GLP-1 regulation by food proteins and protein hydrolysates

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ABSTRACT

Glucagon-like peptide 1 (GLP-1) is an enterohormone with a key role in several processes controlling body homeostasis, including glucose homeostasis and food intake regulation. It is secreted by the intestinal cells in response to nutrients, such as glucose, fat and amino acids. In this review, we analyse the effect of protein on GLP-1 secretion and clearance. We review the literature on the GLP-1 secretory effects of protein and protein hydrolysates, and the mechanisms through which they exert these effects. We also review the studies on protein from different sources that has inhibitory effects on DPP4, the enzyme responsible for GLP-1 inactivation, with particular emphasis on specific sources and treatments, and the gaps there still are in knowledge. There is evidence that the protein source and the hydrolytic processing applied to them can influence the effects on GLP-1 signalling. The gastrointestinal digestion of proteins, for example, significantly changes their effectiveness at modulating this enterohormone secretion in both in vivo and in vitro studies. Nevertheless, little information is available regarding human studies and more research is required to understand their potential as regulators of glucose homeostasis.

INTRODUCTION

The gastrointestinal (GI) tract is responsible for the digestion and absorption of nutrients, and acts as a barrier against luminal pathogens. Moreover, the GI-tract cooperates in controlling the metabolism through hormones secreted from enteroendocrine cells (EEC), which are the body's largest endocrine organ [1]. EECs are capable of responding to luminal content because their apical side has chemosensing machinery such as taste receptors (TASR), G protein-coupled receptors (GPCR), specific transporters and channels. Their secretory products are stored in characterized secretory vesicles, before being secreted through the basolateral membrane by exocytosis [2,3]. When luminal content moves through the GI tract, specific macronutrients stimulate the chemosensing machinery, which leads to the modulation of gut hormone release. Gut hormones exert their effect via vagal nerve or endocrine/paracrine signalling, through the interaction of specific receptors expressed in different tissues of the body. These hormones, which are mainly glucagon-like peptide 1 (GLP-1), cholecystokinin (CCK), peptide YY (PYY), gastric inhibitory polypeptide (GIP), and ghrelin, influence the functioning of the digestive tract, but also modulate insulin secretion from the pancreas, the energy storage of adipose tissue and neuronal signaling in appetite centers in the brain to mediate the regulation of food intake by terminating hunger and inducing satiety.

Since dietary compounds modulate enterohormone secretion, and given the central role of enterohormones in body homeostasis, such an interaction could have beneficial health implications[4]. In this context, protein and protein hydrolysates are currently being studied to determine their effects on GLP-1 modulation, either through secretion or clearance, which may influence the processes regulated by this hormone such as regulation of glycemia homeostasis and food intake control. The nutrient sensing machinery of carbohydrates and lipids is better understood than the detection and pathways followed by protein digestion. The main reasons for this gap in knowledge is the redundant signalling in the gut for the different protein digestion products and the complexity of protein digests[5]. Here we review the literature on this subject in order to determine if the evidence supports differential effects of food proteins on GLP-1 profile. We will introduce the relevance of GLP-1 signalling on

health. Then we will focus on the effects on GLP-1 secretion of proteins and its hydrolysates, and the suggested mechanisms. Finally, we will briefly review the use of protein hydrolysates as DPP4 inhibitors. We compile a significant number of scientific studies to highlight the importance of the different protein sources, the hydrolysis conditions applied to them, and the resulting digestion products.

THE RELEVANCE OF GLP-1 SIGNALLING IN HEALTH

There is evidence to suggest that specific enterohormones administered at physiological concentrations can influence the appetite of rodents and humans (reviewed in [6]). Likewise, the effects of gut hormones on food intake and body weight have been observed in bariatric surgery (such as Roux-en-Y gastric bypass), which induces a huge increase in GLP-1 and PYY secretion and is used to treat obesity[7]. Therefore, the modulation of enterohormone signalling may be an important target in the prevention of obesity and related/associated pathologies. Moreover, endogenous gut hormones regulate appetite physiologically, unlike the drugs that are currently available, which mainly influence the central neurotransmitter systems. Therefore, gut hormone-based therapies might lead to fewer side effects [6].

Furthermore, modulation of endogenous incretin hormones (GLP-1 and GIP) could be an interesting strategy for preventing and/or managing type 2 diabetes mellitus (T2DM) [8]. T2DM is the most common endocrine disorder, characterised by insulin resistance and impaired insulin secretion, and it is one of the fastest growing non-communicable diseases in the world [9]. The main goal in the treatment of T2DM is to keep blood glucose levels within the normal physiological range. In this regard, GLP-1 and GIP are therapeutically interesting peptides because they are important mediators of glycemic homeostasis, as they are responsible for approximately 50-70% of the total insulin secreted after glucose intake [10]. GLP-1, together with GIP, is responsible for the incretin effect, since it binds to GLP-1R in β-cells in the pancreas leading to an increase in intra-cellular calcium and a subsequent insulin secretion in response to glucose [11]. It has also been shown that GLP-1 enhances

markers of proliferation and differentiation, and decreases markers of apoptosis in the pancreas of Zucker diabetic rats [12,13]. Furthermore, GLP-1 improves the glycemic profile by inhibiting glucagon secretion and improves glucose disposal in peripheral tissues[10]. In that way, for patients with T2DM, a non-pharmacological therapeutic approach could be achieved by targeting these incretins (GLP-1 and GIP) through protein and protein hydrolysate based strategies. This approach would be mainly focus on increasing GLP-1 levels rather than stimulating GIP because in these patients the responsiveness of their β-cells to GIP action is decreased [14]. Furthermore, only GLP-1 exerts an appetite supressing effect, while GIP does not seem to do the same[10]. Accordingly, many incretin-based therapies focus on using GLP-1 analogues, promoting endogenous GLP-1 secretion or using dipeptidyl peptidase-4 (DPP4) inhibitors.

DPP4 is a ubiquitous aminodipeptidase that exists essentially as a membrane-anchored cell-surface enzyme [15]. It is expressed throughout the body tissues, such as kidneys, the GI tract, liver, pancreas, and the endothelial and epithelial cells on the vascular bed. Its soluble form is found in plasma and therefore it is in close proximity with hormones circulating in the blood [16,17]. The main activity of DPP4 is to remove N-terminal dipeptides from polypeptides [18], which preferably have a proline or alanine in the second position from the N-terminal. Some of the main DPP4 substrates are GLP-1 and the other incretin hormone GIP, which are peptides with N-terminal Tyr–Ala and His–Ala, respectively [19]. The intact GLP-1 is rapidly hydrolysed by DPP4 into a shorter, inactive form, once it reaches the plasma. GLP-1 has a half-life of 1-2 minutes [18]. Only 25% of the active GLP-1 reaches the portal circulation and subsequently the liver, where a further 40-50% is digested by the DPP4 in hepatocytes. This means that only 15% of the secreted GLP-1 enters the systemic circulation and may reach other tissues, such as the pancreas or the brain [20]. Therefore, DPP4 is responsible for inactivating more than 80% of the secreted GLP-1 [18]. Studies focus not only in the development of DPP4-inhibitory drugs, but also on peptides derived from food sources with DPP4-inhibitory capacity.

Although pharmacological compounds are being studied[21], natural compounds might be used to prevent the development of overweight and obesity-related problems from early preclinical stages through interaction with the enteroendocrine system[22].

DIETARY REGULATION OF GLP-1 SECRETION

Nutrient ingestion is the primary physiological stimulus for inducing GLP-1 secretion by L-cells, located in ileum and colon in human gastrointestinal tract. GLP-1 secretion occurs in a biphasic pattern, which consists of a rapid release in 15-30 min after a meal, followed by a second minor peak that occurs in 60-120 min. EECs have been shown to respond to carbohydrates, lipids and proteins.

Glucose and fat have been reported to be strong GLP-1-secretagogues after they have been ingested [23], or directly administered into the intestine [24,25] or into perfused ileal segments [26]. In the murine model, glucose-stimulated GLP-1 release is blocked using sodium-dependent glucose transporter 1 (SGLT-1) knockout mice and SGLT-1 inhibitor [27,28], which suggests that glucose metabolism uses glucose transport via SGLT-1 to induce GLP-1 secretion. It has also been proposed that sweet taste receptors (T1R2, T1R3) are involved in the glucose-sensing mechanism, but there is still some controversy about whether this is so [29,30]. On the other hand, it has been reported that G-protein-coupled receptors (GPCRs) are activated by dietary fat to stimulate GLP-1 release, including GPR40 and GPR120 by medium-chain fatty acids (MCFAs), long-chain fatty acids (LCFAs) and long-chain unsaturated FAs; and GPR41 and GPR43 by short-chain fatty acids (SCFAs) (reviewed in [31,32]).

Other food components could also modulate GLP-1 secretion. Flavonoid structures, present in several vegetables, also stimulate GLP-1 secretion[33]. In both *ex vivo*[34] and rat models[35], these compounds have been shown to improve the metabolic status altered by a cafeteria diet treatment [36].

EFFECTS OF PROTEINS ON GLP-1 SECRETION

153 Dietary proteins undergo digestion by gastric (pepsin) and pancreatic (chymotrypsin
154 and trypsin) proteases and membrane digestion by peptidases associated with the
155 brush border membrane of enterocytes. The different digestive proteases cleave the
156 peptide bonds at preferential positions. The primary end products
157 are dipeptides and tripeptides, which will enter the cell through peptide transporters.
158 Free amino acids are also released after luminal protein digestion and after peptide
159 hydrolysis within the intestinal cells, and then exit across the basolateral
160 membrane via specific amino acid transporters.

