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Review

Proteins and bioactive peptides

Mechanisms of action on diabetes management

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Abstract

Type 2 diabetes mellitus (T2DM) is a major metabolic, multi-causal and heterogeneous disorder, characterised by chronic hyperglycaemia, which causes significant morbidity and mortality, with a considerable burden on health-care resources. The number of deaths due to T2DM highlights the importance of controlling the disease and its complications. It has been demonstrated that some proteins, protein hydrolysates, bioactive peptides and amino acids can control glucose levels directly or indirectly. Bioactive peptides have been identified in a range of food ingredients and offer the potential for incorporation into functional and nutraceutical foods. In this review, we discuss the possible mechanisms by which these compounds exert their action on glucose control such as modulating insulin production, incretin secretion, dipeptidyl peptidase-4 inhibition, regulation of glucose uptake in peripheral tissue and inhibition of some enzymes related with glucose absorption. Peptides such as IPAVF, PGVGGPLGPIGPCYE, CAYQWQRPVNRIR, PACGGFYISGRPG, WV, GPAE, GPGA, LP, IP, KLPGF and LI have shown potential for regulating blood glucose. Bioavailability and delivery of bioactive peptides are also discussed.

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Introduction

The incidence of non-transmittable diseases has increased not only in industrialised countries but also in developing nations, affecting their economies [1]. Diabetes mellitus is one of these and consists of a metabolic disorder, characterised by chronic hyperglycaemia and alterations in the metabolism of fats and proteins, as well as an increase in β-cell apoptosis. Type 2 diabetes mellitus (T2DM) is a heterogeneous metabolic disorder characterised by both insulin deficiency and peripheral insulin resistance. The oxidative stress in T2DM, a consequence of sev-

eral abnormalities including hyperglycaemia, plays a key role in the pathogenesis complications including atherosclerosis, retinopathy, nephropathy and neuropathy [2].

In non-diabetic individuals, when blood glucose levels increase, pancreas β-cells secrete insulin, promoting glucose uptake into the tissues and normalising the blood glucose level. However, in T2DM patients, this regulation is affected, resulting in high levels of blood glucose, particularly in the postprandial state [3]. In the treatment of diabetes, the first line of action is lifestyle changes. Diet is a key element and aims to reduce energy consumption and stimulate energy expenditure with the intention of reducing the fat mass, which is associated with insulin resistance. In addition, a diet containing foods that have beneficial effects beyond traditional nutrition is recommended [4].

There are several food ingredients that are known to be beneficial for T2DM patients. In this review, we specifically address proteins, protein hydrolysates, bioactive peptides and amino acids in T2DM control. Bioactive peptides from enzymatic hydrolysis of proteins, which usually range from 2 to 20 amino acid residues, may be an alternative to control T2DM [5, 6]. Bioactive peptides are inactive when they are part of the protein, but once released by digestion or by enzymatic hydrolysis, they may produce a health benefit, improving the function of the cardiovascular, endocrine, immune and nervous systems in addition to nutrient utilisation [5]. Bioactive peptides could express their function in the intestinal tract or inside the body after being absorbed.

The objective of this review is to present the possible mechanisms by which amino acids, bioactive peptides and proteins act on glucose control and also their bioavailability and delivery.

Absorption and bioavailability of bioactive peptides

The use of potential bioactive peptides, to a large degree, depends on their bioavailability after oral administration, ensuring their effect on the target organ [7]. Peptides that resist the digestive process and arrive intact in the intestine can have a local function or may be able to cross the epithelium, enter the blood stream and have a systemic effect. Therefore, it is important to study peptide stability in digestion, absorption, distribution, metabolism and excretion.

Small intestine epithelial cells are the primary site of absorption of nutrients such as glucose and amino acids. Most oligopeptides are extracellularly hydrolysed by enzymes in the brush border membrane (BBM) of the intestinal epithelium and cytoplasm, producing amino acids that can be absorbed in the intestinal mucosa. Some peptides are able to resist enzymatic attack, and it has been suggested that this ability is due to their amino acid composition [8]. For example, peptides with proline (P) and hydroxyproline (Hyp) residues resist degradation by digestive enzymes. Dipeptides and oligopeptides from casein and gelatin that contain proline residues in the C-terminal resist hydrolysis from peptidases [7].

