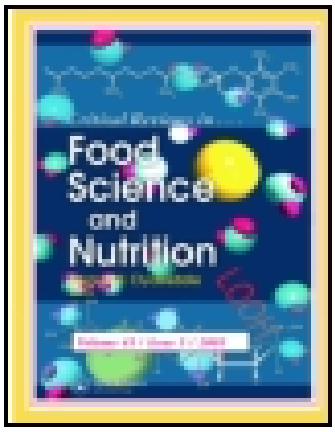

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Antihypertensive Peptides of Animal Origin: A Review

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Antihypertensive Peptides of Animal Origin: A Review

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Abstract

Many bioactive peptides trigger certain useful antihypertensive activities in the living body system and there is a mounting worldwide interest in the therapeutic potential of these bioactive peptides for exploitation in vivo against the hypertension. Studies suggest the antihypertensive properties for many bioactive peptides of animal origin with underlying mechanisms ranging from inhibition of angiotensin-converting enzyme to additional mechanisms to lower blood pressure such as opioid-like activities and mineral-binding and antithrombotic properties. Antihypertensive peptides are the most extensively studied of all the bioactivities induced by food protein hydrolysates, highlighting their importance in human health and disease prevention and treatment. There exist enormous opportunities for the production of novel peptide-based products in biopharmaceutical manufacturing industries for the treatment, prevention and mitigation of hypertension. Numerous products have already struck on the global market and many more are in process. This article focuses on antihypertensive peptides

identified in the meat, fish, blood, milk, dairy products, and egg and their probable application as novel ingredients in the development of functional food products as dietary treatment of hypertension.

Keywords

Bioactive peptides, antihypertensive peptides, meat, fish, blood, milk, dairy products, egg

1. Introduction

Hypertension, which is estimated to affect one-third of the Western population, is highly prevalent and a major risk factor for development of stroke, coronary heart disease, heart failure, and end-stage renal disease in the United States and throughout the world (Egan et al. 2010; Kearney et al. 2005; Lawes et al. 2001). The worldwide statistics of hypertension has shown that in 2000 the estimated total number of adults with hypertension was 972 million which is predicted to increase by about 60 percent to a total of 1.56 billion in 2025 (Kearney et al. 2005). It is the second leading preventable cause of mortality in the United States, after smoking, and blood pressure-associated diseases are the leading causes of morbidity and mortality in the United States and worldwide (Danaei et al. 2009, Appel et al. 2011). Stroke is second only to ischaemic heart disease as a cause of death, and over a third of stroke deaths occur in developing countries (Benamer and Grosset 2009). Being the leading cause of cardiovascular diseases worldwide (Hajjar et al. 2006), hypertension is responsible for more deaths than any other risk factor for cardiovascular disease and is predicted to become the leading cause of death and disability worldwide by 2020 (Kris-Etherton PM et al. 2009). Researchers have predicted that there will be a relative increase of 24 percent in the prevalence of hypertension in developed countries from 2000 to 2025 (Tu et al. 2008). Hypertension is therefore a global high-priority public health challenge requiring urgent attention with prevention, detection, treatment and control.

The relationship between hypertension and cardiovascular disease is well established (Hajjar and Kotchen 2003, Himmelmann et al. 1998, Kannel 1993, Kannel 1995). The Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood

Pressure reports that mortality from both ischemic heart disease and stroke increases linearly with increased blood pressure for persons between the ages of 40 and 90 years (Chobanian et al. 2003). For 40- to 70-year-olds, mortality from a myocardial infarction (MI) or cerebrovascular accidents doubles for each 20-mm Hg increase in systolic blood pressure above 115 mm Hg (Lewington et al. 2002, Chobanian et al. 2003).

Despite increased awareness of the importance of controlling blood pressure, the prevalence of hypertension is increasing in the United States and one out of every three Americans has hypertension (Thaker et al. 2005). According to the most recent National Health and Nutrition Examination Survey data presented at the 2005 American Society of Hypertension scientific meeting, hypertension was indicated as a worsening public health problem (Thaker et al. 2005) with 4.7 percent increase from previously reported data (Hajjar and Kotchen 2003). The American Heart Association (AHA) reports that 33.6 percent of Americans 20 years and older have hypertension with similar prevalence among men and women (34.4% and 32.6%, respectively) and additional 37.4 percent of adults have prehypertension. About 77.9 million adults have high blood pressure in the United States alone and was listed as a primary or contributing cause of death in about 348,102 of the more than 2.4 million U.S. deaths in 2009 and projections show that by 2030, prevalence of hypertension will increase 7.2 percent from 2013 estimates (American Heart Association 2013). These significant risks have targeted hypertension, and more recently, prehypertension as important public health goals.

2. Management of Hypertension

2.1 Through Drugs

Being an important regulator of blood pressure, drugs that inhibit the renin-angiotensin system, either by inhibiting angiotensin-converting enzyme (ACE; EC 3.4.15.1) or by blocking angiotensin (AT1) receptors, are widely used in the treatment of hypertension. Many ACE-inhibitory drugs have been used in the treatment of high blood pressure such as captopril, enalapril, alacepril, and lisinopril. These synthetic inhibitors however, have certain side effects, which may range from mild such as cough, taste disturbances and skin rashes to serious side effects, such as proteinuria and blood dyscrasias, especially when captopril is given in high dosage or to patients with renal failure (The Lancet Editorial 1980). While high blood pressure can be safely treated in most individuals, side effects of drugs are relatively frequent and may have notable effects on quality of life and compliance with the prescribed regimens.

2.2 Through Lifestyle Changes

The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines recommend early intervention for prehypertension (Chobanian et al. 2003). Besides the antihypertensive drugs, lifestyle modifications, including weight loss, quitting smoking, reducing sodium and alcohol intake, increasing physical activity and changing diet are recommended for both treatment and prevention (Krousel-Wood et al. 2004). A healthy lifestyle, including diet, is important in both preventing increased blood pressure over the lifespan and management of blood pressure in people with hypertension. Evidence-based national guidelines recommend diets low in salt and high in fruits, vegetables, and low-fat dairy products to reduce blood pressure in hypertensive subjects and decrease hypertension risk in healthy individuals (Chobanian et al. 2003, Whelton et al. 2002).

