



REVIEWS: CURRENT TOPICS

The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease

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Abstract

Vascular diseases such as atherosclerosis, stroke or myocardial infarction are a significant public health problem worldwide. Attempts to prevent vascular diseases often imply modifications and improvement of causative risk factors such as high blood pressure, obesity, an unfavorable profile of blood lipids or insulin resistance. In addition to numerous preventive and therapeutic drug regimens, there has been increased focus on identifying dietary compounds that may contribute to cardiovascular health in recent years. Food-derived bioactive peptides represent one such source of health-enhancing components. They can be released during gastrointestinal digestion or food processing from a multitude of plant and animal proteins, especially milk, soy or fish proteins. Biologically active peptides are considered to promote diverse activities, including opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic and antihypertensive actions. By modulating and improving physiological functions, bioactive peptides may provide new therapeutic applications for the prevention or treatment of chronic diseases. As components of functional foods or nutraceuticals with certain health claims, bioactive peptides are of commercial interest as well. The current review centers on bioactive peptides with properties relevant to cardiovascular health.

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1. Introduction

Biologically active peptides are food-derived peptides that exert — beyond their nutritional value — a physiological, hormone-like effect in humans. They are found in milk, egg, meat and fish of various kinds as well as in many plants. Bioactive peptides are inactive within the sequence of their parent protein and can be released by enzymatic hydrolysis either during gastrointestinal digestion or during food processing (e.g., cheese ripening and milk fermentation). They usually contain 2–20 amino acid residues per molecule, but in some cases may consist of more than 20 amino acids. Following digestion, bioactive peptides can either be absorbed through the intestine to enter the blood circulation intact and exert systemic effects, or produce local effects in the gastrointestinal tract. Depending on the sequence of amino acids, these peptides can exhibit diverse activities,

including opiate-like, mineral binding, immunomodulatory, antimicrobial, antioxidant, antithrombotic, hypocholesterolemic, and antihypertensive actions [1–4]. Many of the known bioactive peptides are multifunctional and can exert more than one of the effects mentioned [5–7]. Because of their health-enhancing potential and safety profiles they may be used as components in functional foods or nutraceuticals. However, milk proteins are currently the main source of several biofunctional peptides and daily intake of milk and milk products has proved to be physiologically important to both neonates and adults [8–10].

This review centers on bioactive peptides with properties relevant to cardiovascular health including effects on blood pressure, oxidative stress, hemostasis, appetite and lipid metabolism.

2. Cardiovascular disease and nutraceuticals

Cardiovascular disease (CVD) is the single leading cause of death for both males and females in the United States

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and other technologically advanced countries in the world. In lesser-developed countries it generally ranks among the top five causes of death. The World Health Organization estimates that by 2020, heart disease and stroke will have surpassed infectious diseases to become the leading cause of death and disability worldwide [11]. Consequently, there has been an increased focus on improving diet and lifestyle as a strategy for CVD risk reduction. Current dietary advice to reduce risk of CVD includes substituting saturated fat with carbohydrate without changing the protein content. In the USA, the DASH-diet (Dietary Approaches to Stop Hypertension), which is rich in fruits, vegetables and low-fat dairy products, is recommended to meet this objective. The DASH-diet is based on a reduced intake of saturated fat that is replaced by carbohydrate. Such high-carbohydrate, low-protein diets are known to reduce blood pressure and low-density lipoprotein (LDL) cholesterol [8,12], but also to reduce high-density lipoprotein (HDL) cholesterol levels and to raise fasting triglycerides [13,14]. Recent evidence suggests that an increased consumption of protein, particularly plant protein, may further lower the risk of hypertension and CVD [15,16]. The OmniHeart (Optimal Macro-Nutrient Intake to Prevent Heart Disease) trial demonstrated that partial substitution of carbohydrate with protein sources low in saturated fat can lower blood pressure, improve lipid levels, facilitate short-term weight loss and reduce the risk of CVD [17]. The mechanisms by which protein could exert its beneficial effects include an increased intake of biologically active amino acids or peptides [18,19].

These and other findings have increased the awareness of the critical link between diet and health. Moreover, they have led to the development of nutritionally enhanced food products designed to suit specific health concerns, particularly with relevance to the management of lifestyle-related diseases. Such foods, termed functional foods or nutraceuticals, are generally defined as products that have been satisfactorily demonstrated to have positive effects on one or more functions in the body, beyond their nutritional properties, in a way which is relevant to either an improved state of health and well-being and/or a reduction of disease risk. They should remain as foods or beverages and not in pharmaceutical forms such as tablets or capsules. As part of a normal dietary regimen they must demonstrate their effects in amounts that are reasonably expected to be consumed in the diet. The physiologically active components of functional foods are either added or enriched by modification of the usual manufacturing process. Consequently, increasing attention has focused on identifying dietary compounds, from plants (i.e., phytochemicals) as well as animals (i.e., zoochemicals), for promotion of specific health benefits [20,21]. Based on observations made in human studies over the last several years such as the already mentioned OmniHeart trial, bioactive peptides have gained scientific interest as constituents of nutraceuticals for their potential in disease prevention and health improvement.

