Food-derived dipeptidyl-peptidase IV inhibitors as a potential approach for glycemic regulation – current knowledge and future research considerations

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Abstract

Background: Diabetes, which currently affects 1 in 11 adults, is considered one of the biggest worldwide health crises of the 21st century. Over the last decade, synthetic inhibitors of the enzyme dipeptidyl-peptidase IV (DPP-IV) have emerged as an effective pharmaceutical approach for the management of type 2 diabetes. These molecules exert their beneficial effect by preventing the inactivation of gut-derived hormones that play a pivotal role in glycemic regulation. More recently, food components have been suggested as sources of DPP-IV inhibitors with the potential to help manage blood glucose levels.

Scope and approach: This review examines the sources, production, molecular characteristics and modes of action of food-derived DPP-IV inhibitors. Insights into the needs for future research to validate their efficacy and to establish their application in the management of type 2 diabetes are also discussed.

Key findings and conclusions: To date, hydrolysates of protein from a variety of food commodities, including both plant and animal sources, have been shown to be able to inhibit the activity of the DPP-IV enzyme. Moreover, a number of peptides, either isolated from these hydrolysates or synthetically produced, as well as non-protein-derived compounds such as polyphenols, have also been identified as DPP-IV inhibitors. These food-derived constituents present different degrees of potency and modes of action on the DPP-IV enzyme. While their effectiveness in humans is currently unknown, findings from *in vitro* and animal studies conducted to date warrant further research to evaluate their potential as functional food ingredients for glycemic regulation.

Keywords: Dietary constituent; dipeptidyl-peptidase IV inhibitor; phenolic compound; peptide; protein hydrolysate; type 2 diabetes

1. Introduction

Diabetes and its complications are major causes of mortality, accounting for 14.5% of global all-cause mortality among adults aged 20 to 79 years old (International Diabetes Federation, 2015). In spite of the increasing awareness of the social and economic impacts of diabetes and the development of new treatments, the incidence and prevalence of this multifactorial disorder have been unrelentingly rising. The International Diabetes Federation estimates that 415 million people (1 in 11 adults) are currently living with diabetes and predicts that, if the present demographic growth continues, 642 million (1 in 10 adults) will be affected with this metabolic disorder by 2040.

Characterized by hyperglycemia resulting primarily from defects in insulin secretion and insulin action (DeFronzo, 2009), type 2 diabetes is the most prevalent form of diabetes, accounting for about 90% of cases diagnosed (International Diabetes Federation, 2015).

While the exact causes leading to the development of type 2 diabetes are still unknown, a number of risk factors, including physical inactivity, excess body weight, and unhealthy diet, have been identified (International Diabetes Federation, 2015). Inadequate glycemic control can lead to an array of serious and debilitating microvascular (e.g. retinopathy, nephropathy, neuropathy) and macrovascular (e.g. cardiovascular diseases such as stroke and heart attack) complications (Fowler, 2008). Therefore, developing effective strategies to restore and maintain blood glucose homeostasis is of primary importance.

Type 2 diabetic patients have access to a number of pharmacologic therapies that are based primarily upon increasing insulin availability, either by direct administration of insulin or via agents promoting insulin secretion, improving insulin sensitivity, delaying gastrointestinal absorption of carbohydrates and/or increasing glucose excretion (DeFronzo, Triplitt, Abdul-Ghani, & Cersosimo, 2014). Of the twelve classes of glucose-lowering drugs currently available for the management of diabetes, inhibitors of the enzyme dipeptidyl-peptidase IV (DPP-IV) are among the newest agents to have been introduced to the type 2 diabetes pharmacopeia. These synthetic inhibitors, which can be used either as monotherapy or in combination with other anti-diabetic drugs (Craddy, Palin, & Johnson, 2014), exert their glucose-lowering effect by preventing the degradation of gut-derived hormones that play a pivotal role in glycemic regulation (Filippatos, Athyros, & Elisaf, 2014).

Diet is well recognized to play an important role in the prevention and management of diabetes. Over the past few decades, numerous studies have reported putative associations between the consumption of certain foods, or their constituents, and the incidence of diabetes (Lacroix & Li-Chan, 2014a). Moreover, compelling findings from *in vitro* as well as animal and clinical studies have shown that some dietary factors, such as peptides and phenolic compounds, can help regulate blood glucose levels (Lacroix & Li-Chan, 2014a). Recent research has suggested that one of the plausible mechanisms of action underlying the anti-diabetic effect of various food commodities could reside in the ability of their constituents to inhibit the DPP-IV enzyme. In particular, several food proteins, such as those found in milk, egg and fish, were shown by *in silico* analysis to contain within their sequences peptides able to inhibit the activity of the DPP-IV enzyme (Lacroix & Li-Chan, 2012a). The discovery that dietary factors may be sources of natural DPP-IV inhibitors that could potentially complement pharmacotherapy in the regulation of blood glucose levels has truly captured the attention of the scientific community. As a result, there has been an emergence of literature from research groups around the world describing the production and identification of DPP-IV inhibitors from a variety of food commodities.

The objectives of this review are to describe the role of DPP-IV in blood glucose regulation and to investigate the potential of dietary constituents to serve as natural inhibitors of this enzyme. The sources, production, molecular characteristics and modes of action of these food-derived inhibitors will be discussed and the needs for further research to validate their efficacy and potential commercialization for application in the management of type 2 diabetes will be examined.

2. DPP-IV, the incretin hormones and their roles in blood glucose regulation

First described in 1966 (Hopsu-Havu & Glenner, 1966), dipeptidyl-peptidase IV (DPP-IV; EC 3.4.14.5), also originally known as lymphocyte cell surface marker CD26 or as the adenosine deaminase (ADA)-binding protein, is a 110 kDa glycoprotein existing primarily as a membrane-anchored cell-surface enzyme (Filippatos et al., 2014). DPP-IV belongs to the prolyl oligopeptidase family, a group of structurally related enzymes that preferentially remove N-terminal dipeptides from substrates (Thoma et al., 2003), and is known to take part in a number of biological processes as both a regulatory protease and a binding protein (Zhong, Rao, & Rajagopalan, 2013). While *in vivo* the enzyme is responsible for cleaving the amide bond that releases a dipeptide from the amino terminal end of a number of molecules such as neuropeptides, chemokines and regulatory peptides (Zhong et al., 2013), DPP-IV is more widely known for its catalytic activity against the incretin hormones glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GIP is a 42 amino acid long peptide derived from the proGIP gene, while GLP-1 is produced from processing of the proglucagon gene to yield primarily two active forms, glycine-extended GLP-$1_{7–37}$ and GLP-$1_{7–36}$ amide, the latter being the most abundant in human plasma (Tasyurek, Altunbas, Balci, & Sanlioglu, 2014).

Secreted from the neuroendocrine L and enteroendocrine K cells respectively in response to intake of nutrients (**Figure 1**), GLP-1 and GIP exert glucose lowering effects by engagement of G-protein-coupled receptors (GLP-1R and GIP-R) that are expressed on pancreatic β- and α-cells as well as peripheral tissues (Tasyurek et al., 2014). By way of its action on the pancreatic β-cells, GLP-1 promotes insulin secretion as well as insulin gene transcription and biosynthesis. This incretin hormone has also been suggested to have trophic effects on pancreatic β-cells and has been reported to inhibit glucagon release, suppress appetite and food intake, as well as retard gastric emptying (Phillips & Prins, 2011; Tasyurek et al., 2014). Like GLP-1, GIP stimulates insulin secretion in a glucose-dependent manner. Besides its insulinotropic effect, the GIP hormone also elicits glucagon release and is involved in fat metabolism (Tasyurek et al., 2014).

Being responsible for approximately 50-70% of the total insulin secreted following glucose intake, the incretin hormones are important mediators of glycemic homeostasis (Baggio & Drucker, 2007). However, once secreted, GLP-1 and GIP are rapidly hydrolyzed by the DPP-IV enzyme into shorter and inactive molecules (**Figure 1**). The discovery that DPP-IV is responsible for the inactivation of more than 95% of the secreted GLP-1 has drawn considerable attention to this enzyme as a target for the management of type 2 diabetes (Thoma et al, 2003). Consequently, extensive efforts have been put towards the development of small molecules able to inhibit the activity of DPP-IV and therefore prolong the activity of the incretins. This growing body of research has led to the discovery of a number of DPP-IV inhibitors that were designed based on molecular modeling and knowledge from X-ray crystallographic data of the amino acid residues forming the enzyme's active site, including the catalytic triad (Havale & Pal, 2009). Since the launch in 2006 of the first DPP-IV inhibitor, sitagliptin, at least 10 other synthetic inhibitors have been approved for the management of type 2 diabetes (Deacon & Lebovitz, 2016). While these molecules, often referred to as 'gliptins', all

competitively and reversibly bind to the active site of DPP-IV, they differ greatly in their pharmacodynamic as well as their pharmacokinetic properties. Nevertheless, meta-analyses have shown no major differences between them in terms of their ability to improve blood glucose regulation (Craddy et al, 2014).

3. Dietary proteins as precursors of DPP-IV inhibitory peptides

Over the past few years, proteins from a variety of food commodities have been studied for their potential to serve as sources of inhibitors against the DPP-IV enzyme. Complementary to empirical methods, computer-assisted techniques have also been employed to predict the potential of dietary proteins as precursors of DPP-IV inhibitors and therefore assist in the selection of the best proteins to produce these bioactive peptides (Lacroix & Li-Chan, 2012a; Nongonierma & FitzGerald, 2014a; Udenigwe, Gong, & Wu, 2013). These *in silico* investigations, which are based on the occurrence within the food protein molecule of peptide sequences reported to possess DPP-IV inhibitory activity, have revealed the presence of potential DPP-IV inhibitors within the sequences of a variety of proteins from both plant and animal sources. Among the proteins investigated, those from milk (Lacroix & Li-Chan, 2012a; Udenigwe et al., 2013; Nongonierma & FitzGerald, 2014a), collagen (Lacroix & Li-Chan, 2012a), as well as canola, chicken egg, oat and wheat (Nongonierma & FitzGerald, 2014a) were suggested to be particularly promising sources of DPP-IV inhibitory peptides.