2.3 Through Food-derived Peptides

Besides the recommendation of general lifestyle changes, efforts have been made to produce functional foods that contain components which have a blood pressure reducing effect, and could be a supplement or alternative to the pharmaceutical treatment of hypertension. Therefore, any food component with the ability to reduce blood pressure could contribute to the prevention or treatment of cardiovascular diseases (Houston 2007, Thomas et al. 2007, Rudkowska and Jones 2007). Table-1 shows bioactive peptides from various animal proteins with antihypertensive activity. Many food-derived peptides have been identified as potential antihypertensive agents. Bioactive peptides with angiotensin-I-converting enzyme (ACE) inhibitory and antihypertensive effects have been the focus of special attention. They have been isolated from several food sources, biochemically characterized, and currently, some commercial food products with clinically proven effects are used (Yamamoto et al. 2003). Specifically, milk and dairy products have been shown to have good effects (Lopez-Fandin et al. 2006). Table-2 shows some of the commercially available dairy products with antihypertensive claims based on bioactive peptides.

The association between milk consumption and blood pressure was reported in the analysis of a cross-sectional study with over 10,000 persons during first National Health and Nutrition Examination Survey (NHANES I). In this study, low consumption of milk products was associated with a high incidence of hypertension (McCarron et al. 1984). Milk-derived peptides have been identified as potential antihypertensive agents. Through fermentation, peptides that have an ACE-inhibiting and thus a blood pressure-lowering effect can be derived from milk proteins (Yamamoto et al. 1999). Some of these peptides have also been found to have

opioid receptor binding properties (Meisel 1998). Table-3 shows some antihypertensive peptides derived from casein. Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP) are the best-characterized peptides found in fermented or enzymatically treated milk. Over twenty five human studies have been performed linking the consumption of products containing both IPP and VPP with significant reductions in blood pressure (Xu 2008, Boelsma and Kloek 2009). Fifteen of the blood pressure studies have been done in Japanese subjects. Ten studies have been performed in Caucasians, that is, in Finnish subjects (Seppo et al. 2002, Seppo et al. 2003, Tuomilehto et al. 2004, Jauhiainen et al. 2005), Dutch subjects (Engberink et al. 2008, van der Zander et al. 2008a, van der Zander et al. 2008b, de Leeuw et al. 2009), Scottish subjects (van Mierlo et al. 2009) and American subjects (Neutel et al. 2006). Effective dosages range from 3.07 mg/d (1.60 mg IPP and 1.47 mg VPP) to 52.5 mg/d (30 mg IPP and 22.5 mg VPP) (Jauhiainen et al. 2005, Sano et al. 2005).

A fermented milk product with the biologically active peptides valyl-prolyl-proline (Val-Pro-Pro) and isoleucyl-prolyl-proline (Ile-Pro-Pro) was shown to lower blood pressure in spontaneously hypertensive rats, which constitute an accepted model for human essential hypertension (Nakamura et al. 1995b). It was suggested that small peptides are absorbed from the gastrointestinal tract without being decomposed by digestive enzymes (Masuda et al. 1996). Two other peptides (Tyr-Pro and Lys-Val-Leu-Pro-Val-Pro-Gln) that were purified and characterized from fermented milk were also shown to have ACE-inhibitory activity in spontaneously hypertensive rats (Yamamoto et al. 1999, Maeno et al. 1996). Nurminen et al (2000) found that α-lactorphin (Tyr-Gly-Leu-Phe) also reduced blood pressure in normotensive and spontaneously hypertensive rats (Nurminen et al. 2000). In a placebo-controlled study of

hypertensive patients, sour milk fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae* reduced systolic and diastolic blood pressure in an 8-wk intervention (Hata et al. 1996). In an 8-wk study (Seppo et al. 2002), the milk product containing the bioactive tripeptides Val-Pro-Pro and Ile-Pro-Pro reduced blood pressure in mildly hypertensive subjects. In a placebo-controlled study, the same milk product also tended to lower systolic blood pressure in hypertensive subjects (Seppo et al. 2003). Seppo et al. (2003) reported a blood pressure-lowering effect in hypertensive subjects while studying the long-term blood pressure-lowering effect of *L. helveticus* LBK-16 H fermented milk, in normal daily use, in hypertensive subjects during a 21-wk intervention period.

Recently, MPH1 (ortensVida™) and MPH2 were developed from casein hydrolysates with different bioactive peptide profiles based on their *in vitro* ACE inhibitory potential. IPP was identified as the peptide contributing to ACE inhibitory effects in MPH1 whereas Methionine-Alanine-Proline (MAP), IPP, and Leucine-Proline-Proline (LPP) were responsible for ACE inhibitory effects in MPH2. These products were reported to contain a negligible amount of minerals contrasting with all other lactotripeptide-based products tested before (Allender et al. 1996, Griffith et al. 1999, Jee et al. 2002, van Mierlo et al. 2006, Whelton et al. 1997, Elliott et al. 2008). Boelsma and Kloek (2010) studied the effectiveness of these IPP-rich milk protein hydrolysates on reducing blood pressure as well as safety parameters and tolerability. The authors reported that MPH1, containing IPP and no minerals, exert clinically relevant blood pressure lowering effects in subjects with stage 1 hypertension and recommended that it may be included in lifestyle changes aiming to prevent or reduce high blood pressure.

Thus, many bioactive peptides from various livestock products exhibit certain useful antihypertensive activities in the living body system and there is a global interest in the therapeutic potential of these bioactive peptides for the treatment and prevention of hypertension. Many new functional food products have been launched in the markets globally with antihypertensive claims. The results from extensive randomized, blinded and placebo-controlled clinical trials are used to scientifically support claimed effects of these functional foods.

3. Production of Antihypertensive Peptides

Bioactive peptides produced by enzymatic hydrolysis with digestive enzymes; by means of microbial activity of fermented foods; and through the action of enzymes derived from proteolytic microorganisms are the most common methods to produce antihypertensive peptides from precursor proteins. Once the structure of antihypertensive peptides is known, it is also possible to synthesize them. Chemical synthesis; recombinant DNA technology; and enzymatic synthesis are the three main approaches that are available at present (Korhonen and Pihlanto 2003a). For production of bioactive peptides, milk proteins are the best known commercial source at present, however, lack of suitable large-scale technologies has been a limitation in the commercial production of milk derived bioactive peptides. Many techniques are available for the enrichment of peptides with a specific molecular weight range and even nanofiltration and ultrafiltration techniques are now employed for industrial production of specific bioactive peptides. At present, the membrane separation techniques seem to provide the best technology available industrially (Korhonen 2002; Kitts and Weiler 2003; Pouliot et al. 2006; Korhonen and Pihlanto 2007).