3. Antihypertensive (ACE inhibitory) peptides

Elevated blood pressure is one of the major independent risk factors for CVD [22,23]. Angiotensin I-converting enzyme (ACE) plays a crucial role in the regulation of blood pressure as it promotes the conversion of angiotensin I to the potent vasoconstrictor angiotensin II as well as inactivates the vasodilator bradykinin (Fig. 1). By inhibiting these processes, synthetic ACE inhibitors have long been used as antihypertensive agents. In recent years, some food proteins have been identified as sources of ACE inhibitory peptides and are currently the best known class of bioactive peptides [24]. These nutritional peptides have received considerable attention for their effectiveness in both the prevention and the treatment of hypertension. The main sources of ACE inhibitory peptides are dairy products and fish, but they are also derived from plant (e.g., soy, wheat), meat and egg. Potent ACE inhibitory peptides from caseins and whey proteins are termed casokinins and lactokinins, respectively [2,24–26].

Numerous studies in spontaneously hypertensive rats (SHR) as well as in hypertensive human volunteers have been performed to determine the antihypertensive effects of food-derived ACE inhibitors. These *in vivo* studies have demonstrated that several ACE inhibitory peptides significantly reduce blood pressure, either after intravenous or oral administration. ACE inhibitory peptides with documented *in vivo* antihypertensive effects are listed in Table 1. An important observation from these trials is that the peptides being studied have little or no effect on blood pressure of normotensive subjects suggesting that they exert no acute hypotensive effect. Therefore, ACE inhibitory peptides could be applied as initial treatment in mildly hypertensive individuals or as supplemental treatment. They would also represent a low-cost alternative treatment for hypertension. Another advantage is that these peptides have not been associated with the harmful side effects reported for synthetic ACE inhibitors such as dry cough, skin rashes and angioedema, probably due to the lower ACE inhibitory activity determined *in vitro* [24,38].

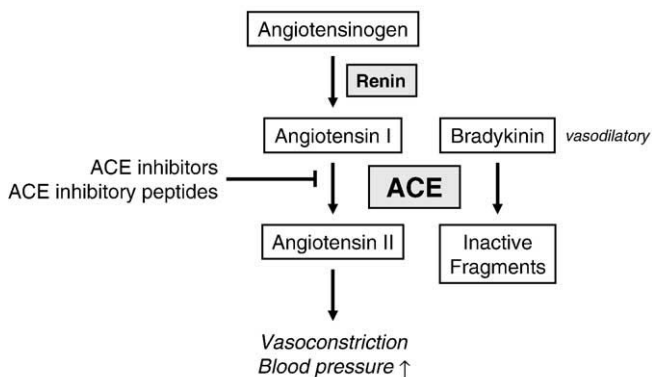


Fig. 1. The renin–angiotensin system.

Table 1
Examples of ACE inhibitory peptides with in vivo antihypertensive effects

Origin	Sequence/name	IC ₅₀ (μmol/L)	Subjects	Reference
Milk (β-casein)	VPP	9.0	SHR, humans	[27,28]
	IPP	5.0		
Milk (β-lactoglobulin)	IPA (β-lactosin A)	141.0	SHR	[29,30]
	ALPM (β-lactosin B)	928.0		
Fish (sardine muscle)	VY	26.0	SHR, humans	[31,32]
Fish (bonito muscle)	LKPNM	2.4	SHR	[33]
	LKP	0.32		
Meat (chicken muscle)	LKP	0.32	SHR	[34]
	IKW	0.21		
	LAP	3.5		
	MNPPK (myopentapeptide A)	945.5		
Meat (porcine muscle)	ITTNP (myopentapeptide B)	549.0	SHR	[35]
	LW	6.8		
Egg (ovalbumin)	NWGPLY	21	SHR	[36]
Soy (glycinin)	IAP	2.7	SHR	[37]

