A comprehensive review on the gluco-regulatory properties of food-derived bioactive peptides

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 A B S T R A C T

Diabetes mellitus, a group of metabolic disorders characterized by persistently elevated blood glucose levels, affects millions of people worldwide and is on the rise. Dietary proteins, from a wide range of food sources, are rich in bioactive peptides with anti-diabetic properties. Notable examples include AGFAGDAPR, a black tea-derived peptide, VRIRLLQRFNKRS, a β-conglycinin-derived peptide, and milk-derived peptide VPP, which have shown anti-diabetic effects in diabetic rodent models through various pathways including improving beta-cell function, suppression of alpha-cell proliferation, inhibiting food intake, increasing portal cholecystokinin concentration, enhancing insulin signaling and glucose uptake, and ameliorating adipose tissue inflammation. Despite the immense research on gluco-regulatory properties of bioactive peptides, incorporation of these bioactive peptides in functional foods or nutraceuticals is widely limited due to the existence of several challenges in the field of peptide research and commercialization. Ongoing research in this field, however, is fundamental to pave the road for this purpose.

1. Introduction

Diabetes mellitus, a group of metabolic disorders characterized by persistent hyperglycemia, affects millions of people worldwide and is on the rise. Dietary proteins, from a wide range of food sources, are rich in bioactive peptides with anti-diabetic properties. Notable examples include AGFAGDAPR, a black tea-derived peptide, VRIRLLQRFNKRS, a β-conglycinin-derived peptide, and milk-derived peptide VPP, which have shown anti-diabetic effects in diabetic rodent models through various pathways including improving beta-cell function, suppression of alpha-cell proliferation, inhibiting food intake, increasing portal cholecystokinin concentration, enhancing insulin signaling and glucose uptake, and ameliorating adipose tissue inflammation. Despite the immense research on gluco-regulatory properties of bioactive peptides, incorporation of these bioactive peptides in functional foods or nutraceuticals is widely limited due to the existence of several challenges in the field of peptide research and commercialization. Ongoing research in this field, however, is fundamental to pave the road for this purpose.

Abbreviations: Akt, Protein kinase B; AMPK, AMP-activated protein kinase; C/EBP-α, CCAAT enhancer binding protein alpha; CCK, Cholecystokinin; CCK-1R, CCK type 1 receptor; cGMP, cyclic guanosine-monophosphate; DPP-IV, Dipeptidyl peptidase IV; ERK1/2, Extracellular signal regulated kinase 1/2; GIP, Glucose-dependent insulinotropic polypeptide; GLP-1, Glucagon-like peptide 1; GLUT, Glucose transporter; IRS-1, Insulin receptor substrate-1; MAPK, Mitogen activated protein kinase; PISK, Phosphatidylinositol 3-kinase; PPARY, Peroxisome proliferator associated receptor gamma; TZD, Thiazolidinediones.

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Food proteins from different sources including animal and plant (Antony & Vijayan, 2021; Kehinde & Sharma, 2020) as well as edible insects (Lee et al., 2021) have been increasingly acknowledged for their potential benefits towards management of many chronic diseases including diabetes. The physiological properties of food proteins are carried out by bioactive peptides; the protein fragments encrypted within the parent protein with positive effects on body functions and/or human health beyond their nutritional value (Korhonen & Pihlanto, 2006). The physiological effects of bioactive peptides include antihypertensive, antioxidant, anti-inflammatory, antiatherogenic, opioid, antimicrobial, antithrombotic, immunomodulatory, and mineral binding properties (Chakrabarti, Jahandideh, & Wu, 2014; Erdmann, Cheung, & Schroder, 2008; Guha & Majumder, 2019; Nagaoka, 2019; Udenigwe & Aluko, 2012). These peptides can be released enzymatically, during food processing, or by microbial fermentation (Korhonen & Pihlanto, 2006; Wu, Jahandideh, Yu, & Majumder, 2015). Protein hydrolysates and bioactive peptides have also a great potential to regulate glucose metabolism (de Campos Zani, Wu, & Chan, 2018).

Here, we review the involvement of protein hydrolysates and bioactive peptides in the regulation of blood glucose and insulin sensitivity, categorize the food-derived bioactive peptides based on their mechanisms of action, and discuss the challenges and opportunities of the peptide discovery and research.

2. Antidiabetic potential of bioactive peptides

Effective diabetic management requires a comprehensive approach to reduce blood glucose, normalize β-cell functions, and improve insulin sensitivity through weight management, diet, or medication (Knowler et al., 2002; Van Gaal & Scheen, 2015). Dietary proteins have a great satiety effect coming from stimulating gut hormones secretion, increasing energy expenditure, and enhancing gluconeogenesis (Westterper-Plantenga, 2008). Protein intake positively affects blood glucose, insulin secretion, and body fat (Layman et al., 2003). Although the beneficial effects of protein intake on energy homeostasis and glycemia management have been mainly attributed to amino acid composition (Westterp-Plantenga, Lemmens, & Westterp, 2012), the role of bioactive peptides as potential molecules accounting for these positive effects has been more recognized in recent years (Caron, Domenger, Dhlulster, Ravallec, & Cudennec, 2017). The beneficial effects of milk protein-derived peptides on glycemia control have been well characterized (recently reviewed in (Horner, Drummond, & Brennan, 2016)). Diverse studies have also shown the protective effects of cereals (barley, oat, rice, and wheat), pseudocereals (amaranth, quinoa, and buckwheat), and legumes (soy, pea, and some types of beans) against diabetes. Administration of Alcalase oat protein hydrolysate (MW < 5 kDa; 1 g/kg BW) to streptozotocin (STZ)-induced diabetic mice reduced blood glucose through affecting food intake, insulin secretion and sensitivity, and glycogenesis (Wang et al., 2019; Zhang, Wang, Liu, & Sun, 2016). FLQPNLDEH, DLELQNNVFPH, and TPNAGVASAGGAGGKH were...
identified as the major peptides in oat protein hydrolysate (Zhang et al., 2016). Marine-derived bioactive peptides with glucoregulatory effects have also been commercialized and are available in the market. Nutripeptin™, a product containing cod hydrolysate has been marketed to improve glycemic index. Fortidium Liquamen® a white fish (Molva molva) autolysate containing fish oil and vegetable oil, is another example of commercialized food supplements with benefits on oxidative stress, glycemic index, psychological, and nervous balance (Guerrard et al., 2010). Different mechanisms are involved in the glucoregulatory properties of bioactive peptides including carbohydrate digestion, gut hormone release, insulin secretion and function, glucose uptake, and adipose tissue modification (Fig. 1).

