Available online at www.sciencedirect.com
ScienceDirect
Journal of Nutritional Biochemistry 19 (2008) 643–654
Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

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

Kati Erdmann, Belinda W.Y. Cheung, Henning Schröder *

Department of Pharmaceutics, College of Pharmacy, University of Minnesota, Minneapolis, MN 55455, USA

Received 2 October 2007; accepted 26 November 2007

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.

Keywords: Antihypertensive; Antioxidant; Antithrombotic; Bioactive peptides; Cardiovascular disease; Hypocholesterolemic/hypotriglyceridemic

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

* Corresponding author. Tel.: +1 612 625 5695; fax: +1 612 624 6695.
E-mail address: schro601@umn.edu (H. Schröder).

0955-2863/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2007.11.010

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].

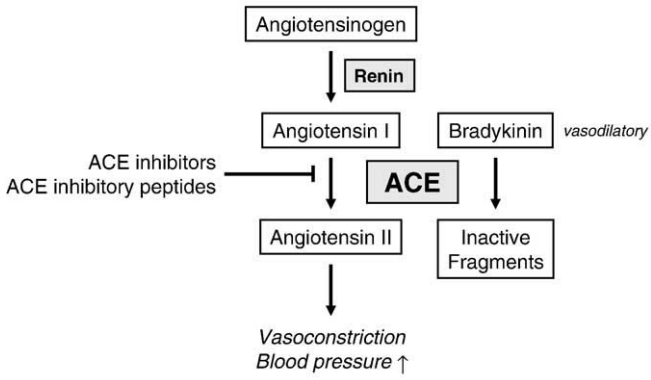

Fig. 1. The renin–angiotensin system.

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		
Meat (porcine muscle)	MNPPK (myopentapeptide A)	945.5	SHR	[35]
	ITTNP (myopentapeptide B)	549.0		
Egg (ovalbumin)	LW	6.8	SHR	[34]
Soy (glycinin)	NWGPLV	21	SHR	[36]
Wheat (gliadin)	IAP	2.7	SHR	[37]

The potency of an ACE inhibitor is usually expressed as an IC_{50} 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 IC_{50} 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 I_2 [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]
	Sugar content	Positive correlation with antithrombotic activity	[51]
Hypocholesterolemic	Low ratios of methionine–glycine and lysine–arginine in the dietary protein	Favors a hypocholesterolemic effect	[52–54]
	High amounts of hydrophobic amino acids	Hydrophobic peptides can bind bile acids and thereby enhance the fecal steroid excretion	[55,56]
Antiobesity	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 (lipoxygenase-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 singulet 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 lipoxygenase, 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 peroxyradical 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 lipoxygenase-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

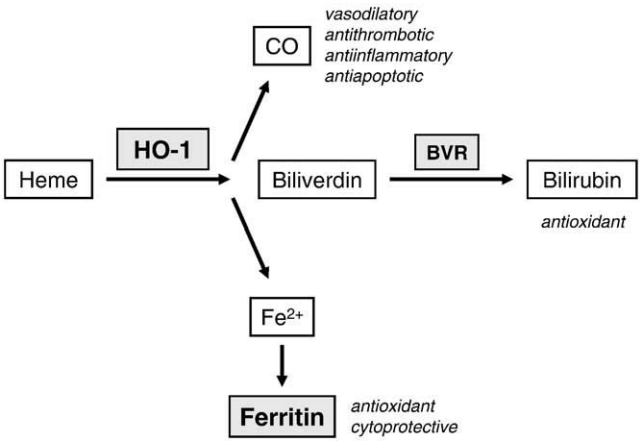

Fig. 2. The heme oxygenase (HO) enzyme reaction: HO-1 is an inducible enzyme that catalyzes the degradation of the toxic heme moiety. This process leads to generation of carbon monoxide (CO), free iron and biliverdin; the latter is subsequently converted to bilirubin by biliverdin reductase (BVR) [79,80]. 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 MAIPPKKNQDK, 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 (Ile^{108}, Lys^{112}, Asp^{115}) 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. Hypocholesterolemic 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 LPYRP and IAVPGEVA inhibited 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) 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 7α-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 constituently 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]. CKK 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)	IAVPGEVA	[115]
	Soy (β-conglycinin)	Several peptide fragments (e.g., sequence corresponding to the residues 127–150)	[117]
	Milk (β-lactoglobulin)	IIAEK (lactostatin)	[126]
Hypotriglyceridemic	Blood (globin)	VVYP, VYP, VTL	[122]
Antiobese	Soy (β-conglycinin)	VRIRLLQRFNKRS	[58]
	Milk (κ-casein)	Caseinoglycomacropeptide	[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.