Peptides can be transported through the intestinal epithelium (Fig. 1) by a specific carrier via paracellular transport or transcellular or transcytotic routes [8]. Smaller peptides, di- and tripeptides, can be absorbed intact and hydrolysed later. It has been demonstrated that these peptides can be absorbed by H+-coupled oligopeptide transporters (POT)

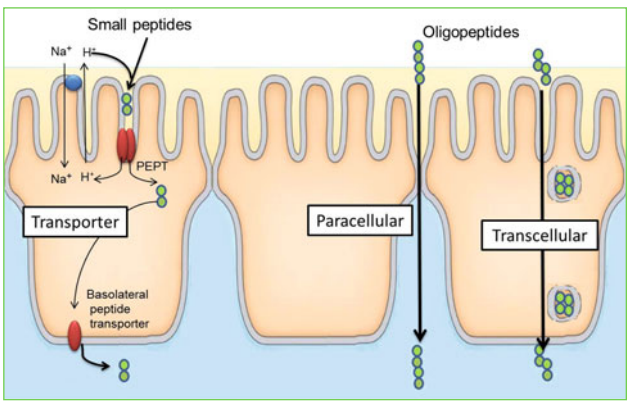


Figure 1 Routes through which peptides can be carried in the intestinal epithelium

across the cells. The proton-coupled oligopeptide transporter SLC15 family consists of four distinct isoforms: peptide transporters 1 and 2 (PEPT1/SLC15A1 and PEPT2/SLC15A2) and peptide-histidine transporters 1 and 2 (PHT1/SLCA4 and PHT2 SLCA3). PEPT1, expressed in the small intestine and located in the BBM, as a member of the POT family, uses a transmembrane electrochemical proton gradient as a broad-specificity transport force. PEPT1 facilitates the transport of small digestion-resistant peptides from the enterocytes into the bloodstream [9, 10]. The bioactive dipeptide derivative anserine is absorbed intact at the intestinal epithelium by the transporter PEPT1 [11]. The tripeptide GP-Hyp is partially hydrolysed on the apical membrane, but the dipeptide P-Hyp is transported across the apical membrane of the cell via PEPT1 [12].

The human intestinal peptide transporter 1 (HPT1), also known as the liver-intestine cadherin, a non-SLC member, is related to proteins of the cadherin family, and it has been demonstrated to facilitate transport of peptide-based drugs [12, 13].

Paracellular transport is an aqueous pathway involving diffusion (through passive diffusion) of the peptide in the extracellular space between adjacent cells, which is restricted by tight junctions of the cells [8, 14]. Quirós et al. suggested the paracellular route to be the intestinal absorption route of the pentapeptide HLPLP, an antihypertensive peptide [15].

Peptides can also enter through the transcytosis route, which implies endocytosis, by which the cell membrane forms a vacuole to transport the peptide [8, 16].

Once the peptides reach their target organs, these organs may then exert their action. The insulinotropic effect of an enzymatic bovine whey protein hydrolysate in β-cells and ob/ob mice and the ability of the peptides to cross the intestinal epithelium in an *in vitro* caco-2 cell model suggest the transport of di- and tripeptides via PEPT1 [17].