161 GLP-1 release is activated by luminal intestinal chemosensors, which could be
162 reached by peptides of different sizes, mixed with free amino acids.
163
164 Studies in human, animal and enteroendocrine cells have shown increased GLP-1
165 secretion by free amino acids such as L-Phenylalanine, L-alanine and L-glutamine
166 [37,38] and L-asparagine [39]. The effect of glutamine has been confirmed in healthy,
167 obese and diabetic humans [40,41]. Tolhust et al. [42] demonstrated this effect in
168 isolated mouse L cells and reported that the mechanisms were associated with an
169 increase in cAMP and cytosolic Ca^{2+} levels. They also found evidence to suggest
170 that electrogenic sodium coupled amino acid uptake is responsible for initiating
171 membrane depolarisation and voltage gated Ca^{2+}, while a second pathway increases
172 intracellular cAMP levels. Young et al. [43] also reported similar results with L-
173 proline, L-serine, L-alanine, L-glycine, L-histidine, L-cysteine and L-methionine in
174 STC-1 cell line.
175
176 When analysing the effects of protein on GLP-1 release, many studies focus on the
177 effects of protein hydrolysates, produced by the hydrolysis of food protein with
178 commercial enzymes (summarized in Tables 1-3). Sometimes, especially in *in vitro*
179 studies, these are digestive enzymes that simulate intestinal digestion. However,
180 many different hydrolysates are obtained through treatment with enzymes other than
181 pepsin, chymotrypsin or trypsin. Protein hydrolysis can have two main benefits: 1)
182 protein will be more quickly digested after intake and 2) bioactive peptides[44–52]

might be released. Thus, the degree of protein digestion may impact the capability of protein to stimulate GLP-1 release, as discussed below.

In vitro studies on the STC-1 cell line showed a clear stimulation by whole dairy proteins (whey, casein, alpha-lactalbumin, beta-lactoglobulin (BLG)) [53–55]. Moreover, the stimulation of GLP-1 by whey protein BLG in STC-1 cells was partially lost when treated with trypsin (BLG 7.3-fold increase and hydrolysates 2-5.8-fold increase, all versus vehicle control), and totally lost when digested with chymotrypsin for 60 minutes or more [54]. In the same cell line, the stimulatory effects of whey protein on GLP-1 were lost after extensive hydrolysis with microbial (not described) enzymes, or after a simulated gastrointestinal digestion that included a 90-minute treatment with pepsin and a 150-minute treatment with corolase PP [56]. Another study showed that treating whey or casein with trypsin or DPP4 for 30 minutes did not lead to any loss of GLP-1 stimulatory properties [53].

In humans, dairy protein is one of the most studied protein sources involving GLP-1 secretion. Intraduodenal infusion of whey protein hydrolysate has stimulated plasma GLP-1 in lean and obese subjects [57], reduced glucose concentration and supressed energy intake [58] compared with saline. In these studies, hydrolysed rather than intact, whey protein was selected because it more closely resembles partially digested protein. Also in patients with T2DM, a whey preload increased GLP-1 secretion, lowered plasma glucose levels and increased the insulin response[59,60] compared with water and sucralose respectively. It has been shown that whey, casein and casein hydrolysates increase GLP-1 secretion [61–63]. However, there is no agreement about whether there are any differences between their effect on GLP-1 secretion. Hall et al showed that 120 minutes after being ingested, whey protein induced a 2-fold increase in postprandial GLP-1 levels compared with casein protein [62]. On the other hand, when comparing whey, casein, and their hydrolysates, Calbet et al. showed that the release of GLP-1 was not influenced by the source or hydrolysis process [63]. Also, a commercially available whey protein hydrolysate showed a higher GLP-1 release 30 minutes after an oral glucose tolerance test

(OGTT) than did casein glycomacropeptide (CGMP), but not compared to whey isolate or alpha-lactalbumin enriched whey [64] (iAUC $_{30min}$ median; 593 (hydrolysate), 270 (CGMP); $P = 0.045$). Thus, the studies performed with whey and whey hydrolysates do not show any differences in the effects of the two sources in terms of GLP-1 secretion. Calbet et al suggested that this is because the dairy protein hydrolyzes rapidly in the intestine and there is a subsequent rise in peripheral amino acids independent of the fractionation [63].

Other protein sources have also been shown to stimulate GLP-1 release *in vivo*. A similar rise in rat plasma GLP-1 levels, comparable to that caused by dairy protein, has been observed after pea-protein meals [65]. Furthermore, also in rats, pea protein and pea protein hydrolysate have been shown to similarly stimulate GLP-1 release, although the hydrolysate showed stronger eating-inhibitory properties [66] (total energy intake: 63±6 kJ, 46±3 kJ, 67±5 kJ after pea protein, the hydrolysate and the control respectively). *In vitro* studies with STC-1 cells showed that intact pea protein increases GLP-1 release. On the other hand, various pea protein hydrolysates obtained by enzymatic hydrolysis with subtilisin were tested, and only one of them maintained its GLP-1 secretory capacity [53].

Cereal protein has also been shown to stimulate GLP-1. Corn protein zein (a major corn protein) hydrolysate attenuated glycemia in rats under IPGTT, associated with enhanced secretions of GLP-1 and GIP [67] compared with water. *In vitro* (GLUTag cells), zein hydrolysate was shown to stimulate GLP-1 release more than albumin egg, country bean and meat hydrolysates [68]. However, the type of hydrolysis was different in the various sources, so the effect of the protein source *per se* cannot be concluded from this paper. The stimulation of GLP-1 secretion by corn zein hydrolysate in GLUTag cells is not affected by treatment with pepsin/pancreatin for 60 minutes, although it is reduced after pronase treatment [67] compared with the positive control, KCl 70mM. The authors suggested that the hydrolysate is not further cleaved by pepsin treatment (the degree of hydrolysis was only 8.6%).

Oral administration of rice protein hydrolysates also increased total GLP-1 in plasma, and improved glycaemic response in rats [69] (the control used was 2 g kg^{-1} of glucose solution). In the same study, rice protein hydrolysates (degree of hydrolysis 5-10%) stimulated GLP-1 in GLUTag cells with the potency depending on the enzyme and the time of digestion [69] compared with the blank treatment. The effect of the whole rice protein was not assessed. The authors found that GLP-1 secretion was weaker after 60 min digests with pepsin in rice endosperm protein hydrolysates than after 30 min digests, which suggests that oligo- or larger peptides, rather than small peptides or free amino acids, might be responsible for this stimulation. The results for wheat protein were just the opposite.In GLUTag cells, a low-molecular fraction of wheat protein hydrolysate enhanced GLP-1 secretion while a high-molecular fraction did not [70]. The low molecular fraction of wheat protein hydrolysate had a glucose-lowering effect mediated by GLP-1 in rats [70] after an oral administration compared with NaCl 0.9%. Also, in another study in a distal enteroendocrine cell model (GLUTag cells), the effect of wheat hydrolysate on the stimulation of GLP-1 secretion was largely enhanced by pepsin/pancreatin digestion relative to the blank[71].

For other protein sources, *in vitro* studies also showed that GLP-1 secreting activity of digested protein was greater than that of the original source. In a study performed with cuttlefish (*Sepia officinalis*) viscera, a hydrolysate (obtained from digestion with cuttlefish hepato-pancreatic enzymes) was found to exert GLP-1- secreting action while the undigested protein did not [72]. These results were found with the samples solubilized in saliva, but they were subjected to further *in vitro* simulated gastrointestinal digestion (including treatment with pepsin and pancreatin). Results showed that gastrointestinal digestion increased the GLP-1 secretory effects of both the hydrolysate and the initially undigested protein, leading to no differences between the hydrolysate and the non-hydrolysate gastrointestinally-digested samples. Also, intestinal digested bovine haemoglobin protein had a greater effect on GLP-1 release than partially digested protein (saliva and gastric digest) in STC-1 cells [73].

Taken together, all these studies prove that several protein sources increase GLP-1 secretion, which is associated to benefits such as food intake or glucose homeostasis regulation. *In vivo* studies do not fully clarify whether previous hydrolysis of the protein sources with commercial enzymes leads to stronger GLP-1-secreting effects. *In vitro* data show that many protein sources, including purified proteins, activate GLP-1 release. However, digestion as it might physiologically happen upon protein intake might stimulate or reduce the effect of the undigested protein, depending on the original source. This suggests that some high molecular weight peptides might reach enteroendocrine cells and activate GLP-1 secretion, while in other cases the lower molecular weight peptides or the amino acids released after digestion are responsible for the secretion.

Mechanisms involved in the effects of protein as GLP-1 secretagogue

The mechanisms through which the proteins and peptides released after protein hydrolysis (either "synthetic" or simulated digestion) act as secretagogues are still not fully understood, but several pathways have been shown to be involved. Studies on the mechanisms through which protein and protein hydrolysates stimulate GLP-1 secretion are carried out using *in vitro* (i.e. enteroendocrine cell lines such as STC-1 and GLUTag) and *ex vivo* (i.e. perfused intestine and intestinal explants) models, and also primary cultures.

Many of the studies that focus on the mechanisms that stimulate GLP-1 secretion use commercial meat peptones, that is meat hydrolysates produced by the digestion of meat with proteolytic enzymes which lead to a complex mixture of partially metabolised proteins.

With this protein source, it seems that one key player in the oligopeptide-stimulation of GLP-1 release is peptide transporter 1 (PepT1) **(figure 1)**. Meat peptone was shown to stimulate GLP-1 secretion in mouse colonic primary culture through PepT1-dependent uptake, followed by an increase in intracellular calcium, and activation of calcium-sensing receptor (CaSR) [(74)]. Very recently Modvig et al. used

isolated perfused rat small intestine to study GLP-1 secretion stimulated by meat peptone. The sensory mechanisms underlying the response depended on di/tripeptide uptake through PepT1 and subsequent basolateral activation of the amino acid sensing receptor (CaSR) **(figure 2)**. CaSR might also be activated by free amino acids taken up from the intestinal lumen by different amino acid transporters [75].