Despite the knowledge that DPP-IV inhibitory peptides can be potentially generated from food proteins, only a limited number of studies have identified the DPP-IV inhibitory peptides that were actually released upon hydrolysis of these proteins (**Table 1** and section 3.1). In fact, a large number of the DPP-IV inhibitory peptides reported in the literature have been discovered through chemical synthesis of sequences that can be found within dietary proteins, and not empirically from the protein hydrolysates *per se* (**Table 2** and section 3.2).

3.1 Protein hydrolysates and their constituent peptides with DPP-IV inhibitory activity

Enzymatic hydrolysis has been the primary approach to generate DPP-IV inhibitory peptides from dietary proteins. A variety of common food commodities, including milk, egg, fish, corn, as well as less common ones such as amaranth, quinoa and hemp, have been explored to produce protein hydrolysates and peptides with DPP-IV-inhibiting properties (**Table 1** & **Figure 2**). To date, proteins from cow's milk have been the most extensively investigated sources of DPP-IV inhibitors, hydrolysates of dairy ingredients and proteins from both the casein and whey fractions of milk having been reported in a number of studies to inhibit DPP-IV activity *in vitro*. Collagen from fish and mammals has also attracted notable attention as a potential source of DPP-IV inhibitory peptides partly due to its high content in proline residues, an amino acid often occurring in peptides reported to present DPP-IV inhibitory activity (**Tables 1** & **2**).

As shown in **Table 1** and **Figure 2**, a variety of enzymes have been employed to produce DPP-IV inhibitory peptides from dietary proteins, including food-grade proteinases such as Alcalase, Flavourzyme, and Protamex derived from microorganisms, as well as enzymes obtained from animal (e.g. pepsin, trypsin, Corolase PP) and plant (e.g. papain, protease from pumpkin) sources. Additionally, protein digests produced by *in vitro* simulated gastrointestinal digestion have also been reported to be able to inhibit DPP-IV activity, therefore suggesting that DPP-IV inhibitory peptides might be generated *in vivo* during the digestion process.

Fractionation of DPP-IV-inhibiting protein hydrolysates and digests and analysis of the most potent fractions by mass spectrometry have allowed the identification of a number of peptide sequences contributing to the observed inhibitory activity. As shown in **Table 1**, these peptides vary widely in terms of their length (2 to 17 amino acids long), amino acid composition and potency (half-maximal inhibitory concentrations (IC_{50}) values ranging from 5 µM to >20 000 µM). Similar to the hydrolysates whose IC_{50} values ranged from µg/mL to mg/mL (**Figure 2**), the identified DPP-IV inhibitory peptides show much weaker effect on DPP-IV activity than the synthetic DPP-IV inhibitors currently used for the treatment of type 2 diabetes (IC_{50} in the nM range) (Hunziker, Henning, & Peters, 2005).

3.2 Peptides with DPP-IV inhibitory activity

In addition to the peptides isolated from protein hydrolysates presented in **Table 1**, many other peptides able to inhibit the DPP-IV enzyme have been identified (**Table 2**). These peptides, which were not isolated from hydrolysates but either are likely to occur or else have actually been identified in the sequence of dietary proteins, were synthetically produced and studied for their effect on DPP-IV activity.

As shown in **Table 2**, many of the peptides studied and reported to have DPP-IV inhibitory activity are dipeptides. In addition to the 70 dipeptides with DPP-IV inhibitory activity, 43 other peptides varying from three to ten amino acids in length and that can be found in the sequences of commonly consumed proteins such as those from milk and soy, have been shown to be able to inhibit the DPP-IV enzyme. With IC_{50} values ranging from 4 µM to >20,000 µM, the potency of these peptides is similar to that of the peptides that were identified in protein hydrolysates (**Table 1**). The tripeptide IPI (also known as diprotin A), which can be found in the sequence of κ-casein, is a well-known DPP-IV inhibitor and the most potent (IC_{50} = ~ 4 µM) of currently known peptides. Although not as effective as diprotin A, the peptides WR, IPIQY and WCKDDQNPHS occurring in the sequence of lactoferrin, κ-casein, and α-lactalbumin, respectively, are among the most potent food protein-derived DPP-IV inhibitors that have been reported to date (**Table 2**). None of the DPP-IV inhibitory peptides discovered so far have been as effective as the gliptins at inhibiting the enzyme.

Unlike the peptides listed in **Table 1**, which were actually isolated from and identified to occur in protein hydrolysates, it is unknown whether the peptides presented in **Table 2**

could in fact be released from dietary proteins during digestion or enzymatic treatment with proteases.

3.3 Structural characteristics and mode of action of DPP-IV inhibitory peptides

Investigations to elucidate the possible structure-activity relationship of DPP-IV inhibitory peptides have suggested that the specific amino acid sequence is the predominant factor determining DPP-IV inhibitory activity. Various physicochemical properties of the peptides, including length, isoelectric point, hydrophobicity and net charge, have not been found to be correlated with their DPP-IV inhibitory activity (Lacroix & Li-Chan, 2014b; Nongonierma, Mooney, Shields, & FitzGerald, 2014). *In silico* studies based on the alignment of peptide sequences with known DPP-IV inhibitory activity have shown that potent inhibitory peptides generally contain a branched-chain amino acid or an aromatic residue with a polar group in the side-chain (primarily tryptophan) at their N-terminal and/or a proline residue at their P_1 position (Nongonierma & FitzGerald, 2014a; Tulipano, Faggi, Nardone, Cocchi, & Caroli, 2015). A recent analysis by Lan et al. (2015) of a peptide library containing 337 dipeptides also revealed the presence of a tryptophan residue at the N-terminal position of several of the most potent peptides. The authors pointed out, however, that although the residue at the N-terminal position seems to have a major impact on a dipeptide's ability to inhibit DPP-IV, the C-terminal amino acid also affects its potency likely because both residues are involved in the interaction with the enzyme.

In vitro kinetic assays and molecular docking models have been used to investigate the interaction between peptides and the DPP-IV enzyme. As shown in **Table 2**, different peptides have been found to exhibit different inhibition behaviors, including competitive, un-competitive, non-competitive and mixed-type modes of action, therefore indicating that they exert their effect by binding either at the active site and/or outside the catalytic center of the enzyme. Early work on the catalytic properties of DPP-IV by Yan, Ho, & Hou (1992) revealed that dipeptides of the general structure Xaa-Pro, Pro-Xaa, or Xaa-Ala (where Xaa represents any amino acid) generally inhibit the enzyme in a competitive manner. A competitive mode of action has also been observed for most food-derived peptides having a proline at their P_1 position (**Tables 1** & **2**). The DPP-IV enzyme is known to preferentially act on substrates bearing proline or other small uncharged residues such as serine and alanine at their penultimate amino acid position (Engel et al., 2003). It has been proposed that, similarly to diprotin A, which exhibits a competitive mode of action but has been shown to actually be a substrate with a low turnover rate (Rahfeld, Schierhorn, Hartrodt, Neubert, & Heins, 1991), food protein-derived peptides with a proline at their P_1 position may act as substrates for the enzyme, their apparent competitive behavior being a kinetic artifact resulting from their substrate-mimicking structure. On the other hand, the majority of peptides containing a tryptophan amino acid at their N-terminal display un-competitive or non-competitive inhibition toward the DPP-IV enzyme (**Table 2**). Computational modeling of a Trp-Arg-Xaa tripeptide library suggested that the side chain of the N-terminal tryptophan interacts with the Phe357 residue located within the enzyme's hydrophobic S2 pocket (Lan, Ito, Ito, & Kawarasaki, 2014). Similarly, binding of the tryptophan-containing dipeptide Trp-Val was proposed to

occur at a secondary site located near the enzyme's active site (Nongonierma, Mooney, Shields, & FitzGerald, 2013).

It can be noted that there is some discrepancy in the mode of action reported by different research groups for the same peptide (**Table 2**). This variability may result from differences in the experimental conditions used for the enzyme kinetics assay, such as the type of substrate and enzyme specie, as well as in the methods for data analysis, such as linear versus non-linear regression models. Porcine and recombinant human DPP-IV are the most common species that have been used to assess the inhibitory activity of molecules. Although the enzyme sequence is highly conserved among mammalian species, the porcine and human DPP-IV enzymes are not identical and different inhibition patterns between the two species have been reported (Lacroix & Li-Chan, 2015; Stöckel-Maschek et al., 2003).

4. Other food constituents with DPP-IV inhibitory activity

While the vast majority of food-derived inhibitors reported to date in the literature to have DPP-IV inhibitory activity are derived from proteins, studies on DPP-IV inhibitors from other dietary constituents are starting to emerge. The beneficial effect of phenolic and polyphenolic compounds on blood glucose regulation has been widely reported and a number of mechanisms, including enhancement of insulin secretion and alleviation of oxidative stress, have been proposed to explain their beneficial effect (Lacroix & Li-Chan, 2014a). In the past few years, researchers have begun investigating DPP-IV inhibition as another putative mechanism of action underlying the anti-diabetic properties of these compounds.

González-Abuín and colleagues (2012) reported that grape seed-derived procyanidins, which have previously been shown to have an anti-hyperglycemic effect in insulin resistant animals (Montagut et al., 2010; Pinent et al., 2004), were able to modulate both DPP-IV activity and expression *in vitro* and *in vivo*. Similarly, inhibition of the DPP-IV enzyme activity both *in vitro* and in rodents was observed by Parmar et al (2012) in an investigation into the effect of naringin, a flavonoid commonly found in orange peels and previously shown to have hypoglycemic effect (Jung, Lee, Jeong, & Choi, 2004). Anthocyanins from blueberry-blackberry wine blends have also been reported to be able to inhibit enzymes involved in carbohydrate regulation, including DPP-IV (Johnson, de Mejia, Fan, Lila, & Yousef, 2013). In a follow-up investigation, the authors assessed the effect of twenty-seven phenolic compounds commonly found in citrus, berries, grapes, soybeans and other plant foods on DPP-IV activity (Fan, Johnson, Lila, Yousef, & de Mejia, 2013). Sixteen of the tested phytochemicals were able to inhibit the enzyme, having IC_{50} values that ranged from 0.6 nM for resveratrol to 10.36 μM for eriocitrin. Thirteen of these had IC_{50} values lower than that of diprotin A (IC_{50} = 4.21 μM), with resveratrol, luteolin, apigenin, flavone, naringenin and genistein being the most potent (**Figure 3**). Interestingly, Fan et al. (2013) observed that most of the glycosylated flavonoids with two sugar moieties (e.g. naringin, rutin, narirutin, hesperidin and neohesperidin) did not decrease DPP-IV activity. The authors hypothesized that the presence of bulky sugar groups on the flavonoid core skeleton might cause steric

hindrance preventing their binding to the enzyme's active sites. Thus, while the aglycone of naringin, naringenin, was able to inhibit DPP-IV, no inhibition of DPP-IV by naringin was observed by Fan et al. (2013), which is contradictory to the results reported by Parmar et al (2012). This discrepancy might be attributable to differences in the experimental conditions, such as the type of substrate or enzyme specie, used in the two studies to assess the inhibitory activity.