The potency of an ACE inhibitor is usually expressed as an IC₅₀ value, which is the inhibitor concentration leading to 50% inhibition of ACE activity. The majority of milk protein-derived ACE inhibitors have moderate inhibitory potencies, usually within an IC₅₀ range of 100–500 μmol/L [39]. Due to the incomplete and often unknown bioavailability of the ACE inhibitory peptides following oral administration, it is difficult and unreliable to predict the in vivo antihypertensive effect based on measured inhibitory activity in vitro. In order to produce antihypertensive effects in vivo the peptides have to be absorbed intact through the intestine and reach the cardiovascular system in an active form. In this regard, specific structural properties play an important role. Most of the ACE inhibitory peptides are short peptides with only two to nine amino acids. It has been demonstrated that di- or tripeptides, especially those with C-terminal proline or hydroxyproline residues, are generally resistant to degradation by digestive enzymes [40,41]. In addition, short peptides consisting of two or three amino acids are absorbed more rapidly than free amino acids [42,43]. The ACE inhibitory tripeptides IPP and VPP, for example, were detected in the aorta of SHR, following oral administration of fermented milk [44]. Larger peptides (10–51 amino acids) present in the diet can also be absorbed intact through the intestine and produce biological effects, although the potency of the peptides decreases as the chain length increases [45]. However, binding to ACE appears to be strongly influenced by the C-terminal sequence of the peptides. It has been postulated that proline, lysine or arginine is preferred as C-terminal residue and thus contributes to the ACE inhibitory potency [4]. Furthermore, studies in SHR revealed that dipeptides with a C-terminal tyrosine residue produced a slow but prolonged decrease in systolic blood pressure compared to dipeptides with phenylalanine at the C-terminal. In contrast, dipeptides with C-terminal phenylalanine caused a more rapid reduction and a shorter duration of action [46]. Table 2 presents examples of structural properties of selected biofunctional peptides.

In vivo comparative studies with captopril, a clinically used ACE inhibitor, have shown that ACE inhibitory peptides with antihypertensive effect exhibit higher in vivo activity than would be expected from their in vitro activity. The exact mechanisms underlying this observation have not yet been identified. Fujita and Yoshikawa [33] suggested that bioactive peptides have higher tissue affinities and are subject to a slower elimination than captopril.

However, these peptides may influence blood pressure by mechanisms other than the established ACE inhibition. The release of vasodilatory substances like prostaglandin I₂ [59], NO [60] or CO [61] could also contribute to the blood pressure-lowering effects of various ACE inhibitory peptides. Recently, Nurminen et al. [62] reported that the antihypertensive effect of α-lactorphin, a peptide with opiate-like and ACE inhibitory properties, was mediated through the vasodilatory action of binding to opioid receptors. Furthermore, inhibition of chymase by ACE inhibitory peptides has been suggested to provide an additional antihypertensive effect as well [63].

4. Antioxidant peptides

Oxidant stress, the increased production of reactive oxygen species (ROS) in combination with outstripping endogenous antioxidant defense mechanisms, is another significant causative factor for the initiation or progression of several vascular diseases. ROS can cause extensive damage to biological macromolecules like DNA, proteins and lipids [64]. Specifically, the oxidative modification of LDL results in the increased atherogenicity of oxidized LDL [65,66]. Therefore, prolonged production of ROS is thought to contribute to the development of severe tissue injury [67].

Dietary consumption of antioxidants appears to provide further benefits to the endogenous antioxidant defense strategies in the fight against oxidative stress [68–70]. In addition to the well-known dietary antioxidants like vitamin C, vitamin E, polyphenols and carotenoids, other dietary

Table 2
Structural properties of selected biofunctional peptides

Activity	Structural elements	Remarks	Reference
ACE inhibitory	Pro or hydroxy-Pro as C-terminus	Usually resistant to degradation by digestive enzymes	[40,41]
	Pro, Lys or Arg as C-terminus	Preferred C-terminal residues with contribution to the ACE inhibitory potency	[4]
	Tyr or Phe as C-terminus	Dipeptides with a C-terminal Tyr produced a higher antihypertensive effect compared to dipeptides with C-terminal Phe	[46]
Antioxidant	High amounts of His and hydrophobic amino acids	Contribution to the antioxidant potency	[47,48]
	Peptides with a Pro-His-His sequence	Peptides with a Pro-His-His sequence showed the greatest antioxidant activity among all tested peptides	[49]
Antithrombotic	Ile ¹⁰⁸ , Lys ¹¹² and Asp ¹¹⁵ residues of casoplatelin	Important for the antithrombotic activity	[50]
Hypocholesterolemic	Sugar content	Positive correlation with antithrombotic activity	[51]
	Low ratios of methionine–glycine and lysine–arginine in the dietary protein	Favors a hypocholesterolemic effect	[52–54]
Antiobesity	High amounts of hydrophobic amino acids	Hydrophobic peptides can bind bile acids and thereby enhance the fecal steroid excretion	[55,56]
	Peptide length	Peptide length influences CCK-releasing activity (different among each dietary protein)	[57]
	Multiple Arg residues	Necessary condition for CCK release through direct binding to intestinal cells	[58]

compounds have generated particular interest as defenses against oxidative damage. Recent studies have shown that peptides with antioxidant properties can be released from food sources such as milk casein [71], whey protein [72], egg [73,74] and soy protein [75]. Table 3 lists some examples of bioactive peptides derived from different protein sources.