It has been pointed out that it is difficult to determine the PepT1-dependent oligopeptide-sensing pathway in GLUTag and STC-1 cell lines, because the expression of endogenous PepT1 is lower than in native L cells [74]. Therefore, the effects of peptones observed in both cells lines may be due to the free amino acids that some of these peptones contain, as has been suggested in an *in vitro* study on the effects of salmon hydrolysate [76] carried out in GLUTag cells. However, other studies on these cell lines do not share this view. As mentioned above, GLP-1 secretion is activated by dairy proteins [53–55], low molecular weight wheat (with less than 1% free aminoacids) [70], intact pea-protein [53] or peptin-resistant zein hydrolysate [67]. Furthermore, three synthetic peptide sequences, ANVST, TKAVEH and KAAT, were reported to be able to enhance GLP-1 secretion in STC-1 [77]**.** The authors concluded that the incretin effect of proteins is associated with the amino acid profile, but the specific amino acid motif that triggers GLP-1 secretion stimulation was not determined. Thus, receptor or peptide transporters other than PepT1 expressed in STC-1 and GLUTag cells might be involved in the peptide stimulation of GLP-1. For instance, one of the mediators suggested was the G protein-coupled receptor family C group 6 subtype A (GPRC6A) [70] **(figure 3)**.

Protein hydrolysates are also detected by the umami receptor (T1R1-T1R3 heterodimer[78] (**figure 4**) and G protein-coupled receptor 92/93 (GPR92/93) [79], which leads to the release of the gut-derived satiety factor cholecystokinin. There is no direct evidence of umami stimulation and GLP-1 secretion, but the T1R1 receptors were coexpressed with GLP-1-expressing STC-1 cells [80], which suggests that umami receptors play a role in GLP-1 signalling.

An increase in intracellular calcium has been reported to be a pathway activated by protein hydrolysates to mediate GLP-1 secretion. Pais et al. [37] reported that meat peptone-stimulated GLP-1 secretion from primary L cells was also associated with calcium influx through voltage gate calcium channels (VGCC) **(figure 3)**. In NCI-H716 human enteroendocrine cells, tetrapeptides, but not single amino acids or any of the dipeptides, tripeptides and pentapeptides tested were found to induce a robust and selective $[Ca^{2+}]_i$ response associated with increased secretion of GLP-1 [81]. Moreover, these effects were not observed in either STC-1 or in GLUTag rodent cells. Interestingly, in the same paper, the authors showed that casein protein hydrolysate elicited an increase in GLP-1 without modulating intracellular calcium.

It has been suggested that GLP-1 secretion is mediated by other intracellular pathways such as extracellular signal-regulated kinase 1/2 (ERK1/2), mitogen-activated protein kinase MAPK and p38 MAPK, activated by peptones and mixtures of essential amino acids in NCI-H716 cells [82].

Altogether, the studies show that which signalling pathways are involved in GLP-1 secretion by different peptide mixtures will depend on the peptide length, the sequences and/or the amino acid composition, and whether there are free amino acids in the mixture. Furthermore, the model studied has to be carefully considered since there are differences in the expression of key genes (such as pepT-1) and some effects might depend on the vectoriality of the system (the capacity to differentiate basolateral and apical processes).

Protein bioactivity on GLP-1 clearance

Like the studies on the effects of protein on GLP-1 secretion, most of the studies on the effects of protein on DPP4 inhibition are performed with protein hydrolysates. Over the past few years, bioactive peptides have shown their potential as DPP4 inhibitors, a research area that is currently expanding. *In vitro* simulated gastrointestinal digestion has been reported to produce DPP4-inhibitory protein hydrolysates [83,84]. Also hydrolysis with a range of enzymes is used to release DPP4

inhibitory peptides [69,85–90]. Thus, a wide range of protein sources have been used to obtain hydrolysates, for which DPP4 inhibitory activity has been screened mainly *in vitro*.

Research has shown that the amino acid sequence plays a much greater role in DPP4 inhibitory activity than other physicochemical parameters such as length, isoelectric point, hydrophobicity and net charge [91,92]. DPP4 preferentially cleaves substrates that bear proline or alanine at their P1 position (Xaa-Pro and Xaa-Ala; where Xaa represents any amino acid) and also acts on substrates that bear other residues, such as glycine, serine, valine and leucine [93]. Hydrophobic and basic residues at the P_2 position enhance the affinity for cleavage compared with acidic residues[94]. The presence of tryptophan residue at the N-terminal position increases the susceptibility to cleavage. Although the residues at the N-terminal position may have a major impact by inhibiting DPP4, the authors pointed out that the C-terminal amino acid also affects the potency of DPP4 because it is involved in the interaction with the enzyme[95].

To date, some studies have been carried out on the *in vivo* DPP4 inhibitory effects of the hydrolysates and peptides from dietary proteins. Peptides derived from milk and bean proteins, which have been shown to inhibit the activity of DPP4 *in vitro,* were also found to have glycemic effects on mice [96,97] as plasma glucose levels decreased after an OGTT. A β-casein-derived peptide LPQNIPPL found in Gouda-type cheese with *in vitro* DPP4 inhibitory effects has also been tested with animal models. Oral administration of this octapeptide resulted in 1.8-fold lower postpandrial glucose under the curve; however, insulin plasma levels did not differ [98]. In these studies, the authors did not measure plasma DPP4 activity so it is not known whether the lower blood glucose was caused by inhibition of DPP4 activity. Chicken feet hydrolysates with DPP4 inhibitory activity *in vitro* improved hyperglycemia in diet and aged models of glucose homeostasis impairment [99].

As well as hydrolysates from milk and bean protein, in *in vivo* models hydrolysate from the egg protein lysosyme has also shown a 25% reduction in blood serum DPP4 activity and a trend towards higher serum GLP-1 levels after 90 min in diabetic rats undergoing chronic treatment [100]. Streptozotocin-induced diabetic rats were used to evaluate the effects of porcine skin gelatin hydrolysates [48], atlantic salmon skin gelatin [47], and halibut and tilapia skin gelatin [49]. In all these studies, diabetic animals showed reduced blood glucose levels during OGTT, increased plasma insulin and active GLP-1 levels, and reduced plasma DPP4 activity after a chronic treatment with these proteins compared with water. Diabetic rats treated for 42 days with a daily dose of 300 mg/kg of porcine skin gelatin showed their plasma glucose AUC reduced from 30.000 to 28.000 mg*min/dL, insulin levels increased 2-fold, active GLP-1 levels reduced from 15 to 13.5 pM and DDP4 activity reduced by half [48]. In another study in which the animals were treated for 35 days with a daily dose of 300 mg/kg of Atlantic salmon skin gelatin hydrolysate, blood glucose levels were reduced to less than 200 mg/dL during OGTT, insulin levels increased 3-fold, active GLP-1 levels increased 1.6-fold and DPP4 activity was reduced from 115.5 to 82.6% (lower than in normal rats).[47]. When these animals received a 30 day treatment involvinga daily dose of 750 mg/kg of halibut (HSGH) or tilapia skin gelatin hydrolysate (TSGH) the plasmatic glucose was lower than 200 mg/dL in the TSGH treated group. When TSGH was administered, insulin levels were 1.56 g/L, higher than that of HSGH (1.14 g/L) and the diabetic control group (0.43 g/L). The active GLP-1 plasmatic levels of the diabetic control rats (5.14 pM) were lower than those for TSGH treated group (13.32 pM) and for HSGH treated group (7.37 pM) and the DPP4 activity reduced from 115.5 in the diabetic group to 86.6 and 71.6% in the HSGH and TSGH treated groups respectively[49]. Moreover, rodents receiving halibut and tilapia skin gelatin hydrolysates also showed increased total GLP-1 levels. Therefore, the findings of this study suggest that these hydrolysates exert their anti-hyperglycemic effect via dual actions of DPP4 inhibition and GLP-1 secretion enhancement. Similarly, the ileal administration of zein protein hydrolysate to rats was found to potentiate the incretin effect when administered prior to an intraperitoneal glucose tolerance test, resulting in decreased glucose

concentration, increased insulin levels, decreased plasma DPP4 activity, and increased total and active GLP-1 secretion compared with water [101]. Rice-derived peptides were likewise found to act via dual action. Oral administration increased plasma GLP-1 levels compared with water during an intraperitoneal glucose tolerance test, and ileal administration reduced plasma DPP4 activity and increased the ratio of active GLP-1 to total GLP-1 [69] in rats. *In vitro* studies also showed dual mechanisms for protein hydrolysates, both enhanced GLP-1 secretion and inhibited DPP4, as has been shown for the cuttlefish (*Sepia officinalis*) viscera protein hydrolysate and bovine haemoglobin hydrolysate [72,77], whey proteins [56], and chicken feet hydrolysate [99]. Therefore, these two mechanisms might also take part *in vivo* for some protein sources, leading to an increase in active GLP-1 and improve glycemia.

Human studies, although limited, offer some evidence that food-derived peptides, mostly from dairy protein, act as DDP4 inhibitors [102]. It was shown that a whey preload, consumed before the breakfast meal, reduced glucose levels by 28% and increased insulin and total GLP-1 levels by 105% and 141% respectively, compared with water. Nevertheless, no significant differences in plasma DPP4 activity was found. This could be interpreted as whey protein acting as an endogenous inhibitor of DPP4 in the proximal small intestine, but not in the plasma (intestinal DPP4 activity was not assessed)[60]. Further studies are needed to examine the potential of casein and whey-derived peptides, as well as peptides derived from other sources, to act with DPP4 inhibitors in human subjects.

CONCLUSIONS

Food proteins target the enteroendocrine system. They directly enhance GLP-1 release from enteroendocrine cells. Current studies suggest that the source of the protein might lead to differences in GLP-1 secretion, although there is not enough literature to enable the different proteins to be compared. The effect of gastrointestinal digestion can also enhance or decrease GLP-1 secreting capacity depending on the protein type. Thus, it is important to consider this digestion when discussing the effects of protein on GLP-1 secretion *in vitro*. In addition, peptides

with DPP4 inhibitory effects can be released during the digestion process, which could modulate the life span of target enterohormones. However, whether this hydrolysis remains important after intestinal digestion *in vivo* remains to be clarified. Thus, the use of protein/protein hydrolysates to ameliorate situations of glucose derangements is promising, but more research, specifically human studies, is required to define the most effective sources/treatments.