The anthocyanins cyanidin 3, 5-diglucoside isolated from the juice of aronia berries (Kozuka et al., 2015) and cyanidin 3-glucoside found in blackberry-blueberry wine blend (Fan et al. 2013) have both been reported to have DPP-IV inhibitory activity (IC_{50} = 5.5 µM and 0.42 µM, respectively). Phytochemicals found in extracts from Greek oregano, rosemary, marjoram and Mexican oregano were also recently found to be able to inhibit the DPP-IV enzyme in a study by Bower, Hernandez, Berhow, & de Mejia (2015). Among the compounds identified in the most potent fractions obtained from Mexican oregano and rosemary, cirsimaritin, hispidulin and naringenin (**Figure 3**), with IC_{50} values of 0.43, 0.49 and 2.5 µM, respectively, were the most effective inhibitors.

To date, little is known on the structural characteristics of polyphenolic compounds that determine the mechanism of their inhibitory effect on DPP-IV. Findings from computational modeling have suggested that polyphenols could modulate enzymatic activity by interacting, mainly through hydrogen bonding, with key amino acid residues in the binding sites (S1 containing the catalytic triad, S2 and/or S3 pockets) of the enzyme's catalytic region (Bower et al., 2015; Fan et al., 2013; Parmar et al., 2012). Resveratol and flavone, for example, were found to interact with all three active sites of the enzyme and to behave as competitive inhibitors (K_i = 0.2 µM and 18.6 µM, respectively). Luteolin and apigenin, on the other hand, only showed interactions with the S2 and S3 sites and were reported to act on DPP-IV in a non-competitive manner (K_i = 4.9 µM and 7.9 µM, respectively) (Fan et al., 2013).

In addition to phenolic compounds, tocopherol has also been suggested to be able to inhibit DPP-IV activity *in vitro*. A green sea urchin gonad tissue extract with *in vivo* glucose lowering effect was reported to have DPP-IV inhibitory activity (IC_{50} = 20 µg/mL) in a study by Pozharitskaya et al. (2015). The authors speculated that tocopherol present in the fraction was responsible for the observed inhibition of DPP-IV. While tocopherol isomers have been previously shown to be able to interact with DPP-IV in docking experiments (Bharti et al., 2013), the actual effect of tocopherol on DPP-IV activity has not been determined. Therefore, it is unknown whether the DPP-IV inhibitory activity of the gonad tissue extract was due to its content of tocopherol and/or other constituents. Additionally, probiotic *Lactobacillus* strains were also recently shown to be able to inhibit the DPP-IV enzyme (Zeng et al., 2016). Zeng et al. (2016) evaluated the DPP-IV inhibitory activity of cell-free excretory supernatants and cell-free extracts of 21 strains and found that all were able to cause some inhibition of the enzyme, the greatest effect being observed with the cell-free extract of *L. paracasei* NL41, an isolate from cheese. As for the study on gonad tissue, it is unclear what constituents in the cell-free excretory supernatants or extracts of lactobacilli are responsible for the observed inhibition of DPP-IV nor how they affect the enzyme activity.

5. *In vivo* effect of food-derived DPP-IV inhibitors

While the DPP-IV inhibitory activity of food-derived constituents has been shown in numerous *in vitro* studies, literature on the efficacy of these natural inhibitors *in vivo* is still rather sparse. Nonetheless findings from the few animal studies conducted so far are promising (**Table 3**).

The ileal administration of a zein protein hydrolysate produced using papain to rats prior to an intraperitoneal glucose tolerance test (IPGTT) was shown to enhance insulin secretion 2.4 fold at 15 min and to decrease blood glucose levels compared to control rats (Mochida, Hira, & Hara, 2010). Moreover, the administration of the zein hydrolysate caused increases in both total and active GLP-1 concentrations, the latter response being found to be correlated with changes in insulin and glucose levels. To assess whether the increase in active GLP-1 concentration was due to the ability of the hydrolysate to inhibit DPP-IV, the authors also measured the plasma DPP-IV activity and observed a significant inhibition of the enzyme compared to the control group. These findings suggested that zein-derived peptides exert their anti-hyperglycemic effect via their ability to induce GLP-1 secretion and inhibit DPP-IV activity.

The administration of rice-derived peptides was likewise found to potentiate the incretin effect in rats in a study by Ishikawa et al. (2015). Animals that were orally given rice endosperm or bran protein hydrolysate showed an attenuated glycemic response and increased plasma GLP-1 concentrations during an IPGTT. Moreover, plasma DPP-IV activity was found to be reduced and the ratio of active GLP-1 to total GLP-1 increased following the ileal administration of the hydrolysates.

Peptides derived from bean proteins with *in vitro* DPP-IV inhibitory effect were also found to have a glucose lowering effect in mice in a patent by Tominaga et al. (2012). Animals that received azuki bean, tora bean, otebo bean or soybean hydrolysates produced by treatment with the protease Umamizyme G showed reduced increases in blood glucose levels during a oral glucose tolerance test (OGTT) compared to those who did not received the bean hydrolysates. Since no other metabolic parameters were measured, it is unknown whether the blood glucose lowering effect observed was caused by the inhibition of DPP-IV activity.

In addition to hydrolysates from plant proteins, a hydrolysate produced by Alcalase treatment of the egg protein lysozyme was found to reduce DPP-IV activity in diabetic rats in a study by Wang et al. (2012). Animals that were orally administered the hydrolysate showed a 25% reduction of blood serum DPP-IV activity after 90 min. A trend towards higher (1.4 fold) serum GLP-1 was also observed. However, 15 weeks of treatment with the hydrolysate or the synthetic inhibitor vildagliptin did not significantly improve metabolic parameters such as blood glucose, serum insulin, cholesterol concentrations and percent Hb1Ac. On the other hand, the administration of the egg protein hydrolysate was found to attenuate renal damage development and prevent aortic endothelial dysfunction.

Huang, Hung, Jao, Tung, & Hsu (2014) studied the effect of daily administration of a < 1 kDa fraction of porcine skin gelatin hydrolysate (PGH) on DPP-IV activity as well as active GLP-1, insulin and glucagon levels of streptozotocin-induced rats. Rodents that received a daily dose of hydrolysate or commercial DPP-IV inhibitor sitagliptin showed reduced blood glucose levels during an OGTT compared to control diabetic rats. Moreover, after 42 days, both the PGH- and sitagliptin-treated rats showed reduced DPP-IV activity (50% and 75% reduction, respectively) as well as increased plasma insulin (about 6–8 fold) and active GLP-1 (~ 10% increase) concentrations, compared to the diabetic control group. On the other hand, no significant difference in plasma glucagon levels between the control and PGH-treated diabetic rats was observed. Similar *in vivo* anti-diabetic effects of hydrolysates of Atlantic salmon skin gelatin (Hsieh, Wang, Hung, Chen, & Hsu, 2015), halibut skin gelatin and tilapia skin gelatin (Wang et al., 2015) were also recently reported by the same research group. Diabetic animals administered the Atlantic salmon skin gelatin hydrolysate had lower blood glucose levels during an OGTT, increased plasma insulin and active GLP-1 concentrations as well as greater insulin-to-glucagon ratios than the control diabetic rodents after 5 weeks of treatment, and a ~30% reduction in plasma DPP-IV activity compared to the diabetic control group (Hsieh, Wang, Hung, Chen, & Hsu, 2015). Similarly, rodents receiving the halibut and tilapia skin gelatin hydrolysates showed reduced plasma DPP-IV activity, increased total and active plasma GLP-1 levels as well as increased insulin concentrations, these effects being more pronounced in the animals treated with tilapia than halibut skin gelatin hydrolysate. Findings from these studies suggest that the blood glucose lowering effect of the fish skin gelatin hydrolysates could be due to their ability to inhibit the DPP-IV enzyme, therefore reducing the degradation of GLP-1 and enhancing insulin secretion.

As well as hydrolysates from corn, rice, bean, egg, pork and fish proteins, peptides with *in vitro* DPP-IV inhibitory activity from milk proteins have also been studied for their effect on blood glucose regulation in rodents. Uchida, Ohshiba, & Mogami (2011) investigated the glycemic effect in mice of a β-lactoglobulin hydrolysate with *in vitro* DPP-IV inhibitory activity. Oral administration of the trypsin-treated β-lactoglobulin 30 min prior to an OGTT resulted in a significantly greater decrease in plasma glucose levels over a 180 min postprandial period compared to control mice that only received an aqueous buffer. This reduction, however, was less pronounced than in mice that received sitagliptin phosphate hydrate. Since the authors did not measure plasma DPP-IV activity, it is unknown whether the glucose lowering effect observed was caused by the DPP-IV inhibitory activity of the β-lactoglobulin hydrolysate. A β-casein-derived peptide LPQNIPPL identified in gouda-type cheese and shown to be able to inhibit the activity of DPP-IV *in vitro* was also tested in an animal model (Uenishi, Kabuki, Seto, Serizawa, & Nakajima, 2012). Rodents that received the octapeptide as part of an OGTT had lower postprandial glucose areas under the curve than animals that did not receive the peptide. Plasma insulin concentrations, on the other hand, did not differ significantly between the LPQNIPPL-treated rats and control group throughout the postprandial period. As in the study by Uchida et al. (2011), plasma DPP-IV activity was not determined.