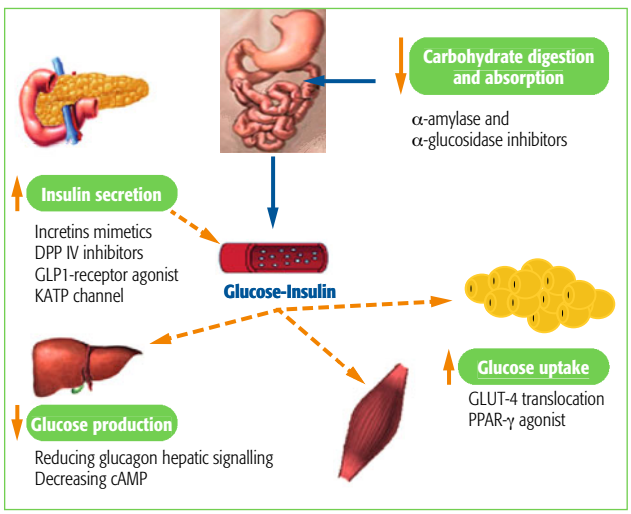


Figure 2 Mechanisms by which amino acids, peptides, protein hydrolysates and proteins could control glucose levels; inhibiting some enzymes related to glucose absorption, incretin secretion, DPP-IV inhibition and regulation of glucose uptake in peripheral tissue

Hypoglycaemic effects of protein, bioactive peptides and amino acids

The goal in T2DM is to keep blood glucose levels normal. This can be achieved with diet, exercise and the use of medications [18]. A healthy diet can help to prevent and control diabetes, and the dietary pattern for diabetes prevention and control converges on the consumption of fruits and vegetables, whole grains, dairy products, nuts and legumes [19]. In addition, the consumption of dairy products, a good source of protein, has been associated with a moderately lower risk of diabetes. The American Diabetes Association [20] does not recommend reducing the amount of dietary protein for people with diabetes and kidney disease, and the Diabetes and Nutrition Study Group of European Association for the Study of Diabetes [21] concluded that there is insufficient evidence to recommend protein restriction for those with T2DM and incipient nephropathy.

There are different mechanisms by which amino acids, peptides, protein hydrolysates and proteins can control glucose levels including inhibition of α-glucosidase, α-amylase and dipeptidyl peptidase-IV (DPP-IV) inhibitors, incretin and insulin secretion, increase of glucose uptake and decrease of glucose production (Fig. 2). Table 1 shows peptides

Source	Enzyme used	Amino acid sequence	Mechanism of action	Inhibition	Model	Reference
***In vitro* models**						
β-Lactoglobulin	Trypsin (EC 3.4.21.4)	IPAVF	DPP-IV inhibition	IC_{50}: 44.7 μM	Enzymatic assay	[55]
α-Lactoalbumin, β-lactoglobulin, lactoferrin, bovine serum albumin, whey protein isolate	Pepsin (EC 3.4.23.1)	–	DPP-IV inhibition	α-Lactoalbumin hydrolysate showed the best IC_{50}: 0.036 mg/ml	Enzymatic assay	[42]
β-Lactoglobulin, whey protein isolate	Pepsin (EC 3.4.23.1)	–	α-Glucosidase inhibition	IC_{50} 3.5±0.4 mg/ml and 4.5±0.6, respectively	Enzymatic assay	[56]
Whey protein	Pancreatic enzyme preparation	–	Insulin secretion	–	BRIN-BD11 β-cells	[17]
Casein	Gastrointestinal preparation Protease XXIII Orientase	WV	DPP-IV inhibition	IC_{50} 65.69±2.95 μM	Enzymatic assay	[43]
Tuna cooking juice		PGVGGPLGPIGPCYE, CAYQWQRPVNRIR, PACGGFYISGRPG	DPP-IV inhibition	IC_{50} 116.1, 78.0 and 96.4 μM, respectively	Enzymatic assay	[57]
Salmon skin gelatin	Alcalase (EC 3.4.21.62), Bromelain (EC 3.4.22.33), Flavourzyme (EC 3.4.11.1)	GPAE, GPGA	DPP-IV inhibition	IC_{50} 49.6 and 41.9 μM, respectively	Enzymatic assay	[58]
Egg albumin	Alcalase (EC 3.4.21.62)	RVPSLM, KLPGF	α-Glucosidase inhibition and α-amylase inhibition	α-Glucosidase inhibition IC_{50} of 23.1 and 59.5, and α-amylase inhibition IC_{50} N.D. and 120.0 μmol, respectively	Enzymatic assay	[24, 25]
Silk cocoon	Protease N (3.4.24.28)	GEY, GYG	α-Glucosidase inhibition	IC_{50} 2.7 and 1.5 mg/ml, respectively	Enzymatic assay	[26]
Dry-cured ham		AA, KA, AAATP	DPP-IV inhibition	IC_{50} 9.4, 6.3 and 6.5 mM, respectively	Enzymatic assay	[41]
Defatted rice bran	Umamizyme G, Bioprase SP	LP, IP	DPP-IV inhibition	IC_{50} 2.4±0.17 and 0.41±0.07 mM, respectively	Enzymatic assay	[59]
Amaranth	Trypsin 7 (EC 3.4.21.4) pancreatin	–	DPP-IV inhibition	IC_{50} of 1.1 mg/ml	Enzymatic assay	[46]
Soy protein	Pepsin (EC 3.4.23.1) and pancreatin	–	Glucose uptake in muscular cells via AMK activation	–	Glucose uptake in L6 myocytes	[60]
Flaxseed	Trypsin (EC 3.4.21.4) and pronase (EC 3.4.24.4)	–	Glucose uptake in muscular cells		Glucose uptake in L6 myocytes	[61]
***In vivo* models**						
Whey protein	Pancreatic enzyme preparation	–	Insulin secretion	–	Heterozygous ob/wild-type mice	[17]
Whey protein		LI, L	Glut 4 translocation	–	Male Wistar rats	[51]
Chum salmon			β-cells protection reducing oxidative stress and inflammation	–	Diabetic male Sprague–Dawley rats	[62]