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REFERENCES

1. Rehfeld JF. The New Biology of Gastrointestinal Hormones. physological Rev. 1998;78(4):1087–108.
2. Gunawardene AR, Corfe BM, Staton CA. Classification and functions of enteroendocrine cells of the lower gastrointestinal tract. Int. J. Exp. Pathol. 2011 Aug;92(4):219–31.
3. Sternini C, Anselmi L, Rozengurt E. Enteroendocrine cells: a site of “taste” in gastrointestinal chemosensing. Curr. Opin. Endocrinol. diabetes Obes. 2008;15(1):73–8.

4. Pinent M, Blay M, Serrano J, et al. Effects of Flavanols on the Enteroendocrine System: Repercussions on Food Intake. Crit. Rev. Food Sci. Nutr. 2015 Jun;57(2):326–34.

5. Santos-Hernández M, Miralles B, Amigo L, et al. Intestinal Signaling of Proteins and Digestion-Derived Products Relevant to Satiety. J. Agric. Food Chem. American Chemical Society; 2018 Oct 3;66(39):10123–31.

6. Murphy KG, Bloom SR. Gut hormones and the regulation of energy homeostasis. Nature. 2006 Dec;444(7121):854–9.

7. Le Roux CW, Aylwin SJB, Batterham RL, et al. Gut hormone profiles following bariatric surgery favor an anorectic state, facilitate weight loss, and improve metabolic parameters. Ann. Surg. [Internet]. Lippincott, Williams, and Wilkins; 2006 Jan [cited 2020 Sep 2];243(1):108–14. Available from: /pmc/articles/PMC1449984/?report=abstract

8. Kreymann B, Williams G, Ghatei MA, et al. Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet (London, England). 1987 Dec;2(8571):1300–4.

9. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. 2010. p. 4–14.

10. Baggio LL, Drucker DJ. Biology of Incretins: GLP-1 and GIP. Gastroenterology. 2007;132(6):2131–57.

11. Vilsbøll T, Holst JJ. Incretins, insulin secretion and Type 2 diabetes mellitus. Diabetologia. 2004 Mar;47(3):357–66.

12. Pick A, Clark J, Kubstrup C, et al. Role of apoptosis in failure of b-cell mass compansation for insulin resistance and b-cell defects in the male Zucker diabetic fatty rat. Diabetes. 1998;47(3):358–64.

13. Farilla L, Hui H, Bertolotto C, et al. Glucagon-like peptide-1 promotes islet cell growth and inhibits apoptosis in Zucker diabetic rats. Endocrinology. 2002;143(11):4397–408.

14. Nauck MA. Incretin-based therapies for type 2 diabetes mellitus: properties, functions, and clinical implications. Am. J. Med. Elsevier Inc.; 2011;124(1 Suppl):S3-18.

15. Filippatos TD, Athyros VG, Elisaf MS. The pharmacokinetic considerations and adverse effects of DDP-4 inhibitors. Expert Opin. Drug Metab. Toxicol. 2014;10(6):787–812.

16. Yu DMT, Yao T, Chowdhury S, et al. The dipeptidyl peptidase IV family in cancer and cell biology. FEBS J. 2010;277(5):1126–44.

17. Mentlein R. Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. Regul. Pept. 1999. p. 9–24.

18. Thoma R, Löffler B, Stihle M, et al. Structural basis of proline-specific exopeptidase activity as observed in human dipeptidyl peptidase-IV. Structure. 2003;11(8):947–59.

19. Havale SH, Pal M. Medicinal chemistry approaches to the inhibition of dipeptidyl peptidase-4 for the treatment of type 2 diabetes. Bioorg. Med. Chem. 2009 Jan;17(5):1783–802.

20. Holst JJ. The Physiology of Glucagon-like Peptide 1. Physiol. Rev. 2007 Oct;87(4):1409–39.

21. Khera R, Murad MH, Chandar AK, et al. Association of Pharmacological Treatments for Obesity With Weight Loss and Adverse Events. Jama. 2016 Jun;315(22):2424.

22. Serrano J, Casanova-Martí À, Blay MT, et al. Strategy for limiting food intake using food components aimed at multiple targets in the gastrointestinal tract. Trends Food Sci. Technol. 2017;68:113–29.

23. Elliott RM, Morgan LM, Tredger JA, et al. Glucagon-like peptide-1(7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: Acute post-prandial and 24-h secretion patterns. J. Endocrinol. 1993;138(1):159–66.

24. Rocca AS, Brubaker PL. Role of the vagus nerve in mediating proximal nutrient-induced glucagon- like peptide-1 secretion. Endocrinology. 1999;140(4):1687–94.

25. Roberge N, Brubaker L. Regulation of Intestinal Proglucagon-Derived Peptide Secretion by Glucose-Dependent Insulinotropic Peptide in a Novel Enteroendocrine Loop. Endocrinology. 1993;133(1):233–40.

26. Cordier-Bussat M, Bernard C, Levenez F, et al. Peptones stimulate both the secretion of the incretin hormone glucagon- like peptide 1 and the transcription of the proglucagon gene. Diabetes. 1998;47(7):1038–45.

27. Gorboulev V, Schürmann A, Vallon V, et al. Na +-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes. 2012;61(1):187–96.

28. Kuhre RE, Frost CR, Svendsen B, et al. Molecular mechanisms of glucose-stimulated GLP-1 secretion from perfused rat small intestine. Diabetes. 2015;64(2):370–82.

29. Steinert RE, Gerspach AC, Gutmann H, et al. The functional involvement of gut-expressed sweet taste receptors in glucose-stimulated secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). Clin. Nutr. Elsevier Ltd; 2011;30(4):524–32.

30. Kokrashvili Z, Mosinger B, Margolskee RF. T1r3 and ??-gustducin in gut regulate secretion of glucagon-like peptide-1. Ann. N. Y. Acad. Sci. 2009. p. 91–4.

31. Hirasawa A, Hara T, Katsuma S, et al. Free fatty acid receptors and drug discovery. Biol. Pharm. Bull. 2008;31(October):1847–51.

32. Reimann F. Molecular mechanisms underlying nutrient detection by incretin-secreting cells. Int. Dairy J. Elsevier Ltd; 2010;20(4):236–42.

33. Domínguez Avila JA, Rodrigo García J, González Aguilar GA, et al. The Antidiabetic Mechanisms of Polyphenols Related to Increased Glucagon-Like

572 Peptide-1 (GLP1) and Insulin Signaling. Molecules. 2017;22(6):1–16.

573 34. Casanova-Martí À, Serrano J, Blay MT, et al. Acute selective bioactivity of
574 grape seed proanthocyanidins on enteroendocrine secretions in the
575 gastrointestinal tract. Food Nutr. Res. 2017 Jan;61(1):1321347.

576 35. González-Abuín N, Martínez-Micaelo N, Margalef M, et al. A grape seed
577 extract increases active glucagon-like peptide-1 levels after an oral glucose
578 load in rats. Food Funct. England; 2014 Sep;5(9):2357–64.

579 36. Gonzalez-Abuin N, Martinez-Micaelo N, Blay M, et al. Grape-seed
580 procyanidins prevent the cafeteria diet-induced decrease of glucagon-like
581 peptide-1 production. J. Agric. Food Chem. American Chemical Society;
582 2014;

583 37. Pais R, Gribble FM, Reimann F. Signalling pathways involved in the
584 detection of peptones by murine small intestinal enteroendocrine L-cells.
585 Peptides. 2016;77:9–15.

586 38. Reimann F, Williams L, Da Silva Xavier G, et al. Glutamine potently
587 stimulates glucagon-like peptide-1 secretion from GLUTag cells.
588 Diabetologia. 2004;47(9):1592–601.

589 39. Mace OJ, Schindler M, Patel S. The regulation of K- and L-cell activity by
590 GLUT2 and the calcium-sensing receptor CasR in rat small intestine. J.
591 Physiol. 2012;590(Pt 12):2917–36.

592 40. Greenfield JR, Farooqi IS, Keogh JM, et al. Oral glutamine increases
593 circulating glucagon-like peptide 1 , glucagon , and insulin concentrations in
594 lean , obese , and type 2 diabetic subjects 1 – 4. Am. J. Clin. Nutr.
595 2009;1:106–13.

596 41. Samocha-Bonet D, Wong O, Synnott EL, et al. Glutamine reduces
597 postprandial glycemia and augments the glucagon-like peptide-1 response in
598 type 2 diabetes patients. J Nutr. 2011;141(7):1233–8.

599 42. Tolhurst G, Zheng Y, Parker HE, et al. Glutamine triggers and potentiates

glucagon-like peptide-1 secretion by raising cytosolic Ca2+and cAMP. Endocrinology. 2011;152(2):405–13.

43. Young SH, Rey O, Sternini C, et al. Amino acid sensing by enteroendocrine STC-1 cells: role of the Na+-coupled neutral amino acid transporter 2. Am J Physiol Cell Physiol. 2010;298:1401–13.

44. Mine Y, Li-Chan ECY, Jiang B. Biologically Active Food Proteins and Peptides in Health: An Overview. Bioact. Proteins Pept. as Funct. Foods Nutraceuticals. Wiley-Blackwell; 2010. p. 3–11.

45. Bhat ZF, Kumar S, Bhat HF. Bioactive peptides of animal origin: a review. J. Food Sci. Technol. 2015. p. 5377–92.

46. Suleria HAR, Gobe G, Masci P, et al. Marine bioactive compounds and health promoting perspectives; innovation pathways for drug discovery. Trends Food Sci. Technol. 2016. p. 44–55.

47. Hsieh CH, Wang TY, Hung CC, et al. Improvement of glycemic control in streptozotocin-induced diabetic rats by Atlantic salmon skin gelatin hydrolysate as the dipeptidyl-peptidase IV inhibitor. Food Funct. Royal Society of Chemistry; 2015;6(6):1887–92.

48. Huang SL, Hung CC, Jao CL, et al. Porcine skin gelatin hydrolysate as a dipeptidyl peptidase IV inhibitor improves glycemic control in streptozotocin-induced diabetic rats. J. Funct. Foods. Elsevier Ltd; 2014;11(C):235–42.