Besides hydrolysates and protein-derived peptides, phenolic compounds with *in vitro* DPP-IV inhibitory activity have also been tested for their effect in rodents. In a study by González-Abuín et al. (2012), the impact of grape seed-derived procyanidins on DPP-IV activity and expression was determined using different animal models. Administration of the procyanidins to healthy and diet-induced obese rats led to decreases in both intestinal DPP-IV activity and gene expression. The animals' plasmatic DPP-IV activity, however, was unchanged. Additionally, long-term intake of grape seed-derived procyanidins in healthy animals was found to increase plasma insulin-to-glucose ratios in an OGTT, but had no effect when glucose was administered intraperitoneally. In genetically obese rats, only DPP-IV gene expression was reduced by the treatment. Since the grape seed procyanidin extract is composed of a mixture of compounds of various structures, the authors suggested that the discrepancy observed between intestinal and plasma DPP-IV activities could be due to intestinal permeability and differences between the forms in which the procyanidins reach the intestine and the forms in which they enter the systemic circulation. On the other hand, decreased serum DPP-IV activity was observed in rats treated with the flavonoid naringin (Parmar et al., 2012). In fact, the administration of this flavonoid commonly found in orange peel was more effective at inhibiting plasma DPP-IV than the DPP-IV inhibitor sitagliptin. Animals receiving both treatments also showed lower random blood glucose levels and increased insulin concentrations compared to those in the control group.

The glycemic effect of a sea urchin gonad tissue extract with *in vitro* DPP-IV inhibitory activity has also been investigated in rodents (Pozharitskaya et al., 2015). Diabetic mice administered the extract rich in tocopherol showed reduced fasting serum glucose levels after 5 days of treatment as well as a decrease in plasma malondialdehyde levels (a biomarker of lipid peroxidation) and an increase in reduced glutathione concentrations (an endogenous tripeptide that acts as an antioxidant) compared to diabetic control animals. Since the animals' plasmatic DPP-IV activity was not determined, it is unclear whether the antidiabetic effects of the gonad tissue extract resulted from its action on the DPP-IV enzyme (Pozharitskaya et al., 2015).

While findings from these few studies in rodents have suggested that the administration of protein-derived peptides and phenolic compounds with *in vitro* DPP-IV inhibitory activity can help regulate blood glucose levels and, in some instances, lower plasma DPP-IV activity in these animals, there is currently no data on the effect of food-derived DPP-IV inhibitors in humans.

6. Future considerations and research needed

As illustrated in the previous sections, numerous studies have demonstrated the *in vitro* DPP-IV inhibitory activity of food-derived constituents including protein hydrolysates, peptides and phenolic compounds, and the limited number of animal studies conducted to date have shown promising outcomes attributed to these natural sources of DPP-IV inhibitors. However, the potential opportunities for developing food-derived DPP-IV inhibitors as nutraceuticals or functional food ingredients that can be a complementary approach for management of hyperglycemia can only be established by conducting

further research to demonstrate *in vivo* clinical efficacy of food-derived DPP-IV inhibitors and to provide the practical knowledge necessary for their commercialization.

6.1 Bioavailability of food-derived DPP-IV inhibitors and possible food-drug interactions

In order to prevent the inactivation of the incretin hormones, food-derived DPP-IV inhibitors have to resist degradation by gastric, pancreatic, and small intestinal brush border membrane enzymes and be absorbed in the lumen. Although some of the peptides reported in **Tables 1** and **2** may reach the small intestine unchanged, it is likely that many of the peptides identified to have *in vitro* DPP-IV inhibitory activity will be broken down *in vivo* during the digestion process. In an *in silico* study, Nongonierma et al. (2014) used an amino acid clustering model and a peptide cutter tool from the Expert Protein Analysis System (ExPASy) to predict the stability of short peptides (2 to 5 amino acids long) with *in vitro* DPP-IV inhibitory activity. Among the 42 sequences investigated, 23 were predicted to be unstable; several of the tryptophan-containing dipeptides and most of the peptides containing more than two amino acids were predicted to not be resistant to digestion. For unstable peptides to be effective *in vivo*, their digestion products would have to also be able to inhibit the activity of DPP-IV. Using an *in vitro* digestion system with pepsin and pancreatin Huang, Jao, Ho, & Hsu (2012) studied the effect of the digestion process on the DPP-IV inhibitory activity of three tuna-derived peptides (13-15 amino acids long). The peptide digests obtained were found to have the same or greater DPP-IV inhibitory activity than the un-digested peptides suggesting that hydrolysis of these oligopeptides may improve their inhibitory activity. The DPP-IV inhibitory capacity of hydrolysates such as those obtained from cuttlefish viscera and proteins from hemp, pea, rice and soy was also found to be mostly retained or slightly increased following *in vitro* digestion in studies by Cudennec et al. (2015) and Nongonierma & FitzGerald (2015), respectively. Research on protein digestion and absorption has demonstrated that dipeptides and tripeptides can cross the intestinal endothelium and reach the systemic circulation intact (Miner-Williams, Stevens, & Moughan, 2014). On the other hand, findings on the absorption of oligopeptides and macromolecules are conflicting. While some bioactive peptides, such as lunasin (a 43-amino acid peptide possessing anti-inflammatory and anti-cancer properties), have been found to pass into the systemic circulation (Dia, Torres, De Lumen, Erdman, & de Mejia, 2009), it is believed that most oligopeptides or proteins that are intestinally absorbed are broken down by cytosolic peptidases (Miner-Williams et al., 2014)

As for peptides, to be effective *in vivo* phenolic compounds also need to retain their activity through the digestion process and be absorbed in the lumen. A number of factors may impact the stability and absorption of polyphenols, including their interaction with other food constituents or salivary proteins, their molecular structure and isomeric configuration. Most polyphenols are known to have a low oral bioavailability (Rein et al., 2012). Studies on the metabolic fate of procyanidins, for example, have suggested that they are primarily degraded into monomers such as epicatechin and catechin in the stomach, and that methylated and glucuronidated dimers and monomers are the primary metabolites found in the plasma (Zhang et al., 2016b). Changes in the form of a phenolic

compound during digestion are likely to affect its interaction with the DPP-IV enzyme and therefore its inhibitory activity.

As most of the food-derived DPP-IV inhibitors identified so far have shown a weaker potency than the synthetic drugs, they do not have the potential to replace pharmacotherapy in the management of type 2 diabetes, but could be a complementary approach to help regulate blood glucose levels in at risk, pre-diabetic or diabetic individuals. Clinical trials are needed to assess not only the effectiveness and metabolic fate of food-derived DPP-IV inhibitors, but should also consider potential interactions of the inhibitors with anti-diabetic drugs. A recent *in vitro* study on the interactions between the synthetic DPP-IV inhibitor sitagliptin and whey protein hydrolysate or whey protein-derived peptides has suggested that the combination of these molecules may have an additive effect on DPP-IV inhibition (Nongonierma & FitzGerald, 2013b). Additional research is warranted to evaluate the potential additive, synergistic or antagonistic effects between DPP-IV inhibitory peptides and glucose-lowering drugs.

Although the specific interactions between gliptins, or other anti-diabetic agents, and phenolic compounds with DPP-IV inhibitory activity have not yet been investigated, the co-administration of dietary polyphenols and drugs have been the object of a number of studies. Phenolic compounds have been shown to affect drug metabolism by altering the expression and activity of drug-metabolizing enzymes, such as the oxidizing enzyme CYP3A4, which may lead to an increase or a decrease in drug plasma concentration and therefore may cause toxicity or treatment failure, respectively (Basheer & Kerem, 2015; Rein et al., 2012). Therefore, further investigation into the potential interactions between DPP-IV inhibiting polyphenols and pharmaceuticals is warranted.

6.2 Commercialization considerations and challenges

In addition to establishing clinical efficacy and safety of food-derived DPP-IV inhibitors, further research is required to propose strategies that will enable production that is economically feasible. Many of the known DPP-IV inhibitory peptides have been discovered through chemical synthesis of sequences that can be found within dietary proteins, and not empirically by producing and isolating them from the protein hydrolysates *per se*. Although knowledge on the identity and characteristics of specific food constituents with potent DPP-IV inhibitory activity is necessary, it is also of great importance to determine the best dietary sources of these inhibitors and the methods to optimize their production from those sources. This could be achieved by exploring integrated approaches combining *in silico* analysis to predict promising sources of DPP-IV inhibitors, *in vitro* assays to validate the release and biological activity, and experimental designs to optimize the generation of potent protein hydrolysates.

Unlike synthetic drugs that typically consist of well-defined molecular entities, the target final product for food-derived bioactives is not usually a single pure active compound. This is because the large-scale production of individual peptides or phenolic compounds from foods is not technically or financially realistic. On the other hand, while food protein hydrolysates or polyphenol extracts are much cheaper and easier to produce in

large quantities, making them more practical from a commercial point of view, they would contain a complex mixture of molecules, many of which may not present any biological activity. This may represent a limitation in terms of the doses of the crude hydrolysates that would be needed to potentiate an effect *in vivo*. Many of the hydrolysates reported to have DPP-IV inhibitory activity (**Figure 2**) have not undergone fractionation processes to yield peptide preparations with higher bioactivity. Even though findings from animal models conducted using these crude hydrolysates showed beneficial effects on glycemic regulation (**Table 3**), the doses used in these studies may not be what humans could realistically consume. Therefore, considerations should be made during the development stage of food-derived DPP-IV inhibitors to designing schemes for efficient fractionation of the hydrolysates and extracts using techniques such as membrane and/or liquid chromatographic processes, which would yield products that are enriched in the active constituent peptides or phenolic compounds.

Besides cost, yield and efficacy, a number of other factors must be taken into consideration for the development and commercialization of food-derived DPP-IV inhibitors, including their stability during storage and their sensory properties. Phenolic compounds as well as bioactive peptides and hydrolysates have been reported to often present a bitter taste that may limit their application as functional food ingredients (Gaudette & Pickering, 2013; Li-Chan, 2015). Assessing and ensuring the consistency of composition and activity of hydrolysates or phenolic extracts represent another important challenge to the commercialization of food-derived DPP-IV inhibitors. While the identity of individual peptides or phenolic compounds can be confirmed using techniques such as mass spectrometry or nuclear magnetic resonance, routinely determining the composition and effectiveness of hydrolysates or phenolic extracts containing complex mixtures of molecules, is a much more difficult task. For quality assurance, standardized methods to assess the activity and/or the presence of specific active peptides or polyphenols within the hydrolysates or extracts must be developed.