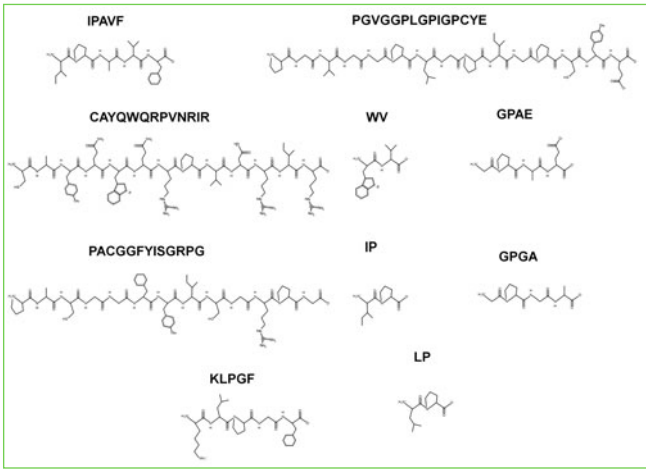
Table 1 Potential anti-diabetic activity associated with protein hydrolysates and peptides

with the potential to control glucose levels by different mechanisms of action and Fig. 3 shows the chemical structure of some of these peptides.

α-Glucosidase and α-amylase inhibitors

α-Glucosidase inhibitors delay carbohydrate digestion via competitive inhibition of α-glucosidase enzymes located in the brush border of the enterocytes that line the intestinal villi [22].

This process prevents breakdown of disaccharides and oligosaccharides into absorbable monosaccharides. Glucose absorption is completed over a longer



Sequence	Molecular Mass (Daltons)	Isoelectric point	Net charge	Hydrophibicity (kcal/mol)	Activity
CAYQWQR PVNRIR	1688.8657	11.05	3	11.96	DPP-IV inhibitor
PGVGGPLG PIGPCYE	1411.6783	3.01	-1	14.28	DPP-IV inhibitor
PACGGFYIS GRPG	1280.5953	8.93	1	11.99	DPP-IV inhibitor
KLPGF	560.3312	9.93	1	9.03	α-glucosidase and α-amylase inhibitor
IPAVF	545.3203	5.3	0	5.25	DPP-IV inhibitor
GPAE	372.1639	3.21	-1	13.32	DPP-IV inhibitor
WV	303.1578	5.46	0	5.35	DPP-IV inhibitor
GPGA	300.1429	5.36	0	10.84	DPP-IV inhibitor
LP	228.1469	5.35	0	6.79	DPP-IV inhibitor
IP	228.1469	5.34	0	6.92	DPP-IV inhibitor