Some peptides derived from hydrolyzed food proteins exert antioxidant activities against enzymatic (lipoygenase-mediated) and nonenzymatic peroxidation of lipids and essential fatty acids [49,76–78]. The exact mechanisms behind these effects are not fully understood. The antioxidant properties of these peptides have been suggested to be due to metal ion chelation, free radical scavenging and singlet oxygen quenching [2]. However, through the investigation of synthetic histidine-containing peptides, Chen et al. [47] demonstrated that none of these properties can be correlated solely with the antioxidant activity of the tested peptides. Therefore, overall antioxidant action is most likely attributed to the cooperative effects of the mechanisms mentioned [47]. Another plausible mode of action may be the induction of genes which protect cells from damage by ROS. Previously, our laboratory has shown that the biofunctional dipeptide MY, derived from sardine muscle, stimulates expression of the antioxidant defense proteins heme oxygenase-1 (HO-1) and ferritin in endothelial cells (Fig. 2). These genomic actions of MY have been associated with a sustained cellular protection from oxidative stress [61]. The antioxidant activity of whey-derived peptides and whey itself has been linked with the presence of cysteine-rich proteins which promote the synthesis of glutathione, a potent intracellular antioxidant [39]. In a study by Rival et al. [85], caseins and

casein-derived peptides were found to inhibit lipoygenase, an enzyme which catalyzes the peroxidation of unsaturated fatty acids such as linoleic acid.

The antioxidant activity has been attributed to certain amino acid sequences [71]. High amounts of histidine and some hydrophobic amino acids are related to the antioxidant potency [48]. The activity of histidine-containing peptides is thought to be connected to hydrogen-donating ability, lipid peroxy radical trapping, and/or the metal ion chelating ability of the imidazole group [86]. The addition of a leucine or proline residue to the N-terminus of a histidine–histidine dipeptide would enhance antioxidant activity. According to Chen et al. [49], peptides with a Pro-His-His sequence showed the greatest antioxidant activity among all tested peptides and had synergistic effects with nonpeptidic antioxidants. The hydrophobicity of the peptide also appears to be an important factor for its antioxidant activity due to increased accessibility to hydrophobic targets (e.g., lipophilic fatty acids) [47]. Furthermore, there is some evidence that the antioxidant effect of certain amino acids is greater when they are incorporated in dipeptides [87,88]. For example, the constituent amino acids of the histidine containing dipeptide carnosine and related agents are far less effective antioxidants than their parent proteins [89]. As another example, milk casein has been reported to inhibit the lipoygenase-mediated lipid autoxidation, whereas the free amino acids could not substitute for casein as the antioxidant [90]. The results suggest a crucial role of the peptide bond and/or specific structural features of the peptides regarding antioxidant potency. In contrast, recent results on the antioxidant effect of whey protein hydrolysates indicated

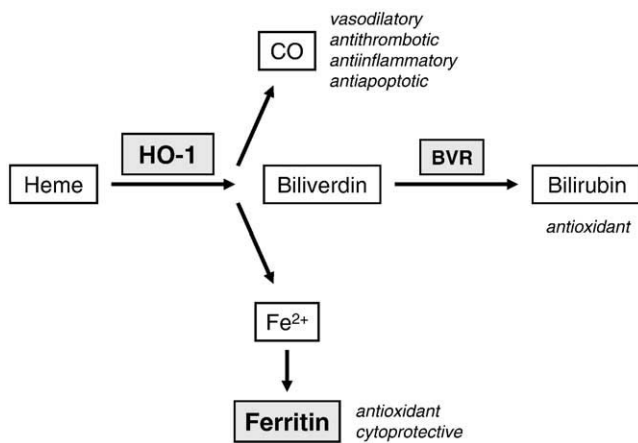


Fig. 2. The heme oxygenase (HO) enzyme reaction: HO-1 is an inducible enzyme that catalyzes the degradation of the toxic heme moiety. This process leads to generation of carbon monoxide (CO), free iron and biliverdin; the latter is subsequently converted to bilirubin by biliverdin reductase (BVR) [79,80]. CO and bilirubin have been causally linked to a higher resistance against cardiovascular disease [81–83]. Furthermore, the HO-1-dependent release of free iron during heme catabolism results in the up-regulation of ferritin protein expression [84]. Induction of HO-1 and ferritin is considered to be an adaptive and beneficial response to oxidative stress in a wide variety of cells.

that the peptide linkage or structural peptide conformation can also attenuate the antioxidant activity of the constituent amino acids [72]. Thus, peptide conformation can lead to both synergistic and antagonistic effects in regard to the antioxidant activity of free amino acids. Further research examining the structure–activity relationship in peptides is needed.