7. Conclusion

The inhibition of the DPP-IV enzyme is an established effective approach for the management of type 2 diabetes. The recent discovery that food-derived constituents have the ability to act as DPP-IV inhibitors has raised great interest in their potential to play a role in regulating glycemia. To date, a considerable amount of research conducted in this area has focused on the identification of dietary constituents with DPP-IV inhibitory activity, leading to the discovery of a number of protein hydrolysates, protein-derived peptides and other food constituents, such as phenolic compounds, able to inhibit the enzyme. Although these natural inhibitors have not been studied in humans and most show lower potency than the synthetic drugs, findings from both *in vitro* and animal studies suggest their potential as functional food ingredients to complement pharmacotherapy in the management of blood glucose levels. The next step in the investigation of food-derived DPP-IV inhibitors is to gain critical information on the bioavailability and bioaccessibility of these bioactive molecules, clinical evidence of their efficacy in humans and possible interaction with currently prescribed drugs. Ongoing research must also be conducted to establish the strategies that will achieve economically

feasible production of food-derived DPP-IV inhibitors, which may be developed for use as dietary ingredients to reduce the risk of developing hyperglycemia or in conjunction with anti-diabetic drugs that are currently approved for glycemic regulation in type 2 diabetes.

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Figure 1. Schematic representation of the generation, following food intake, of the incretins GLP-1 and GIP from proglucagon and proGIP, respectively, and their cleavage by the enzyme DPP-IV. GLP-1 is the product obtained by the post-translational processing of the proglucagon gene by PC1/3 in the intestinal L cells while GIP is derived from the modification of proGIP by PC1/3 in the enteroendocrine K cells. Both GIP and GLP-1 are substrates for the DPP-IV enzyme that hydrolyzes them into shorter and inactive molecules. DPP-IV, dipeptidyl-peptidase IV; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; GLP-2, glucagon-like peptide-2; GRPP, glicentin-related pancreatic polypeptide; IP, intervening peptide; PC, prohormone convertase.

Figure 2. Examples of milk (**A**), animal (**B**) and plant (**C**) protein hydrolysates reported to have *in vitro* DPP-IV inhibitory activity. BSA, bovine serum albumin; GI, gastrointestinal; *S.* collagenase, *Streptomyces* collagenase; WP, whey protein; WPC, whey protein concentrate; WPI, whey protein isolate. IC_{50} values were obtained from the references in Table 1 that are marked with an asterisk (*).

Figure 3. Examples of phenolic compounds reported to have potent DPP-IV inhibitory activity. IC_{50} values, shown in parentheses, were obtained from Bower et al. (2014)[a] and Fan et al. (2013)[b].

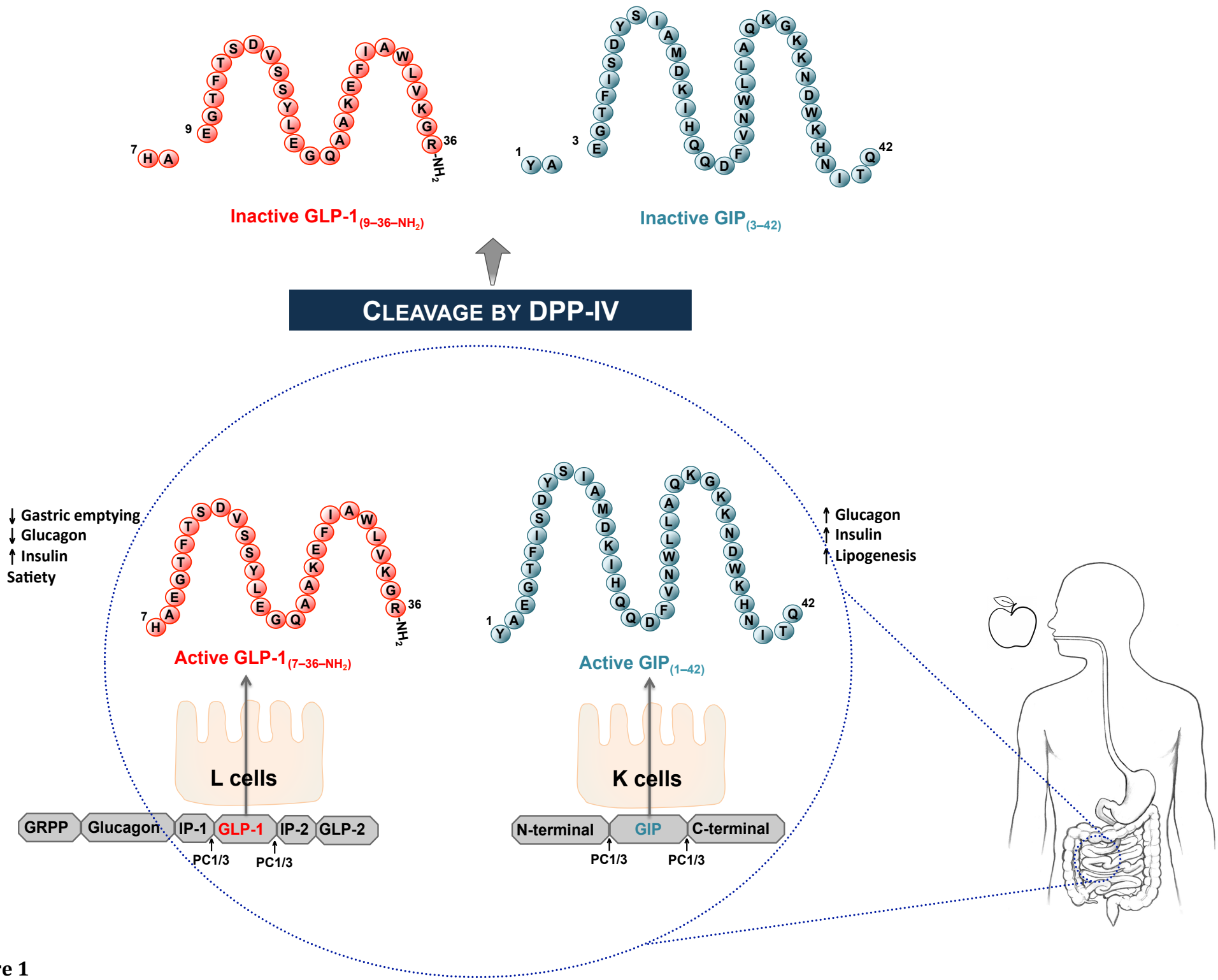

Inactive GLP-1(9–36–NH2)
Inactive GIP(3–42)
CLEAVAGE BY DPP-IV
↓ Gastric emptying
↓ Glucagon
↑ Insulin
Satiety
Active GLP-1(7–36–NH2)
Active GIP(1–42)
↑ Glucagon
↑ Insulin
↑ Lipogenesis
L cells
K cells
GRPP
Glucagon
IP-1
GLP-1
IP-2
GLP-2
PC1/3
PC1/3
N-terminal
GIP
C-terminal
PC1/3
PC1/3

Figure 1

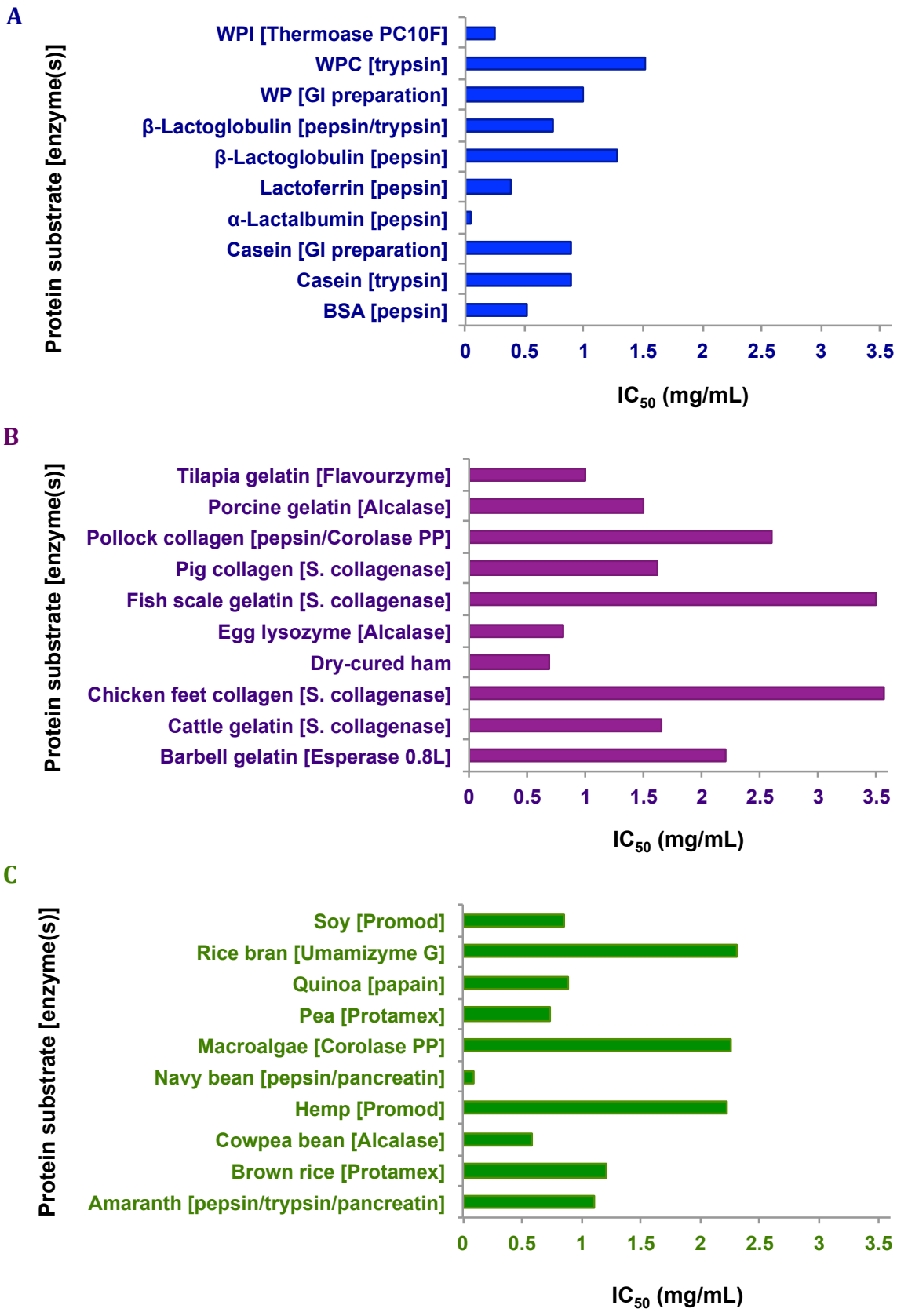
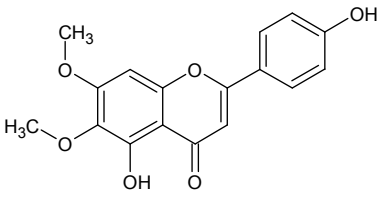
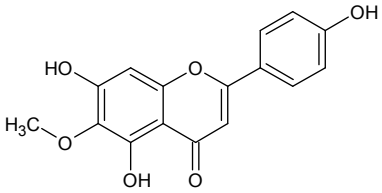