Suitable technologies have to be developed to isolate the active peptide fractions from the hydrolysates of various proteins of animal origin and to incorporate them into the model foods that will retain their physiological activity for a required period of time. The production of bioactive peptides during food processing, for example, by the use of specific bacterial enzymes or genetically transformed microorganisms, is of interest for future research (Hartmann and Meisel 2007).

4. Antihypertensive Peptides from Meat

People are showing a growing interest in potential uses of bioactive molecules in food and health care sectors (McCann et al. 2005). Meat has been used as a valuable protein source for the production of bioactive peptides with special emphasis on the production of ACE inhibitory bioactive peptides. Food-based strategies to control high blood pressure would be an excellent intervention to improve health and wellness and decrease health care costs. Naturally occurring peptides with ACE inhibitory activity have been identified from the proteolytic degradation products of food proteins that can be of animal (e.g. dairy, fish, meat and eggs), plant (e.g. soy, rice and garlic) or microbial (e.g. yeast) origin (FitzGerald and Meisel 2000; Hong et al. 2008). Bioactive peptides have been identified from the hydrolysis of skeletal muscle proteins including myosin, tropomyosin, troponin, actin and collagen (Vercruysse et al. 2005). A large number of studies have shown that peptides originating from food proteins have ACE inhibitory activity *in vitro* and can decrease blood pressure in laboratory animal studies (FitzGerald and Meisel 2000; Hong et al. 2008). However, evidence also exists for the ability of these peptides to decrease blood pressure in human populations.

Arihara et al. studied eight different enzymatic hydrolyzates of porcine skeletal muscle proteins by using exogenous enzymes for their ACE inhibitory activity and reported that the thermolysin digest had the most potent inhibitory activity and the two identified ACE inhibitory peptides, Met-Asn-Pro-Pro-Lys and Ile-Thr-Thr-Asn-Pro, had significant blood pressure-reducing effect in spontaneous hypertensive rats (Nakashima et al. 2002). Saiga et al. (2003) evaluated chicken breast meat extract treated with an Aspergillus protease and gastric proteases (trypsin, chymotrypsin, and intestinal juice) and observed ACE inhibitory effect in both the extract and hydrolysate of the extract whereas three ACE inhibitory peptides having common sequence of Gly-X-X-Gly-X-X-Gly-X-X had been identified. The strongest ACE inhibitory activity was observed with Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe peptide. Fu-Yuan et al. (2008) reported the highest ACE inhibitory activity by hydrolysate obtained by alkalase enzyme while evaluating the hydrolysates of chicken leg bones. Jang and Lee (2005) and Kazunori et al. (2003) reported that a peptide with Val-Leu-Ala-Gln-Tyr-Lys sequence and RMLGQTPT amino acid sequence, respectively from hydrolysates of sarcoplasmic protein extracts of beef and pepsin hydrolysate of porcine skeletal troponin C, respectively showed a very strong ACE inhibitory ability.

Two ACE inhibitory pentapeptides from the thermolysin digestion of porcine myosin were reported by Arihara et al. (2001). These were named myopentapeptides A and B. The amino acid sequence of myopentapeptide A was found to be MNPPK, which corresponded to positions 79–83 on the myosin heavy chain, while myopentapeptide B, with the amino acid sequence ITTNP, corresponded to positions 306–310 on the myosin heavy chain. Another ACE inhibitory octapeptide VKKVLGNP was discovered by Katayama et al. (2007) following the

digestion of crude myosin light chain with pepsin corresponding to positions 47–54 on the myosin light chain. Porcine skeletal muscle was also found to be a source of a novel antihypertensive peptide M6 with the amino acid sequence KRVITY corresponded to positions 191–196 on the myosin heavy chain when crude myosin B was hydrolyzed with pepsin (Katayama et al. 2003). A novel ACE inhibitory peptide from the pepsin hydrolysate of crude porcine troponin with the amino acid sequence KRQKYDI corresponding to amino acid positions 198–204 of troponin T was described by Katayama et al. (2008).

5. Antihypertensive Peptides from Fish

ACE inhibitory peptides have been found in various fish species, including shellfish, tuna, bonito, salmon and sardine (Joseph et al. 2011). There have been several reports of crude fish protein hydrolysates containing ACE inhibitory peptides. ACE inhibitory peptides were released following the hydrolysis of purified catfish protein with the commercial enzyme Protamex® with the majority being found in the soluble protein fraction (Theodore and Kristinsson 2007). A crude enzyme extract from the viscera of sardine was used to hydrolyze the protein contained within the head and viscera of the fish species known as sardinelle. The resulting hydrolysate contained a high concentration of peptides displaying low hydrophobicity with molecular masses between 200 and 600 Da (Joseph et al. 2011). Kawasaki et al. (2000) reported that with the sardine dipeptide, valyl-tyrosine, systolic and diastolic blood pressures were found to decrease 7.4-9.7 and 4.5-5.2 mm Hg, respectively, in human patients. Pacific Hake fish protein subjected to simulated gastrointestinal digestion was reported to contain ACE inhibitory activity (Samaranayaka et al. 2010).

The fish-derived bioactive peptides have enormous potential and have been utilized in the production of pharmaceutical products with an active functional role and effect on health; for example, blood pressure lowering capsules have been manufactured that contain Katsuobushi Oligopeptide LKPNM (in single-letter amino acid code) from thermolysin-treated dried bonito (a fish from tuna family), which is converted into its active form (LKP) by digestive enzymes (Vasotensin 120T^{TM} by Metagenics, USA; PeptACE^{TM} Peptides 90 by Natural Factors, USA) (Hartmann and Meisel 2007).

6. Antihypertensive Peptides from Blood

Almost 30 percent of the blood produced from slaughtered animals is utilized by the food industry (Gatnau et al. 2001). However, biological safety issues surrounding the blood collected from slaughtered animals (Hsieh and Ofori 2011) together with the religious constraints and negative consumer perception for direct blood consumption have contributed to its limited use in food applications. Therefore, exploration of novel methods of blood utilization is the need of the time. Finding new applications for blood components represents an important challenge for scientists (Silva and Silvestre 2003) and it is necessary to develop procedures and applications that will permit the utilization of animal blood on a larger scale, both to eliminate a sizable pollution hazard and prevent the loss of a potentially valuable material (Hyun and Shin 1998). Many studies in the search for alternative ways to use slaughterhouse blood have suggested extraction of bioactive peptides from blood components as one of the prospective alternatives. The recovery and extraction of bioactive compounds from blood has been seen as an opportunity to add economic value and generate new applications for slaughterhouse blood.