References

[1] Hartmann R, Meisel H. Food-derived peptides with biological activity: from research to food applications. Curr Opin Biotechnol 2007;18:163–9.

[2] Kitts DD, Weiler K. Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. Curr Pharm Des 2003;9:1309–23.

[3] Kovacs-Nolan J, Phillips M, Mine Y. Advances in the value of eggs and egg components for human health. J Agric Food Chem 2005;53: 8421–31.

[4] Meisel H. Biochemical properties of regulatory peptides derived from milk proteins. Biopolymers 1997;43:119–28.

[5] Korhonen H, Pihlanto A. Food-derived bioactive peptides — opportunities for designing future foods. Curr Pharm Des 2003;9:1297–308.

[6] Meisel H. Multifunctional peptides encrypted in milk proteins. Biofactors 2004;21:55–61.

[7] Meisel H, FitzGerald RJ. Biofunctional peptides from milk proteins: mineral binding and cytomodulatory effects. Curr Pharm Des 2003;9: 1289–95.

[8] Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. N Engl J Med 1997; 336:1117–24.

[9] Pfeuffer M, Schrezenmeir J. Bioactive substances in milk with properties decreasing risk of cardiovascular diseases. Br J Nutr 2000; 84(Suppl 1):S155–9.

[10] Severin S, Wenshui X. Milk biologically active components as nutraceuticals: review. Crit Rev Food Sci Nutr 2005;45:645–56.

[11] Lopez AD, Murray CC. The global burden of disease, 1990–2020. Nat Med 1998;4:1241–3.

[12] Svetkey LP, Simons-Morton D, Vollmer WM, Appel LJ, Conlin PR, Ryan DH, et al. Effects of dietary patterns on blood pressure: subgroup analysis of the Dietary Approaches to Stop Hypertension (DASH) randomized clinical trial. Arch Intern Med 1999;159: 285–93.

[13] Jeppesen J, Schaaf P, Jones C, Zhou MY, Chen YD, Reaven GM. Effects of low-fat, high-carbohydrate diets on risk factors for ischemic heart disease in postmenopausal women. Am J Clin Nutr 1997;65: 1027–33.

[14] Obarzanek E, Sacks FM, Vollmer WM, Bray GA, Miller III ER, Lin PH, et al. Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) Trial. Am J Clin Nutr 2001;74:80–9.

[15] Stamler J, Elliott P, Kesteloot H, Nichols R, Claeys G, Dyer AR, et al. Inverse relation of dietary protein markers with blood pressure. Findings for 10,020 men and women in the INTERSALT Study. INTERSALT Cooperative Research Group. INTERnational study of SALT and blood pressure. Circulation 1996;94:1629–34.

[16] Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Speizer FE, et al. Dietary protein and risk of ischemic heart disease in women. Am J Clin Nutr 1999;70:221–7.

[17] Appel LJ, Sacks FM, Carey VJ, Obarzanek E, Swain JF, Miller III ER, et al. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. JAMA 2005;294:2455–64.

[18] Appel LJ. The effects of protein intake on blood pressure and cardiovascular disease. Curr Opin Lipidol 2003;14:55–9.

[19] Elliott P. Protein intake and blood pressure in cardiovascular disease. Proc Nutr Soc 2003;62:495–504.

[20] Hasler CM. The changing face of functional foods. J Am Coll Nutr 2000;19:499S–506S.

[21] Roberfroid MB. A European consensus of scientific concepts of functional foods. Nutrition 2000;16:689–91.

[22] Harris T, Cook EF, Kannel W, Schatzkin A, Goldman L. Blood pressure experience and risk of cardiovascular disease in the elderly. Hypertension 1985;7:118–24.

[23] Kannel WB, Higgins M. Smoking and hypertension as predictors of cardiovascular risk in population studies. J Hypertens Suppl 1990;8: S3–S8.

[24] FitzGerald RJ, Murray BA, Walsh DJ. Hypotensive peptides from milk proteins. J Nutr 2004;134:980S–8S.

[25] Vercruysse L, Van Camp J, Smagghe G. ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: a review. J Agric Food Chem 2005;53:8106–15.