A
Protein substrate [enzyme(s)]
WPI [Thermoase PC10F]
WPC [trypsin]
WP [GI preparation]
β-Lactoglobulin [pepsin/trypsin]
β-Lactoglobulin [pepsin]
Lactoferrin [pepsin]
α-Lactalbumin [pepsin]
Casein [GI preparation]
Casein [trypsin]
BSA [pepsin]
0 0.5 1 1.5 2 2.5 3 3.5
IC50 (mg/mL)
B
Protein substrate [enzyme(s)]
Tilapia gelatin [Flavourzyme]
Porcine gelatin [Alcalase]
Pollock collagen [pepsin/Corolase PP]
Pig collagen [S. collagenase]
Fish scale gelatin [S. collagenase]
Egg lysozyme [Alcalase]
Dry-cured ham
Chicken feet collagen [S. collagenase]
Cattle gelatin [S. collagenase]
Barbell gelatin [Esperase 0.8L]
0 0.5 1 1.5 2 2.5 3 3.5
IC50 (mg/mL)
C
Protein substrate [enzyme(s)]
Soy [Promod]
Rice bran [Umamizyme G]
Quinoa [papain]
Pea [Protamex]
Macroalgae [Corolase PP]
Navy bean [pepsin/pancreatin]
Hemp [Promod]
Cowpea bean [Alcalase]
Brown rice [Protamex]
Amaranth [pepsin/trypsin/pancreatin]
0 0.5 1 1.5 2 2.5 3 3.5
IC50 (mg/mL)

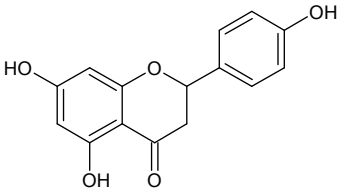
Figure 2.



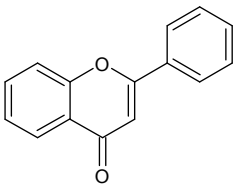
Cirsimaritin
(0.43 μM)[a]



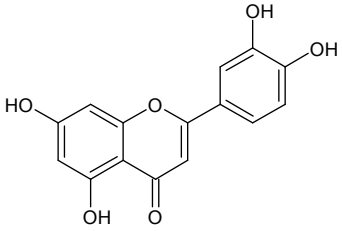
Hispidulin
(0.49 μM)[a]



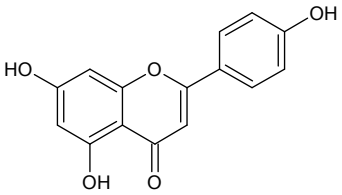
Naringenin
(2.5 μM)[a] (0.24 μM)[b]



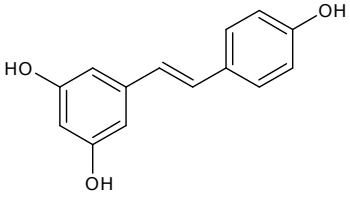
Flavone
(0.17 μM)[b]



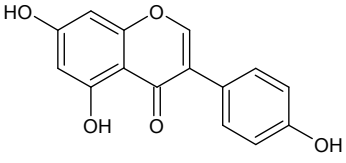
Luteolin
(0.12 μM)[b]



Apigenin
(0.14 μM)[b]



Resveratrol
(0.0006 μM)[b]



Genistein
(0.048 μM)[b]

Figure 3

Table 1. Protein hydrolysates, and peptides isolated from them, reported to have *in vitro* DPP-IV inhibitory activity

Protein source		Treatment or enzyme used	DPP-IV inhibitory peptide identified[a]		Reference
			Sequence	IC_{50} (μM)	
Amaranth		Trypsin, *in vitro* SGID with pepsin/pancreatin/trypsin	—	—	Velarde-Salcedo et al., 2013*
Beans	Azuki, tora, otebo	Koji mold, Umamizyme G	—	—	Tominaga et al., 2012
	Cowpea	Germination followed by treatment with Alcalase	—	—	de Souza Rocha, Hernandez, Chang, & de Mejía, 2014*
	Black, pinto, red, navy, great northern	*In vitro* SGID with pepsin/pancreatin	—	—	Mojica, Chen, & de Mejía, 2015*
	Pinto Durango, Negro 8025	Alcalase, bromelain	—	—	Oseguera-Toledo, de Mejia, & Amaya-Llano, 2015
Brewers' spent grain		Alcalase 2.4L, Flavourzyme 500L, Prolyve 1000, Protex 6L, Protamex, Corolase PP, Corolase L10, Promod 144MG, Promod 439, Promod 24P, trypsin 250	—	—	Connolly, Piggott, & FitzGerald, 2014
Cow's milk	α-Lactalbumin	*In vitro* SGID with pepsin/trypsin	—	—	Tulipano, Faggi, Nardone, Cocchi, & Caroli, 2015
		Pepsin	WLAHKALCSEKLDQ LAHKALCSEKL LCSEKLDQ TKCEVFRE IVQNNDSTEYGLF ILDKVGINY LKPTPEGDL	141 165 186 166 337 263 45	Lacroix & Li-Chan, 2013*; Lacroix & Li-Chan, 2014b
	β-Lactoglobulin	Trypsin	VAGTWY	174	Uchida, Ohshiba, & Mogami, 2011
		Serine protease isolated from Asian pumpkin (*Cucurbita ficifolia*)	—	—	Konrad et al., 2014
		In vitro SGID with pepsin/trypsin	—	—	Tulipano et al., 2015*
		Pepsin	—	—	Lacroix & Li-Chan, 2013*
	Lactoferrin	Pepsin	—	—	Lacroix & Li-Chan, 2013*
		Food-grade commercial gastrointestinal preparation	—	—	Nongonierma & FitzGerald, 2013a

Bovine serum albumin	Pepsin	—	—	Lacroix & Li-Chan, 2013*
Skin milk powder	*In vitro* SGID with pepsin/pancreatin	—	—	Lacroix & Li-Chan, 2012b
Sodium caseinate/casein	*In vitro* SGID with pepsin/pancreatin, Validase BNP-L, Umamizyme K, Thermolysin, Protin SD-NY10, Protamex, N“Amano”K, Flavourzyme, Corolase PP, bromelain, A“Amano”2, Alcalase	—	—	Lacroix & Li-Chan, 2012b
	Food-grade commercial gastro-intestinal preparation	—	—	Nongonierma & FitzGerald, 2013a*
	Alkalase, Protease N, Flavourzyme, bromelain, Scintillase CS150L, papain, sequential treatment of trypsin PTN 6.0 and Corolase LAP F	APFPEVF APFPE HPIK GPFPIIV LPLP EMPFPK LPVP PFP PQSVLS YVPEPF MPLW LPQYL LPVPQ GPFP PLLQ VPYPQ VPLGTQ LPVPQK KVLP LPL IPI	120 (6%)[b] 120 (6%)[b] (9%)[b] (9%)[b] (11%)[b] (12%)[b] (15%)[b] (20%)[b] (20%)[b] (20%)[b] (23%)[b] (26%)[b] (28%)[b] (34%)[b] (37%)[b] (51%)[b] (55%)[b] (63%)[b] (98%)[b] 5 (100%)[b] 5 (100%)[b]	Boots, 2009
	Newlase F, Umamizyme, Promod 278, Alcalase	—	—	Van Amerongen et al., 2009
	Alcalase 2.4 L, trypsin, Flavourzyme, pepsin, Neutrase	—	—	Zhang et al., 2016a*
Milk protein concentrate	*In vitro* SGID with pepsin/pancreatin	—	—	Lacroix & Li-Chan, 2012b

	Whey protein concentrate or isolate	Trypsin	VAGTWY TPEVDDEALEK IPAVF IPAVFK VLVLDTDYK	174.0 319.5 44.7 143.0 424.4	Silveira, Martínez-Maqueda, Recio, & Hernández-Ledesma, 2013*
		Protease N	—	—	Boots, 2009
		Serine protease isolated from Asian pumpkin (*Cucurbita ficifolia*)	—	—	Konrad et al., 2014
		In vitro SGID with pepsin/pancreatin, Validase BNP-L, Umamizyme K, Thermolysin, Protin SD-NY10, Protamex, N“Amano”K, Flavourzyme, Corolase PP, bromelain, A“Amano”2, Alcalase, pepsin	WLAHKAL WLAHKALCSEKLDQ LAHKALCSEKL TKCEVFRE LKPTPEGDL LKPTPEGDLEIL IPAVFKIDA	286 141 165 166 45 57 191	Lacroix & Li-Chan, 2012b; Lacroix & Li-Chan, 2014b
		Thermoase PC10F, sequential treatments of Thermoase PC10F/Accelerzyme CGP, Thermoase PC10F/Peptidase R & Thermoase PC10F/ProteAX	IQKVAGTW LKPTPEGDLE LKPTPEGDLEIL LKALPMH LKGYGGVSLPE WLAHKAL	329 42 57 193 486 286	Lacroix, Meng, Cheung, & Li-Chan, 2016*
		Food-grade commercial gastrointestinal preparation	—	—	Nongonierma & FitzGerald, 2013a*
		Corolase PP, *in vitro* SGID with pepsin/Corolase PP	—	—	Nongonierma & FitzGerald, 2013b; Power-Grant et al., 2015
		Food-grade protease preparation from *Carica* papaya latex (papain) and its microbially-derived alternative (papain-like enzyme)	—	—	Le Maux, Nongonierma, Barre, & FitzGerald, 2016
Cuttlefish (*Sepia officinalis*) viscera		Crude protease extracts from smooth hound (*M. mustelus*) intestine and cuttlefish (*S. officinalis*) hepato-pancreas, *in vitro* SGID with pepsin/pancreatin	—	—	Cudennec et al., 2015
Goat’s milk	Casein	Trypsin preparation containing some chymotrypsin	MHQPPQPL SPTVMFPPQSVL INNQFLPYPY	350.41 676.31 40.08	Zhang, Chen, Ma, & Chen, 2015