2.1. α-glucosidase and α-amylase inhibitors

The enzymes α-amylase and α-glucosidase are involved in the carbohydrate digestion; hydrolyzing complex carbohydrates into mono- saccharides to be transported across the intestinal mucosa in the small intestine. While α-amylase breaks down long chain carbohydrates, α-glucosidase located in the brush border of the enterocytes lining the intestinal villi, facilitate the breakdown of disaccharides and oligosaccharides into absorbable monosaccharides. Inhibitors of α-amylase and α-glucosidase prolong the overall carbohydrate digestion time and reduce postprandial hyperglycaemia (Ross, Guive, & Wang, 2004). Natural sources of α-glucosidase inhibitors are believed to lack the common side effects associated with synthetic α-glucosidase inhibitors including flatulence, abdominal cramping, vomiting, and diarrhea (Chaudhry et al., 2017). α-amylase and α-glucosidase inhibitory peptides have been identified from a variety of food sources, as reviewed elsewhere (Yan, Zhao, Yang, & Zhao, 2019), but notable examples include KLPFG from egg albumin with α-glucosidase inhibitory activity (IC₅₀ values of 59.5 μM) and RCMALFSLGDGAAMQQQLPQYWY from cumin seeds with α-amylase inhibitory activity (IC₅₀ values of 0.04 μM). Bioinformatic tools have been recently employed for the identification of bioactive peptides with anti-diabetic properties. For example, five peptides with inhibitory activities against α-amylase were identified from pinto bean using a phage display technique to study protein-ligand interactions and receptor binding sites (Ngoh, Lim, & Gan, 2016). α-amylase inhibitory peptides were also identified from cumin (Cuminum cyminum) seed using an integrated bioinformatics-phage display approach (Siow, Lim, & Gan, 2017). This approach was shown to be very efficient in discovering peptides with α-amylase inhibitory properties; two novel α-amylase inhibitory peptides were identified from the 56 unknown peptides initially found in the cumin seed protein hydrolysate (Siow et al., 2017). Another bioinformatics-assisted approach involving PeptideRanker and Pepsite2 software was used to identify peptides with α-amylase inhibitory activity from pinto bean (Ngoh & Gan, 2018) as well as rambutan (Nephelium lappaceum L.) and pulasan (Nephelium mutabile) seed proteins (Evaristus, Wan Abdullah, & Gan, 2018). α-amylase inhibitory peptides have the capability to occupy the catalytic and substrate binding sites of the enzyme and prevent the α-amylase from binding or hydrolyzing the substrate (carbonhydrate polymers). α-amylase inhibitory peptides may also attach to the starch and prevent it from digestion (Evaristus et al., 2018).

Bioactive peptides with α-glucosidase inhibitory properties have also been identified from milk (Konrad et al., 2014; Lacroix & Li-Chan, 2013), fish (Henriques et al., 2021), and egg proteins (Yu et al., 2011; Yu, Yin, Zhao, Liu, & Chen, 2012; Zambrowicz et al., 2015). Table 1 summarizes recent food-derived bioactive peptides with α-glucosidase and α-amylase inhibitory activities. Six weeks treatment of type 2 diabetic Goto-Kakizaki rats with PEPDIA, a milk protein hydrolysate containing dipeptides with α-glucosidase inhibitory effect, has been reported to improve glycemic control in these rats (Boulier, Auger, & Romelard, 2021). Plant proteins are another source to produce α-glucosidase inhibitory peptides. Brewers’ spent grain protein hydrolysates contain peptides with α-glucosidase as well as DPP-IV inhibitory activity (Connolly, Piggott, & FitzGerald, 2014). The proteolytic activity of the enzymes used for protein hydrolysis highly affects the bioactivity of peptides. Tryptic digests of the brewers’ spent grain protein isolate showed the highest inhibition of α-glucosidase while Corolase PP hydrolysates exhibited the highest DPP-IV inhibitory potential. Alcalase and Prolyve 1000 hydrolysates on the other hand, exhibited the most potent angiotensin converting enzyme (ACE) inhibitory effects (Connolly et al., 2014). Walnut (Juglans mandshurica Maxim.) protein isolates prepared by alkali-soluble acid precipitation and separated into different molecular weight fractions; <3 kDa, 3–10 kDa, and >10 kDa were tested for their α-glucosidase activity in vitro. The 3–10 kDa fraction exhibited α-glucosidase inhibitory rate of 61.7% and raised extracellular glucose consumption in insulin-resistant HepG2 cells. Administration of this peptide fraction to diabetic mice exhibited antidiabetic effects through reducing fasting blood glucose and increasing insulin secretion, liver glucokinase, and glycogen levels (Wang et al., 2018). Edible insect protein hydrolysates have also been recently reported to exert α-amylase and α-glucosidase inhibitory activities (Hall, Reddivari, & Liceaga, 2020; Yoon, Wong, Chae, & Auh, 2019). Alcalase-treated silkworm pupae and the Flavourzyme and Alcalase mixture-treated mealworm larvae were shown to inhibit α-glucosidase activity in vitro (Yoon et al., 2019). The cationic peptide fraction of cricket protein hydrolysate has been recently reported to exert α-amylase and α-glucosidase inhibitory activities with IC₅₀ values of 18.5 and 13.9 μg/mL, respectively (Hall et al., 2020). The inhibitory mechanism of α-glucosidase inhibitors has been attributed to the hydrophobic interactions of these compounds with the active site of the enzyme (Bharatham, Bharatham, Park, & Lee, 2008). In summary, peptides with α-amylase and/or α-glucosidase inhibitory effects have been identified from various food proteins which can potentially mitigate postprandial hyperglycemia. The effectiveness of such peptides on improving blood glucose levels need to be further evaluated in animal models as well as diabetic patients.

2.2. Peptides inhibiting glucose absorption

The products of carbohydrate digestion, namely glucose and galactose, are transported across the enterocytes through the intestinal brush-border membrane. This process occurs in two stages involving active sodium-glucose cotransporter 1 (SGLT1) and facilitative glucose transporter 2 (GLUT2). Thirty minutes after food consumption, the products of digestion reach the apical membrane of the jejunum where their absorption into the epithelial cells occurs predominantly by the SGLT1. GLUT2, on the other hand, is considered to provide basolateral exit of these hexoses from epithelial cells into the circulation (Chen, Tuo, & Dong, 2016; Roder et al., 2014). Therefore, factors influencing SGLT1 and GLUT2 activities will also alter glucose absorption and metabolism. SGLT1 is also shown to play an important role in intestinal glucose sensing and incretin secretion (Roder et al., 2014). Food-derived peptides targeting transporters involved in glucose absorption are less explored. Alcalase hydrolysate of black bean protein reduced postprandial glucose in an oral glucose tolerance test in normal healthy rats (Mojica, Gonzalez de Mejia, Granados-Silvestre, & Menjivar, 2017). This protein hydrolysate further reduced fasting glucose in hyperglycemic rats receiving two intraperitoneal injections of streptozotocin (Mojica et al., 2017). Oral administration of rice albumin to healthy rats has been reported to suppress blood glucose elevation in response to both starch and glucose loading (Ina et al., 2016). Tryptic digests of rice albumin containing indigestible high molecular weight (14 kDa) and low molecular weight (2 kDa) fractions, also suppress postprandial glucose elevation in healthy rats by inhibiting glucose uptake into small intestinal epithelial cells (Ina et al., 2020). The inhibitory mechanisms of these fractions were different; the high molecular weight fraction worked like dietary fibers by adsorbing glucose and retarding its diffusion rate while the low molecular weight fraction inhibited the expression of SGLT1 (Ina et al., 2020).
Table 1
Some examples of food-derived bioactive peptides with antidiabetic properties (α-amylase, α-glucosidase, and DPP-IV inhibition).