Over the past decades a new emerging area has been developing intensively, focused on obtaining bioactive peptides from blood fractions (Wanasundara et al. 2002; Nedjar-Arroume et al. 2008; Wei and Chiang 2009; Parés et al. 2011; Toldrá et al. 2012). Several studies conducted during the last decade, predominantly using bovine and porcine blood, have indicated that bioactive peptides from blood sources have angiotensin I-converting (ACE)-inhibitory activity. Several peptides derived from food proteins including blood have been shown to exert ACE-inhibitory activity and are considered to be milder and safer than synthetic drugs (Yu et al. 2006). Furthermore, the activities reported for natural peptides with ACE-inhibitory activity usually have other bioactivities and are easily absorbed (Lantz et al. 1991; Korhonen and Pihlanto 2003a; Yu et al. 2006; Adje et al. 2011).

7. Antihypertensive Peptides from Milk

Several epidemiological studies have shown a link between milk consumption and hypertension as evident from the fact that individuals with low milk consumption have a higher incidence of hypertension (McCarron et al. 1984). This association was also confirmed by Garcia-Palmieri et al. (1984) reporting that men in Puerto Rico who drank no milk have twice the incidence of hypertension than the men who drank a litre or more of milk per day. Epidemiological studies have also shown a link between protein consumption and hypertension as reported by the Honolulu Heart Program (Reed et al. 1985) and the Intersalt studies (Stamler et al. 1996) that blood pressure was lower in populations that had high protein intake. More direct evidence of the ability of ACE inhibitory peptides to reduce blood pressure in humans has been reported in several clinical trials. Consumption of a fermented milk (Seppo et al. 2003) high in bioactive peptides can decrease systolic blood pressure 6.7±3.0 mm Hg and diastolic blood

pressure 3.6±1.9 mm Hg in human patients with high blood pressure while fermented milk tablets containing bioactive peptides decreased systolic blood pressure 11.2±3.0 mm Hg but did not significantly decrease diastolic blood pressure (Aihara et al. 2005). While these studies show that peptide originating from food proteins have promise in decreasing blood pressure, other studies have not been so promising showing that lactopeptides (Van der Zander et al. 2008a) and milk supplemented with whey peptides (Lee et al. 2007) did not decrease blood pressure in human populations. The inability of ACE inhibitory peptides to decrease blood pressure in some human studies could be due to the loss of their efficacy in the gastrointestinal (GI) tract. Most clinical studies on ACE inhibitory peptides have utilized peptides derived from the hydrolysis of proteins by microbial proteases. These peptides are susceptible to further proteolytic hydrolysis in the stomach and intestine from acids or from enzymes including pepsin, chymotrypsin and various peptidases (FitzGerald and Meisel 2000). In addition, naturally occurring microbes in the GI tract produce additional proteases and peptidases that will catalyze further peptide hydrolysis.

Many ACE-inhibitory peptides have been isolated and identified by *in vitro* enzymatic digestion of milk proteins or by chemical synthesis of peptide analogs (Gobbetti et al. 2002, 2004). Table-4 shows some antihypertensive peptides derived from caseins by proteolytic action. The ACE-inhibitors derived from milk proteins account for different fragments of casein, named *casokinins* (Meisel and Schlimme 1994), or whey proteins, named *lactokinins* (FitzGerald and Meisel 2000). Antihypertensive peptides have been found in processed dairy products like milk, cheese, etc. without any intentional functional role. Lactotripeptides isoleucine-proline-proline (Ile-Pro-Pro) and valine-proline-proline (Val-Pro-Pro) have been found from sour milk (Nakamura et al. 1995a, b). Also several cheeses from Swiss origin contain the same tripeptides.

The concentration of Ile-Pro-Pro and Val-Pro-Pro seems to increase in the course of ripening process, reaching 100 mg/kg after 4-7 months (Sharma et al. 2011).

The antihypertensive effect of CN-derived peptides contained in dairy products has been shown by many recent *in vivo* and *in vitro* studies (FitzGerald and Meisel 2000; Gobbetti et al. 2004, 2007). Nakamura et al. (1995a, b) reported the ACE-inhibitory peptides from Calpis, which is a Japanese soft drink manufactured from skim milk fermented by *Lactobacillus helveticus* and *S. cerevisiae*. Two potent ACE inhibitory peptides from β-casein, f84-f86, which corresponds to Val-Pro-Pro, and f74-f76, which corresponds to Ile-Pro-Pro and one from κ-casein, f108-f110, which corresponds to Ile-Pro-Pro were purified. Single oral administration of sour milk containing these two tripeptides to spontaneously hypertensive rats (SHR) with dosage of 5 ml/kg of body weight significantly decreased the systolic blood pressure from 6 to 8 h after administration (Nakamura et al. 1995a, b). Antihypertensive effect of these chemically synthesized peptides was also observed from 2 to 8 h after administration and the effects were dose dependent. Yamamoto et al. (1994) reported release of Val-Pro-Pro and Ile-Pro-Pro peptides from α_{s1}- and β-CN from the milk inoculated with *Lb. helveticus.* Maeno et al. (1996) identified a β-casein-derived antihypertensive peptide from the casein hydrolysate using the same proteinase. The antihypertensive effect of this peptide was dose dependent in SHR at a dosage level from 0.2-2 mg/kg of body weight. This peptide did not show strong ACE inhibitory activity as such, but a corresponding synthetic hexapeptide deleted by Gln (Lys-Val-Leu-Pro-Val-Pro) exhibited strong ACE inhibitory activity as well as antihypertensive effect in SHR. This suggests possible activation of the peptides in the digestive tract. It has been demonstrated that a tetrapeptide isolated from β-lactoglobulin f142-f145, termed "β-lactosin B" had significant anti-

hypertensive activity when administered orally to spontaneously hypertensive rats (Murakami et al. 2004). A study in normotensive and mildly hypertensive human volunteers reported that twice daily ingestion of 10 g of a tryptic digest of casein for 4 weeks had an antihypertensive effect (Sekiya et al. 1992).