		Alcalase 2.4 L, trypsin, Flavourzyme, pepsin, Neutrase	—	—	Zhang, Chen, et al., 2016a
Gouda-type cheese		Prepared with microbial rennet and cheese starter	VPITPT LPQNIPP PQNIPPL VPITPTL FPGPIPN PGPIHNS IPPLTQTPV VPPFIQPE YPFPGPIPN LPQNIPPL LPQ	130 160 1500 110 260 1000 1300 2500 670 46 82	Uenishi, Kabuki, Seto, Serizawa, & Nakajima, 2012
Hemp		Corolase L10, Promod 144MG, Protamex, *in vitro* SGID with pepsin/Corolase PP	—	—	Nongonierma & FitzGerald, 2015*
Macroalgae (*Palmaria palmata*)	Aqueous, alkaline and combined aqueous & alkaline protein extracts	Alcalase 2.5 L, Flavourzyme 500L, Corolase PP	ILAP LLAP MAGVDHI	43.40 53.67 159.37	Harnedy & FitzGerald, 2013*; Harnedy, O'Keeffe, & FitzGerald, 2015
Pea		Corolase L10, Promod 144MG, Protamex, *in vitro* SGID with pepsin/Corolase PP	—	—	Nongonierma & FitzGerald, 2015*
Lysozyme		Alcalase, Newlase F, Promod 278, Umamizyme, pepsin	—	—	Van Amerongen et al., 2009*
Quinoa		Food-grade protease preparation from *Carica* papaya latex (papain) and its microbially-derived alternative (papain-like enzyme)	—	—	Nongonierma, Le Maux, Dubrulle, Barre, & FitzGerald, 2015*
Rice	Bran	Umamizyme G, Bioprase SP	IP LP	410 2370	Hatanaka et al., 2012*
		Pepsin, papain	—	—	Ishikawa et al., 2015
	Brown	Corolase L10, Promod 144MG, Protamex, *in vitro* SGID with pepsin/Corolase PP	—	—	Nongonierma & FitzGerald, 2015*
	Endosperm	Pepsin	—	—	Ishikawa et al., 2015
Skin or scale collagen/gelatin	Alaska pollock	Trypsin and *in vitro* SGID with pepsin/corolase PP	—	—	Guo et al., 2015*
	Atlantic salmon	Alcalase, bromelain, Flavourzyme	GPAE	49.6	Li-Chan, Hunag, Jao, Ho, & Hsu,

			GPGA	41.9	2012
	Barbel (*barbus callensis*) fish	Esperase 0.8L, Savinase 16L, Alcalase 2.4 L, trypsin, Izyme G, Protamex, Neutrase 0.8L, Peptidase	—	—	Sila et al., 2015*
	Deer	Pepsin, sequential treatments with pepsin/Alcalase or pepsin/trypsin	GPGSPGGPL GPVGXAGPPGK GPM(O)GPXGVK GPVGPSGPXGK GPAGPXGVXGL	1638.3 83.3 226.9 93.7 318.1	Jin, Yan, Yu, & Qi, 2015
	Halibut, hake, tilapia, milkfish	Flavourzyme 1000L	SPGSSGPQGFTG GPVGPAGNPGANGLN PPGPTGPRGQPGNIGF IPGDPGPPGPPGP LPGERGRPGAPGP GPKGDRGLPGPPGRDGM	101.6 81.3 146.7 65.4 76.8 89.6	Wang et al., 2015*
	Pork, cattle, fish, chicken feet	*Streptomyces* collagenase	GAX GPA GPX	> 20000 5030 2510	Hatanaka, Kawakami, & Uraji, 2014*
	Pork	Alcalase	GPX GPAG	45.3 41.1	Hsu, Tung, Huang, & Jao, 2013*
		Alcalase, Flavourzyme	—	—	Huang, Hung, Jao, Tung, & Hsu, 2014
Soy		Corolase L10, Promod 144MG, Protamex, *in vitro* SGID with pepsin/Corolase PP	—	—	Nongonierma & FitzGerald, 2015*
Spanish dry-cured ham		--	AAAAG AAATP ALGGA LVSGM	8130 6470 >10000 >10000	Gallego, Aristoy, & Toldrá, 2014*
Tuna cooking juice		Protease XXIII, Orientase 90N	PGVGGPLGPIGPCYE CAYQWQRPVDRIR PACGGFWISGRPG	116.1 78.0 96.4	Huang, Jao, Ho, & Hsu, 2012
Zein		Papain	—	—	Mochida, Hira, & Hara, 2010

* IC_{50} values of hydrolysates reported in these studies are shown in **Figure 2**

M(O), Met(O); SGID, simulated gastrointestinal digestion; X, hydroxyproline

[a] Only peptides identified in the hydrolysates, and for which IC_{50} values against DPP-IV were determined, are reported.

[b] Values from Boots (2009) are reported in percent relative potency, with 100% and 6% relative potency corresponding to $IC_{50} \approx 5$ µM and ≈ 120 µM, respectively

Table 2. DPP-IV inhibitory peptides discovered through chemical synthesis of sequences that can occur within food proteins

Peptide sequence	Identified parent protein(s)[a]	Inhibitory activity		Mode of action	Reference
		IC_{50} (μM)	K_i (μM)		
AA		9400	—	ND	Gallego, Aristoy, & Toldrá, 2014
AL		882.13	—	Competitive	Nongonierma & FitzGerald, 2013a
AP		7950	—	ND	Hatanaka et al., 2012
AW	α_{s1}-CN, BSA	>6000	—	ND	Nongonierma & FitzGerald, 2013c
DP		—	200	Competitive	Brandt et al., 1995
EK		3216.73	—	Competitive	Nongonierma & FitzGerald, 2013a
FA		94	—	ND	Lan et al., 2015
FL		399.58	—	Competitive	Nongonierma & FitzGerald, 2013a
FP		3630	—	ND	Hatanaka et al., 2012
		—	548.8	Non-competitive	Lacroix & Li-Chan, 2015[b]
GA		—	599	Competitive	Yan, Ho, & Hou, 1992
GF		—	3200	Competitive	Brandt et al., 1995
GL		2615.03	—	Competitive	Nongonierma & FitzGerald, 2013a
GP		9690	—	ND	Gallego et al., 2014
HA			2350	ND	Bella, Erickson, & Kim, 1982
HL		143.19	—	Competitive	Nongonierma & FitzGerald, 2013a
HP		2820	—	ND	Hatanaka et al., 2012
IA		88	—	ND	Lan et al., 2015
IV		—	40	Competitive	Brandt et al., 1995
KA		6270	—	ND	Gallego et al., 2014
KP		2540	—	ND	Hatanaka et al., 2012
LA	β-Lg	454	—	ND	Tulipano, Cocchi, & Caroli, 2012
		91	—	ND	Lan et al., 2015
LF		—	140	Competitive	Brandt et al., 1995
LL		—	340	ND	Bella et al., 1982
LW	α_{s1}-CN	993.4	—	Competitive	Nongonierma & FitzGerald, 2013c
		98	—	ND	Lan et al., 2015
MA		—	500	ND	Bella et al., 1982
ML		91	—	ND	Lan et al., 2015
MM		93	—	ND	Lan et al., 2015
MP		870	—	ND	Hatanaka et al., 2012
MW		1691.4	—	ND	Nongonierma & FitzGerald, 2013c
NH		69	—	ND	Lan et al., 2015
NP	α_{s1}-CN, α_{s2}-CN, β-Lg, α-La	>20000	—	ND	Nongonierma & FitzGerald, 2013d
PA		—	800	ND	Bella et al., 1982
		—	103	Competitive	Yan et al., 1992
PF		—	85.6	Competitive	Yan et al., 1992
PG		—	9000	Competitive	Brandt et al., 1995
		—	112	Competitive	Yan et al., 1992
PI		—	105	Competitive	Yan et al., 1992
PL		>10000	—	ND	Gallego et al., 2014
PM		—	102	Competitive	Yan et al., 1992
PP		5860	—	ND	Hatanaka et al., 2012
QP	β-CN, α_{s2}-CN, κ-CN, Lf	>4000	—	ND	Nongonierma & FitzGerald, 2013d
RP		2240	—	ND	Hatanaka et al., 2012
SL		2517.08	—	Competitive	Nongonierma & FitzGerald, 2013a
SP		5980	—	ND	Hatanaka et al., 2012
TH		49	—	ND	Lan et al., 2015
TP		2370	—	ND	Hatanaka et al., 2012
TW		84	—	ND	Lan et al., 2015
VA		168.24	—	Competitive	Nongonierma & FitzGerald, 2013a