| Source | Treatments | Identified peptides | Mechanism of action | Model | Reference |
|--------|------------|---------------------|---------------------|-------|-----------|
| Sardine muscle | Subtilisin, trypsin, flavourzyme | Peptides < 1400 Dalton, NAPNPR, YACSVR | DPP-IV inhibition | In vitro | Rivero-Pino et al. (2020) |
| Sardine muscle | Alkaline protease | VW, YYP | α-glucosidase inhibition | In vitro | Matsui, Oki, & Osajima (1999) |
| Antarctic krill protein | Corolase PP | KVEFLP, PAL | DPP-IV inhibition | In vitro | Ji, Zhang, & Ji (2017) |
| Salmon gelatin | Corolase PP | GGPAGFAV, GPVA, PP, GF, arginine, tyrosine | DPP-IV inhibition, antioxidant | In vitro | Neves et al. (2017) |
| Casein (derived from bovine and camel milk) | Alcalase, pronase, and simulated gastrointestinal digestion | FLWPEYGAIL, ACGP, DGLHIPPL | α-amylase inhibition | In vitro | Mudgil et al. (2021) |
| Casein (derived from bovine and camel milk) | Alcalase, pronase, and simulated gastrointestinal digestion | LPTGWLM, MFE, GPAHCLL | α-glucosidase inhibition | In vitro | Bava et al. (2021) |
| Egg yolk protein (defatted) | Pepsin | YIEAVNVSPRAQF, YINQMPQKSL, YINQMPQKSREA, VTGRFAGHPAAQ | DPP-IV inhibition, antioxidant activity | In vitro | Zambrowicz et al. (2015) |
| Egg white proteins | Alcalase | RVPSLM, TPSPR | α-glucosidase inhibition | In vitro | Yu et al. (2011) |
| Brewers' spent grain protein-enriched isolate | Alcalase and simulated gastrointestinal digestion | LDLI, ILLGAQDGL | DPP-IV inhibition | In vitro | Connolly et al. (2017) |
| Chickpea Protein | Pepsin and pancreatin | PHPATSGGGL, YVDDSSTPTLT, SPOQSPFATPLW, YVDDSSTPTLT | DPP-IV, α-amylase, and α-glucosidase inhibition | In vitro | Chandrasekaran, Luna-Vital, and de Mejia (2020) |
| Bromalin | GKAAPGSGGTVK, KMTAGSGVTD, GATGASLGSGPSPLL | α-amylase and α-glucosidase inhibition | In vitro | Vilcacundo, Martinez-Villaluenga, Miralles, and Hernandez-Ledesma (2019) |
| Kiwicha protein | Pepsin and pancreatin | FLISCLL, SVFDEELS, DFHILE, NRPET, HVIKPPS | α-amylase and DPP-IV inhibition | In vitro | de Souza Rocha et al. (2015) |
| Common bean | Germination, alcalase hydrolysis, and simulated gastrointestinal digestion | RGPLVPNPPKFL | α-amylase and DPP-IV inhibition | In vitro | Olagunju, Omoba, Enujugba, Alashi, and Akoko (2021) |
| Pigeon pea protein | Thermolase | Peptide fractions of < 1, 1–3, 5–10, >10 kDa | α-amylase and α-glucosidase inhibition | In vitro | Siow and Gan (2016) |
| Quinoa protein (11S seed storage globulin B) | Simulated gastrointestinal digestion | IQAEGGLT, DKDVPK, GEHGSDGNV | DPP-IV, α-amylase, and α-glucosidase inhibition | In vitro | Vilcacundo, Martinez-Villaluenga, and Hernandez-Ledesma (2017) |
| Cummin seeds | Protamex | FFRSCLLSGDAAAAGKALLPLPYW, RCMACLSSDAAAGQQLPLPYW, DPAQNPYPYWTAYLVFRH | α-amylase inhibition | In vitro | Siow and Gan (2016) |
| Soy protein | Alkaline proteinase | LLPLPVLK, SWRL, WRL | α-glucosidase inhibition | In vitro | Wang et al. (2019) |
| Silk worm pupae protein | In-silico digestion and screening | SQSPA, QPGR, QPPT, NSPR | α-glucosidase inhibition | Quantitative structure-activity relationship modeling | Zhang et al. (2016) |
| Cricket protein | Alcalase and simulated gastrointestinal digestion (pepsin, bile salts and pancreatin) of the supernatant | Twenty eight peptides were identified but none of the peptides were verified against α-amylase and α-glucosidase enzymes | α-amylase and α-glucosidase inhibition | In vitro | Hall et al. (2020) |
| Mealworm larvae, cricket and silkworm pupae proteins | Flavourzyme and alcalase | Not determined | α-glucosidase inhibition | In vitro | Yoon et al. (2019) |
| Lesser mealworm protein | Thermolysin | Not determined | DPP-IV inhibition | In vitro | Lacroix, Daulos Teran, Fogliano, and Wichers (2019) |
| Cricket | Alcalase and simulated gastrointestinal digestion (pepsin, bile salts, and pancreatin) | Not determined | DPP-IV inhibition | In vitro | Hall, Johnson, and Liceaga (2018) |
| Housefly larvae | Water extraction | Protein fractions of > 6kDa molecular weight | DPP-IV inhibition | In vitro | Li et al. (2017) |
2.3. Insulinotropic peptides

The impairment of glucose-stimulated insulin secretion is a hallmark of β-cell failure in type 2 diabetes. β-cells are involved in insulin secretion by continually monitoring and responding to dietary nutrients to best meet the needs of the organism. While glucose is the primary stimulus for insulin secretion, specific amino acids such as Arg and Glu and fatty acids also regulate insulin secretion (Newsholme & Krause, 2012). The mixed nutrient sensing and outputs of glucose, amino acid, and fatty acid metabolism generate the metabolic coupling factors which activate signals to promote insulin biosynthesis as well as the movement of insulin containing vesicles to the cell surface and insulin release (Newsholme & Krause, 2012). Primary metabolic coupling factors in β-cells include ATP, NADPH, glutamate, long chain acyl coenzyme A, and diacylglycerol. Failure to generate enough coupling factors in a coordinated manner may underlie the failure of β-cell to secrete insulin during the pathogenesis of type 2 diabetes (Newsholme & Krause, 2012). Mitochondria play a key role in insulin secretion by generating ATP and other coupling factors. A rise in the ATP/ADP ratio and suppression of the ATP-sensitive potassium (KATP) channels activates the voltage-gated Ca2+ channels, which eventually lead to stimulation of insulin granule exocytosis (Jensen et al., 2008). Insulinotropic properties of food proteins have long been known (Calbet & MacLean, 2002; Floyd, Fajans, Conn, Knopf, & Rull, 1966; Lang et al., 1999; van Loon et al., 2003). The generation of certain peptides during protein digestion as well as composition and concentration of released amino acids are important factors in stimulating insulin secretion (Schmid et al., 1992). L-arginine is one such amino acid with strong insulin secretagogue properties. This amino acid also has a synergic effect for nutrient-dependent insulin secretion (Krause et al., 2011). In addition to the acute effects on β-cells and insulin secretion, amino acids may impact on insulin secretion and cellular integrity by influencing gene expression in β-cells following chronic exposure (Newsholme & Krause, 2012). Branched-chain amino acids and hydrophilic peptides have been reported to exert insulinotropic properties (Nongonierma et al., 2013). Similarly, Leu, Ile, Val, Lys, and Thr, generated after whey protein ingestion, were shown to have the strongest correlation with insulin response in healthy subjects (Nilsson, Stenberg, Frid, Holst, & Bjorck, 2004). The enzymatic hydrolysis of whey proteins has been reported to enhance insulin secretion in pancreatic BRIN-BD11 β-cells, improve blood glucose clearance, and restore the glucose-induced pancreatic islet capacity to secrete insulin in ob/ob mice (Gaudel et al., 2013). Fermented soybean, from which Meju—the Korean product used in many foods, also affects glucose homeostasis (Kwon, Hong, Lee, Sung, & Park, 2007; Yang, Kwon, Kim, Kang, & Park, 2012). Kwon and co-workers found that both isoflavonoid and peptide fractions of fermented meju affected adipocyte differentiation and insulin secretion (Kwon et al., 2011). Water extracts of 60-day fermented meju, mostly containing peptides of 15 KDa molecular weight, promoted insulin-stimulated glucose uptake and adipocyte differentiation in 3T3-L1 adipocytes. This fraction also enhanced glucose-stimulated insulin secretion and moderately enhanced β-cell proliferation in Min6 insulinoma cells. The specific sequence of peptides, however, was not identified in this fraction (Kwon et al., 2011).