[26] Yamamoto N. Antihypertensive peptides derived from food proteins. Inc Biopoly 1997;43:129–34.

[27] Nakamura Y, Yamamoto N, Sakai K, Takano T. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I_converting enzyme. J Dairy Sci 1995;78:1253–7.

[28] Seppo L, Jauhiainen T, Poussa T, Korpela R. A fermented milk high in bioactive peptides has a blood pressure_lowering effect in hypertensive subjects. Am J Clin Nutr 2003;77:326–30.

[29] Abubakar A, Saito T, Kitazawa H, Kawai Y, Itoh T. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. J Dairy Sci 1998;81:3131–8.

[30] Murakami M, Tonouchi H, Takahashi R, Kitazawa H, Kawai Y, Negishi H, et al. Structural analysis of a new anti_hypertensive peptide (beta_lactosin B) isolated from a commercial whey product. J Dairy Sci 2004;87:1967–74.

[31] Kawasaki T, Seki E, Osajima K, Yoshida M, Asada K, Matsui T, et al. Antihypertensive effect of valyl_tyrosine, a short chain peptide derived from sardine muscle hydrolyzate, on mild hypertensive subjects. J Hum Hypertens 2000;14:519–23.

[32] Matsufuji H, Matsui T, Ohshige S, Kawasaki T, Osajima K, Osajima Y. Antihypertensive effects of angiotensin fragments in SHR. Biosci Biotechnol Biochem 1995;59:1398–401.

[33] Fujita H, Yoshikawa M. LKPNM: a prodrug-type ACE-inhibitory peptide derived from fish protein. Immunopharmacology 1999;44: 123–7.

[34] Fujita H, Yokoyama K, Yoshikawa M. Classification and antihypertensive activity of angiotensin I_converting enzyme inhibitory peptides derived from food proteins. J Food Sci 2000;65:564–9.

[35] Nakashima Y, Arihara K, Sasaki A, Mio H, Ishikawa S, Itoh M. Antihypertensive Activities of Peptides Derived from Porcine Skeletal Muscle Myosin in Spontaneously Hypertensive Rats. J Food Sci 2002;67:434–7.

[36] Kodera T, Nio N. Identification of an Angiotensin I_converting Enzyme Inhibitory Peptides from Protein Hydrolysates by a Soybean Protease and the Antihypertensive Effects of Hydrolysates in Spontaneously Hypertensive Model Rats. J Food Sci 2006;71: C164–73.

[37] Motoi H, Kodama T. Isolation and characterization of angiotensin I_converting enzyme inhibitory peptides from wheat gliadin hydrolysate. Nahrung/Food 2003;47:354–8.

[38] FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr 2000;84(Suppl 1): S33–7.

[39] Meisel H. Biochemical properties of peptides encrypted in bovine milk proteins. Curr Med Chem 2005;12:1905–19.

[40] Matsufuji H, Matsui T, Seki E, Osajima K, Nakashima M, Osajima Y. Angiotensin I-converting enzyme inhibitory peptides in an alkaline protease hydrolyzate derived from sardine muscle. Biosci Biotechnol Biochem 1994;58:2244–5.

[41] Vermeirssen V, Van Camp J, Verstraete W. Bioavailability of angiotensin I converting enzyme inhibitory peptides. Br J Nutr 2004;92:357–66.

[42] Gardner ML. Gastrointestinal absorption of intact proteins. Annu Rev Nutr 1988;8:329–50.