VL		74	—	ND	Lan et al., 2015
VP		880	—	ND	Hatanaka et al., 2012
		93	350	Competitive	Hikida, Ito, Motoyama, Kato, & Kawarasaki, 2013; Lan et al., 2015
VR	β-CN, κ-CN, β-Lg, Lf, BSA	826.1	—	Non-competitive	Nongonierma & FitzGerald, 2013d
VV			620	ND	Bella et al., 1982
WA	Lf	92.6	—	Non-competitive	Nongonierma & FitzGerald, 2013c
		48	50	Competitive	Hikida et al., 2013; Lan et al., 2015
WC	α-La, Lf	420.0	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WE	β-Lg, Lf	>2000	—	ND	Nongonierma & FitzGerald, 2013c
WF	Lf	>3000	—	ND	Nongonierma & FitzGerald, 2013c
WG	BSA	>8000	—	ND	Nongonierma & FitzGerald, 2013c
WI	α_{s2}-CN, Lf	138.7	—	Non-competitive	Nongonierma & FitzGerald, 2013c
		89	—	ND	Lan et al., 2015
WK	Lf	40.6	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WL	α-La	43.6	—	Competitive	Nongonierma & FitzGerald, 2013c
		—	200.2	Mixed	Lacroix & Li-Chan, 2015[b]
WM	β-CN	243.1	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WN	Lf	148.5	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WP		4530	—	ND	Hatanaka et al., 2012
		44.5	—	Non-competitive	Nongonierma & FitzGerald, 2013c
		44	40	Competitive	Hikida et al., 2013; Lan et al., 2015
WQ	κ-CN, Lf	120.3	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WR	Lf	37.8	—	Non-competitive	Nongonierma & FitzGerald, 2013c
		—	11.5	Un-competitive	Lacroix & Li-Chan, 2015[b]
		20	—	Mixed	Lan et al., 2014; Lan et al., 2015
WS	BSA, Lf	643.5	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WT	Lf	482.1	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WV		65.69	—	Non-competitive	Nongonierma & FitzGerald, 2013a
		—	137.9	Mixed	Lacroix & Li-Chan, 2015[b]
		37	—	ND	Lan et al., 2015
WW		554.8	—	Non-competitive	Nongonierma & FitzGerald, 2013c
WY	β-Lg, α_{s1}-CN	281.0	—	Non-competitive	Nongonierma & FitzGerald, 2013c
YP	β-CN, α_{s1}-CN, κ-CN, Lf	658.1	—	Competitive	Nongonierma & FitzGerald, 2013d
		3170	—	ND	Hatanaka et al., 2012
YT		>6000	—	ND	Nongonierma, Mooney, Shields, & FitzGerald, 2014
IPA	β-Lg	49	—	Competitive	Tulipano, Sibilia, Caroli, & Cocchi, 2011
IPI		4.7	2.6	Competitive	Lacroix & Li-Chan, 2014b; Lacroix & Li-Chan, 2015[b]
	κ-CN	4.23	—	Competitive	Nongonierma & FitzGerald, 2013a
IQP	α_{s2}-CN	>4000	—	ND	Nongonierma & FitzGerald, 2013c
LQP		1181.1	—	ND	Nongonierma et al., 2014
PPG	Bovine collagen	2252.68	—	ND	Lafarga, O'Connor, & Hayes, 2014
PPL	BSA	390.14	—	ND	Lafarga et al., 2014
VGL		>5000	—	ND	Nongonierma & FitzGerald, 2014b
VPL		47	—	ND	Maruyama, Ohmori, & Nakagami, 1996
		—	7.6	ND	Umezawa et al., 1984
WRA		690	—	Un-competitive	Lan et al., 2014
WRD		376	160	Un-competitive	Lan et al., 2014
WRE	Soybean β-amylase	350	130	Un-competitive	Lan et al., 2014
WRF		413	—	Un-competitive	Lan et al., 2014
WRG		473	—	Un-competitive	Lan et al., 2014
WRH		670	—	Un-competitive	Lan et al., 2014
WRI		730	—	Un-competitive	Lan et al., 2014

WRK		406	—	Un-competitive	Lan et al., 2014
WRL		903	—	Un-competitive	Lan et al., 2014
WRM		673	—	Un-competitive	Lan et al., 2014
WRN		403	—	Un-competitive	Lan et al., 2014
WRP		780	—	Un-competitive	Lan et al., 2014
WRQ		720	—	Un-competitive	Lan et al., 2014
WRR		570	—	Un-competitive	Lan et al., 2014
WRS		483	—	Un-competitive	Lan et al., 2014
WRT		526	—	Un-competitive	Lan et al., 2014
WRW		487	—	Un-competitive	Lan et al., 2014
WRY		640	—	Un-competitive	Lan et al., 2014
WWW		216.0	—	Non-competitive	Nongonierma et al., 2014
YPY		243.7	—	Competitive	Nongonierma & FitzGerald, 2014b
FLQP	β-CN	65.3	—	Competitive	Nongonierma & FitzGerald, 2013d
		—	120.9	Competitive	Lacroix & Li-Chan, 2015[b]
VLGP	β-CN	580.4	—	Competitive	Nongonierma & FitzGerald, 2013d
VRGP	β-CN	>3000	—	ND	Nongonierma & FitzGerald, 2013d
WIQP	α_{s2}-CN	237.3	—	Non-competitive	Nongonierma & FitzGerald, 2013d
YPYY	κ-CN	194.4	—	Competitive	Nongonierma & FitzGerald, 2014b
IPIQY	κ-CN	35.2	—	Competitive	Nongonierma & FitzGerald, 2014b
		—	13.4	Competitive	Lacroix & Li-Chan, 2015[b]
LPLPL	β-CN	325.0	—	Competitive	Nongonierma & FitzGerald, 2014b
LPYPY	κ-CN	108.3	—	Competitive	Nongonierma & FitzGerald, 2014b
		—	47.0	Competitive	Lacroix & Li-Chan, 2015[b]
HPINHR	Caprine α_{s1}-CN	452.2	—	Competitive	Zhang et al., 2016a
GPFPILV	Caprine β-CN	163.7	—	Competitive	Zhang et al., 2016a
IPAVFKIDAL	β-Lg	—	107.2	Mixed	Lacroix & Li-Chan, 2015[b]
LAHKALCSEK	α-La	—	223.9	Non-competitive	Lacroix & Li-Chan, 2015[b]
LCSEKLDQWL	α-La	—	82.0	Mixed	Lacroix & Li-Chan, 2015[b]
LPEWVCTTFH	α-La	—	75.2	Competitive	Lacroix & Li-Chan, 2015[b]
WCKDDQNPHS	α-La	—	13.3	Un-competitive	Lacroix & Li-Chan, 2015[b]

α_{s1}-CN, α_{s1}-casein; α_{s2}-CN, α_{s2}-casein; α-La, α-lactalbumin; β-CN, β-casein; β-Lg, β-lactoglobulin; BSA, bovine serum albumin; κ-CN, κ-casein; Lf, lactoferrin; ND, not determined.

[a] Only parent proteins specified by the authors are mentioned.

[b] K_i value and mode of inhibition reported are those determined using porcine DPP-IV

Table 3. *In vivo* studies on the glycemic regulatory effect of food-derived constituents with *in vitro* DPP-IV inhibitory activity

Study	Animal model	Food constituent	Method of administration	Treatment (dose; duration)	Main findings
Mochida, Hira, & Hara, 2010	Male Sprague Dawley rats	Zein protein hydrolysate	Ileal	500 mg/rat; once	Increased insulin concentrations and decreased blood glucose levels during IPGTT; increased total and active GLP-1 concentrations; decreased plasma DPP-IV activity
Uchida, Ohshiba, & Mogami, 2011	C57BL/6 mice	β-lactoglobulin hydrolysate	Oral	300 mg/kg bw; once	Decreased plasma glucose levels during a OGTT; decreased plasma glucose AUC_{120min}
González-Abuín et al., 2012	Healthy female Wistar rats, diet-induced obese Wistar rats, Zucker fa/fa rats	Grape seed extract enriched in procyanidins	Oral	25–35 mg/kg bw/day; 19–60 days	Decreased intestinal, but not plasmatic, DPP-IV activity and expression in healthy and diet-induced obese rats; increased plasma insulin-to-glucose ratios during OGTT, but not during IPGTT, in healthy rats; reduced DPP-IV gene expression, but not DPP-IV activity, in genetically obese rats
Parmar et al., 2012	Male Wistar albino rats	Flavonoid naringin	Oral	40 mg/kg bw twice daily; 10 days	Decreased serum DPP-IV activity; lower random blood glucose levels; increased insulin levels; no change in fasting serum glucose concentrations
Tominaga et al., 2012	C57BL/6J SPF male mice	Azuki, tora, otebo and soy beans hydrolysates	Oral	4g/kg bw; once	Decreased plasma glucose levels during OGTT (greater decrease with azuki bean hydrolysate)
Uenishi, Kabuki, Seto, Serizawa, & Nakajima, 2012	Female Sprague Dawley rats	β-lactoglobulin-derived peptide LPQNIPPL	Oral	300 mg/kg bw; once	Decreased plasma glucose AUC_{120min} during OGTT; no change in insulin plasma concentrations
Wang et al., 2012	Male obese Zucker diabetic fatty rats	Lysozyme hydrolysate	Oral	1 g/kg bw/day; 15 weeks	25% reduction of serum DPP-IV activity after 90 min administration; increased (but not significant) GLP-1 levels; improvement of certain parameters of renal inflammation and structural damage; no significant changes in metabolic parameters such as blood glucose and serum insulin
Huang, Hung, Jao, Tung, & Hsu, 2014	Streptozotocin-induced male Sprague Dawley rats	Porcine skin gelatin hydrolysate (<1 kDa fraction)	Oral	300 mg/day; 42 days	Decreased plasma glucose AUC_{180min} during OGTT; 50% reduction in plasma DPP-IV activity; 8 fold increase in plasma insulin concentrations; ~10% increase in active GLP-1 concentrations; no difference in plasma glucagon levels

Hsieh, Wang, Hung, Chen, & Hsu, 2015	Streptozotocin-induced male Sprague Dawley rats	Atlantic salmon skin gelatin hydrolysate	Oral	300 mg/day; 5 weeks	Decreased plasma glucose AUC_{180min} during OGTT; increased plasma insulin concentrations; increased active GLP-1 concentrations; increased insulin-to-glucagon ratios; 33% reduction in plasma DPP-IV activity
Ishikawa et al., 2015	Male Sprague Dawley rats	Rice endosperm and bran protein hydrolysates	Oral and ileal	0.1–2 g/kg; once (oral) and 500 mg; once (ileal)	No significant difference in plasma glucose AUC_{120min} during OGTT; reduced glycemic response under IPGTT; increased GLP-1 concentrations under IPGTT; reduced plasma DPP-IV activity and increased active GLP-1 to total GLP-1 ratio following ileal administration of the hydrolysates
Pozharitskaya et al., 2015	Streptozotocin-induced male mice	Urchin gonad tissue extracts (ESD1, ESD2, ESD3)	Intragastric	1.8 mg/kg bw/day; 10 days	Decreased fasting serum glucose levels in ESD2-treated group; reduced blood malondialdehyde and increased reduced glutathione concentrations with all three extracts.
Wang et al., 2015	Streptozotocin-induced male Sprague Dawley rats	Halibut (HSGH) and tilapia (TSGH) skin gelatin hydrolysates (<1.5 kDa fraction)	Oral	750 mg/kg bw/day; 30 days	Decreased plasma glucose AUC_{180min} during OGTT; reduced plasma DPP-IV activity (greater reduction with TSGH); increased total and active GLP-1 concentrations (greater increase with TSGH); increased plasma insulin levels (greater increase with TSGH)

AUC, area under the curve; bw, body weight; DPP-IV, dipeptidyl-peptidase IV; GLP-1, glucagon-like peptide-1; IPGTT, intraperitoneal glucose tolerance test; OGTT, oral glucose tolerance test