2.4. Incretin mimetic peptides

Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) released by the intestinal K and L cells, respectively, after meal ingestion are known as incretins (Pais, Gribble, & Reimann, 2016). These peptide hormones regulate postprandial insulin secretion (Drucker & Nauck, 2006). Destruction of pancreatic islets in diabetic patients results in the reduced effect of incretins (Nauck et al., 2011). Administration of incretins to diabetic patients has been reported to increase β-cell proliferation along with insulinotropic effects without the risk of hypoglycaemia (Nauck & Meier, 2010). Enhancement of endogenous GLP-1 secretion by dietary factors is a promising strategy for the prevention of hyperglycaemia in type 2 diabetes. Amino acids, particularly glutamine, stimulate GLP-1 secretion and increase plasma GLP-1 level in humans (Greenfield et al., 2009; Samocha-Bonet et al., 2011). Wheat protein hydrolyzed by bacterial protease contains a glutamine rich low molecular weight fraction which increases GLP-1 secretion through activation of the Ca2+/calmodulin-dependent kinase II pathway in GLUTag cells (enteroendocrine L cell line) (Kato, Naknishi, Tani, & Tsuda, 2017). The effectiveness of this treatment on incretin secretion and regulation of blood glucose was further confirmed in normal healthy Sprague Dawley rats (Kato et al., 2017). Oligopeptides including LGG and GF along with the non-metabolizable peptide transporter-1 (PPT1) substrate glycin-sarcosine, have been reported to enhance GLP-1 release in murine primary L cells. Mechanistic experiments revealed that PPT1 and activation of Ca2+ channels are involved in the glycine-sarcosine-stimulated GLP-1 secretion (Dia-kogianni et al., 2013). The ileal administration of zein hydrolysate (prepared by papain) enhanced GLP-1 secretion while attenuated the glucose-included hyperglycaemia by enhancing insulin secretion in normal healthy rats (Mochida, Hira, & Hará, 2010). In a follow-up study, the anti-hyperglycemic effect of this treatment was shown in normal and diabetic rats. The oral administration of the zein hydrolysate attenuated hyperglycaemia by stimulating GLP-1 and GIP secretion in normal rats. The involvement of increased GLP-1/GIP secretion was determined using GLP-1/GIP receptor antagonists. This treatment also effectively reduced the glyemic response under oral glucose tolerance test in diabetic rats which was accompanied by increased GLP-1 and insulin secretion (Higuchi, Hira, Yamada, & Hará, 2013).

2.5. DPP-IV inhibitors

Incretins are rapidly cleaved and inactivated after release into the circulation by the action of the serine protease dipeptidyl peptidase IV (DPP-IV) (Irwin & Flatt, 2013). DPP-IV inhibitors are a class of oral antidiabetic drugs which can extend the half-life of the endogenous GLP-1 and GIP, and in turn prolong the insulin response (Green, Flatt, & Bailey, 2006). The potential of proteins from nine different food commodities as the precursors of DPP-IV inhibitory peptides was evaluated through an in-silico approach (Lacroix & Li-Chan, 2012). Milk caseins and bovine meat and salmon collagens were reported to be the best sources to produce DPP-IV inhibitors, whereas oat proteins were the least promising sources. Among the 2256 fragments from 34 proteins with peptide sequences matching the reported DPP-IV inhibitory peptides in the literature, dipeptides GA, GP, and PG were the most frequently occurring sequences (Lacroix & Li-Chan, 2012). IPI has been reported as the most potent DPP-IV inhibitory peptide with the IC50 of 5 μM, which is present in the primary sequence of several food proteins (Nongonierma & FitzGerald, 2014). Bioactive peptides with DPP-IV inhibitory effect have been reported to contain proline especially on the second N-terminus and flanked by leucine, valine, or phenylalanine (Harnedy-Rothwell et al., 2020; Rivero-Pino, Espeso-Carpio, & Guadix, 2020). Bovine α-lactalbumin hydrolysates generated by alcalase contain DPP-IV inhibitory peptides, ELKDLKGY and ILKDVGINY. These peptides could form hydrogen bonds, pi-cation interactions, and salt bridges with DPP-IV enzyme as shown by molecular docking studies (Gao, Gong, & Mao, 2020). Camel milk also contains peptides with DPP-IV inhibitory effects, as well as positive effects on insulin receptor activation and glucose uptake (Ashraf et al., 2021). Subjection of the alcalse hydrolysates of the brewers’ spent grain protein to the simulated gastrointestinal digestion improved DPP-IV inhibitory activity and two novel DPP-IV inhibitory peptides of ILDL and ILLPGAQDGL were identified (Connolly, O’Keefe, Nongonierma, Piggott, & FitzGerald, 2017). Although food proteins have been suggested to be precursors for DPP-IV inhibitory peptides (Liu, Cheng, & Wu, 2019), most of the studies in this area have been performed through in-silico or in vitro assays using biochemical tools involving purified porcine or human DPP-IV enzymes.
and a standard substrate for bioactivity measurements (Lammi et al., 2018). Despite being advantageous, these methods provide insufficient characterization of the peptides’ activity before performing expensive in vivo studies. Potential degradation of peptides by membrane associated peptidases can affect bioactive peptides’ activity which is not considered in in vitro assays. IPVDM is a boarfish-derived peptide with DPP-IV inhibitory activity. While the IC$_{50}$ value of 21.7±µM has been reported for the in vitro assay, the IC$_{50}$ value of this peptide for the cell-based assay is 44.3 µM. Similarly, GPSI, was shown to exert significantly lower activity in the cell-based assay (−312.5 µM) when compared to the in vitro assay (72.8 µM) (Harney-Rothwell et al., 2020). To fill the gap between the biochemical assays and in vivo studies, Lammi and co-workers have recently developed fast and sensitive DPP-IV assays using human intestinal cells and human serum. These assays were further validated on previously identified soy (IAPTGVA) and lupin (LTPPAGSAED) peptides with known DPP-IV inhibitory activity (Lammi et al., 2018). This experimental approach which combines in-situ and ex vivo DPP-IV assays seems to be promising for identifying food-derived peptides with DPP-IV inhibitory effect in a more realistic fashion compared to the in vitro biochemical assays. Only a few studies have explored the physiological effects of DPP-IV inhibitory peptides in vivo.

The antidiabetic potential of Atlantic salmon skin gelatin hydrolysate with in vitro DPP-IV inhibitory activity has been assessed in streptozotocin-induced diabetic rats (Hsieh, Wang, Hung, Chen, & Hsu, 2015). The 5-week oral administration of this treatment at a single dose of 300 mg/day in diabetic rats reduced blood glucose levels during an oral glucose tolerance test, inhibited plasma DPP-IV activity, and increased plasma GLP-1 and insulin levels (Hsieh et al., 2015). Tilapia skin gelatin hydrolysate has been shown to improve glucose tolerance and increase GLP-1 and insulin secretion in streptozotocin-induced diabetic rats (Wang et al., 2015). LPQNNPI is a gouda cheese-derived octapeptide with a high DPP-IV inhibitory activity. Administration of this peptide to female rats improved blood glucose response after an oral glucose tolerance test. However, plasma DPP-IV activity or concentration of incretin hormones were not reported (Unishi, Kabuki, Seto, Serizawa, & Nakajima, 2012), which makes it difficult to understand the mechanisms underlying these effects in vivo. Ileal administration of a zein hydrolysate attenuated the hyperglycemia in normal healthy Sprague Dawley rats by enhancing active GLP-1 concentration, insulin secretion, and reducing plasma DPP-IV activity (Mochida et al., 2010). A low molecular weight fraction (<1 kDa) of a Flavourzyme porcine skin gelatin hydrolysate shows in vitro DPP-IV inhibitory activity. The daily administration of this fraction to streptozotocin-induced diabetic rats at the dose of 300 mg/day improved glucose tolerance, reduced plasma DPP-IV activity, increased GLP-1 and insulin levels, and reduced glucagon content in these rats. GFPPLPD, GGKPSSMT, and GGHLFFC were the peptides identified from porcine skin gelatin hydrolysate (Huang, Hung, Jao, Tung, & Hsu, 2014). AGFAGDDAPR is a Chinese black tea-derived peptide with in vitro DPP-IV inhibitory properties. Administration of this peptide (400 mg/day) to streptozotocin-induced diabetic mice for 57 days enhanced blood GLP-1 and insulin concentration, improved beta-cells function, and suppressed proliferation of alpha-cells compared to the diabetic control mice (Lu et al., 2019). Therefore, DPP-IV inhibitory peptides have a great potential for glycemic control through enhancing GLP-1 and insulin secretion.

