLP-1, and showed a significant increase in active GLP-1 with multiple skin gelatin hydrolysates. However, no study has yet examined how the peptides are eliciting this effect. For example, free amino acids such as glutamine are known to potently stimulate incretin hormone secretion from intestinal cells,[42,43] but it is not known if the increased hormone release seen with these FPH is due to further peptide breakdown to free amino acids, or if the peptides remain intact and bioactive when passing through the gastrointestinal tract. This point is reinforced by the work of Harnedy et al.[31,32] where a simulated gastrointestinal digestion model showed some improved bioactivities, but again it is unclear if this was due to the formation of potentially more potent bioactive peptide fragments or increased free amino acid release. Production of more bioactive peptide fragments could prove an interesting development for future uses of such peptides, as the chronic effect of a more stable peptide which remains intact will likely be much more effective than that of free amino acids which will be excreted relatively quickly. Furthermore, in the case of T2DM, ingestion of free amino acids in the fasting state may encourage hyperglycemia through an increase in substrate availability for gluconeogenesis.[44]

The use of DPP-4 inhibitors has rapidly grown in popularity partly due to being an orally available treatment.[45,46] This is also reflected in the level of research conducted into food-derived peptides as DPP-4 inhibitors.[47,48] There are numerous studies examining the in vitro potency of FPH as DPP-4 inhibitors (summarized in Table 1). Although the main body of this research has been in vitro models, there are limited numbers of in vivo models examining plasma DPP-4 enzyme activity. However, both Hsieh et al.[49] and Wang et al.[42] showed a promising reduction in plasma DPP-4 activity after long-term hydrolysate administration. While it is interesting to see that the DPP-4 inhibition does translate to animal models, the dosage used was relatively high (300 mg per day and 750 mg kg^{-1} BW per day, respectively) thus it may be difficult to see a corresponding reduction translating to human studies. Furthermore, many studies have shown reduction in blood glucose but have not specifically measured plasma DPP-4 activity. Some of the effect witnessed may be due to reduced DPP-4 activity but this potential mechanism was not measured directly and further research is needed in this area.

3.2. Regulation of Glucose Uptake

Although some of the anti-diabetic effect may be due to the mechanisms discussed in Section 3.1, there is also a promising body of research demonstrating the ability of FPH in enhancing glucose uptake and reducing lipid accumulation in vitro. Increased glucose uptake is a vital part of blood glucose control in T2DM, as there is increased hepatic glucose production and associated elevated blood glucose, resulting in glucotoxicity and eventually β-cell dysfunction.[50] A fractionated hydrolysate of salmon frame exhibited glucose uptake equal to that of insulin in rat skeletal muscle (L6) cells.[51] Harnedy et al. also showed an enhancement in glucose uptake in mouse adipose (3T3-L1) cells with both salmon and blue whiting protein hydrolysates.[31,32] Furthermore, Zhu et al., while showing no significant change in plasma insulin in diabetic patients, showed an improvement in insulin sensitivity with 6 weeks of treatment with marine collagen peptides.[52] However, the understanding of the mechanisms of action of glucose uptake by FPH is limited. Insulin receptor (IR), insulin receptor substrate-1/2 (IRS - 1/2), phosphoinositide-3-kinase (PI3K), and protein kinase B (Akt) all play important regulatory roles in glycemic control through increasing insulin sensitivity. None of the studies carried out to date have examined upregulation of these pathways, nor have changes in insulin sensitivity been reported in any of the in vivo studies. Ayabe et al. showed an in vitro increase in glucose uptake and in vivo plasma glucose reduction but did not further investigate the in vivo mechanism of action.[52] Understanding the extent of glucose uptake enhancement in animal models will add further weight to the in vitro studies already carried out. Furthermore, this analysis combined with information regarding the upregulation of glucose transporters such as GLUT2 and GLUT4 (which are primary regulators of glucose uptake) could play a key role in understanding the wider effect of these hydrolysates on blood glucose homeostasis.