49. Wang TY, Hsieh CH, Hung CC, et al. Fish skin gelatin hydrolysates as dipeptidyl peptidase IV inhibitors and glucagon-like peptide-1 stimulators improve glycaemic control in diabetic rats: A comparison between warm- and cold-water fish. J. Funct. Foods. Elsevier Ltd; 2015;19:330–40.

50. Hsieh CC, Hernández-Ledesma B, Fernández-Tomé S, et al. Milk proteins, peptides, and oligosaccharides: Effects against the 21st century disorders. Biomed Res. Int. 2015.

51. Nongonierma AB, Fitzgerald RJ. Biofunctional properties of

628 caseinophosphopeptides in the Oral Cavity. Caries Res. 2012. p. 234–67.

629 52. Udenigwe CC, Aluko RE. Food protein-derived bioactive peptides:
630 Production, processing, and potential health benefits. J. Food Sci. 2012.

631 53. Geraedts MCP, Troost FJ, Fischer M a JG, et al. Direct induction of CCK and
632 GLP-1 release from murine endocrine cells by intact dietary proteins. Mol.
633 Nutr. Food Res. 2011;55(3):476–84.

634 54. Gillespie AL, Calderwood D, Hobson L, et al. Whey proteins have beneficial
635 effects on intestinal enteroendocrine cells stimulating cell growth and
636 increasing the production and secretion of incretin hormones. Food Chem.
637 Elsevier Ltd; 2015;189:120–8.

638 55. Gillespie AL, Green BD. The bioactive effects of casein proteins on
639 enteroendocrine cell health, proliferation and incretin hormone secretion.
640 Food Chem. [Internet]. Elsevier Ltd; 2016;211:148–59. Available from:
641 http://dx.doi.org/10.1016/j.foodchem.2016.04.102

642 56. Power-Grant O, Bruen C, Brennan L, et al. In vitro bioactive properties of
643 intact and enzymatically hydrolysed whey protein: targeting the enteroinsular
644 axis. Food Funct. Royal Society of Chemistry; 2015;6(3):972–80.

645 57. Hutchison AT, Feinle-Bisset C, Fitzgerald PCE, et al. Comparative effects of
646 intraduodenal whey protein hydrolysate on antropyloroduodenal motility, gut
647 hormones, glycemia, appetite, and energy intake in lean and obese men. Am.
648 J. Clin. Nutr. 2015 Dec;102(6):1323–31.

649 58. Ryan AT, Feinle-Bisset C, Kallas A, et al. Intraduodenal protein modulates
650 antropyloroduodenal motility, hormone release, glycemia, appetite, and
651 energy intake in lean men. Am. J. Clin. Nutr. 2012;96(3):474–82.

652 59. Watson LE, Phillips LK, Wu T, et al. Title: Differentiating the effects of
653 whey protein and guar gum preloads on postprandial glycemia in type 2
654 diabetes. Clin. Nutr. [Internet]. Churchill Livingstone; 2019 Dec 1 [cited 2020
655 Sep 1];38(6):2827–32. Available from: https://pubmed-ncbi-nlm-nih-
656 gov.sabidi.urv.cat/30583967/

60. Jakubowicz D, Froy O, Ahrén B, et al. Incretin, insulinotropic and glucose-lowering effects of whey protein pre-load in type 2 diabetes: A randomised clinical trial. Diabetologia [Internet]. Springer Verlag; 2014 Jul 10 [cited 2020 Sep 1];57(9):1807–11. Available from: https://link-springer-com.sabidi.urv.cat/article/10.1007/s00125-014-3305-x

61. Bendtsen LQ, Lorenzen JK, Gomes S, et al. Effects of hydrolysed casein, intact casein and intact whey protein on energy expenditure and appetite regulation: A randomised, controlled, cross-over study. Br. J. Nutr. 2014;112(8):1412–22.

62. Hall WL, Millward DJ, Long SJ, et al. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. Br. J. Nutr. 2003;89(2):239–48.

63. Calbet JAL, Holst JJ. Gastric emptying, gastric secretion and enterogastrone response after administration of milk proteins or their peptide hydrolysates in humans. Eur. J. Nutr. 2004;43(3):127–39.

64. Mortensen LS, Holmer-Jensen J, Hartvigsen ML, et al. Effects of different fractions of whey protein on postprandial lipid and hormone responses in type 2 diabetes. Eur. J. Clin. Nutr. Nature Publishing Group; 2012;66(7):799–805.

65. Overduin J, Guérin-Deremaux L, Wils D, et al. NUTRALYS(®) pea protein: characterization of in vitro gastric digestion and in vivo gastrointestinal peptide responses relevant to satiety. Food Nutr. Res. Taylor & Francis; 2015 Jan;59:25622–31.

66. Häberer D, Tasker M, Foltz M, et al. Intragastric infusion of pea-protein hydrolysate reduces test-meal size in rats more than pea protein. Physiol. Behav. Elsevier Inc.; 2011;104(5):1041–7.

67. Higuchi N, Hira T, Yamada N, et al. Oral Administration of Corn Zein Hydrolysate Stimulates GLP-1 and GIP Secretion and Improves Glucose Tolerance in Male Normal Rats and Goto-Kakizaki Rats. Endocrinology. 2013 Sep;154(9):3089–98.

686 68. Hira T, Mochida T, Miyashita K, et al. GLP-1 secretion is enhanced directly
687 in the ileum but indirectly in the duodenum by a newly identified potent
688 stimulator, zein hydrolysate, in rats. Am. J. Physiol. Gastrointest. Liver
689 Physiol. 2009 Oct;297(4):G663-71.

690 69. Ishikawa Y, Hira T, Inoue D, et al. Rice protein hydrolysates stimulate GLP-1
691 secretion, reduce GLP-1 degradation, and lower the glycemic response in rats.
692 Food Funct. 2015 Aug;6(8):2525–34.

693 70. Kato M, Nakanishi T, Tani T, et al. Low-molecular fraction of wheat protein
694 hydrolysate stimulates glucagon-like peptide-1 secretion in an
695 enteroendocrine L cell line and improves glucose tolerance in rats. Nutr. Res.
696 Elsevier Inc.; 2017;37:37–45.

697 71. Chen W, Hira T, Nakajima S HH. Wheat gluten hydrolysate potently
698 stimulates peptide-YY secretion and suppresses food intake in rats. Biosci
699 Biotechnol Biochem. 2018;80(11):1992–9.

700 72. Cudennec B, Balti R, Ravallec R, et al. In vitro evidence for gut hormone
701 stimulation release and dipeptidyl-peptidase IV inhibitory activity of protein
702 hydrolysate obtained from cuttlefish (Sepia officinalis) viscera. Food Res. Int.
703 2015;78:238–45.

704 73. Caron J, Domenger D, Belguesmia Y, et al. Protein digestion and energy
705 homeostasis: How generated peptides may impact intestinal hormones? Food
706 Res. Int. 2016;88(Part B):310–8.

707 74. Diakogiannaki E, Pais R, Tolhurst G, et al. Oligopeptides stimulate glucagon-
708 like peptide-1 secretion in mice through proton-coupled uptake and the
709 calcium-sensing receptor. Diabetologia. 2013;56(12):2688–96.

710 75. Modvig IM, Kuhre RE, Holst JJ. Peptone-mediated glucagon-like peptide-1
711 secretion depends on intestinal absorption and activation of basolaterally
712 located Calcium-Sensing Receptors. Physiol. Rep. 2019;7(8):1–13.

713 76. Harnedy PA, Parthsarathy V, McLaughlin CM, et al. Atlantic salmon (Salmo
714 salar) co-product-derived protein hydrolysates: A source of antidiabetic

peptides. Food Res. Int. 2018;106(November 2017):598–606.

77. Caron J, Cudennec B, Domenger D, et al. Simulated GI digestion of dietary protein: Release of new bioactive peptides involved in gut hormone secretion. Food Res. Int. 2016;89:382–90.

78. Raka F, Farr S, Kelly J, et al. Metabolic control via nutrient-sensing mechanisms: role of taste receptors and the gut-brain neuroendocrine axis. Am. J. Physiol. Endocrinol. Metab. 2019;317(4):E559–72.

79. Choi S, Lee M, Shiu AL, et al. Identification of a protein hydrolysate responsive G protein-coupled receptor in enterocytes. Am. J. Physiol. - Gastrointest. Liver Physiol. 2007;292(1):98–112.

80. Wang H, Murthy KS, Grider JR. Expression patterns of l-amino acid receptors in the murine STC-1 enteroendocrine cell line. Cell Tissue Res. Springer; 2019 Dec 1;378(3):471–83.

81. Le Nevé B, Daniel H. Selected tetrapeptides lead to a GLP-1 release from the human enteroendocrine cell line NCI-H716. Regul. Pept. Elsevier B.V.; 2011;167(1):14–20.

82. Reimer R a. Meat hydrolysate and essential amino acid-induced glucagon-like peptide-1 secretion, in the human NCI-H716 enteroendocrine cell line, is regulated by extracellular signal-regulated kinase1/2 and p38 mitogen-activated protein kinases. J. Endocrinol. 2006;191(1):159–70.

83. Lacroix IME, Li-Chan ECY. Dipeptidyl peptidase-IV inhibitory activity of dairy protein hydrolysates. Int. Dairy J. 2012;25(2):97–102.

84. Mojica L, Chen K, de Mejía EG. Impact of Commercial Precooking of Common Bean (Phaseolus vulgaris) on the Generation of Peptides, After Pepsin-Pancreatin Hydrolysis, Capable to Inhibit Dipeptidyl Peptidase-IV. J. Food Sci. 2015;80(1):H188–98.

85. Lacroix IME, Li-Chan ECY. Inhibition of dipeptidyl peptidase (DPP)-IV and α-glucosidase activities by pepsin-treated whey proteins. J. Agric. Food

Chem. 2013;61(31):7500–6.