2.6. Satiogenic peptides

Altered satiety signaling is involved in the development of obesity and type 2 diabetes (Hellström, 2013). Various gut hormones including incretins have important physiological roles in the regulation of hunger and satiety. A decrease in the concentrations of ghrelin, the orexigenic gut hormone, along with an increase in anorexigenic peptides such as cholecystokinin (CCK), peptide YY (PYY), and GLP-1 are important changes that occur postprandially. Acting in concert on the brain, these hormones induce an eventual decrease in hunger and increase in satiety leading to meal termination (Field, Chaudhri, & Bloom, 2010). Pharmacological interventions enhancing the gut hormone signaling are considered potential treatments for obesity and type 2 diabetes.

Dietary proteins are believed to induce satiety feeling potentially through increasing the concentration of satiety hormones and energy expenditure (Veildhorst et al., 2008). Amino acid composition of ingested proteins has also been suggested to play a major role in the satiety-induced effects of high-protein diets (Mellinkoff, Frankland, Boyle, & Greipel, 1956). CCK is released from 1 cells of the small intestine in response to fat and protein and stimulates CCK1 receptors on vagal afferents in the brainstem and hypothalamus (Blevins, Stanley, & Redelberger, 2000). Food proteins contain peptides with stimulating effects on CCK secretion. The potential of chicken, pork, beef, beef liver, and egg white protein hydrolysates on CCK release from STC-1 cells was examined in one study (Sufian et al., 2006). Chicken and pork pepsin hydrolysates were shown to bind to the rat small intestinal brush border membrane and release CCK from STC-1 cells in a dose-dependent manner (Sufian et al., 2006). Orogastic administration of these protein hydrolysates to normal healthy rats suppressed 60-min food intake only in the porcine meat hydrolysate group (Sufian et al., 2006).

Marine-derived proteins and bioactive peptides also have strong satiety effects. Blue whiting (Micromesistius poutassou) muscle hydrolysate consisting of short peptides in range of 1000 Da enhanced CCK secretion in STC-1 cell line (Cudennec, Ravallec-Ple, Couroux, & Fouchereau-Peron, 2008). The administration of blue whiting hydrolysate to rats (normal healthy) reduced the short-term food intake along with an increase in the CCK and GLP-1 plasma levels. Chronic administration of this marine hydrolysate also decreased body weight gain in these rats (Cudennec, Fouchereau-Peron, Perry, Duclos, & Ravallec, 2012). Administration of a protein hydrolysate from smooth hound (Mustelus mustelus) to Wistar rats (normal healthy) for 21 days reduced the body weight compared to the control group (Bougatef et al., 2010). Despite the reduction in body weight, no significant changes in plasma CCK levels after thirty minutes of the oral administration of this hydrolysate was observed (Bougatef et al., 2010). Legume proteins from different sources such as soy (Nakajima, Hira, Eto, Asano, & Hara, 2010; Nishi, Hara, & Tomita, 2003; Nishi, Hara, Asano, & Tomita, 2003; Sufian et al., 2011), and some under-utilized beans such as Country bean and Yard long bean (Sufian, Hira, Asano, & Hara, 2007) are other potential candidates with beneficial effects on gut hormone release and appetite control. Jang and co-workers reported the anti-obesity effects of an isoflavone-free peptide mixture derived from black soybean (Rhzynchosia volubilis) in high fat diet-fed mice (Jang et al., 2008). This treatment reduced food intake through activation of the leptin-like signaling in hypothalamus and reduced body weight gain in mice fed a high fat diet for 13 weeks. Interestingly, identification of a hepta peptide IPPGVYPY in the plasma 30 min after oral administration of 1 g black soybean peptide mixture suggests the potential role of absorbed peptides in the observed physiological effects in vivo (Jang et al., 2008). Intraduodenal infusion of pepsin hydrolyzed soybean β-conglycinin suppressed food intake in normal healthy rats. The suppression of food intake by β-conglycinin hydrolysate was abolished by an intravenous injection of a selective peripheral CCK receptor antagonist. The infusion of β-conglycinin hydrolysate into the rat duodenum strongly suppressed gastric emptying with marked increase in portal CCK level. Further experiments revealed that β-conglycinin hydrolysate binds to components of the rat intestinal cell membrane directly and stimulates CCK release from these cells (Sufian et al., 2006). A considerable increase in protein structures has been reported to play a role in CCK release (Nishi, Hara, Hira, & Tomita, 2001). The fragment from 51 to 65 of the β-conglycinin’s β-subunit with the sequence of VRRLRQFNRKRS had the strongest binding activity to the rat small intestinal mucosal cells. Intraduodenal infusion of this peptide to normal healthy rats inhibited food intake and markedly increased portal CCK concentration. Different model peptides with Arg and Gly residues were constructed to further explore the structure requirements for the observed effects among which only GRGRGGRG had strong
binding affinity (Nishi, Hara, Asano, et al., 2003). Although food-derived peptides with CKR release stimulation are capable of controlling appetite, it should be noted that CKR release per se is not efficient in reducing long-term food intake and weight loss (Hellstrom, 2013).

2.7. Peptides improving peripheral glucose uptake

2.7.1. Activating phosphatidylinositol 3-kinase/protein kinase B (PI3K/Akt) pathway

Insulin signals signaling events that control the metabolic fate of nutrients. The phosphatidylinositol 3-kinase (PI3K) and the mitogen activated protein kinase (MAPK) pathways are the major signaling cascades mediating most metabolic and transcriptional effects of insulin (Laviola et al., 2006). The MAPK pathway mainly controls the mitogenic, growth, and cell differentiation processes in cells; phosphorylation and activation of Extracellular signal regulated kinase 1/2 (ERK1/2) in this pathway plays a direct role in cell proliferation and differentiation (Boucher, Kleinridders, & Kahn, 2014; Laviola et al., 2006). The PI3K pathway, being the other major signaling cascade of insulin, is responsible for most metabolic effects of insulin. Activation of the protein kinase B (Akt) in this pathway leads to the translocation of glucose transporters from intracellular sites to the plasma membrane for glucose uptake. Glucose transporters facilitate the transport of glucose passively across the cell membrane. Glucose transporter 4 (GLUT4) is the main isoform which mediates glucose uptake into muscle and fat cells (Bilous & Donnelly, 2010). Peripheral glucose uptake is impaired in insulin resistance and type 2 diabetes (Goodpaster et al., 2014). Indeed, the disruption of intracellular signaling pathways involved in GLUT4 translocation leads to insufficient channels for glucose uptake and accumulation of glucose extracellularly in insulin resistance and type 2 diabetes (Shulman, 2000).