Hata et al. (1996) reported that the blood pressure of hypertensive patients decreased significantly after 4 and 8 weeks of daily ingestion of 95mL of sour milk, which contained the two tripeptides, and that resulted in ingested dosage of ACE-inhibitory peptides of 1.2 to 1.6 mg/day. ACE-inhibitory peptides f46–53, f58–61, f103–105, and f122–125 were identified and purified from the hydrolysates of caprine β-Lg that was digested with thermolysin (Gobbetti et al. 2007). Very highly active ACE-inhibitory peptides were found, which were corresponding to casokinins such as α_{s1}-CN f23–27 and f1–9, β-CN f60–68 and f177–183, and α_{s2}–CN f174–181 and f174–179, having IC_{50} values lower than 20 μ mol/L (Saito et al. 2000; Meisel 2001). Maruyama and Suzuki (1982) and Meisel and Schlimme (1994) reported that tryptic hydrolysates of casein correspond to f23–24 and f23–27 of α_{s1}-casein, as well as to f177–183 and f193–202 of β–bovine casein. Recently, Perpetuo et al. (2003) showed that the new ACE-inhibition peptides Glu-Met-Pro-Phe-Pro-Lys (f108–113) and Tyr-Pro-Val-Glu-Pro-Phe-Thr-Glu (f114–121) were generated by tryptic hydrolysis of γ-casein.

Antihypertensive peptides have been produced as well as identified by the hydrolysis of isoelectric casein with pepsin. Peptides corresponding to the sequences RYLGY, AYFYPEL, and YQKFPQY were the most potent ACE-inhibitory peptides found in these hydrolysates and have *in vitro* radical scavenging activity and also exerted significant antihypertensive activity when administered orally in spontaneously hypertensive rats (SHR) at doses of 5 mg/kg of body

weight. It must be highlighted that the antihypertensive effect found for peptides RYLGY and AYFYPEL is comparable to the activity of Val-Pro-Pro, an antihypertensive peptide already included in functional foods (Sharma et al. 2011).

Mizuno et al. (2004) conducted a study among patients with high-normal blood pressure and mild hypertension, who took different doses of casein hydrolysate produced with *Aspergillus oryzae* containing IPP and VPP. Volunteers consuming a 1.8 mg daily dose of IPP and VPP exhibited a significant decrease in systolic blood pressure (SBP) after 6 weeks and in those receiving either 2.5 or 3.6 mg, this benefit was already observed at 3 weeks. Moreover a significant difference in SBP between the placebo group and IPP and VPP group receiving 3.6 mg was also observed. Two different human studies with Evolus reported statically significant decrease in SBP of 10 and 6.7 mm Hg compared with controls (acidified milk and a mixed strain of *Lactococcus*-fermented milk) following 8 and 21 weeks ingestion of 150 ml, respectively, by mildly hypertensive volunteers (Sharma et al. 2011). Two other commercial products, a casein hydrolysate (Casein DP, Kanebo, Ltd., Japan and C12 peptide, DMV, Netherlands), and a whey protein hydrolysate (Biozate, Davisco, US) were also claimed to lower blood pressure in humans (Sharma et al. 2011; FitzGerald et al. 2004).

The blood pressure lowering effect of peptides from milk proteins has been confirmed in different animal models of human hypertension (Jakala and Vapaatalo 2010) and there is some evidence for their beneficial effect on vasculature as well. Clinical evidence for the antihypertensive effect of the lactotripeptides is more controversial and the products containing lactotripeptides offer a valuable option as a non-pharmacological and nutritional treatment of elevated blood pressure (Jakala and Vapaatalo 2010). ACE inhibitory peptides derived from milk

and dairy products are not as potent as the drugs used for hypertension treatment, but hold a promise as safe and natural therapeutic agent without any adverse side effect (Sharma et al. 2011). The antihypertensive potential of milk protein-derived peptides is dependent on the ability of these peptides to reach their target site without being degraded and inactivated by the action of intestinal or plasma peptidases. Resistance to peptidase degradation may be a prerequisite for an antihypertensive effect during the oral ingestion and the intravenous infusion of ACE inhibitory hydrolysates/peptides (Sharma et al. 2011).

8. Antihypertensive Peptides from Dairy Products

Bioactive peptides generated during milk fermentation with the starter cultures traditionally employed by the dairy industry is now an established fact and peptides with antihypertensive activities can be found in the end-products, such as various cheese varieties and fermented milks (Gobbetti et al. 2002; Korhonen and Pihlanto 2003b; Korhonen and Pihlanto-Leppa¨ la¨ 2004; Korhonen and Pihlanto 2006; FitzGerald and Murray 2006). Thus, when ingested as part of the daily diet, these traditional dairy products may carry specific health effects under certain conditions. Table-5 shows some of the commercially available dairy products with peptide based antihypertensive claims.

There have been numerous reports regarding the identification and isolation of antihypertensive peptides found in different cheese. Although bioactive peptides with ACE-inhibitory activity (Smacchi and Gobbetti 1998; Saito et al. 2000; Gomez-Ruiz et al. 2004), β-casomorphin (Muehlenkamp and Warthesen 1996; Jarmolowska et al. 1999), and calcium-binding phosphopeptides (Ferranti et al. 1997) have been identified in cheese, the stability of these peptides depend on several factors like pH, salt, and type of enzymes present under the

conditions of cheese ripening. Muehlenkamp and Warthesen (1996) suggested that β-casomorphin might be degraded under conditions common for cheddar cheese. Gomez–Ruiz et al. (2004), on the other hand, reported that the ACE-inhibitory activity of peptides was not severely affected by simulated gastrointestinal digestion. The ACE-inhibitory activity of several commercially available fermented milks and fresh cheeses was analyzed by Herna´ndez-Ledesma et al. (2004a) who found that most of these products showed moderate ACE-inhibitory activity and the activity of the products remained stable or increased after simulated gastrointestinal digestion with pepsin and Corolase PP (from pig pancreas, showing mainly trypsin and chymotrypsin activities). Herna´ndez-Ledesma et al. (2004b) reported the similar results with a number of infant formulas tested for potential ACE-inhibitory activity. These studies may draw an inference that digestion process may promote the formation of bioactive peptides from the proteins and oligopeptides present in dairy products and that at least part of the active peptides survive this process (Korhonen 2009).