5. Antithrombotic peptides

Another complication related to CVD is the inclination to develop thrombosis due to abnormalities in coagulation. Increased occurrence of thrombosis has been linked to platelet hyperreactivity, high levels of hemostatic proteins (e.g., fibrinogen), defective fibrinolysis and hyperviscosity of the blood [91,92]. Hence, antithrombotic drugs are commonly used to reduce platelet aggregation and enhance fibrinolysis.

Indeed, it has been proved that there is a significant amount of molecular similarities between the mechanisms involved in milk clotting, defined by the interaction of κ -casein with chymosin, and blood clotting, defined by the interaction of fibrinogen with thrombin [93]. To date, food-derived peptides with antithrombotic properties are mainly the result of enzymatic hydrolysis of bovine κ -casein. Recently, antithrombotic peptides have been isolated from human and sheep κ -casein as well [51,94].

The main antithrombotic peptide MAIPPCKKNQDK, isolated from the soluble C-terminal fragment (caseinoglycomacropeptide) of bovine κ -casein, corresponds to the residues 106 to 116 of κ -casein and is termed casoplatelin.

This undecapeptide inhibits both the aggregation of ADP-activated platelets as well as the binding of human fibrinogen γ -chain to its receptor region on the platelet surface. Smaller fragments of this peptide, known as casoplatelins, can also affect platelet function although they have much lower inhibitory activity than the complete fragment [95].

Three amino acid residues (Ile¹⁰⁸, Lys¹¹², Asp¹¹⁵) of the aforementioned undecapeptide seem to be important for the antithrombotic effect, because they are homologous in positions to the γ -chain sequence of human fibrinogen. Therefore, antithrombotic activity is influenced by the competition for platelet receptors between casoplatelin and the γ -chain of human fibrinogen [50]. Furthermore, a correlation between sugar level and antithrombotic activity has been suggested since the human κ -caseinoglycomacropeptide, which is richer in sugars than that in bovine, is reportedly more potent [51].

It is thought that milk protein-derived antithrombotic peptides are absorbed intact into the bloodstream. Human and bovine κ -caseinoglycomacropeptides, two antithrombotic peptides derived from the corresponding κ -caseins, have been detected at physiologically active concentrations in the plasma of newborn children following ingestion of breast milk or cow milk-based formula, respectively [51].

Furthermore, a peptide derived from human lactoferrin, KRDS, which holds structural similarities to fibrinogen α -chain, has been shown to inhibit platelet aggregation but to a lesser extent than the fibrinogen analogue, RGDS [96,97]. It is likely that KRDS and RGDS have different mechanisms of action and/or their binding sites are different and sequence specific. Inhibition of platelet aggregation by KRDS has been associated with an inhibition of the release of the dense granule protein serotonin, whereas RGDS did not exhibit a similar inhibition [98].

In vivo antithrombotic activities have been shown for the κ -casein-derived undecapeptide [99] as well as for the lactoferrin-derived tetrapeptide [98]. In addition, no detectable toxic effect has been reported. Hence, caseinoglycomacropeptide could potentially be used to treat or prevent thrombosis. In contrast, the RGDS sequence has been found to induce detachment of endothelial cells in vitro and therefore serious concerns exist regarding the toxicity of this sequence in vivo. The related peptide KRDS does not appear to have the same potential detrimental effects as RGDS possibly due to their different modes of action [100].

6. Hypcholesterolemic and hypotriglyceridemic peptides

An unfavorable profile of blood lipids is another important risk factor for the genesis of various CVDs. Many studies have found a positive correlation between hypercholesterolemia and/or hypertriglyceridemia and the likelihood for developing CVD [92,101,102]. Not surprisingly, treatment for hyperlipidemia-accelerated diseases

often includes the improvement of serum lipid distribution through diet modifications.

It is generally known that several dietary proteins can improve blood lipid profiles. To date, hypocholesterolemic properties have been reported for soy [103,104], whey [105,106] and fish protein [52], capable of altering the plasma profile from atherogenic to cardioprotective. In contrast, bovine casein tends to cause species-dependent hypercholesterolemia and atheromatous plaques in animal studies [107,108]. The exact mechanisms responsible for the hypocholesterolemic effects have not been fully identified, but evidence suggests that the specific amino acid composition of dietary proteins probably influences the effect of the protein source on plasma cholesterol levels. It has been reported that dietary proteins with low ratios of methionine–glycine and lysine–arginine, such as soy and fish protein, favor a hypocholesterolemic effect [52–54]. In contrast, bovine casein tends to elevate cholesterol levels probably due to its high ratios of methionine–glycine and lysine–arginine [107].