The potential benefits of amino acids and bioactive peptides on enhancing glucose uptake have been reported in literature. Leu and Ile are the two amino acids accelerating glucose uptake (Oei et al., 2005; Nishitani et al., 2002). Daily administration of aglycin, a 37-amino-acid polypeptide isolated from soybean, at dose of 50 mg/kg/day for 4 weeks, was reported to effectively control hyperglycemia and enhance oral glucose tolerance in streptozotocin/high-fat-diet-induced diabetic mice (Lu et al., 2012). While insulin signaling was perturbed in skeletal muscle in these diabetic mice, aglycin restored insulin signaling by maintaining insulin receptor and insulin receptor substrate 1 (IRS-1) expression at both mRNA and protein levels. This peptide further enhanced phosphorylation of IRS-1 and Akt and increased membrane GLUT4 protein expression thereby increasing glucose uptake in skeletal muscle (Lu et al., 2012). Agents that block the renin angiotensin system have shown glucoregulatory potential in the context of metabolic syndrome (Jahandideh & Wu, 2020). The work from our group has shown the insulin sensitizing and mimetic properties of different food-derived antihypertensive peptides (Chakrabarti, Jahandideh, Davidge, & Wu, 2018; Jahandideh et al., 2019; Jahandideh, Chakrabarti, Davidge, & Wu, 2017). VPP and IPP, the casein-derived tripeptides with well-known antihypertensive properties (Kim, Kim, & Choie, 2008; Nakamura et al., 2011) also enhanced insulin signaling and contributed toward the prevention of insulin resistance in the presence of tumor necrosis factor in 3T3-F442A adipocytes (Chakrabarti, Jahandideh, Liu, & Wu, 2018). Egg white hydrolysate prepared by thermolysin and pepsin with antihypertensive properties (Jahandideh et al., 2016) also enhanced insulin effects on the upregulation of protein kinase B/Akt phosphorylation as well as increased ERK1/2 phosphorylation to a level similar to that of insulin in 3T3-F442A adipocytes (Jahandideh et al., 2017). Administration of this protein hydrolysate to insulin resistant rats further increased insulin sensitivity, improved oral glucose tolerance, and reduced systemic inflammation. EWH exhibited its insulin sensitizing effects through potentiating insulin induced Akt phosphorylation in muscle and adipose tissue (de Campus Zani, Jahandideh, Wu, & Chan, 2019; Jahandideh et al., 2019).

2.7.2. Activating AMP-activated protein kinase (AMPK) pathway

AMP-activated protein kinase (AMPK) is an evolutionarily conserved serine/threonine kinase known as a master regulator of metabolism (Ruderman & Prentki, 2004). Activation of this nutrient-sensing kinase occurs when cellular energy levels are low and results in restoration of normal energy levels by stimulating processes that generate ATP (such as fatty acid oxidation) and inhibiting those that use ATP (like triglyceride and protein synthesis) (Ruderman, Carling, Prentki, & Cacicedo, 2013). While AMPK pathway is impaired in animals and humans with type 2 diabetes (Coughlan, Valentine, Ruderman, & Saha, 2014), activators of this pathway can improve insulin sensitivity by stimulating glucose uptake in skeletal muscle, enhancing fatty acid oxidation in adipose tissue, and reducing hepatic glucose production (Zhang, Zhou, & Li, 2009).

Protein hydrolysates and bioactive peptides have been reported to activate AMPK pathway. Black soybean peptides were shown to activate AMPK in vitro (in myotubes) and in vivo (Jang et al., 2008). These peptides also restored insulin signaling in normal and insulin resistant HepG2 cells by stimulating Akt serine phosphorylation, forkhead transcription factor, Foxo1, and glycogen synthase kinase-3β (Jang et al., 2010). Oral administration of black soybean peptides to diabetic (db/db) mice showed antidiabetic effects partially through suppression of hepatic endoplasmic reticulum stress (Jang et al., 2010). The black soy peptide supplementation has also been reported to have a modest effect on reducing fasting glucose and improving glucose tolerance in Korean adults with prediabetes (fasting glucose ≥ 110 mg/dL) in a double-blind randomized placebo-controlled trial (Kwak et al., 2010). The low molecular weight (300–500 Da) fractions of soybean peptides have been reported to improve glucose uptake in L6 muscle cells in the presence of insulin. These charged peptides also activated AMPK in muscle cells, however, no increase in glucose uptake was observed in the absence of insulin in these cells (Roblet et al., 2014). IAVPGEVA, IAVPTGVA, and LPYPP, the peptides derived from soy glycinin hydrolysate, have been reported to activate AMPK pathway in hepatic cells. These peptides also increased glucose uptake in hepatic cells via activation of Akt (Lammi, Zanoni, Arnoldi, & Vistoli, 2015). Although this study demonstrates the potential of these peptides for enhanced glucose uptake via distinct Akt and AMPK pathways, the major effect of AMPK activation in hepatic cells is to inhibit glucose production (Coughlan et al., 2014), use of muscle cells or fat cells rather than HepG2 cells would be more relevant. Treatment of L6 myotubes with dipeptide WH significantly increased phosphorylation and activation of AMPKα, GLUT4 translocation to the plasma membrane, and glucose uptake into L6 myotubes. It has also been shown that activation of AMPKα occurs after transportation of WH into cells via the peptide transporter (Soga, Ohashi, Taniguchi, Matsui, & Tsuda, 2014).

2.8. Peptides promoting adipocyte differentiation

Adipose tissue has an important role in controlling whole-body glucose and lipid homeostasis in both normal and disease states by sequestering fat and producing various hormones and cytokines. Chronic excess calorie intake and the inability to generate new fat cells (adipocytes) may cause ectopic fat deposition, resulting in peripheral insulin resistance, particularly in skeletal muscle (Guilherme, Virbasius, Puri, & Czech, 2008). Decreased expression of adipogenic genes has been reported in obese subjects with type 2 diabetes (Dubois et al., 2006). Adipocyte differentiation generates new adipocytes with higher capacity for fat storage, and functional adipose tissue in proper proportion to body size is required for the normal insulin sensitivity and glucose homeostasis (Longo et al., 2011). Two key transcription factors in this process are the peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer binding protein (C/EBP). PPAR-γ activates genes involved in adipocyte differentiation and fatty acid sequestration (Abrahamian et al., 2013). Several food-derived peptides have been reported to increase adipocyte differentiation in vitro and in vivo.
vivo. Milk-derived peptides, IPP and VPP, enhance adipocyte differentiation through upregulation of PPARγ and C/EBP-α. The effect of these peptides on adipocyte differentiation and adiponectin release was similar to insulin. IPP and VPP further exerted anti-inflammatory effects by inhibiting the cytokine mediated activation of the pro-inflammatory NF-κB pathway in adipocytes (Chakrabarti & Wu, 2015). Oral administration of VPP to C57BL/6J mice ameliorated diet-induced chronic inflammation in adipose tissue (Aihara, Osaka, & Yoshida, 2014; Sawada et al., 2015). High fat feeding resulted in the accumulation of activated monocytes and pro-inflammatory macrophages in the stromal vascular fractions of the adipose tissue, while VPP supplementation significantly reduced the pro-inflammatory status in adipose tissue (Aihara et al., 2014). VPP administration has also been reported to improve insulin sensitivity, reduce TNF-α and IL-1β expression, and macrophage accumulation and activation in diet induced obese mice (Sawada et al., 2015). Chlorellla protein hydrolysate has shown favorable effects on glucose tolerance and insulin sensitivity in high-fat fed mice (Noguchi, Yanagita, Rahman, & Ando, 2016). Smaller adipocytes, lower triglycerides levels in liver, increased serum MCP-1 as well as MCP-1 mRNA expression in adipose tissue was correlated well with enhanced glucose tolerance and insulin sensitivity in chlorella hydrolysate-treated mice as compared to the control group. Considering the role of MCP-1 in development of inflammation and macrophage infiltration, less adipose tissue inflammation has been suggested as the key mechanism for the observed beneficial effects of chlorella-derived peptides in obese mice (Noguchi et al., 2016). In a similar vein, rice bran protein hydrolysate promoted the gene expression of PPARγ in adipose tissue of high carbohydrate-high fat fed rats similar to pioglitazone. Serum adiponectin was enhanced while adipose tissue inflammatory markers were decreased in rats treated with rice protein hydrolysate (Boonlohl et al., 2015). We have recently shown the beneficial effects of egg white hydrolysate on adipocyte differentiation and insulin sensitivity in cell culture (Jahandideh et al., 2017, 2018) and animal studies (Jahandideh et al., 2019). Administration of egg white hydrolysate in insulin resistant rats reduced adipocyte size and increased PPARγ2 protein abundance in the adipose tissue, as well as reduced inflammation in these rats (Jahandideh et al., 2019). Despite the well-documented beneficial effects of enhanced PPARγ expression and adipocyte differentiation on insulin sensitivity, inhibition of adipocyte differentiation has been associated with beneficial outcomes. DJVDKIEI, an octapeptide derived from boiled tuna, has been reported to inhibit C/EBP-α expression in 3T3-L1 adipocytes (Kim, Kim, Choi, Lee, & Nam, 2015). However, it should be noted that inhibition of adipocyte differentiation per se without affecting whole body energy balance is not beneficial for adipose tissue health and function. Inhibition of adipocyte differentiation could possibly result in the generation of hypertrophied adipocytes with less buffering capacity for circulating fats, and hence redistribution of body fat into non-adipose peripheral tissues in physiological conditions (Kim & Park, 2011). This would eventually lead to the development of insulin resistance in these tissues. Moreover, the hypertrophied adipocytes showed pro-inflammatory state due to the endocrine characteristics of the adipose tissue (Guilherme et al., 2008), which would result in the inactivation of insulin signaling and development of systemic insulin resistance. Whey peptides have been reported to promote adipocyte differentiation, PPARγ and PPAR6 activation to increase lipid storage and oxidation, respectively. In myotubes, whey peptides ameliorate palmitate-induced inflammation, diacylglycerol accumulation and increase sequestration of fatty acids in the triglyceride pool, thereby countering insulin resistance (D’Souza et al., 2020). Table 2 summarizes the antiadipic potential of protein hydrolysates/bioactive peptides through cell-based, animal studies as well as clinical trials.