86. Silveira ST, Martínez-maqueda D, Recio I, et al. Dipeptidyl peptidase-IV inhibitory peptides generated by tryptic hydrolysis of a whey protein concentrate rich in b -lactoglobulin. Food Chem. Elsevier Ltd; 2013;141(2):1072–7.

87. Nongonierma AB, FitzGerald RJ. Dipeptidyl peptidase IV inhibitory properties of a whey protein hydrolysate: Influence of fractionation, stability to simulated gastrointestinal digestion and food-drug interaction. Int. Dairy J. Elsevier Ltd; 2013;32(1):33–9.

88. Konrad B, Anna D, Marek S, et al. The evaluation of dipeptidyl peptidase (DPP)-IV, α-glucosidase and angiotensin converting enzyme (ACE) inhibitory activities of whey proteins hydrolyzed with serine protease isolated from asian pumpkin (Cucurbita ficifolia). Int. J. Pept. Res. Ther. 2014;20(4):483–91.

89. Boots J-WP. protein hydrolysate enriched in peptides inhibiting DPP-IV and their use. United States;

90. Connolly A, Piggott CO, FitzGerald RJ. In vitro α-glucosidase, angiotensin converting enzyme and dipeptidyl peptidase-IV inhibitory properties of brewers' spent grain protein hydrolysates. Food Res. Int. Elsevier Ltd; 2014;56:100–7.

91. Lacroix IME, Li-Chan ECY. Isolation and characterization of peptides with dipeptidyl peptidase-IV inhibitory activity from pepsin-treated bovine whey proteins. Peptides. 2014;54:39–48.

92. Nongonierma AB, Fitzgerald RJ. An in silico model to predict the potential of dietary proteins as sources of dipeptidyl peptidase IV (DPP-IV) inhibitory peptides. FOOD Chem. Elsevier Ltd; 2014;165:489–98.

93. Lambeir A, Durinx C, Scharpé S, et al. Dipeptidyl-peptidase IV from bench to bedside: an update on structural properties, functions, and clinical aspects of the enzyme DPP IV. Crit. Rev. Clin. Lab. Sci. 2003;40(3):209–94.

772 94. Power O, Nongonierma AB, Jakeman P, et al. Food protein hydrolysates as a
773 source of dipeptidyl peptidase IV inhibitory peptides for the management of
774 type 2 diabetes. Proc. Nutr. Soc. [Internet]. Cambridge University Press; 2014
775 Feb [cited 2020 Sep 2];73(1):34–46. Available from:
776 https://doi.org/10.1017/S0029665113003601

777 95. Lan VTT, Ito K, Ohno M, et al. Analyzing a dipeptide library to identify
778 human dipeptidyl peptidase IV inhibitor. Food Chem. Elsevier Ltd;
779 2015;175:66–73.

780 96. Tominaga Y, Yokota S, Tanaka H, et al. DIPEPTIDYL PEPTIDASE-4
781 INHIBITOR. united states;

782 97. Uchida M, Ohshiba Y, Mogami O. Novel dipeptidyl peptidase-4-inhibiting
783 peptide derived from β-lactoglobulin. J. Pharmacol. Sci. 2011;117(1):63–6.

784 98. Uenishi H, Kabuki T, Seto Y, et al. Isolation and identification of casein-
785 derived dipeptidyl-peptidase 4 (DPP-4)-inhibitory peptide LPQNIPPL from
786 gouda-type cheese and its effect on plasma glucose in rats. Int. Dairy J.
787 Elsevier Ltd; 2012;22(1):24–30.

788 99. Casanova-Martí À, Bravo FI, Serrano J, et al. Antihyperglycemic effect of a
789 chicken feet hydrolysate via the incretin system: DPP-IV-inhibitory activity
790 and GLP-1 release stimulation . Food Funct. Royal Society of Chemistry;
791 2019;10(7):4062–70.

792 100. Wang Y, Landheer S, van Gilst WH, et al. Attenuation of Renovascular
793 Damage in Zucker Diabetic Fatty Rat by NWT-03, an Egg Protein
794 Hydrolysate with ACE- and DPP4-Inhibitory Activity. Tomé D, editor. PLoS
795 One. Public Library of Science; 2012 Oct;7(10):e46781.

796 101. Mochida T, Hira T, Hara H. The corn protein, zein hydrolysate, administered
797 into the ileum attenuates hyperglycemia via its dual action on glucagon-like
798 peptide-1 secretion and dipeptidyl peptidase-IV activity in rats.
799 Endocrinology. 2010;151(7):3095–104.

800 102. Horner K, Drummond E, Brennan L. Bioavailability of milk protein-derived

bioactive peptides: A glycaemic management perspective. Nutr. Res. Rev. 2016;29(1):91–101.

103. Daniel H, Spanier B, Kottra G, et al. From bacteria to man: Archaic proton-dependent peptide transporters at work. Physiology. 2006;21(2):93–102.

104. Wauson EM, Lorente-Rodríguez A, Cobb MH. Minireview: Nutrient sensing by G protein-coupled receptors. Mol. Endocrinol. 2013/07/02. Endocrine Society; 2013 Aug;27(8):1188–97.

105. Kinnamon SC. Umami taste transduction mechanisms. Am. J. Clin. Nutr. 2009;90(3):753–5.

Figure 1: The intestinal transporter form PEPT1 (SLC15A1) is located in apical membranes with a functional coupling to the apical Na^+/H^+ antiporter (NHE-3) for pH recovery from the peptide-transport-induced intracellular acid load. Adapted from Daniel and col. [103]

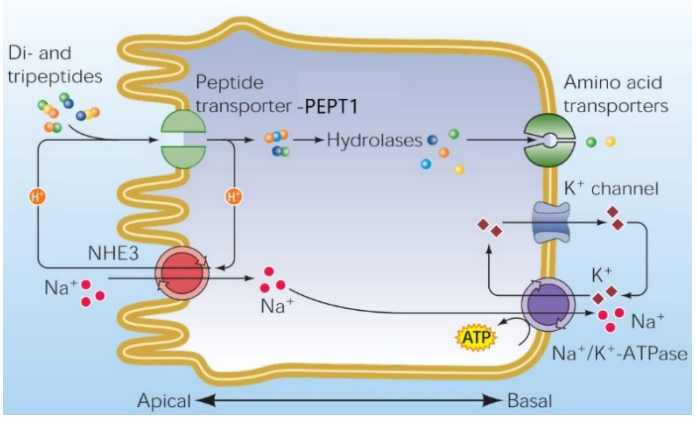
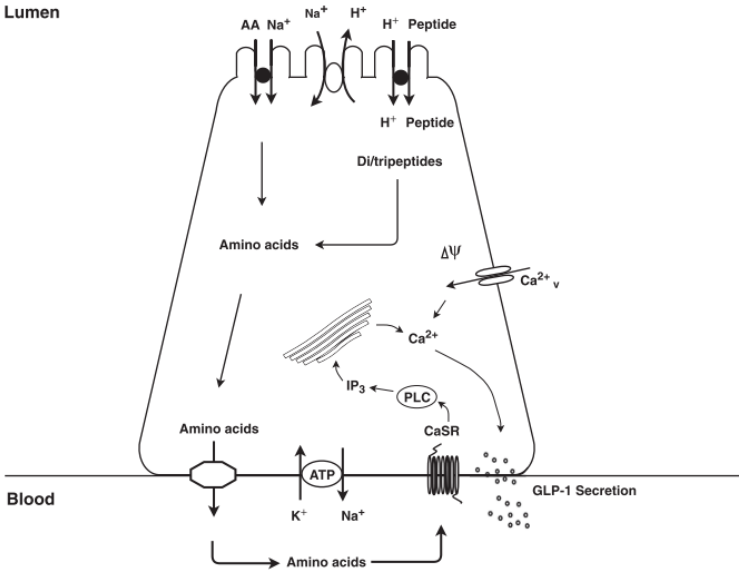


Figure 2. Illustration of the endocrine L cell and the proposed mechanisms by which peptone stimulates GLP-1 release. Di/tripeptides are taken up by PepT1 and are degraded by cytosolic peptidases to their respective amino acids. Intracellular amino acids are then transported to the interstitial side through basolateral amino acid transporters, wherefrom they stimulate the L cells by activating amino acid sensors, like CaSR, situated on the basolateral. Adapted from Modvig IM and col. [75]



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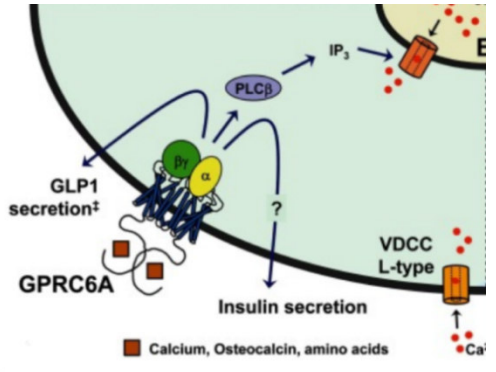
826 **Figure 3:** Signaling through GPRC6A in β- or Gut Cells. GPC6A can be directly
827 activated by amino acids and use calcium as an allosteric regulator. Adapted from
828 Wauson EM and col.[104]

829



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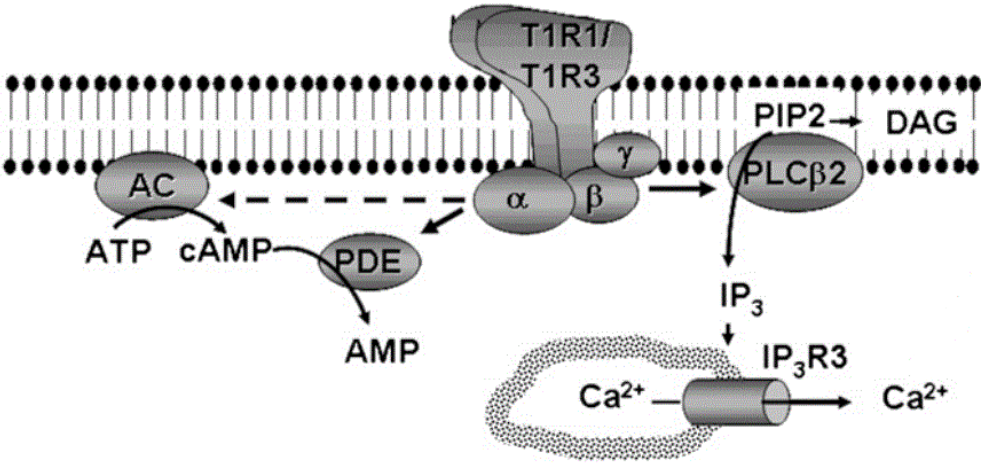
832 **Figure 4**: The T1R1/T1R3 heterodimer is coupled to a heteromeric G protein, where
833 the Gbc subunit appears to mediate the predominant leg of the signalling pathway.
834 Ligand-binding activates Gbg, which results in activation of phospholipase C B,
835 which produces inositol trisphosphate (IP3) and diacylglycerol. IP3 activates the

IP3R3 which results in the release of $Ca2^{+}$ from intracellular stores. Adapted from Kinnamon SC [105]



839 **Table 1**. Stimulation of GLP-1 secretion by protein and protein hydrolysates in human.