Of the limited number of peptides reported to have hypocholesterolemic effects, dietary soy protein has received the most attention. Published data offer a range of possible mechanism of action in soy protein's ability to reduce total plasma cholesterol including induction of LDL receptor expression, increase of bile acid synthesis and excretion as well as decrease in steroid absorption from the intestine. In addition, changes in the endocrine status such as alteration in the insulin–glucagon ratio and in thyroid hormone concentrations have also been reported [109]. Although these effects have not been attributed to specific soy constituents, several studies suggest that peptides derived from soy protein may be the bioactive components. When amino acid mixtures mimicking soy protein were fed to rats or rabbits, the resulting blood cholesterol levels were significantly lowered but not as low as those that were fed with the intact protein [110,111]. It has also been shown that soy protein hydrolysates reduce total cholesterol levels more effectively than intact soy protein [104,112]. Several more reports indicate that hydrophobic peptides derived from soy protein can bind bile acids thereby enhancing fecal steroid excretion which may contribute to the hypocholesterolemic activity [55,56].

Recently, LPYPR, a peptide derived from soy glycinin, was found to produce serum cholesterol-lowering effects in mice following oral administration [113]. LPYPR is structurally homologous to enterostatin (VPDPR), an endogenous peptide exhibiting hypocholesterolemic and anorectic effects [114]. Another glycinin-derived peptide with cholesterol-lowering activity is IAVPGEVA [115]. In vitro measurements have shown that both LPYPR and IAVPGEVA inhibited 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) which is a known key enzyme in cholesterol biosynthesis. Investigations on the structure–activity relationship revealed that the hydrophobic region of both peptides is a required structural element for their biological activity. The maximum length of the hydrophobic

sequence was stated to be four amino acids. Moreover, the proline residue seems to be a key component and can be located at both the C-terminus and in any other position of the amino acid sequence except the N-terminus [116].

Several peptide fragments obtained from the subunits of β -conglycinin are considered to possess hypocholesterolemic activity. The regulation of cholesterol homeostasis has been proposed to be due to the activation of LDL receptors and LDL degradation in liver cells at least in vitro. Preliminary data evidently suggest a positive modulation of LDL receptor induced by a specific sequence corresponding to the positions 127–150 of β -conglycinin [117]. Duranti et al. [118] reported a marked up-regulation of liver β -very low-density lipoprotein (VLDL) receptors and a significant decrease in plasma triglycerides in hypercholesterolemic rats after oral treatment with the α' -subunit of β -conglycinin. They hypothesized that peptides arising from digestion elicit the biological effect [118].

As for the observed hypotriglyceridemic activity, there is some evidence that dietary protein may affect lipogenesis in the liver. Iritani et al. [119] have shown that oral administration of soy protein to rats reduced the concentrations of triglycerides in plasma and more prominently in liver. These effects were associated with significant reductions in the activities of hepatic lipogenic enzymes indicating that soy protein reduces liver triglycerides or fat in part by inhibiting hepatic fatty acid synthesis [119]. Furthermore, dietary soy protein appeared to cause a stimulation of lipolysis and fatty acid utilization [120]. In mice, Moriyama et al. [121] demonstrated that β -conglycinin reduced serum triglyceride levels by the acceleration of β -oxidation, suppression of fatty acid synthesis and increased fecal excretion of triglycerides. They concluded that some specific β -conglycinin peptides might be responsible for these multiple events [121]. Remarkable hypotriglyceridemic activities in different animal species were also achieved by the administration of hydrolyzed globin. VVYP, VYP and VTL were identified as the effective constituent peptides. The hypotriglyceridemic effect of these peptides was associated with decreased intestinal fat absorption as well as an enhanced lipolysis of triglycerides [122]. The hypotriglyceridemic effect of hydrolyzed globin has also been demonstrated in humans [123].

Numerous studies have shown that milk whey protein, in contrast to milk casein, decreases serum cholesterol similar to soy protein [106,124,125]. This effect was more marked with the whey peptide fraction than with the intact whey protein [124]. Nagaoka et al. [126] identified IIAEK as the hypocholesterolemic peptide derived from bovine milk β -lactoglobulin. In animal studies, IIAEK, which is also termed lactostatin, exhibited a greater hypocholesterolemic effect than β -sitosterol. Following oral administration to rats, total serum cholesterol levels were significantly lower, whereas HDL concentration and atherogenic index (HDL cholesterol/total cholesterol) were significantly higher than in the group fed with β -sitosterol. These effects have been speculated to

be at least in part due to a decrease of micellar solubility of cholesterol which leads to lower intestinal cholesterol absorption [126]. Recent data have shown that lactostatin is capable of inducing the gene transcription of human cholesterol 7 α -hydroxylase (CYP7A1), a cholesterol-metabolizing enzyme, resulting in hypocholesterolemic effects. This new site of action involves Ca and MAPK-dependent signaling pathways. Further results imply that the C-terminal side of lactostatin, especially the glutamyl–lysine sequence, is crucial for the induction of human CYP7A1 transcription. However, an amino acid mixture constitutively equivalent to lactostatin failed to induce the CYP7A1 gene [127].