[43] Webb Jr KE. Intestinal absorption of protein hydrolysis products: a review. J Anim Sci 1990;68:3011–22.
[44] Masuda O, Nakamura Y, Takano T. Antihypertensive peptides are present in aorta after oral administration of sour milk containing these peptides to spontaneously hypertensive rats. J Nutr 1996;126:3063–8.
[45] Roberts PR, Burney JD, Black KW, Zaloga GP. Effect of chain length on absorption of biologically active peptides from the gastrointestinal tract. Digestion 1999;60:332–7.
[46] Suetsuna K. Isolation and characterization of angiotensin I converting enzyme inhibitor dipeptides derived from *Allium sativum* L (garlic). J Nutr Biochem 1998;9:415–9.
[47] Chen HM, Muramoto K, Yamauchi F, Fujimoto K, Nokihara K. Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. J Agric Food Chem 1998;46:49–53.
[48] Pena-Ramos EA, Xiong YL, Arteaga GE. Fractionation and characterisation for antioxidant activity of hydrolysed whey protein. J Sci Food Agric 2004;84:1908–18.
[49] Chen HM, Muramoto K, Yamauchi F, Nokihara K. Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. J Agric Food Chem 1996; 44:2619–23.
[50] Fiat AM, Levy-Toledano S, Caen JP, Jolles P. Biologically active peptides of casein and lactotransferrin implicated in platelet function. J Dairy Res 1989;56:351–5.
[51] Chabance B, Jolles P, Izquierdo C, Mazoyer E, Francoual C, Drouet L, et al. Characterization of an antithrombotic peptide from kappa-casein in newborn plasma after milk ingestion. Br J Nutr 1995;73: 583–90.
[52] Wergedahl H, Liaset B, Gudbrandsen OA, Lied E, Espe M, Muna Z, et al. Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion of HDL cholesterol, and lowers acyl-CoA: cholesterol acyltransferase activity in liver of Zucker rats. J Nutr 2004;134:1320–7.
[53] Morita T, Oh-hashi A, Takei K, Ikai M, Kasaoka S, Kiriyama S. Cholesterol-lowering effects of soybean, potato and rice proteins depend on their low methionine contents in rats fed a cholesterol-free purified diet. J Nutr 1997;127:470–7.
[54] Kritchevsky D, Tepper SA, Czarnecki SK, Klurfeld DM. Atherogenicity of animal and vegetable protein Influence of the lysine to arginine ratio. Atherosclerosis 1982;41:429–31.
[55] Iwami K, Sakakibara K, Ibuki F. Involvement of post-digestion 'hydrophobic' peptides in plasma cholesterol-lowering effect of dietary plant proteins. Agric Biol Chem 1986;50:1217–22.
[56] Making S, Nakashima H, Minami K, Moriyama R, Takao S. Bile acid-binding protein from soybean seed: isolation, partial characterization and insulin-stimulating activity. Agric Biol Chem 1988;52: 803–9.
[57] Nishi T, Hara H, Hira T, Tomita F. Dietary protein peptic hydrolysates stimulate cholecystokinin release via direct sensing by rat intestinal mucosal cells. Exp Biol Med (Maywood) 2001;226:1031–6.
[58] Nishi T, Hara H, Asano K, Tomita F. The soybean beta-conglycinin beta 51–63 fragment suppresses appetite by stimulating cholecystokinin release in rats. J Nutr 2003;133:2537–42.
[59] Fujita H, Usui H, Kurahashi K, Yoshikawa M. Isolation and characterization of ovokinin, a bradykinin B1 agonist peptide derived from ovalbumin. Peptides 1995;16:785–90.
[60] Matoba N, Usui H, Fujita H, Yoshikawa M. A novel anti-hypertensive peptide derived from ovalbumin induces nitric oxide-mediated vasorelaxation in an isolated SHR mesenteric artery. FEBS Lett 1999;452:181–4.
[61] Erdmann K, Grosser N, Schipporeit K, Schroder H. The ACE inhibitory dipeptide Met-Tyr diminishes free radical formation in human endothelial cells via induction of heme oxygenase-1 and ferritin. J Nutr 2006;136:2148–52.