3. Challenges on bioactive peptides research

The classical workflow for the production and discovery of bioactive peptides involves several steps as outlined in Fig. 2. This approach starts with identifying a suitable protein source, followed by steps to release bioactive peptide fragments, initial screening for a targeted bioactivity, multi-step fractionation procedures, identification of the peptide sequence, and ends with bioactivity validation using chemically synthesized pure peptides (Li-Chan, 2015). This classic approach for bioactive peptide discovery is often time consuming and costly. Moreover, commercialization of peptide discovery is challenging due to the low yield and purity of the peptides after extensive fractionation and isolation. Therefore, a feasible workflow that allows researchers to overcome these challenges is needed. Bioinformatics, referring to applied computational methods to manage, curate, and interpret information on biological systems, is becoming increasingly important in the discovery of food-derived bioactive peptides. These tools facilitate bioactive peptide discovery through the prediction of peptides’ biological activity and optimization of classical procedures for their production. Therefore, bioinformatics provides a cost-effective strategy in the discovery of bioactive peptides by reducing steps in the traditional workflow (Li-Chan, 2015; Sánchez-Rivera, Martínez-Maqueda, Cruz-Huerta, Miralles, & Recio, 2014). Artificial intelligence (AI) which includes machine learning approaches, has been recently employed in the discovery of food-derived bioactive peptides (Kennedy et al., 2020; Reit et al., 2019). An AI approach to discover a functional ingredient capable of modulating glucose levels was utilized by Chauhan and co-workers.

Following prediction of pea (P. sativum) as an optimal plant source of bioactive peptides with glucoregulatory properties, NRT_N0G5JU (PepTifiForce™) was produced, and its activity and safety was validated in human skeletal muscle cells. The antiadipic effects of this peptide were then further confirmed in a diabetic murine model followed by a clinical trial carried in a prediabetic population (Chauhan et al., 2021).

Considering the potential effect of exopeptidases on peptide digestion by these methods can further improve their power in predicting the release of peptides with both in vitro and in vivo biological properties (Sato, 2018). After discovery and production of peptides either through in-silico or classical approaches, evaluation of the biological activity of peptides in vivo is critical. Many studies in this area rely on in vitro and cell-based assays for identification of peptides and assessment of biological activities. In vivo assessment of bioactive peptides has been done, but these are uncommon, and clinical studies for validation of bioactive peptides effectiveness in humans are even more scarce. The sparse data on bioavailability and metabolic fate of bioactive peptides in vivo is a major challenge in the field of bioactive peptides’ research. Additionally, information on absorption, distribution, metabolism, and excretion of bioactive peptides is critical. Susceptibility of the peptides to degradation by gastric, pancreatic, and small intestinal brush border membrane enzymes has not been considered in majority of the research on bioactive peptides especially for the peptides which exert their bioactivity via the systemic circulation. Considering the harsh condition of the gastrointestinal tract during digestion as well as food matrix and interactions between food components (especially between peptides and polyphenols) (Perez-Gregorio, Soares, Mateus, & de Freitas, 2020), it is likely that only minute quantities of the bioactive peptides pass into the systemic circulation, which may be insufficient to induce biological activity. Indeed, food-derived peptides usually present in the body as di- or tripeptides at concentrations of up to 100 μM (Sato, 2017, 2018). Whether ingestion of these peptides or the hydrolysate containing them would result in the release of peptides which are active at these concentrations, or whether the peptides are converted to inactive forms post-oral delivery is an important issue to consider. To account for the shortcomings of the in vitro activity-guided fractionation approach for peptides’ bioavailability, Sato has recently proposed a new approach for identification of bioactive peptide in the target organ first followed by an examination of their in vitro and in vivo activities (Sato, 2018). Pre-identification of peptides present in the in vitro exopeptidase digests of food peptide is helpful for identification of the food-derived peptides in the body.

Due to the bitter taste of peptides, successful implementation of
Table 2  
Some examples of food-derived bioactive peptides/protein hydrolysates with antidiabetic properties (cell-based, in vivo, and human studies).