During cheese ripening a great variety of peptides are formed and many of them have been shown to exert biological activities. Calcium phosphopeptides (CPPs) have been identified in Comte´ and Cheddar cheese in the beginning (Roudot-Algaron et al. 1994; Singh et al. 1997) and now recently in Herrgard cheese (Ardo et al. 2007). Secondary proteolysis during cheese ripening, moreover, may lead to the formation of other bioactive peptides, with occurrence of bioactivity dependent on the ripening stage of the cheese. Higher ACE-inhibitory activities have been reported by Meisel et al. (1997) in middle-aged Gouda cheese than in short-termed or long-termed ripened cheese. Products having a low degree of proteolysis, such as yogurt, fresh cheese and quark exhibited low ACE-inhibitory activity that was also in agreement with the findings of

Ryhanen et al. (2001), who observed that ACE-inhibitory peptides developed gradually during cheese ripening and their concentration was highest at the age of 13 weeks in a Gouda-type cheese, declining slowly thereafter. Saito et al. (2000) on the other hand, detected the highest ACE-inhibitory activity in Gouda cheese aged two years among the several cheese varieties studied. Several peptides were isolated and identified from 8-month-old Gouda cheese and two peptides derived from α_{s1}-casein f(1–9) and β-casein f(60–68), respectively, showed potent ACE-inhibitory activity. Some ACE-inhibitory activity was observed in Manchego cheese that was at least 15 days old (Gomez-Ruiz et al. 2002) with ACE-inhibitory activity decreasing during the first 4 months, increasing when proteolysis advanced, and decreasing again in 12-month-old cheese. Corresponding to the sequences of sheep α_{s1}-, α_{s2}- and β-casein, 22 peptide fragments were altogether identified in the chromatographic fractions.

The presence of ACE-inhibitory peptides of low molecular mass was found in several ripened cheeses by Meisel et al. (1997). They further observed that the ACE-inhibitory activity increased as proteolysis developed, while the ACE-inhibitory effect decreased when the cheese maturation exceeded a certain level during proteolysis. Gagnaire et al. (2001) reported 28 peptides out of 91 identified peptides in Emmental cheese with various bioactivities (e.g. antimicrobial, mineral-carrying, antihypertensive, immunostimulatory) *in vitro*. Cathepsin D originating from milk and cell-envelope proteinase from thermophilic starters seem to be involved in the hydrolysis of α_{s1}-casein in Emmental cheese in addition to the action of plasmin which is involved in the hydrolysis of β- and α_{s2}-caseins. Furthermore, throughout the ripening period, peptidases released from both starter and nonstarter LAB seem to contribute to the formation of bioactive peptides. Active opioid peptides were not detected in mature Cheddar

cheese (Muehlenkamp and Warthesen 1996) due to probable degradation during the ripening process. However, small quantities of β-casomorphin-3 were found in Edam cheese by Sabikhi and Mathur (2001) during ripening whereas longer casomorphins were not detectable. 44 hard, semi-hard and soft cheese samples of Swiss origin were investigated by Bu¨tikofer et al. (2007) for occurrence and quantification of IPP and VPP. They observed considerable variations in the amounts of both peptides (0 to 224 mg/kg for VPP and 0 to 95.4 mg/kg for IPP) in different samples with highest amounts of these two peptides in hard and semi-hard cheese varieties, such as Emmental, Hobelka¨ se, Gouda and Gruyere. Many ACE-inhibitory peptides from α_{s1}-casein and β-casein were isolated and identified by Ong et al. (2007) in Cheddar cheese made with starter lactococci. They observed that addition of probiotic cultures (strains *Lb. casei 279* and *Lb. casei LAFTI® L26*) increased the ACE-inhibitory activity of the cheeses during ripening at 4°C, possibly due to increased proteolysis. Parrot et al. (2003) suggested that more bioactive peptides are likely to be formed in the gastrointestinal tract upon ingestion of a piece of cheese besides the generation during ripening process. They demonstrated that consecutive digestion of the water-soluble extract of Emmental cheese with pepsin and trypsin, respectively, induced an increase in ACE-inhibition as compared with undigested water-soluble extract under *in vitro* conditions.

Many studies have reported the occurrence of various antihypertensive peptides in fermented milks, e.g., yoghurt, sour milk and ‘‘*Dahi*”. Rokka et al. (1997) reported ACE-inhibitory peptides in yoghurt and in milk fermented with a probiotic *Lb. casei ssp.* Rhamnosus strain. ACE-inhibitory peptides were reported in yoghurt made from ovine milk by Chobert et al. (2005) and in *kefir* made from caprine milk by Quiros et al. (2005). Ashar and Chand (2004) showed that a new casokinin Ser-Lys-Val-Tyr-Pro (f) was found in bovine fermented milk

product "*Dahi*" which was fermented by *Lb. derbrueckii* ssp. *bulgaricus, Str. thermophilus* and *Lc. lactis* ssp. *lactis biovar. diacetylactis.*

9. Antihypertensive Peptides from Egg

Nowadays egg is known as an important source of many bioactive substances which may find wide application in medicine and food production (Trziszka et al. 2006). It has been reported that certain egg white-derived peptides can play a role in controlling the development of hypertension by exerting vasorelaxing effects (Davalos et al. 2004). Table-6 shows some peptide sequences derived from egg proteins with antihypertensive properties. Many antihypertensive peptides derived from egg proteins by enzymatic hydrolysis have been described (Miguel et al. 2004; Sakanaka and Tachibana 2006). The sequences FRADHPFL (ovokinin) and RADHPFL (ovokinin 2-7), that have shown endothelium-dependent vasodilator activity, were obtained at first. Davalos et al. (2004) reported that the enzymatic hydrolysis of crude albumen proteins with pepsin resulted in the production of peptides with strong antioxidant activities. The egg white peptide Tyr–Ala–Glu–Glu–Arg–Tyr–Pro–Ile–Leu, which was shown previously to possess ACE-inhibitory activity, also exhibited a high radical-scavenging activity. These results would suggest that the combined antioxidant and ACE-inhibitory properties of albumen hydrolysates, or the corresponding peptides, would make a useful multifunctional preparation for the control of cardiovascular diseases, in particular hypertension.

It has been shown that orally-administered ovokinin; an octapeptide (Phe-Arg-Ala-Asp-His-Pro-Phe-Leu) isolated from the pepsin hydrolysate of ovalbumin, significantly lowered systolic blood pressure in spontaneously hypertensive rats (SHRs) (Fujita et al.1995b). A hexapeptide obtained by chymotrypsin digestion of ovalbumin called ovokinin (2–7) also

exhibited antihypertensive properties. Synthetic analogs of ovokinin (2–7), such as Arg-Pro-Phe-His-Pro-Phe and Arg-Pro-Leu-Lys-Pro-Trp, are also reported to exhibit strong hypotensive activity in SHRs after oral administration (Matoba et al. 2001; Yamada et al. 2002). The replacement of amino acids in the ovokinin (2-7) peptide has resulted in enhanced anti-hypertensive activity, with the most potent derivative resulting in 100-fold more potent anti-hypertensive activity (Matoba et al. 2001; Mine and Kovacs-Nolan 2005). Two angiotensin I converting enzyme (ACE)-inhibitory peptides were also identified in ovalbumin by peptic (OA 183-184) and tryptic (OA 200-218) digestions (Mine and Kovacs-Nolan 2005). Miguel et al. (2005) examined peptides with ACE-inhibitory properties produced by enzymatic hydrolysis of crude egg white. Peptide sequences RADHPFL, YAEERYPIL, and IVF with IC_{50} values of 4.7, 6.2, and 33.11 μ mol/L, respectively, derived after 3h incubation with pepsin followed by ultrafiltration exhibited potent ACE-inhibitory activity and antihypertensive activity.