7. Antiobesity peptides

In many industrial countries obesity is a serious health issue that has been associated with higher incidence of CVD and related disorders [128]. Hyperinsulinemia, insulin resistance and abnormalities in lipid metabolism have all been linked to obesity. Lipoprotein profile obtained in obese subjects revealed a pattern of higher levels of triglycerides, elevated LDL-cholesterol and low HDL-cholesterol. Restriction of caloric intake and increasing physical exercise are recommended for the treatment of adiposity. Both weight loss and exercise can also improve insulin resistance and associated dyslipidemia [129,130].

It is generally accepted that protein is the most satiating macronutrient [131,132]. Besides inducing the feeling of satiety, a high-protein diet promotes thermogenesis leading to a faster rate of caloric metabolism. Diets rich in protein are known to suppress food intake and facilitate short-term weight loss even more effectively than high-carbohydrate diets and thus can be used in the management of obesity. However, some evidence suggests that different sources of dietary protein in low-calorie diets produce varying effects on metabolism and therefore strongly influence weight loss. For example, the ingestion of plant protein such as soy is effective in reducing body weight and in improving

cardiovascular risk factors. In contrast, the regular intake of protein sources rich in saturated fat and cholesterol such as red meat and eggs may increase the risk of CVD [131,133]. Nevertheless, reliable information on the long-term effects of high-protein diets on overall health is not yet available.

Ingestion of soy, casein and whey protein has all been shown to hold antiobesity or anorectic properties with the effect of soy protein in reducing body weight more superior than that of casein and whey protein [134–136]. The mechanisms by which proteins exert anorectic actions are still unclear. Several studies speculate that peptides released from dietary proteins during digestion can initiate several satiety signals from the gut and thus prevent further food intake. Because these peptides act at the intestinal site they do not need to be absorbed into the systemic circulation.

Pupovac and Anderson [137] conclude that the induction of satiety by peptides derived from soy and casein protein is mediated by independent activation of both opioid and cholecystokinin (CCK)-A receptors. The pivotal role of opioid and CCK-A receptors in the regulation of food intake is well recognized. Peptides with opioid-like activities affect food intake by the delay of gastric emptying and intestinal transit [4]. CCK is an important physiologic endocrine factor that regulates appetite and gastric emptying. The stimulation of CCK release contributes to appetite suppression in the central nervous system as well as in the periphery [138,139].

In regard to soy protein, it has been shown that the decline in body fat and food intake was more significant with the soy peptide fraction than with the intact protein indicating that hydrolysis of soy protein is important in its effect of weight reduction [135,140]. Furthermore, amino acid mixtures simulating soy and casein protein were not effective in releasing CCK from mucosal cells [57,141,142]. These results indicate that peptides released from dietary proteins contribute to the initiation of satiety signals. Nishi et al. [57] hypothesize that the peptide length might be an important factor in CCK-releasing activity. The optimal peptide size seems to be different among each dietary protein. Well-

Table 3
Examples of bioactive peptides derived from food

Activity	Origin	Sequence/name	Reference
Antioxidant	Fish (sardine muscle)	MY	[61]
	Soy (β -conglycinin)	LLPHH	[75]
	Milk (casein)	YFYPEL	[71]
	Milk (β -lactoglobulin)	MHIRL, YVEEL, WYSLAMAASDI	[72]
	Egg (egg white)	YAEERYPIL	[73]
Antithrombotic	Milk (κ -casein)	MAIPPKKNQDK (casoplatelin) and smaller fragments	[95]
	Milk (lactoferrin)	KRDS	[96,97]
Hypocholesterolemic	Soy (glycinin)	LPYPR	[113]
	Soy (glycinin)	IAPVGEVA	[115]
	Soy (β -conglycinin)	Several peptide fragments	[117]
		(e.g., sequence corresponding to the residues 127–150)	
Hypotriglyceridemic	Milk (β -lactoglobulin)	IAEK (lactostatin)	[126]
	Blood (globin)	VVYP, VYP, VTL	[122]
Antiobese	Soy (β -conglycinin)	VRIRLLQRFNKRS	[58]
	Milk (κ -casein)	Caseinoglycomacropptide	[143,144]

digested soy protein for instance is more effective than less-digested one on CCK liberation. In contrast, larger peptides of casein hydrolysate seem to be involved in the direct stimulation of CCK release [57].