4. Anorexigenic Potential of Fish Protein Hydrolysates

Obesity is another chronic non-communicable metabolic disease which affects 650 million people globally.[53] There are a wide number of obesity-related co-morbidities, including cardiovascular diseases, cancer, and T2DM.[54] Obesity accounts for ≈80% of risk of development of T2DM.[55,56] Inflammation, adipokines and overaccumulation of lipids in pancreatic, liver, and muscle tissue is thought to play a role in the molecular link between development of both obesity and T2DM.[57,58] The above are all accentuating factors of the β-cell dysfunction and insulin resistance commonly associated with T2DM.[50,58,59]

Many of the entero-insular axis hormones which play a role in diabetes also play a role in appetite control, such as GLP-1, GIP, cholecystokinin (CCK), and peptide-YY (PYY).[60–62] However, pharmaceutical targeting of some of these hormones (namely CCK and PYY) has been largely unsuccessful due to receptor expression in the nervous system, resulting in increased mental health concerns.[63,64] This has made functional foods an attractive option as the adverse effects seen with high dose pharmacotherapies should not occur in lower dose oral treatments,

Table 2. Summary of research on bioactivities from fish hydrolysates relevant to obesity.

Source	Study type	Effect	Reference
Alaskan pollock[b)]	In vivo	↓ Weight gain over 3 days, and in NPY and AgRP expression	[66]
Blue whiting[b)] brown shrimp[c)]	In vitro	↑ CCK secretion	[86]
Blue whiting[b)]	In vitro and in vivo	↑ GLP-1 and CCK secretion. ↓ food intake and weight gain. ↑ plasma GLP-1 and CCK	[39]
Common cuttlefish[d)]	In vitro	IC_{50} of 1 mg mL^{-1}. ↑ GLP-1 and CCK secretion	[40]
Common smoothhound[b)]	In vivo	↓ Weight gain after 21 days. ↔ food intake, plasma insulin and CCK	[65]
Ocellate spot skate[a)]	In vivo	↓ Serum leptin	[107]
Skate[a)]	Human	↓ Body fat and body fat mass	[108]
Yellow catfish	In vivo	↓ Weight gain. ↓ total cholesterol at 500 mg kg^{-1} BW	[88]
Blue whiting[b)]	Human	↓ Desire to eat something sweet after 90 min (breakfast at 60 min after capsules). ↔ plasma glucose, insulin, CCK, and GLP-1	[94]
Blue whiting[b)]	Human	↓ Bodyweight, BMI, waist hip, and thigh circumference after 45 days	[95]
Cod	Human	↔ Plasma ghrelin and satiety/fullness scores	[89]

LDL, low-density lipoprotein; TG, triglycerides; NEFA, non-esterified fatty acids; HDL, high-density lipoprotein. Superscript letters indicate the source of the hydrolysate; [a)] Skin; [b)] Muscle; [c)] Head; [d)] Viscera. Lack of superscript letter indicates source was not clarified by the authors.

but they may still alleviate symptoms if used as a preventative measure or early-stage treatment in combination with lifestyle change. Although the complications of obesity alleviate if the individual loses weight, it is often difficult to lose weight and remain motivated to maintain this weight loss. Thus, it is important to identify viable options (e.g., functional foods) which may make this weight loss more achievable and sustainable.

The anorexigenic effect of FPH has received markedly less attention than studies on anti-diabetic action, as represented in **Table 2**. Of the limited in vivo studies which have been carried out, only one has shown an improvement in circulating GLP-1 and CCK, while another has shown that there was no significant change.[40,65] Despite this, many FPH have shown reductions in weight gain both in vivo and in human studies; however, the mechanism through which this is facilitated is poorly characterized. Data from Mizushige et al. interestingly showed a decrease in expression of hypothalamic neuropeptide-Y and agouti-related protein mRNA, both of which play orexigenic roles in brain appetite regulating centers.[66] However, they also showed no significant increase in pro-opiomelanocortin (POMC) or cocaine and amphetamine-related transcript (CART) mRNA expression, which play an anorexigenic role.[66] These data suggest that the decrease in weight gain may be due to antagonism of orexigenic factors rather than enhancement of anorexigenic factors; however, this a singular study and more research is required to better understand these effects. Some of the reduction in food intake may also be due to delayed gastric emptying through protein-carbohydrate binding.[7] In addition, this action on gastric emptying could be either direct or indirect via DPP-4 inhibition and subsequent enhancement of GLP-1 release.

It is also important to note that although not related to appetite regulation by intestinal hormones, many hydrolysates have shown significant improvements in lipid profiles in both in vivo animal and human studies.[51,66–68] Lipid profile plays a role in the pathogenesis of T2DM and obesity, and improvement in lipid profile is an important outcome for reduction in cardiovascular disease risk, which is a common co-morbidity of both obesity and T2DM.