Protein	***Hydrolysis conditions***	***Subjects***	***n***	***Protein dose***	***Secretion***	***Increment versus***	***Ref***
Turkey	Intact protein	Healthy subjects	8	Ingestion of 352 g	↑	Fat isocaloric meal	(23)
Whey	N.D	Obese and lean men	12	Intraduodenal infusion of 24 g	↑	Saline	(57)
				Intraduodenal infusion of 48 g			
Whey	N.D	Healthy men	16	Intraduodenal infusion 8 g	↑	Saline	(58)
				Intraduodenal infusion 24 g			
				Intraduodenal infusion 48 g			
Whey	Intact protein	T2DM subjects of both gender	21	Ingestion of 17 g	↑	Sucralose	(59)
Whey	Intact protein	T2DM subjects of both gender	15	Ingestion of 50 g	↑	Water	(60)
Casein	Intact protein	Overweight to obese men and women	24	Ingestion of 30 g	↑	Time 0	(61)
Whey							
Casein	N.D						

Whey	Intact protein	Healthy men and women	9	Ingestion of 48 g	↑	Casein	(62)
Whey	Alcalase/53ºC/pH 8.0/- + Neutrase/53ºC/pH 7.0/-	Healthy men	6	Stomach infusion of 36 g	↑	Time 0	(63)
	Intact protein						
Casein	Alcalase/53ºC/pH 8.0/- + Neutrase/53ºC/pH 7.0/-						
	Intact protein						
Whey	N.D	T2DM subjects of both gender	11	Ingestion of 45 g	↑	CGMP enhanced whey	(64)

840 Note: *n* indicates the number of subjects per group.

841 Abbreviations: N.D, hydrolysis conditions not described; T2DM, Type 2 Diabetes Mellitus; CGMP, casein glycomacropeptide.

842 ↑ Indicates that GLP-1 secretion is incremented versus de control, specified in each row.

843

844 **Table 2**. Stimulation of GLP-1 secretion by protein and protein hydrolysates i*n vitro*.

Protein	***Hydrolysis conditions***	***Cell line***	***Protein Treatment***	***Secretion***	***Increment versus***	***Ref***
Egg albumin	N.D	STC-1	2 h 2.5-20 mg/ml	↑	KRB	(26)
Meat						
Meat	N.D	Small intestinal cultures	2 h 5.0-50 mg/ml	↑	Saline with 0.1% BSA	(37)
			2 h 0.5 mg/ml	-		
Milk	N.D	Small intestinal cultures	2 h 5.0 mg/ml	↑		
Vegetables						
Casein	Intact protein	STC-1	2 h 1.0 mg/ml	↑	Hanks Buffered Salt Solution buffer	(53)
Codfish						
Egg						
Pea (DPS)						
Wheat						
Whey						

Ovomucoid	Intact protein	STC-1	2 h 1.0 mg/ml	-		
Pea (Pisane)						
Pea (SM)						
Soybean						
Casein hydrolysate	N.D	STC-1	2 h 1.0 mg/ml	-		
Egg	N.D	STC-1	2 h 1.0 mg/ml	↑		
Pea	Subtilisin/-/pH 8.0/- + PSE/-/pH 6.0/-	STC-1	2 h 1.0mg/ml [1]	-		
Pea HP90	N.D	STC-1	2 h 1.0 mg/ml	-		
Wheat						
Yoghurt whey	Intact protein	STC-1 pGIP/neo	3 h 5.0-25 mg/ml	↑	HEPES	(54)
	Intact protein	STC-1 pGIP/neo	3 h 50-100 mg/ml	↓		
Cheese whey	Intact protein	STC-1 pGIP/neo	3 h 5.0-10, 100 mg/ml	-		
			3 h 25-50 mg/ml	↑		

β-Lactoglobulin	Intact protein	STC-1 pGIP/neo	3 h 0.63-10 mg/ml	↑		
			3 h 0.31 mg/ml	-		
	Chymotrypsin/37ºC/pH 7.4/30 min	STC-1 pGIP/neo	3 h 10 mg/ml	↑		
	Trypsin/37ºC/pH 7.4/30-150 min					
	Chymotrypsin/37ºC/pH 7.4/60-150 min	STC-1 pGIP/neo	3 h 10 mg/ml	-		
α-Lactalbumin	Intact protein	STC-1 pGIP/neo	3h 0.31-0.63 mg/ml	-		
			3 h 1.3-10 mg/ml	↑		
Casein	Intact protein	STC-1 pGIP/neo	3 h 0.31-10 mg/ml	↑	HEPES	(55)
α-Casein	Intact protein	STC-1 pGIP/neo	3 h 0.16-5.0 mg/ml	↑		
β-Casein						
κ-Casein	Intact protein	STC-1 pGIP/neo	3 h 0.16-0.31 mg/ml	-		
			3 h 0.63-5.0 mg/ml	↑		
α-Casein	Chymotrypsin/37ºC/pH 7.4/30-150 min	STC-1 pGIP/neo	3 h 5.0 mg/ml	-		
	Trypsin/37ºC/pH 7.4/30-150 min	STC-1 pGIP/neo	3 h 5.0 mg/ml	↑		

	Pepsin/37ºC/pH 2.3/30-150 min					
β-Casein	Chymotrypsin/37ºC/pH 7.4/30-150 min	STC-1 pGIP/neo	3 h 5.0 mg/ml	-		
	Trypsin/37ºC/pH 7.4/30-150 min					
	Pepsin/37ºC/pH 2.3/30-150 min	STC-1 pGIP/neo	3 h 5.0 mg/ml	↑		
Whey	Intact protein	STC-1	4 h 10.0 mg/ml	↑	KRB with 10 mM Glucose	(56)
	Pepsin/37ºC/pH 2/90 min + CorPP/37ºC/pH 7.5/150 min	STC-1	4 h 10 mg/ml	-		
Whey DH32	N.D	STC-1	4 h 10 mg/ml	-		
	Pepsin/37ºC/pH 2/90 min + CorPP/37ºC/pH 7.5/150 min					
Whey DH45	N.D	STC-1	4 h 10 mg/ml	-		
	Pepsin/37ºC/pH 2/90 min + CorPP/37ºC/pH 7.5/150 min					
Corn Zein	Papain/55ºC/pH 7.2/60 min	GLUTag	1 h 10 mg/ml	↑	HEPES	(67)
	Papain/55ºC/pH 7.2/60 min + Pepsin/37ºC/pH 1.85/60 min					

	+ Pancreatin + trypsin/37ºC/pH 8.2/120 min					
	Papain/55ºC/pH 7.2 + Pronase/37ºC/pH 7.0					
Corn Zein	Papain/55ºC/pH 7.0/60 min	GLUTag	1 h 2.0 mg/ml	-	HEPES	(68)
			1 h 5.0-20 mg/ml	↑		
Egg albumin	N.D	GLUTag	1 h 5.0 mg/ml	-		
BSA	Intact protein					
Meat	N.D					
Bean	Pepsin/37C/pH 1.9/10 min					
Rice endosperm	Papain/55ºC/pH 7.2/60 min	GLUTag	1 h 10 mg/ml	↑	HEPES	(69)
	Pepsin/37ºC/pH 1.85/30 min					
	Pepsin/37ºC/pH 1.85/60 min	GLUTag	1 h 10 mg/ml	-		
Rice bran	Papain/55ºC/pH 7.2/60 min	GLUTag	1 h 10 mg/ml	-		
	Pepsin/37ºC/pH 1.85/30 min	GLUTag	1 h 10 mg/ml	↑		

	Pepsin/37ºC/pH 1.85/60 min					
Wheat (770 Da fraction)	N.D	GLUTag	2 h 0.1-0.25 mg/ml	-	Saline	(70)
			2 h 0.5-1.0 mg/ml	↑		
Wheat (7740 Da fraction)	N.D	GLUTag	2 h 1.0 mg/ml	-		
Wheat gluten	N.D	GLUTag	1 h 5 mg/ml	-	HEPES	(71)
			1 h 10 mg/ml	↑		
α-Lactalbumin	N.D	GLUTag	1 h 5 mg/ml	-		
			1 h 10 mg/ml	↑		
Wheat gluten	N.D + Pepsin/37ºC/pH 1.85/30-60 min + Pancreatin/37ºC/pH 8.2/60-120 min	GLUTag	1 h 10 mg/ml	↑		
α-Lactalbumin	N.D + Pepsin/37ºC/pH 1.85/30-60 min + Pancreatin/37ºC/pH 8.2/60-120 min	GLUTag	1 h 10 mg/ml	↑		