Figure 3 Physicochemical properties of potential peptides to control glucose levels in T2DM, including amino acid sequence and structure, molecular mass, net charge, isoelectric point and hydrophobicity activity

period and postprandial hyperglycaemia is reduced. Matsui et al. [23] demonstrated that a sardine muscle hydrolysate or isolated peptides potently inhibited α-glucosidase activity *in vitro*. RVPSLM and KLPGF, albumin-derived peptides [24, 25], as well as GEY and GYG, isolated from silk cocoon hydrolysate [26], inhibited α-glucosidase activity.

Other therapeutic agents used to control postprandial hyperglycaemia of T2DM are the inhibitors of α-amylase (Table 1). Proteins derived from various plants, particularly cereals, legumes such as rice, and wheat and soybean, can act as α-amylase inhibitors. Dolečková-Marešová et al. reported that the octapeptide motif GHWYYRCW, denoted as PAMI, is an α-amylase inhibitor designed *de novo* through combinatorial chemistry [27].

Insulin secretion

Dietary proteins have different insulinotropic effects (Table 2) due to their amino acid profiles or peptides released during digestion. There is epidemiologic evidence of the effects of milk proteins; whey fraction stimulates insulin secretion and prevents hyperglycaemia more than caseins.

Protein hydrolysates offer a very suitable delivery form of peptides and amino acids. Van Loon et al. [28] demonstrated the *in vivo* insulinotropic potential of protein hydrolysates and amino acids co-ingested with carbohydrates, even though a large amount of protein hydrolysate/amino acid mixture (0.35 g/kg/h) and carbohydrates (0.7 g/kg/h) was ingested. It has been proposed that some amino acids, such as L, I, A and R, increase insulin concentration, leading to a plasma glucose lowering effect [28]. The metabolism of some amino acids results in an increase of ATP, which will lead to the closing of the ATP-sensitive K^+ channel, depolarisation of the plasma membrane (PM), activation of the voltage-activated Ca^{2+} channel and insulin exocytosis. Arginine has been reported to be able to directly depolarise the PM [29].

A study in healthy adults showed that whey proteins have an effect on postprandial glucose in a dose-response manner and slightly increase insulin secretion in comparison with controls after an oral glucose load. These effects may be related to increased levels of plasma I, L, V, K and T [30].

Nongonierma et al. found that the most potent insulinotropic whey protein hydrolysate fraction was enriched in branched-chain amino acids and contained hydrophilic peptides [31].

The co-ingestion of protein and protein hydrolysates can be a dietary strategy in T2DM patients to augment postprandial insulin release and attenuate the postprandial high blood glucose concentrations following carbohydrate intake [32].

Incretins

Incretins are peptidic hormones released by intestinal cells to the bloodstream in response to nutrient intake (mainly glucose and fat), where they