[62] Nurminen ML, Sipola M, Kaarto H, Pihlanto-Leppala A, Piilola K, Korpela R, et al. Alpha-lactorphin lowers blood pressure measured by radiotelemetry in normotensive and spontaneously hypertensive rats. Life Sci 2000;66:1535–43.
[63] Yamamoto N, Maeno M, Takano T. Purification and characterization of an antihypertensive peptide from a yogurt-like product fermented by *Lactobacillus helveticus* CPN4. J Dairy Sci 1999;82: 1388–93.
[64] Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci U S A 1993; 90:7915–22.
[65] Steinbrecher UP, Zhang HF, Lougheed M. Role of oxidatively modified LDL in atherosclerosis. Free Radic Biol Med 1990;9: 155–68.
[66] Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1991;88:1785–92.
[67] Machlin LJ, Bendich A. Free radical tissue damage: protective role of antioxidant nutrients. J Faseb 1987;1:441–5.
[68] de Lorgeril M, Salen P, Monjaud I, Delaye J. The 'diet heart' hypothesis in secondary prevention of coronary heart disease. Eur Heart J 1997;18:13–8.
[69] Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872–9.
[70] Simopoulos AP. The Mediterranean diets: What is so special about the diet of Greece? The scientific evidence. J Nutr 2001;131: 3065S–73S.
[71] Suetsuna K, Ukeda H, Ochi H. Isolation and characterization of free radical scavenging activities peptides derived from casein. J Nutr Biochem 2000;11:128–31.
[72] Hernandez-Ledesma B, Davalos A, Bartolome B, Amigo L. Preparation of antioxidant enzymatic hydrolysates from alpha-lactalbumin and beta-lactoglobulin Identification of active peptides by HPLC-MS/MS. J Agric Food Chem 2005;53:588–93.
[73] Davalos A, Miguel M, Bartolome B, Lopez-Fandino R. Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. J Food Prot 2004;67:1939–44.
[74] Ishikawa S, Yano Y, Arihara K, Itoh M. Egg yolk phosvitin inhibits hydroxyl radical formation from the fenton reaction. Biosci Biotechnol Biochem 2004;68:1324–31.
[75] Chen HM, Muramoto K, Yamauchi F. Structural analysis of antioxidative peptides from soybean beta-conglycinin. J Agric Food Chem 1995;43:574–8.
[76] Park PJ, Jung WK, Nam KS, Shahidi F, Kim SK. Purification and characterization of antioxidative peptides from protein hydrolysate of lecithin-free egg yolk. J Am Oil Chem Soc 2001;78:651–6.
[77] Rival SG, Boeriu CG, Wichers HJ. Caseins and casein hydrolysates: 2 Antioxidative properties and relevance to lipoxygenase inhibition. J Agric Food Chem 2001;49:295–302.
[78] Saiga A, Tanabe S, Nishimura T. Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. J Agric Food Chem 2003;51:3661–7.
[79] Abraham NG, Lin JH, Schwartzman ML, Levere RD, Shibahara S. The physiological significance of heme oxygenase. Int J Biochem 1988;20:543–58.
[80] Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase. Proc Natl Acad Sci U S A 1968;61:748–55.
[81] Coceani F. Carbon monoxide in vasoregulation: the promise and the challenge. Circ Res 2000;86:1184–6.
[82] Hopkins PN, Wu LL, Hunt SC, James BC, Vincent GM, Williams RR. Higher serum bilirubin is associated with decreased risk for early familial coronary artery disease. Arterioscler Thromb Vasc Biol 1996; 16:250–5.
[83] Mayer M. Association of serum bilirubin concentration with risk of coronary artery disease. Clin Chem 2000;46:1723–7.
[84] Eisenstein RS, Garcia_Mayol D, Pettingell W, Munro HN. Regulation of ferritin and heme oxygenase synthesis in rat fibroblasts by different forms of iron. Proc Natl Acad Sci U S A 1991;88:688–92.