| Source | Treatments | Identified peptides | Observed effect/Mechanism of action | Model | Reference |
|--------|------------|---------------------|-------------------------------------|-------|-----------|
| Black bean common beans (Black 8025, Pinto Durgo) | Alcalase or bromelain | Peptides < 1 kDa, FFL, QLGGH, LLSSL, WGVFN, RFEFLMLLLQ, LLLLEDRRR, EPHIGK, HVQNO, NDEPASG | DPP-IV inhibition, increase insulin secretion, improve insulin signalling, enhance insulin-induced glucose uptake via Akt modification | Pancreatic β-cells, adipocytes, in vitro | Toledo, de Mejia, Sivaguru, and Amaya-Llano (2016) |
| Black bean protein isolate | Alcalase | AKSPLF, ATNFLPL, FEELN, and LSVSVL | Blocking GLUT2 and SGLT1 (reduce glucose absorption), reduce fasting and postprandial glucose levels | Caco-2 cells, in-silico, healthy and diabetic rats | Mojica et al. (2017) |
| Pea protein | Synthetic peptides | VLP, LLP, LI, LL | Increase hepatic glucose absorption and consumption through IRS-1/PI3K/AKT and p38MAPK pathways. Increase GLUT2 gene expression and protein content (LLP, VA, LL), decrease intracellular ROS and TNF-α | Insulin resistant HepG2 cells | Zhu et al. (2020) |
| Pea protein | Alcalase and neurase | ALP, VLP, LLP, SP | Reducing blood glucose levels, improving glucose tolerance, promoting insulin release and glycogen synthesis, and protecting liver and kidney structures | High fat fed and streptozotocin (STZ)-induced diabetic mice | Wei et al. (2019) |
| Foxtail millet protein isolates | Raw and cooked protein | Not determined | Hypoglycemic effects through rewiring glucose homeostasis, mitigating diabetes-induced gut dysbiosis. The cooked foxtail millet protein isolate affected the GLP-1R/PEP/K/ACT pathway and reversed the weight loss trend and alleviated lipid disorders in diabetic mice | STZ-induced diabetic mice | Fu et al. (2021) |
| Wheat gluten | Commercial protein isolate (HyPep 4601) | Not determined | Suppression of food intake in healthy rats, elevating plasma PYY levels, stimulation of CCK and GLP-1 in enteroendocrine cells | Enteroendocrine cell lines (STC-1 cells and GLUTag cells), and Wistar rats | Chen, Hira, Nakajima, and Hara (2018) |
| Walnut | Neutrase and alcalase | LVRL, LRYL, VILALVLLR | Improve glucose consumption, glucose uptake, and GLUT4 translocation, elevation of p-IRS-1 and p-Akt. Inhibition of glucose-induced insulin resistance by activating IRS-1/PI3K/Akt and Nrf2/HO-1 signaling pathways | HepG2 cells | Wang et al. (2020b) |
| Walnut | Alcalase | LPRLR | α-glucosidase and α-amylase inhibition, improving hepatic insulin resistance, increase glycogen synthesis and glucose uptake, decrease gluconeogenesis via activating the IRS-1/PI3K/Akt and AMPK signal pathways | Glucose induced insulin resistant HepG2 cells | Wang et al. (2020a) |
| Walnut | Neutrase and alcalase | Peptide fractions with 3–10 KDa | α-glucosidase inhibition, increase in extracellular glucose consumption, reduce fasting blood glucose, increase in insulin secretion, liver glucokinase and glycogen levels | Insulin-resistant HepG2 cells, STZ-induced diabetic mice | Wang et al. (2018) |
| Egg white protein | Thermolysin and peptidase | WEKAFKDED, QAMPRFRTVEQ, ERYPIIL, VFKGL | Enhance pre-adipocyte differentiation, show insulin mimetic and sensitizing effects (Akt and ERK1/2 phosphorylation), improve glucose uptake, glucose tolerance, and reduce systemic inflammation, reduce adipocyte size and increased PPAR/2 protein abundance and activity. | 3T3-F442A pre-adipocytes and diet-induced insulin resistant rats | de Campus Zani et al. (2019), Jahandideh et al. (2017), Jahandideh et al. (2018), and Jahandideh et al. (2019) |
| Egg protein (lysozyme) | Alcalase | Not determined | Decrease in glucose and insulin levels | Overweight and obese subjects with impaired glucose tolerance or type 2 diabetes | Plat, Severins, and Mensink (2019) |
| Kioarfish protein | Alcalase, and flavourzyme | Twenty two DPP-IV inhibitory peptides, fifteen insulinotropic peptides. IPVDM and IPV (the most active) | DPP-IV inhibition, insulinotropic activity | In vitro, Caco2 cells and pancreatic BRIN-BD11 cells | Harnedy-Rothwell et al., 2020 |
| Blue whiting | Not determined | | | | Harnedy et al. (2018) |

(continued on next page)
trol and health claims as well as audit purposes in order to develop appropriate standards for manufacturing of such products, their quality consideration of the evolving regulatory environment. One important step—bioactive peptides as functional food ingredients relies on enhancing the organoleptic properties of peptides. Moreover, development of new tools for the prediction and evaluation of peptides bitterness can potentially pave the road in commercialization of bioactive peptides in food industry. Partial least square regression models constructed with the e-tongue and the combination of size exclusion chromatography and reversed-phase HPLC have been used recently for the prediction of bitterness of dairy protein hydrolysates (Newman et al., 2014). Adoption of such models has the potential to reduce the reliance of bioactive peptides on sensory analysis in future studies.

Finally, successful translation of discoveries in the field of bioactive peptides to viable health promoting products requires a thorough understanding of the evolving regulatory environment. One important step would be defining products containing bioactive compounds precisely and avoid using imprecise and overly broad terminology (e.g. functional foods, novel foods, foods for special uses, supplemented foods etc.).

Placing novel formulations of bioactive peptides in their appropriate place within the food-drug continuum is critical in establishing appropriate standards for manufacturing of such products, their quality control and health claims as well as audit purposes in order to develop commercial products (Chakrabarti, Guha, & Majumder, 2018).

**Table 2 (continued)**

| Source | Treatments | Identified peptides | Observed effect/Mechanism of action | Model | Reference |
| --- | --- | --- | --- | --- | --- |
| **Whey protein** | Protease enzymes from *Bacillus subtilis* and *Aspergillus oryzae* | IV, LV, VI, II, LI, LL | Increase glucose uptake and glycogen synthesis | L6 myotubes and isolated skeletal epitrochlearis muscles | Morifuji, Koga, Kawanaka, & Higuchi, 2009 |
| **Casein** | Food grade gastrointestinal enzymes (pepsin and pancreatin) | Not determined | Reducing blood glucose and lipid, more responsive to glucose in glucose-stimulated insulin secretion. In human trial, increase in insulin secretion with a reduction in glucose was observed, while no effect on c-peptide or GIP secretion was noted | Healthy individuals (young and old) | Dale et al. (2018) and Jemsen et al. (2019) |
| **Horn beetle** | Ethanol extract | Not determined | Inhibition of adipogenesis and lipogenesis, reduce serum triglyceride and leptin contents | 3T3-L1 adipocytes; high fat diet-fed mice | Chung, Yoon, Hwang, Goo, and Yun (2014) and Yoon et al. (2015) |
| **Horn beetle** | Dried ethanol extracts | Not determined | Reducing hypothalamic endoplasmic reticulum stress, body weight and appetite through mTOR ad MAPK signaling pathways | High fat diet obese mice | Kin, Yun, Park, Goo, and Soo (2016) |
| **Yellow mealworm larvae** | Water and dried ethanol extracts | Not determined | Inhibition of adipogenesis through AMPK and MAPK signaling, reduce body weight gain, fat mass, adipocyte size as well as hepatic steatosis | 3T3-L1 adipocytes and high fat diet obese mice | Seo et al. (2017) |

4. Conclusions

Development and discovery of bioactive peptides for use in the treatment of diabetes is a growing research field. The recent discoveries in the field of bioactive peptides and their potential effects on pathways and target cells in the management of glucose and energy metabolism presents new opportunities for the use of such peptides in enhancing adipocyte differentiation and insulin signaling, CCK receptor binding and expression, and incretin stimulants, to name a few. However, there is a paucity of evidence related to the efficacy of such bioactive peptides in models of diabetes, and more research is required to validate their potential benefits in vivo. Furthermore, clinical studies are also required to evaluate the physiological effects of anti-diabetic food-derived peptides in human. Lack of scalable, cost-efficient, and consistent techniques to produce bioactive peptides, the unknown impact of the food matrix on absorption and bioavailability of bioactive peptides, and scarce data on pharmacokinetic and pharmacodynamics of bioactive peptides are some of the challenges associated with the commercialization and utilization of food-derived bioactive peptides for their health benefits. Taste and other sensory attributes of the final product containing bioactive peptides also need to be considered for successful adoption of peptides as functional food ingredients that can improve health and promote resilience.
Fig. 2. The classical and in-silico approaches for the production and discovery of bioactive peptides from food proteins. AI: artificial intelligence; RSM: response surface methodology; QSAR: quantity structure activity relationship.

Declaration of Competing Interest

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Author Contributions

Conceived and designed the idea for the manuscript: Forough Jahandideh; Revised the manuscript: Stephane Bourque and Jianping Wu. Wrote the manuscript: Forough Jahandideh et al.; Revised the manuscript: Stephane Bourque and Jianping Wu.

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