Cuttlefish Viscera	Intact protein + Salivary fluid	STC-1	2 h 13 mg/ml	▬	Baseline	(72)
	H^a/50ºC/pH 8.0/4 h + Salivary fluid	STC-1	2 h 13 mg/ml	↑	UCVP + Salivary fluid	
	H^b/50ºC/pH 8.0/4 h + Salivary fluid	STC-1	2 h 13 mg/ml	▬	UCVP + Salivary fluid	
	H^a/50ºC/pH 8.0/4 h + Salivary fluid + Pepsin/37ºC/pH 2.5-3/120 min	STC-1	2 h 13 mg/ml	▬	UCVP + IVD	
	H^b/50ºC/pH 8.0/4 h + Salivary fluid + Pepsin/37ºC/pH 2.5-3/120 min	STC-1	2 h 13 mg/ml	↓	UCVP + IVD	
	H^a/50ºC/pH 8.0/4 h + Salivary fluid + Pepsin/37ºC/pH 2.5-3/120 min + Pancreatin/37ºC/pH 7.0/120 min	STC-1	2 h 13 mg/ml	▬	UCVP + IVD	
	H^b/50ºC/pH 8.0/4 h + Salivary fluid	STC-1	2 h 13 mg/ml	↓	UCVP + IVD	

	+ Pepsin/37ºC/pH 2.5-3/120 min + Pancreatin/37ºC/pH 7.0/120 min					
Bovine haemoglobin	Intact protein + Salivary fluid	STC-1	2 h 13 mg/ml	↑	HEPES	
Bovine haemoglobin	Salivary fluid + Pepsin/37ºC/pH 2.5-3.0/60-120 min	STC-1	2 h 13 mg/ml	↑	HEPES	(73)
	Salivary fluid + Pepsin/37ºC/pH 2.5-3.0/120 min + Pancreatin/37ºC/pH 7.0/30-120 min					
	Salivary fluid + Pepsin/37ºC/pH 2.5-3.0/120 min + Pancreatin/37ºC/pH 7.0/120 min	STC-1	2 h 5.0 mg/ml			
			2 h 10 mg/ml			
Meat	N.D	GLUTag	2 h 1.0-50 mg/ml	↑	Baseline	(74)
Salmon skin gelatin	Alcalase/50ºC/pH 7.0/4 h	GLUTag	2 h 2.5 mg/ml	↑	Glucose 2 mM	(76)
	Alcalase + Flavourzyme /50ºC/pH 7.0/4 h					
	Promod/50ºC/pH 7.0/4 h	GLUTag	2 h 2.5 mg/ml	-		

	Alcalase + Flavourzyme /50ºC/pH 7.0/4 h + Pepsin/37ºC/pH 2.0/90 min + CorPP/37ºC/pH 7.0/150 min	GLUTag	2 h 2.5 mg/ml	-		
Salmon trimmings	Alcalase/50ºC/pH 7.0/4 h	GLUTag	2 h 2.5 mg/ml	-		
	Alcalase + Flavourzyme /50ºC/pH 7.0/4 h	GLUTag	2 h 2.5 mg/ml	↑		
	Promod/50ºC/pH 7.0/4 h	GLUTag	2 h 2.5 mg/ml	↓		
	Alcalase + Flavourzyme /50ºC/pH 7.0/4 h + Pepsin/37ºC/pH 2.0/90 min + CorPP/37ºC/pH 7.0/150 min	GLUTag	2 h 2.5 mg/ml	↑		
Meat	N.D	NCI-H716	2 h 20 mg/ml	↑	KRB with 0.2 % BSA	(82)
Chicken feet	Neutrase/25ºC/pH 7.0/24 h	STC-1	2 h 5 mg/ml	↑	HEPES	(99)
		Ileum explants	1 h 15 mg/ml	↑	KRB with 10 mM Glucose	

Note: The salivary fluid does not contain enzymes.

Pea protein origin: Pisane, from Cosucra; SM, from Nutralys; DPS, from Dutch Protein Services; HP90, from Triballat; DSM, from DSM Food Specialties.

Abbreviations: N.D, hydrolysis conditions not described; DPS, Dutch Protein Services; PSE, Proline-Specific Endoprotease; PSH, Protease from Smooth Hound; KRB, Krebs-Ringer Modified Buffer; BSA, Bovine Serum Albumin; CorPP, a porcine pancreatic enzyme preparation; DH32, 32% degree of hydrolysis; DH45, 45% degree of hydrolysis; H, Hydrolysis; UCVP, Undigested Cuttlefish Viscera Protein; IVD, *In Vitro* Digestion with pepsin and pancreatin, always indicate the same hydrolysis conditions as the protein that is compared to; DMEM, Dulbecco's Modified Eagle Medium.

[a] Hydrolysis with cuttlefish hepatopancreas digestive proteases.

[b] Hydrolysis with cuttlefish smooth hound intestine digestive proteases.

[1] This pea hydrolysate does not stimulate GLP-1 secretion nor the 10kDa permeate. Nevertheless, the supernatant obtained after centrifugation increase GLP1 secretion compared to the control.

↑ Indicates that GLP-1 secretion is incremented versus de control, specified in each row.

↓ Indicates that GLP-1 secretion is reduced versus de control, specified in each row

▬ Indicates that GLP-1 secretion is not altered versus de control, specified in each row.

861 **Table 3**. Stimulation of GLP-1 secretion by protein and protein hydrolysates in animals.

Protein	*Hydrolysis conditions*	*Specie*	*n*	*Protein dose*	*Secretion*	*Increment versus*	*Ref*
Egg albumin	N.D	Wistar male rats	7-9	Jejuno-ileum administration of 25 mg/ml	↑	Saline	(26)
				Jejuno-ileum administration of 50 mg/ml			
				Colon administration of 25 mg/ml			
				Colon administration of 50 mg/ml			
Salmon skin gelatin	Flavourzyme/50ºC/pH 7.0/4 h	Sprague–Dawley male rats[a]	12	5 weeks Oral gavage 300 mg/day	↑	Water	(47)
Porcine skin gelatin	Flavourzyme/50ºC/pH 7.0/6 h	Sprague–Dawley male rats	12	6 weeks Oral gavage 300 mg/day	–	Water	(48)
	Flavourzyme/50ºC/pH 7.0/6 h	Sprague–Dawley male rats[a]		6 weeks Oral gavage 300 mg/day	↑		
Halibut skin gelatin	Flavourzyme/50ºC/pH 7.0/4 h	Sprague–Dawley male rats[a]	11	4 weeks 750 mg/kg/day	↑	Water	(49)
Tilapia skin gelatin	Flavourzyme/50ºC/pH 7.0/6 h	Sprague–Dawley male rats		4 weeks 750 mg/kg/day	–		
Whey	Intact protein	SPF Wistar male rats	9	Oral administration ~ 3g/Kg BW	–	Sucrose	(65)
Pea							
Pea	Intact protein	Sprague–Dawley male rats	10	Intragastric infusion of 136 mg/ml	–	Saline	(66)

	N.D						
Corn Zein	Papain/55ºC/pH 7.2/60 min	Sprague-Dawley male rats	7-10	Oral administration[c] of 2 g/Kg BW	↑	Water	(67)
Meat	N.D	Sprague-Dawley male rats	7-10	Oral administration[c] of 2 g/Kg BW	-		
Corn Zein	Papain/55ºC/pH 7.2/60 min	Goto-Kakizaki male rats	6-7	Oral administration[b] of 2 g/Kg BW	↑		
Whey	Papain/55ºC/pH 7.2/60 min	Goto-Kakizaki male rats	6-7	Oral administration[b] of 2 g/Kg BW	-		
Corn Zein	Papain/55ºC/pH 7.0/60 min	Sprague-Dawley male rats	6-9	Duodenal administration of 100-250 mg/ml	↑	Water	(68)
				Ileal administration of 100 mg/ml	-		
				Ileal administration of 250 mg/ml	↑		
				Duodenal loop administration of 300 mg/ml	-	Time 0	
				Jejunal loop administration of 300 mg/ml			
				Ileal loop administration of 300 mg/ml			
Rice Endosperm	Pepsin/37ºC/pH 1.85/30 min	Sprague-Dawley male rats	4-6	Oral administration of 2 g/Kg BW	↑	Water	(69)
				Oral administration[b] of 0.1-1.0 g/Kg BW	-		
				Oral administration[b] of 2 g/Kg BW	↑		

				Oral administration[c] of 1-2 g/Kg BW	↑		
Rice bran	Pepsin/37ºC/pH 1.85/30 min	Sprague-Dawley male rats	4-6	Oral administration of 2 g/Kg BW	↑		
				Oral administration[b] of 0.1-1.0 g/Kg BW	–		
				Oral administration[b] of 2 g/Kg BW	↑		
Wheat (770 Da fraction)	N.D	Sprague-Dawley male rats	8	Oral administration[c] of 2g/Kg BW	↑	Saline	(70)
Wheat gluten	N.D	Wistar/ST male rats	5-7	Oral administration of 1g/Kg BW	–	Water	(71)
α-Lactalbumin							
Meat	N.D	Wistar male rats	6	Duodenal infusion of 50 mg/ml	↑	Baseline	(75)
Lysozyme	Alcalase/60ºC/pH 8.0/6 h	ZDF male rats	9	Oral administration of 1g/Kg BW	–	Untreated rats	(100)
Corn Zein	Papain/55ºC/pH 7.0/60 min	Sprague-Dawley male rats	6-8	Ileal administration[c] of 250 mg/ml	↑	Water	(101)
Meat	N.D						

862 Note: *n* indicates the number of animals per group.

863 Abbreviations: N.D, hydrolysis conditions not described; BW, Body Weight; ZDF, Zucker Diabetic Fatty.

864 [a] Sprague–Dawley streptozotocin-induced diabetic rats.

865 [b] Changes in plasma GLP-1 after oral administration of the protein under the oral glucose tolerance test (OGTT).

866 [c] Changes in plasma GLP-1 after oral administration of the protein under the intraperitoneal glucose tolerance test (IPGTT).

867 ↑ Indicates that GLP-1 secretion is incremented versus de control, specified in each row.

868 – Indicates that GLP-1 secretion is not altered versus de control, specified in each row.

869