[85] Rival SG, Fornaroli S, Boeriu CG, Wichers HJ. Caseins and casein hydrolysates: 1 Lipoxygenase inhibitory properties. J Agric Food Chem 2001;49:287–94.
[86] Chan KM, Decker EA. Endogenous skeletal muscle antioxidants. Crit Rev Food Sci Nutr 1994;34:403–26.
[87] Alabovsky VV, Boldyrev AA, Vinokurov AA, Shchavratsky V. Effect of histidine-containing dipeptides on isolated heart under ischemia/reperfusion. Biochemistry (Mosc) 1997;62:77–87.
[88] Takenaka A, Annaka H, Kimura Y, Aoki H, Igarashi K. Reduction of paraquat-induced oxidative stress in rats by dietary soy peptide. Biosci Biotechnol Biochem 2003;67:278–83.
[89] Nagasawa T, Yonekura T, Nishizawa N, Kitts DD. In vitro and in vivo inhibition of muscle lipid and protein oxidation by carnosine. Mol Cell Biochem 2001;225:29–34.
[90] Laakso S. Inhibition of lipid peroxidation by casein Evidence of molecular encapsulation of 1,4-pentadiene fatty acids. Biochim Biophys Acta 1984;792:11–5.
[91] Bertina RM. Molecular risk factors for thrombosis. Thromb Haemost 1999;82:601–9.
[92] Grundy SM, Balady GJ, Criqui MH, Fletcher G, Greenland P, Hiratzka LF, et al. Primary prevention of coronary heart disease: guidance from Framingham: a statement for healthcare professionals from the AHA Task Force on Risk Reduction American Heart Association. Circulation 1998;97:1876–87.
[93] Jolles P. Structural aspects of the milk clotting process Comparative features with the blood clotting process. Mol Cell Biochem 1975;7:73–85.
[94] Qian ZY, Jolles P, Migliore-Samour D, Schoentgen F, Fiat AM. Sheep kappa-casein peptides inhibit platelet aggregation. Biochim Biophys Acta 1995;1244:411–7.
[95] Jolles P, Levy-Toledano S, Fiat AM, Soria C, Gillessen D, Thomaidis A, et al. Analogy between fibrinogen and casein Effect of an undecapeptide isolated from kappa-casein on platelet function. Eur J Biochem 1986;158:379–82.
[96] Raha S, Dosquet C, Abgrall JF, Jolles P, Fiat AM, Caen JP. KRDS — a tetrapeptide derived from lactotransferrin-inhibits binding of monoclonal antibody against glycoprotein IIb–IIIa on ADP-stimulated platelets and megakaryocytes. Blood 1988;72:172–8.
[97] Mazoyer E, Levy-Toledano S, Rendu F, Hermant L, Lu H, Fiat AM, et al. KRDS, a new peptide derived from human lactotransferrin, inhibits platelet aggregation and release reaction. Eur J Biochem 1990;194:43–9.
[98] Drouet L, Bal dit Sollier C, Cisse M, Pignaud G, Mazoyer E, Fiat AM, et al. The antithrombotic effect of KRDS, a lactotransferrin peptide, compared with RGDS. Nouv Rev Fr Hematol 1990;32:59–62.
[99] Maubois JL, Leonil J, Trouve R, Bouhallab S. Milk peptides with physiological activities: III Peptides with a cardiovascular effect: antithrombotic and antihypertensive activity. Lait 1991;71:249–55.
[100] Rutherfurd KJ, Gill HS. Peptides affecting coagulation. Br J Nutr 2000;84(Suppl 1):S99–S102.
[101] Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213–9.
[102] Martin MJ, Hulley SB, Browner WS, Kuller LH, Wentworth D. Serum cholesterol, blood pressure, and mortality: implications from a cohort of 361,662 men. Lancet 1986;2:933–6.
[103] Hori G, Wang MF, Chan YC, Komatsu T, Wong Y, Chen TH, et al. Soy protein hydrolyzate with bound phospholipids reduces serum cholesterol levels in hypercholesterolemic adult male volunteers. Biosci Biotechnol Biochem 2001;65:72–8.
[104] Sugano M, Goto S, Yamada Y, Yoshida K, Hashimoto Y, Matsuo T, et al. Cholesterol-lowering activity of various undigested fractions of soybean protein in rats. J Nutr 1990;120:977–85.
[105] Zhang X, Beynen AC. Lowering effect of dietary milk-whey protein v casein on plasma and liver cholesterol concentrations in rats. Br J Nutr 1993;70:139–46.