Bioactive compound, source	Model	Treatment, dose and duration	Study outcome	Reference
L-glutamine	15 T2DM patients (glycated haemoglobin 6.56±0.6%)	Participants ingested a low-fat meal (5% fat) after receiving either water (control), 30 g L-glutamine (Gln-30), 15 g L-glutamine (Gln-15), 100 mg Sitagliptin (SIT) or 100 mg SIT and 15 g L-glutamine (SIT+Gln15).	All Gln treatments enhanced the postprandial insulin response from t=60–180 min. Gln-30 and SIT increased the active GLP-1 AUC compared with control.	[63]
L-glutamine	8 healthy normal-weight volunteers (LEAN), 8 obese with T2DM or impaired glucose tolerance (OB-DIAB) and 8 obese nondiabetic (OB-CON)	Oral glucose (75 g), glutamine (30 g) and water were administered on 3 separate days in random order, and plasma concentrations of GLP-1, GIP, insulin, glucagon and glucose were measured over 120 min.	GLP-1, glucagon and insulin concentrations increased after glutamine ingestion in all groups. Glutamine also increased plasma GIP concentrations but less effectively than glucose.	[64]
L-leucine	13 healthy subjects (6 men and 7 women) were studied on 4 different occasions	They received 25 g glucose or leucine (1 mmol/kg lean mass) or leucine (1 mmol/kg lean mass) and glucose (25 g). Serum leucine, glucose, insulin, glucagon and α-amino nitrogen concentrations were measured at various times over 2.5 h.	Leucine had little effect on serum glucose or insulin concentrations but did increase the glucagon concentration. When leucine was ingested with glucose, it attenuated the serum glucose response and strongly stimulated additional insulin secretion.	[65]
Casein hydrolysate/ L-leucine	11 long-standing T2DM patients and 11 healthy control subjects	Subjects received a beverage containing protein hydrolysate/leucine mixture (PRO) or a placebo (PLA). Glucose concentrations were recorded for 40 h.	In the PRO trial, glucose was significantly lower compared with the PLA trial at 24 h. PRO resulted in a 11±3% decline in the overall glucose response in diabetic patients. In control subjects there were not differences.	[66]
Casein/casein hydrolysate	60 T2DM long-standing male patients	Postprandial plasma insulin and glucose responses were determined after ingesting carbohydrate (0.7 g/kg: CHO) with or without an intact protein (0.3 g/kg: PRO) or its hydrolysate (0.3 g/kg: PROh).	Protein co-ingestion strongly increased postprandial insulin release, 10% greater in CHO+PRO and CHO+PROh. The plasma glucose responses were 22–32% and 23–36% lower in the CHO+PRO and CHO+PROh experiments, respectively.	[32]
Whey protein isolate/soy protein isolate	25 healthy men were investigated in a double-blind cross-over trial	Volunteers drank 300 ml of one of four beverages: 6% w/v whey protein isolate (WPI), whey protein hydrolysate (WPH), soy protein isolate (SPI) and 2.66% WPI or a control (no protein added). Blood samples were collected at different times to determine glucose and insulin.	Only beverages containing 6% (w/v) of whey protein increased insulin response and decreased glucose level compared with control.	[67]
Whey protein/whey protein flavourzyme hydrolysate	10 T2DM male patients. The patients were taking metformin (n=2) and metformin with sulphonylurea derivations (n=8)	They received 7 treatments: whey protein hydrolysates (WPH) beverages (0.1, 0.2 and 0.4 g/kg body weight), whey protein isolate (WPI) beverages (0.1, 0.2 and 0.4 g/kg body weight) and a control (distilled water). Blood samples were taken from 0 to 180 min to measure glucose and insulin concentration.	Ingestion of 0.2 g/kg WPH or 0.4 g/kg WPI promoted insulin secretion. Plasma insulin concentration increased significantly in the 0.4 g/kg WPH trial.	[68]
Gelatin	9 healthy men were studied on 3 d each in a random order	They drank 300 ml of one of these beverages: 50 g glucose (glucose), 30 g gelatin (protein) or 50 g glucose with 30 g gelatin (glucose–protein) in water with [13C]acetate. Blood and breath samples were collected for 3 h to measure insulin, GLP-1 and GIP concentrations and gastric half-emptying time (from $13CO_2$ excretion).	The blood glucose response was less after glucose-protein than after glucose; GIP was lower, and there were no significant differences in plasma insulin or GLP-1. Protein alone stimulated insulin, GLP-1 and GIP without elevating blood glucose. The gastric half-emptying time was greater after glucose-protein than after glucose.	[69]

Table 2 Recent human studies with amino acids, proteins and protein hydrolysates with potential antidiabetic activity

stimulate insulin secretion. They are responsible for over 50% of postprandial insulin secretion. The incretin effect may be reduced in diabetic patients at a chronic stage of the disease, probably due to the destruction of pancreatic islets [33]. Administration of these hormones has an insulinotropic effect and a β-cell proliferating response in the diabetic patient with no risk of hypoglycaemia, a common side effect of classic antidiabetic drugs [34]. However, the half-life of incretins is very short (<2 min) due to the cleavage and inactivation of these proteins by DPP-IV.

Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)

The most important incretin hormones are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) [34]. These are secreted by the L cells of the distal ileum and proximal colon, and the K cells of the duodenum and upper jejunum, respectively. Plasma levels of the incretin hormones rise within minutes of eating. GLP-1 and GIP act on β-cell G-protein-coupled receptors to enhance glucose-stimulated insulin secretion. GLP-1, but not GIP, slow gastric emptying and suppress appetite via central effects [35].

Nilsson et al. found that a whey drink caused a significantly enhanced GIP response (+80%) in healthy subjects in comparison with controls, suggesting that the protein caused an increase of postprandial insulin mediated by increased incretin hormones [36]. Individuals with well-controlled T2DM not taking any medication except for sulphonylurea or metformin, and consuming whey protein before a high-glycaemic-index breakfast, had increased GLP-1 and reduced postprandial glycaemia [37]. The oral administration of a dietary peptide, ZeinH, attenuated hyperglycaemia by stimulating GLP-1 and GIP secretion in normal and diabetic rats [38]. Diapin (GGL), a tripeptide, lowered blood glucose in male T2DM mice, suggesting an action on enteroendocrine L-cells in the gastrointestinal tract to stimulate the secretion of the incretin GLP-1, and also acted on pancreatic β-cells to stimulate insulin secretion [39]. Tetrapeptides such as tetra-glycine, tetra-alanine and GWGG showed a specific elevation of intracellular free calcium in the enteroendocrine NCI-H716 cell line, which promoted the increase of GLP-1 secretion [40].

DPP-IV inhibitor

Synthetic inhibitors against the enzyme DPP-IV, a serine protease implicated in the inactivation of incretin hormones, are currently used for the management of T2DM.

DPP-IV inhibitors can also be produced during food preparation; Gallego et al. identified peptides from dry-cured ham [41]. Their results indicated that AA, KA and AAAT were DPP-IV inhibitors with IC_{50} values of 9.40±0.10, 6.27±0.59 and 6.47 mM, respectively. Lacroix and Li-Chan [42] evaluated 34 proteins and found 2256 peptide sequences matching those reported to present DPP-IV inhibitory activity. GA, GP and PG were the most frequently occurring sequences. Caseins from milk and collagens from bovine meat showed the biggest potential to produce DPP-IV inhibitors.

Peptides containing 2–8 hydrophobic amino acid residues, including P, can inhibit DPP-IV, such as those derived from milk (Table 1). The dipeptides WV, AL, EK, GL, SL, FL and HL have been identified as DPP-IV inhibitors [43]. VA, identified as an inhibitor of DPP-IV, occurs in different milk proteins including α-, β- and γ-casein, β-lactoglobulin and lactoferrin.

Also Uenishi et al. identified inhibitory activity of DPP-IV in the water-soluble fraction of gouda-type cheese, and this activity increased during ripening [44]. Eight peptides were synthesised to determine their DPP-IV inhibitory activity. The peptide that showed the highest activity (IC_{50} 46 μM) was LPQNIPPL. This peptide showed the highest activity when administered orally to female rats in a glucose tolerance test.

Estrada-Salas et al. observed that canary seed peptides

obtained by gastrointestinal digestion showed an inhibition of DPP-IV activity in a dose-dependent manner; the highest inhibition (43.4%) was obtained at the higher peptide concentration (1.4 mg/ml) [45]. In contrast, the non-hydrolysed proteins showed a low inhibition (9.3%). This showed that peptides are released during gastrointestinal digestion and can have an effect on blood glucose levels.

Nongonierma and FitzGerald [43] predicted the release of peptides with DPP-IV inhibition using an *in silico* assay with gastrointestinal enzymes, demonstrating that milk protein-derived peptides, with a P at position 2, could act as DPP-IV inhibitors.