The hydrolysate of egg yolk with a crude enzyme from the genus *Rhizopus* exhibited ACE inhibitory action *in vitro*; and the fraction of the hydrolysate with molecular mass lower than 1 kDa suppressed the development of hypertension in SHR after oral administration for 12 weeks (Yoshii et al. 2001). Serum ACE activity of the hydrolysate-administered SHR groups was significantly lower than that of the control group in a dose-dependent manner, which implied the existence of an ACE-inhibitory mechanism *in vivo* (Yoshii et al. 2001). Miguel et al. (2007) reported that peptides identified in pepsin digested egg-white hydrolysate (such as RADHPFL, IVF, and YAEERYPIL) inhibit ACE *in vitro* and exhibit antihypertensive activity in SHRs at minimum effective doses of 2-4 mg/kg. RADHP and YPI fragments yielded from the stimulated gastrointestinal digestion of RADHPFL and YAEERYPIL exhibited a blood pressure-

lowering effect (Miguel et al. 2007). Peptides released from egg-white proteins on treatment with Alcalase have also been reported to have antihypertensive activity (Liu et al. 2010; Zhipeng et al. 2011). RVPSL exhibited the highest level of ACE inhibitory activity (20 μmol/L) among the ovotransferrin-derived peptides (Liu et al. 2010). Three peptides were also isolated from egg-white hydrolysate by Zhipeng et al. (2011) digested with Alcalase. Peptide sequence QIGLF was observed to be a potential ACE inhibitor with an IC_{50} value of 75 μmol/L and also showed low gastrointestinal enzyme susceptibility and contained a relatively high amount of α-helix (Zhipeng et al. 2011).

Production of food products based on egg-derived ACE-inhibitory peptides may utilize the hydrolysates as such, or after enrichment. The isolation of specific peptides from the total peptide mixture or the enrichment of fractions is a technological challenge in the production of ACE-inhibitory and antihypertensive peptides. Since a common feature of ACE-inhibitory peptides is their relatively small size; fractionation using ultrafiltration and size exclusion chromatography constitutes a useful step for pre-concentration (Fujita et al. 2001). In fact, when the hydrolysate obtained from crude egg white with pepsin treatment for 3h (IC50 = 55.3 μg/ml) was filtered through a 3000 Da cut-off membrane, permeate presented approximately ten times more inhibitory activity than the retentate (IC_{50} = 34.5 and IC_{50} = 298.4 μg/ml, respectively; Miguel et al. 2004).

10. Conclusions

Scientific progress in understanding the relationship between nutrition and health has an increasingly profound impact on consumer's approach to nutrition. Consumers more and more believe that foods contribute directly to their health and are not intended to only satisfy hunger

and to provide necessary nutrients but also to prevent nutrition-related diseases and improve physical and mental well-being of the consumers. With the increased knowledge and scientific credibility, development of functional foods based on the functionality of the antihypertensive peptides has been a subject of growing commercial interest. This antihypertensive peptide based functional food market is a fast growing segment of food industry and is still expanding with increasing awareness and purchasing power of the consumers. Numerous products based on the antihypertensive properties of the bioactive peptides have already struck the market and many products are under development by food companies, exploiting the potential of food-derived antihypertensive peptides. Thus, optimal exploitation of antihypertensive peptides for the development of designer, healthy and functional foods possesses an exciting scientific and technological challenge, while at the same time offering potential for commercially successful applications. However, crucial scientific, technological and regulatory issues have to be resolved before these substances can be optimally exploited for human nutrition and health.

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Table 1: Bioactive peptides from various animal proteins with antihypertensive/ACE-inhibition activity

Protein source	Peptide/sequence	Reference
Casein	Phe-Phe-Val-Ala-Pro Val-Pro-Pro Ile-Pro-Pro	Migliore-Samour et al. (1988) Takano (2002), Nakamura et al. (1995a), Nakamura et al. (1995b)
Whey proteins	Tyr-Pro	Yamamoto et al. (1999)
β-lactoglobulin	Trp-Leu-Ala-His-Lys Ala-Leu-Pro-Met-His-Ile-Arg Tyr-Leu-Leu-Phe Ile-Pro-Ala	Pihlanto-Leppälä et al. (2000) Mullally et al. (1997) Belem et al. (1999) Abubakar et al. (1998)
Porcine skeletal muscle	Ile-Thr-Thr-Asn-Pro Thr-Asn-Pro Met-Asn-Pro-Pro-Lys Ile-Thr-Thr-Asn-Pro RMLGQTPT	Nakashima et al. (2002) Arihara et al. (2001) Nakashima et al. (2002) Kazunori et al. (2003)
Gelatin (bovine skin)	Gly-Pro-Leu Gly-Pro-Val	Kim et al. (2001)
Chicken	Ile-Lys-Trp Leu-Lys-Pro Gly-Phe-Hyp-Gly-Thr-Hyp-Gly-Leu-Hyp-Gly-Phe	Fujita et al. (2000) Saiga et al. (2003)
Egg ovalbumin	Arg-Ala-Asp-His-Pro-Phe Leu-Trp Glu-Arg-Lys-Ile-Lys-Val-Tyr-Leu	Matoba et al. (1999) Fujita et al. (2000) Fujita et al. (1995a)

	Phe-Arg-Ala-Asp-His-Pro-Phe-Leu	
Egg white	Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu	Davalos et al. (2004)
Beef	Leu-Ala-Gln-Tyr-Lys	Jang and Lee (2005)
Mozzarella, Crescenza, Gogonzola (cheese)	β-CN (58–72)	Smacchi and Gobbetti (2000)
Gouda (cheese)	α_{s1}-CN (1-9), β-CN (60-68)	Saito et al. (2000)
Festivo (cheese)	α_{s1}-CN (1-9), α_{s1}-CN (1-7), α_{s1}-CN (1-6)	Rihanen et al. (2001)
Manchengo (ovine cheese)	α_{s1}-CN, α_{s2}-CN, β-CN fragments	Gomez-Ruiz et al. (2002)
Fermented milks, sour milk	β-CN (74-76), β-CN (84-86), κ-CN (108-111)	Nakamura et al. (1995a, b)
Dahi	SKVYP	Ashar and Chang (2004)