[106] Nagaoka S, Kanamaru Y, Kojima T, Kuwata T. Comparative studies on the serum cholesterol lowering action of whey protein and soybean protein in rats. Biosci Biotechnol Biochem 1992;56:1484–5.
[107] Carroll KK, Hamilton RMG. Effects of dietary protein and carbohydrate on plasma cholesterol levels in relation to atherosclerosis. J Food Sci 1975;40:18–23.
[108] Van der Meer R, De Vries HT, Van Tintelen G. The phosphorylation state of casein and the species-dependency of its hypercholesterolaemic effect. Br J Nutr 1988;59:467–73.
[109] Potter SM. Overview of proposed mechanisms for the hypocholesterolemic effect of soy. J Nutr 1995;125:606S–11S.
[110] Nagata Y, Ishiwaki N, Sugano M. Studies on the mechanism of antihypercholesterolemic action of soy protein and soy protein-type amino acid mixtures in relation to the casein counterparts in rats. J Nutr 1982;112:1614–25.
[111] Huff MW, Carroll KK. Effects of dietary proteins and amino acid mixtures on plasma cholesterol levels in rabbits. J Nutr 1980;110:1676–85.
[112] Wang MF, Yamamoto S, Chung HM, Chung SY, Miyatani S, Mori M, et al. Antihypercholesterolemic effect of undigested fraction of soybean protein in young female volunteers. J Nutr Sci Vitaminol (Tokyo) 1995;41:187–95.
[113] Yoshikawa M, Fujita H, Matoba N, Takenaka Y, Yamamoto T, Yamauchi R, et al. Bioactive peptides derived from food proteins preventing lifestyle-related diseases. Biofactors 2000;12:143–6.
[114] Takenaka Y, Utsumi S, Yoshikawa M. Introduction of enterostatin (VPDPR) and a related sequence into soybean proglycinin A1aB1b subunit by site-directed mutagenesis. Biosci Biotechnol Biochem 2000;64:2731–3.
[115] Pak VV, Koo MS, Kasymova TD, Kwon DY. Isolation and identification of peptides from soy 11S-globulin with hypocholesterolemic activity. Chem Nat Compd 2005;41:710–4.
[116] Pak VV, Koo M, Lee N, Kim MS, Kwon DY. Structure–activity relationships of the peptide Ile-Ala-Val-Pro and its derivatives revealed using the semi-empirical AM1 method. Chem Nat Compd 2005;41:454–60.
[117] Lovati MR, Manzoni C, Gianazza E, Arnoldi A, Kurowska E, Carroll KK, et al. Soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. J Nutr 2000;130:2543–9.
[118] Duranti M, Lovati MR, Dani V, Barbiroli A, Scarafoni A, Castiglioni S, et al. The alpha′ subunit from soybean 7S globulin lowers plasma lipids and upregulates liver beta-VLDL receptors in rats fed a hypercholesterolemic diet. J Nutr 2004;134:1334–9.
[119] Iritani N, Nagashima K, Fukuda H, Katsurada A, Tanaka T. Effects of dietary proteins on lipogenic enzymes in rat liver. J Nutr 1986;116:190–7.
[120] Iritani N, Hosomi H, Fukuda H, Tada K, Ikeda H. Soybean protein suppresses hepatic lipogenic enzyme gene expression in Wistar fatty rats. J Nutr 1996;126:380–8.
[121] Moriyama T, Kishimoto K, Nagai K, Urade R, Ogawa T, Utsumi S, et al. Soybean beta-conglycinin diet suppresses serum triglyceride levels in normal and genetically obese mice by induction of beta-oxidation, downregulation of fatty acid synthase, and inhibition of triglyceride absorption. Biosci Biotechnol Biochem 2004;68:352–9.
[122] Kagawa K, Matsutaka H, Fukuhama C, Watanabe Y, Fujino H. Globin digest, acidic protease hydrolysate, inhibits dietary hypertriglyceridemia and Val-Val-Tyr-Pro, one of its constituents, possesses most superior effect. Life Sci 1996;58:1745–55.
[123] Kagawa K, Matsutaka H, Fukuhama C, Fujino H, Okuda H. Suppressive effect of globin digest on postprandial hyperlipidemia in male volunteers. J Nutr 1998;128:56–60.
[124] Nagaoka S. Studies on regulation of cholesterol metabolism induced by dietary food constituents or xenobiotics. J Jpn Soc Nutr Food Sci 1996;49:303–13.
[125] Nagaoka S, Kanamaru Y, Kuzuya Y. Effect of whey protein and casein on the plasma and liver lipid in rats. Agric Biol Chem 1991;55:813–8.