5. Isolation of Bioactive Peptides from Fish Protein Hydrolysates

Given the large body of research on crude fish hydrolysates, there is relatively little work focusing on the isolated peptides which have been identified within these hydrolysates. Identification of bioactive peptides within these hydrolysates is useful for potential pharmacotherapeutic uses. For example, if a specific peptide fraction exhibits greater bioactivity than a crude hydrolysate, then use of this single fraction in a foodstuff (oral supplement) could make the resulting bioactivity more potent due to concentration of the bioactive fractions and removal of potential antagonistic peptides. For pharmacotherapeutic use, the identification of novel specific peptides is an attractive route as peptide therapeutics are rapidly increasing in popularity as discussed previously; however, larger peptides typically have very poor oral bioavailability.[69] The small peptides present in these hydrolysates may have improved oral bioavailability so modification of these peptides to enhance bioactivity, when combined with good oral bioactivity, would be of great interest as a future therapeutic option.[70] In a clinical setting, the ability of these peptides to compare to current peptide treatments (such as Semaglutide [Ozempic], a once-weekly treatment) is unlikely, as these are often heavily modified peptides; however, use of these orally available peptides in a concentrated form could represent a viable option in early-stage/prediabetes treatment where the use of such expensive treatments is not warranted. Furthermore, these peptides could also be modified in future studies which could produce effects similar to some current peptide treatments, and identification of bioactive sequences sheds some light on

Table 3. Summary of available literature on the effects of fish-hydrolysate-derived peptides on hyperglycaemia and obesity.

Source	Study type	Peptide sequence	Mw [Da]	Effect	Reference
Alaskan pollock	In vitro and in vivo	QWR	488.53	↑ Glucose uptake. ↓ plasma glucose with hydrolysate and peptide	[52]
Atlantic salmon[a)]	In vitro	GPAE	372	$IC_{50} \geq 41.9$ μM	[16]
		GPGA	300.29		
Atlantic salmon[a)]	In vitro	YYGYTGAFR	1096.49	$IC_{50} \geq 128.7$ μM	[109]
		LDKVFR	776.45		
		VLATSGPG	700.37		
Boarfish[b)]	In vitro	IPVDM	574.29	$IC_{50} \geq 21.7$ μM	[85]
		APIT	401.24	↑ Insulin secretion	
		VPTP	413.23		
		GPIN	400.22		
		LPVYD	303.66		
		LPVDM	574.29		
		APLER	293.17		
		IPGA	357.21		
		GPSL	373.21		
		GPSI	373.21		
		APVP	383.23		
		VPDPR	292.17		
		APLDK	272.16		
		APLT	401.23		
		MPAVP	257.66		
		GPGI	343.20		
		GPLN	400.22		
		PAVP	383.23		
		GPGL	343.20		
		LPGA	357.21		
		AALP	371.23		
		TPTV	417.27		
Silver carp[c)]	In vitro	IADHFL	714.8	$IC_{50} = 610.1$ μM	[17]
Fish[a)]	In vitro	GP(HP)	269.3	$IC_{50} = 2.5$ mM	[110]
Tuna[d)]	In vitro	PGVGGPLGPIGPCYE	1412.7	$IC_{50} \geq 78$ μM	[15]
		CAYQWQRPVDRIR	1690.8		
		PACGGFYISGRPG	1304.6		

IC_{50}, concentration of condition required to inhibit DPP-4 activity by 50%; HP, hydroxyproline. Superscript letters indicate the source of the hydrolysate; [a)] Skin; [b)] Meat; [c)] Muscle; [d)] Whole fish. Lack of superscript letter indicates source was not clarified by the authors.

factors determining the beneficial effects of small molecular weight peptides.

The key structural components for bioactivity retention are far from clear; however, in general peptides containing a proline in the ultimate or penultimate C-terminal position, or the first, second, third, or fourth position from the N-terminal position has been found to increase DPP-4 inhibitory activity. This is enhanced in the presence of other hydrophobic amino acid residues.[71–73] This area has been reviewed more in-depth elsewhere.[74] Interestingly, many of the peptides with identified bioactivities (**Table 3**) adhere broadly to these hypotheses. Harnedy-Rothwell et al. identified 22 peptides from boarfish which exhibited DPP-4 inhibitory activity in vitro and in situ using a Caco-2 cell model and all sequences contained at least one proline and the sequences are rich in other hydrophobic amino acids such as glycine, alanine, leucine, isoleucine, and valine. All other studies in Table 3 also show an abundance of hydrophobic amino acids. Most peptides have only been screened in vitro, and usually to assess DPP-4 inhibitory activity. Many of these have shown promising bioactivities which correspond with the bioactivity found in their precursor hydrolysates. Interestingly, all of the peptides which have been identified thus far are small peptides, with the largest being 1.7 kDa. This agrees with the aforementioned evidence in relation to how the protein hydrolysis process produces these smaller peptides which have greater bioactivity. As peptides which exhibit DPP-4 inhibitory activity have also often displayed anti-oxidant activity,[74,75] this anti-oxidant effect could also play a cytoprotective effect on pancreatic