Most of the DPP-IV inhibitors are competitive inhibitors with some exceptions. The amaranth 11S globulin peptides interact with the active site pocket of DPP-IV, thereby blocking access to the substrate. Three peptides larger than 13 residues prevented the formation of the dimeric active form of DPP-IV, resulting in enzyme inhibition [46].

Glucose transporter 4 (Glut-4) and its role

The main mechanism for disposal and storage of an exogenous glucose load is insulin-stimulated glucose transport into muscle and fat. The principal glucose transporter protein that mediates this uptake is the isoform GLUT-4. In non-diabetic individuals, 80% of total body glucose uptake occurs in skeletal muscle as a result of insulin binding to insulin receptors, which promotes a signalling cascade that results in translocation of GLUT-4 [47]. GLUT-4 is located on insulin-sensitive tissues such as muscle, adipose and cardiac tissues and plays an important role in glucose transport to these tissues. As a result of insulin resistance, there is a disruption of the intracellular signalling pathways responsible for GLUT-4 translocation from intracellular stores, resulting in insufficient channels for glucose uptake and extracellular glucose accumulation [48].

Han et al. studied the protective effect of β-casomorphin-7 (β-CM-7), a milk-derived bioactive peptide, in streptozotocin-induced diabetic rats [49]. They found that the levels of blood glucose of the β-CM-7 treatment group decreased compared with those of the control group. They also found that the β-CM-7 group had a reduction of advanced glycosylation end products (AGEs), produced during hyperglycaemia, while the content of GLUT-4 increased, explaining the reduction of blood glucose. Morato et al. found that the concentrations of GLUT-1 and GLUT-4 were significantly elevated in PM of the muscle cells in rats after consumption of whey protein and whey protein hydrolysates [50]. The peptide LI and the amino acid I contributed to the translocation of GLUT-4 and the entrance of glucose into the skeletal muscle [51].

Lee et al. studied the effect of silk protein hydrolysates in 3T3-L1 fibroblasts showing an increase of glucose uptake and reduction of the expression of leptin [52]. They suggested that silk peptide E5K6 would enhance insulin-stimulated glucose uptake through upregulation of GLUT-4. Another example is aglycin, a 3742.3-Da soy peptide that resists digestive enzymes and has shown antidiabetic effect *in vivo* when administered orally to experimentally diabetic mice induced by streptozotocin/a high-fat diet. The antidiabetic effect may be due to the enhanced insulin signal at the gene levels of IR and IRS1 (two molecules involved in this insulin signalling pathway) and the increased number of GLUT-4 transport proteins at the cell surface [53]. Finally, cereal storage proteins contain peptides with potential biological activities, which include peptide sequences with DPP-IV inhibition [54].

Conclusions

There is increasing evidence that protein, protein hydrolysates or bioactive peptides, and amino acids could be used as effective nutritional strategies to improve blood glucose homeostasis in T2DM patients. It is believed that enhancing the effect of hormones, such as GIP, GLP-1 and insulin, leads to reduced food intake and increased satiety. Insulin secretion is associated with the glucose-lowering effect and with the control of food intake. One possible mechanism is that bioactive peptides serve as

endogenous inhibitors of DPP-IV in the proximal gut, preventing the degradation of insulinotropic incretins GLP-1 and GIP. Another mechanism may involve branched-chain amino acids, which activate the signalling pathways leading to elevated insulin expression and secretion, or the translocation of glucose transporters to the plasmatic membrane. Nevertheless, future studies should be performed in animal models to determine the precise mechanism and bioavailability of the peptides. There is a need to identify and characterise the metabolic role of bioactive peptides and to further prove their efficacy in clinical trials. At the same time, the challenge to the food industry is to incorporate these bioactive peptides without affecting the taste-sensory profile, convenience, bioavailability and safety.

Conflict of interest

No conflict of interest is expressed by the authors.

Human and Animal rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

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