Nishi et al. [58] identified the peptide VRIRLLQRFNKRS corresponding to the residues 51–63 of β -conglycinin as the bioactive appetite suppressant in soy protein. This peptide interacts directly with the intestinal mucosal cells to stimulate CCK release. Further investigations on the binding activities of several synthetic model peptides indicate that multiple arginine residues are a necessary condition for CCK release through direct binding to brush border membrane [58].

Several studies have shown that satiety associated with casein ingestion involves both opioid and CCK regulation [137,142]. Proteolysis of milk casein releases bioactive peptides relevant to hunger regulation including casomorphins and caseinoglycomacropeptide. Casomorphins are peptides with opioid-like activities that are known to interact with gastric opioid receptors to slow gastrointestinal motility and prevent further food intake [145]. In addition, they influence appetite regulation by modifying the postprandial levels of metabolic hormones involved in satiety [146]. Peptides with opioid activity have been reviewed extensively elsewhere [39,147]. Caseinoglycomacropeptide, a 64-amino acid fragment from the C-terminal end of bovine casein, has been shown to exert CCK-releasing activity via direct reaction with the small intestine and therefore act as an appetite suppressant [143,144]. In addition, caseinoglycomacropeptide has also been found to control food intake in animals at least in part through opioid activity [148].

Protein-induced satiety may also be mediated through the glucagon-like peptide-1 (GLP-1) signaling pathway. In rats as well as in humans it has been demonstrated that activation of GLP-1 receptor is involved in casein- and whey-induced suppression of food intake [149,150]. Peptides released from digested protein appear to provide this satiety signal, because neither intact proteins nor free amino acid mixtures stimulated the release of GLP-1 [151].

Above and beyond the stimulation of satiety through bioactive peptides, lipid-lowering effects as well as increasing metabolic rate are also beneficial in fighting obesity. Another possible mode of action is the modulation of adipose genes that contribute to the homeostasis of metabolism and vascular functions. In the case of soy protein, Nagasawa et al. [152] have shown that it raises adiponectin mRNA expression in mice. Adiponectin is an adipose-specific plasma protein possessing antiatherogenic and anti-insulin-resistance properties [152]. Soy protein may also reduce adiposity by modulating the expression of nuclear transcription factors, specifically the peroxisome proliferator-activated receptors (PPARs) and sterol regulatory element binding proteins (SREBPs) that are principal regulators of fatty acid metabolism and cholesterol homeostasis [121,153,154]. Further evidence suggests that the ingestion of soy and whey protein improves insulin resistance which is a hallmark of obesity [136,154]. It remains to be determined whether specific bioactive peptides are responsible for the abovementioned effects.

8. Conclusion

Bioactive peptides have been shown to possess properties that may be advantageous to cardiovascular health. These effects include the lowering of blood pressure and lipid levels as well as reducing free radical formation. Evidence for beneficial effects of bioactive peptides has also been raised under conditions of obesity and enhanced thrombosis (Table 3). Since CVD is a significant public health problem worldwide, biologically active peptides may be of vital interest in maintaining a healthy population. As part of a food product or as a nutraceutical they have a chronic rather than an acute effect on health. Numerous products that contain bioactive peptides are already on the market (Table 4). Until now, however, most of the claimed physiological effects of bioactive peptides have been observed in vitro or in animal model systems. Human clinical studies are limited or nonexistent and the optimal plasma levels of bioactive peptides have not been

Table 4

Examples of commercially available functional foods carrying bioactive peptides (modified from Hartmann and Meisel [1])

Brand name	Manufacturer	Remarks	Bioactive peptides	Health claim
Calpis	Calpis Co., Japan	Sour milk	VPP; IPP	Hypotensive
Evolus	Valio, Finland	Fermented milk	VPP; IPP	Hypotensive
Casein DP	Kanebo Ltd., Japan	Casein hydrolysate	FFVAPFPEVFGK	Hypotensive
C12 peptide	DMV International, Netherlands	Casein hydrolysate	FFVAPFPEVFGK	Hypotensive
BioZate	Davisco, USA	Whey protein hydrolysate	Whey-derived peptides	Hypotensive
Peptide Soup	NIPPON, Japan	Soup	Bonito-derived peptides	Hypotensive
BioPURE-GMP	Davisco, USA	Whey protein hydrolysate	Glycomacropeptide	Antithrombotic, antimicrobial, anticariogenic
CholesteBlock	Kyowa Hakko, Japan	Soft drink	Soy-derived peptides bound to phospholipids	Hypocholesterolemic

determined. Therefore, further research is needed in order to clarify the relevance and potential therapeutic role of bioactive peptides in humans.

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