beta cells.[76] Furthermore, Ben Henda et al.[77] showed some di- and tri-peptides derived from collagen had anorexigenic benefits by up to 80% cytotoxicity in white adipose cells, which is the type of tissue understood to be the primary source of many adipokines involved in the chronic low-grade inflammation associated with obesity.[78] However, very often the authors did not specify which specific marine source the peptides were derived from, thus it could not be included in the tablature associated with this review.

It has also been hypothesized in various published works that the bioactivity is not dependent on molecular weight, but rather the amino acid sequence. For example, Sasaoka et al.[79] reported a blood glucose lowering effect of a collagen hydrolysate from sturgeon by-products. However, peptide sequence analysis showed a repetitive sequence of Gly-X-Y in each bioactive peptide. This is a commonly occurring sequence in the triple helical region of collagen, and liberation of these sequences during hydrolysis results in collagen hydrolysates from various sources producing consistently potent bioactivity. To date, no specific key structural components have been identified in hydrolysates produced from other fish components such as muscle or bone. This hypothesis is further supported by Mizushige et al.[66] who reported a reduction in food intake with Alaskan pollock protein hydrolysate, whereas there was an increase in appetite when mice were presented with a 10 amino-acid long soybean-derived peptide.[80] However, no specific peptide sequences were identified by Mizushuge et al. to confirm sequence differences.[66] Furthermore, identification of structure-activity relationships is further limited by different structural characteristics resulting in different "primary" bioactivities, that is, the relevant structural properties which affect DPP-4 inhibition may be different to those relevant to insulin or GLP-1 secretion.

One area which has been largely overlooked by the existing literature is the oral bioavailability of these peptides. Sontakke and colleagues have studied how collagen-derived peptides are crossing the intestinal layer in vitro through use of the Caco-2 cell model, which showed that a tri-peptide (GPH) was able to cross the intestinal layer much more efficiently than a di-peptide (PH) derived from breakdown of the original tri-peptide.[81] Furthermore, these workers supported this finding by measuring GPH levels in plasma after oral presentation to mice which reached maximum levels within 1 h and returned to basal levels within 3 h, indicating that there was no postprandial response causing synthesis of endogenous GPH. By showing that GPH can more effectively cross the intestinal layer than PH, it implies that the affinity of peptides for active transporters in the intestinal layer plays a greater role in oral bioavailability than peptide size when all peptides are already small. As mentioned above; however, this has not as yet been studied in FPH-derived peptides and thus further research is needed to assess the bioavailability of small peptides. As Caco-2 cells are derived from a colon adenocarcinoma, they often form tighter junctions than would typically be found in the duodenum where most macromolecules are absorbed. Despite this, they are a relatively accurate cell model and have been used for other food-derived peptides in the past.[82–84]

6. General Discussion

The primary aim of this review was to summarize the available literature regarding FPH role in preventing hyperglycemia and increasing satiety, which could have beneficial impact in diabetes and obesity, respectively. Furthermore, it is the role of this review to elucidate the mechanisms through which FPHs are exerting these effects in the available literature. It is important to firstly address that there are several confounding factors which make it difficult to draw a consistent conclusion—namely the "source material" (the fish used will affect resulting bioactivity, as will the part of the fish used, e.g., muscle, bone, scales, collagen, etc.), the processing methods/enzymes used, and the dosage concentration/frequency.

To clarify the effects, it is likely easiest to deal with the literature according to the level of research involved—in vitro, in vivo, or clinical trials in humans. Naturally, much of the research at this point is based upon in vitro findings. As previously discussed in Section 3.1, a large portion of the in vitro research is DPP-4 inhibitory assay-based. Even with the methodological variability discussed before, FPH are clearly a potent source of DPP-4 inhibitory peptides with IC_{50} values as low as 0.08 mg mL^{-1} (from a salmon gelatin hydrolysate) to 2.21 mg mL^{-1} (from a barbel skin gelatin hydrolysate). Furthermore, most identified bioactive peptides from FPH (Table 3) have exhibited potent DPP-4 inhibitory activity, and although it is difficult to directly compare doses of isolated peptide to that of a crude hydrolysate, it is possible that many of the isolated peptides are more potent than the crude hydrolysate. Thus, Harnedy-Rothwell et al. identified IPVDM as the third-most potent food-derived DPP-4 inhibitor discovered to date, with an IC_{50} of 21.7 μM.[85] Alongside this strong evidence for DPP-4 inhibitory action, several studies have also shown FPH to have strong insulin, GLP-1, and CCK release actions.[31–33,39,40,85,86]