[126] Nagaoka S, Futamura Y, Miwa K, Awano T, Yamauchi K, Kanamaru Y, et al. Identification of novel hypocholesterolemic peptides derived from bovine milk beta-lactoglobulin. Biochem Biophys Res Commun 2001;281:11–7.
[127] Morikawa K, Kondo I, Kanamaru Y, Nagaoka S. A novel regulatory pathway for cholesterol degradation via lactostatin. Biochem Biophys Res Commun 2007;352:697–702.
[128] Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation 1983; 67:968–77.
[129] Howard BV. Insulin resistance and lipid metabolism. Am J Cardiol 1999;84:28J–32J.
[130] Krauss RM, Winston M, Fletcher RN, Grundy SM. Obesity: impact of cardiovascular disease. Circulation 1998;98:1472–6.
[131] Anderson GH, Moore SE. Dietary proteins in the regulation of food intake and body weight in humans. J Nutr 2004;134:974S–9S.
[132] Johnstone AM, Stubbs RJ, Harbron CG. Effect of overfeeding macronutrients on day-to-day food intake in man. Eur J Clin Nutr 1996;50:418–30.
[133] Hu FB. Protein, body weight, and cardiovascular health. Am J Clin Nutr 2005;82:242S–7S.
[134] Aoyama T, Fukui K, Nakamori T, Hashimoto Y, Yamamoto T, Takamatsu K, et al. Effect of soy and milk whey protein isolates and their hydrolysates on weight reduction in genetically obese mice. Biosci Biotechnol Biochem 2000;64:2594–600.
[135] Aoyama T, Fukui K, Takamatsu K, Hashimoto Y, Yamamoto T. Soy protein isolate and its hydrolysate reduce body fat of dietary obese rats and genetically obese mice (yellow KK). Nutrition 2000;16:349–54.
[136] Belobrajdic DP, McIntosh GH, Owens JA. A high-whey-protein diet reduces body weight gain and alters insulin sensitivity relative to red meat in Wistar rats. J Nutr 2004;134:1454–8.
[137] Pupovac J, Anderson GH. Dietary peptides induce satiety via cholecystokinin-A and peripheral opioid receptors in rats. J Nutr 2002;132:2775–80.
[138] Baile CA, McLaughlin CL, Della-Fera MA. Role of cholecystokinin and opioid peptides in control of food intake. Physiol Rev 1986;66: 172–234.
[139] Reidelberger RD. Cholecystokinin and control of food intake. J Nutr 1994;124:1327S–33S.
[140] Nishi T, Hara H, Tomita F. Soybean beta-conglycinin peptone suppresses food intake and gastric emptying by increasing plasma cholecystokinin levels in rats. J Nutr 2003;133:352–7.
[141] Nishi T, Hara H, Kasai T. Guanidinated casein hydrolysate stimulates pancreatic secretagogue release by direct action to the intestine in rats. Proc Soc Exp Biol Med 1998;218:357–64.
[142] Froetschel MA, Azain MJ, Edwards GL, Barb CR, Amos HE. Opioid and cholecystokinin antagonists alleviate gastric inhibition of food intake by premeal loads of casein in meal-fed rats. J Nutr 2001;131: 3270–6.
[143] Pedersen NL, Nagain-Domaine C, Mahe S, Chariot J, Roze C, Tome D. Caseinomacropeptide specifically stimulates exocrine pancreatic secretion in the anesthetized rat. Peptides 2000;21:1527–35.
[144] Beucher S, Levenez F, Yvon M, Corring T. Effects of gastric digestive products from casein on CCK release by intestinal cells in rat. J Nutr Biochem 1994;5:578–84.
[145] Daniel H, Vohwinkel M, Rehner G. Effect of casein and beta-casomorphins on gastrointestinal motility in rats. J Nutr 1990;120: 252–7.
[146] Schusdziarra V, Schick A, de la Fuente A, Specht J, Klier M, Brantl V, et al. Effect of beta-casomorphins and analogs on insulin release in dogs. Endocrinology 1983;112:885–9.
[147] Clare DA, Swaisgood HE. Bioactive milk peptides: a prospectus. J Dairy Sci 2000;83:1187–95.
[148] Beucher S, Levenez F, Yvon M, Corring T. Effect of caseinomacropeptide (CMP) on cholecystokinin (CCK) release in rat. Reprod Nutr Dev 1994;34:613–4.
[149] Aziz A, Anderson GH. Exendin-4, a GLP-1 receptor agonist, interacts with proteins and their products of digestion to suppress food intake in rats. J Nutr 2003;133:2326–30.
[150] Hall WL, Millward DJ, Long SJ, Morgan LM. Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite. Br J Nutr 2003;89:239–48.
[151] Cordier-Bussat M, Bernard C, Levenez F, Klages N, Laser-Ritz B, Philippe J, et al. Peptones stimulate both the secretion of the incretin hormone glucagon-like peptide 1 and the transcription of the proglucagon gene. Diabetes 1998;47:1038–45.
[152] Nagasawa A, Fukui K, Funahashi T, Maeda N, Shimomura I, Kihara S, et al. Effects of soy protein diet on the expression of adipose genes and plasma adiponectin. Horm Metab Res 2002;34:635–9.
[153] Velasquez MT, Bhathena SJ. Role of dietary soy protein in obesity. Int J Med Sci 2007;4:72–82.
[154] Ascencio C, Torres N, Isoard_Acosta F, Gomez_Perez FJ, Hernandez_Pando R, Tovar AR. Soy protein affects serum insulin and hepatic SREBP_1 mRNA and reduces fatty liver in rats. J Nutr 2004; 134:522–9.