Table 2: Commercial dairy products with antihypertensive claims based on bioactive peptides*

Brand name	Type of product	Claimed functional bioactive peptide	Producer
Calpis	Sour milk	VPP, IPP from β-CN and k-CN	Calpis Co., Japan
Evolus	Calcium enriched fermented milk drink	VPP, IPP from β-CN and k-CN	Valio Oy, Finland
BioZate	Hydrolyzed whey protein isolate	β-LG fragments	Davisco, USA
C12 peptide	Ingredient-hydrolysate	Casein derived peptide	DMV International, The Netherlands
Casein DP Peptio Drink	Soft drink	Casein-derived dodecapeptide FFVAPFPEVFGK	Kanebo, Japan

*Source: Korhonen (2009), Korhonen and Pihlanto (2006) and Hartmann and Meisel (2007)

Table 3: Antihypertensive peptides derived from casein

Casein Segment	Peptide Sequence	Reference
α_{s1} (1 – 9)	RPKHPIKHQ	Ryhänen et al. 2001
α_{s1} (102 – 109)	KKYKVPQ	Gomez - Ruiz et al. 2002
α_{s2} (174 – 179)	FALPQY	Tauzin et al. 2002
α_{s2} (174 – 181)	FALPQYLK	Tauzin et al. 2002
β (199 – 204)	GPVRGPFPIIV	Gomez - Ruiz et al. 2002
β (193 – 198)	YQEPVL	Pihlanto - Lepp ä l ä et al. 1998
κ (108 – 110)	IPP	Nakamura et al. 1995a,b
γ (108 – 113)	EMPFPK	Perpetuo et al. 2003
γ (114 – 121)	YPVEPFTE	Perpetuo et al. 2003

Table 4: Antihypertensive peptides derived from caseins by proteolytic action*

Peptide	Source	IC_{50} (μM)**	Dose (mg/kg)	SBP (mm Hg)
FFVAPFPEVFGK	α_{s1}-casein	77	100	-13.0
AVPYPQR	β-casein	15	100	-10.0
TTMPLW	α_{s1}-casein	16	100	-13.6
LKPNM	Aldolase	2.4	60	-23
LKP	Aldolase	0.32	60	-18
IPA	β-lactogloblin	141	8	-31
VYPFPG	β-casein	221	8	-22
GKP	β-microglobulin	352	8	-26
FP	β-casein	315	8	-27
YKVPQL	α_{s1}-casein	22	1	-12.5
VPP	β-casein	9	1.6	-20
IPP	β and κ-casein	5	1	-15.1
YP	α_{s1}, β- and κ-casein	720	1	-27.4

*Source: Wakai and Yamamoto (2012)

**IC_{50}: Peptide concentration that shows 50 percent inhibition of ACE activity

***SBP: systolic blood pressure of spontaneously hypertensive rat

Table 5: Commercial dairy products with peptide based antihypertensive claims*

Product	Dose and duration of treatment	Effect in systolic blood pressure (SBP)	References
Calpis (fermented milk)	95 mL/day for 8 weeks	-14.1 mm Hg	Hata et al. (1996)
	160 g/day for 4 weeks	-5.2 mm Hg	Mizushima et al. (2004)
Ameal Peptide (casein hydrolysate tablets)	1.8 mg IPP + VPP for 6 weeks 2.5 mg IPP + VPP for 6 weeks 3.6 mg IPP + VPP for 6 weeks	-6.3mm Hg -6.7mm Hg -10.1 mm Hg	Mizuno et al. (2005)
Casein hydrolysate drink	200 mL/day for 12 weeks	-4.6mm Hg	Sano et al. (2005)
Powdered fermented milk tablets	6 Tablets/day for 4 weeks	-3.2 mmHg	Aihara et al. (2005)
Casein hydrolysate capsules	4 Capsules/day for 1 week	No change in SBP but improved blood flow	Hirota et al. (2007)
Evolus (fermented milk)	150 mL/day for 8 weeks	-7.3%	Seppo et al. (2002)
	150 mL/day for 21 weeks	-6.7mm Hg	Seppo et al. (2003)
	150 mL/day for 8 to 10 weeks	-1.3mm Hg	Tuomilehto et al. (2004)
	2 x 150 mL/day for 10 weeks	-6.4mm Hg	Jauhiainen et al. (2005)
C12 peptide (a bovine milk protein hydrolysate containing a_{s1}-casein f23–34) ± alginic acid	100 or 200 mg C12 with 877 or 1754 mg alginic acid (Single dose)	-9mm Hg (-6 mm Hg DBP) at 6 h compared to 2 h, at high alginic acid dose	Townsend et al. (2004)
BioZate 1 (a WPI hydrolysate)	20 g/day for 6 weeks	-8mm Hg	Pins and Keenan (2006)

*Source: Korhonen (2009)

Table 6: Peptide sequences derived from egg proteins with antihypertensive properties*

Sequence	Origin	ACE inhibitory activity (IC_{50}, μM)**	References
ERKIKVYL	Peptic digest of ovalbumin	1.2	Fujita et al. (2000)
FFGRCVSP	Peptic digest of ovalbumin	0.4	Fujita et al. (2000)
LW	Peptic digest of ovalbumin	6.8	Fujita et al. (2000)
SALAM	Peptic digest of ovalbumin	229	Miguel et al. (2004), Dávalos et al. (2004)
FRADHPFL (ovokinin)	Peptic digest of ovalbumin	3.2	Fujita et. al. (1995a, b)
RADHPFL	Peptic digest of ovalbumin	6.2	Miguel et al. (2004, 2005)
RADHPF (ovokinin 2-7)	Chymotryptic digest of ovalbumin	>400	Matoba et al. (1999)
RADHP	Pancreatic digest of FRADHPFL, RADHPFL	260	Miguel et al. (2006)
YAEERYPIL	Peptic digest of ovalbumin	4.7	Miguel et al. (2004, 2005)
YPI	Pancreatic digest of YAEERYPIL	>1000	Miguel et al. (2006), Dávalos et al. (2004)

*Source: LoPez-Fandi˜No et. al. (2007)

**IC_{50}: Peptide concentration that shows 50% inhibition of ACE activity