The in vivo efficacy of FPH has also been reasonably well established. Acutely, all studies investigating an anti-hyperglycemic effect of FPH has found a reduction in blood glucose with doses as low as 50 mg kg^{-1} bodyweight,[33] and several exhibiting an increase in plasma insulin [33,34] or GLP-1[34]; however, Iba et al.[34] used extremely high doses of 1.5 g kg^{-1} and 3 g kg^{-1} bodyweight which would be impossible to translate to a human trial. Other studies have shown the anti-hyperglycemic effect to be even more pronounced in a chronic setting, with the lowest dose used being 100 mg kg^{-1} bodyweight.[41,48,68,87] Furthermore, all in vivo studies investigating the satiating effect of FPH have shown a decrease in bodyweight or a reduction in weight gain.[39,65,66,88] These findings are very encouraging, given the wide methodological variation between studies discussed previously. Ayabe et al. also found that a tripeptide derived from Alaskan pollack, QWR, decreased plasma glucose and improved insulin sensitivity at 1 mg kg^{-1} BW in vivo, similarly to the precursor hydrolysate, presumably through enhancement of glucose uptake.[52]

Human trials involving FPH are naturally quite limited, due to the novelty of the area and dependence upon first finding a promising bioactive source in vivo. Nevertheless, there have been multiple studies recently published investigating the anti-diabetic and satiating effect of a cod protein hydrolysate; however, there appears to be no significant effect on plasma hormones, blood glucose, or satiety with one study reporting a reduction in plasma insulin.[35,89–91] Initially this appears to be due to a very low dosage regimen (a maximum of 4 g per day) but a separate study performed by Hovland et al. showed an effect on glucose tolerance by a cod protein supplement through ingestion of

2.5 g per day for 8 weeks.[92] The important differentiation between these studies is likely that Hovland et al. used an unhydrolyzed cod protein supplement, highlighting that the bioactivity of the protein is impacted by the hydrolysis process, and in this case hydrolysis may have negatively impacted the bioactivity. It is also possible that as both supplements were prepared by private companies in encapsulated/liquid form that other processing activities affected the bioactivity. However, Harnedy-Rothwell et al. found that the DPP-4 inhibitory activity of a boarfish hydrolysates was not affected by heat treatment when prepared in a tomato-based product.[93] Despite this interesting confliction between these two research groups, many others have shown more promising effects. A marine collagen peptide fraction administered to T2DM subjects showed marked improvements in fasting blood glucose, HbA_{1c}, and insulin sensitivity.[51] Two other studies investigating blue whiting protein hydrolysates showed beneficial effects on bodyweight, body composition markers, and satiety scores.[94,95] A trial involving skate skin also showed a reduction in bodyweight and fat mass.[87] Generally speaking, human trials involving FPH have been reasonably successful with the exception of results from a single cod protein hydrolysate.

To conclude, it is clear that despite the methodological variation between studies involving FPH, there is significant potential for use of these hydrolysates in the prevention and management of bodyweight and hyperglycemia. If these methodological issues can be addressed (potentially by generating comprehensive in vivo data initially and by determining an effective dose which is achievable in human trials), then there is likely to be a significant increase in interest in the use of FPH as a functional ingredient. Furthermore, many studies have found an effect on bodyweight or blood glucose but have not been able to conclusively identify a mechanism of action through which this is achieved. An increase in prioritizing mechanism of action studies, along with identification of bioactive peptides could yield much more effective supplements (by only including the most potent peptide fractions) and overall improvement in the understanding of the structural features important for anti-hyperglycemic and satiating bioactivity.

Acknowledgements

S.J.S. is funded by a Northern Ireland Department of Education and Learning PhD scholarship and P.A.H.-R. is funded under the National Development Plan 2007–2013, through the Food Institutional Research Measure (FIRM), administered by the Department of Agriculture, Food and Marine, Ireland under grant issue 17/F/260, respectively.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

anorexigenic, anti-diabetic, bioactive peptides, dipeptidylpeptidase-4/IV, fish hydrolysate, incretin

Published online:

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