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Review

A review of meat protein hydrolysates and hypertension

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ABSTRACT

We tested the hypothesis that meat is a source of peptides that are effective at preventing and reducing chronic lifestyle-related diseases (CLSRDs) such as hypertension. This analysis reflects the importance of empowering hypertensive people in quality versus quantity of life issues, and in offering nutritional treatment options rather than medical alternatives. For both hypertensive and normotensive individuals, chemically based medications may have harmful side effects. Functional food rich in antioxidant vitamins, and proteins or biologically active peptides, can lower blood pressure in persons with essential hypertension, possibly by preventing an underlying cause of the condition. Deficiency in consumption of crucial nutrients such as proteins from meat origins, along with abnormalities in carbohydrate and fat metabolism, may underlie the etiology of the clinical course of hypertension. Food derived from meat rich in nutrients may provide physiologically functional peptides, as well as improve digestion, and the metabolism of carbohydrate and fats, thus lowering blood pressure, and normalizing associated biochemical and histopathological changes.

Meat was found to have value, because proteolysis of meat muscle generated a substantial number of multi-amino acid peptides that have nutrafunctional roles, and some of which have strong angiotension-converting enzyme inhibitory activity. This also demonstrates that meat proteins might lead to better nutraceutical therapy that minimizes health problems, and might aid in finding the most effective approaches for meeting the needs of all hypertension patients.

Contents

1. Introduction

Food scientists and nutritionists have a responsibility to conduct ongoing research that widely promotes a healthy society. Proper diet is arguably the best element for improving health, and reducing the risk of some chronic diseases, and may occasionally be able to limit the

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progression of such diseases. Questions about how common diseases are related to lifestyle, the hazards of chronic diseases, and ways to minimize or prevent them through non-pharmacological means are frequently discussed. The lifestyle factors include cigarette smoking, diet (fried foods, red meat), alcohol, sun exposure, environmental pollutants, infections, stress, obesity, and physical inactivity (Anand, et al., 2008). Chronic lifestyle-related diseases (CLSRD) such as cardiovascular diseases and cancer, account for millions of deaths each year, and are the leading causes of mortality in industrialized countries (Murray & Lopez, 1997). The most common type of cardiovascular disease is high blood pressure, also called hypertension, in which the pressure in the arteries is consistently above the normal range (systolic 120 mm Hg and diastolic 80 mm Hg).

Currently, antihypertensive medications such as captopril and analapril, marketed under the trade names Accupril, Altace, Capoten, Lotensin, Monoril, Prinvil, Vasotec and Zestril, stabilize blood pressure without removing the root cause, which is as yet unknown. Consequently, in most cases, hypertension is treated nonspecifically because it is of unknown type, or is diagnosed at an advanced stage. Over the last two decades, numerous studies have investigated food therapy and dietary approaches to prevent chronic lifestyle-related diseases, including hypertension. Diets low in salt, and rich in fruit and vegetables, low-fat dairy products, and high-fiber grains, with lean meat, significantly reduce blood pressure in both hypertensive and normative people.

Meat and dairy products are a good source of valuable biologically active peptides (Korhonen, 2009), as well as other nutrients such as fiber, vitamins and antioxidants (Descalzo, & Sancho, 2008; Pihlanto, 2006) that may confer benefits in reducing CLSRD. Determining the particular proteins that play major regulatory roles in normalizing blood pressure and preventing renal vascular damage is crucial for their application.

In the last four decades, meat proteins have been found to be a common source of bioactive peptides. Specific peptides inhibit angiotension-converting enzymes, and have potential as pharmaceuticals for treating hypertension (Vercruysse, Camp, & Smagghe, 2005). Recently, several food peptides that inhibit angiotensin I-converting enzyme (ACE) have been identified (Arihara, Nakashima, Mukai, Ishikawa, & Itoh, 2001; Kawamura, Takane, Satake, & Sugimoto, 1992), but the inhibitory mechanisms of these peptides has not been reported. Fujita, Yokoyama, and Yoshikawa (2000) and Yokoyama, Chiba, and Yoshikawa (1992) reported that inhibitory peptides from enzymatic digests of chicken breast muscle and ovalbumin were resistant to degradation by ACE activity. Peptides were tentatively classified into three types: inhibitory, substrate peptides, and pro-drug peptides. The resistance of the ACE inhibitory peptide against ACE itself, or against digestive enzymes, was a prerequisite for their action *in vivo*. Substrate-type peptides are cleaved by ACE, decreasing their ACE inhibitory activity. The inhibitory activity of the inhibitor-type peptides is not significantly affected by ACE cleavage. Pro-drug-type peptides show an increase in ACE inhibitory activity after ACE cleavage. Substrate-type peptides do not affect the blood pressure of spontaneously hypertensive rats (SHR), but inhibitor- and pro-drug-type peptides reduce blood pressure (Muguruma et al., 2009). We discovered the substrate-type peptide A5, and the pro-drug-type peptide M6, and found that M6 appears to have greater antihypertensive activity than A5 *in vivo* (Muguruma et al., 2009). Therefore, we undertook a retrospective review of the effects of protein hydrolysate derived from skeletal muscles on cardiovascular disease, specifically high blood pressure, a common disease that affects public health worldwide. In this article, we also review the mechanisms influencing blood pressure, and the possible functions of animal meat peptides on *in vivo* blood pressure.

2. Blood pressure statistics from health reports

Recent indexes indicate that 25% of the Japanese population suffers from high blood pressure (Muguruma et al., 2009). Currently, approximately 72 million Americans have high blood pressure (MNT, 2010). An analysis of the global impact of hypertension from Sweden's Karolinska University Hospital (Ostergren 2007), an estimated 1 billion people, or 14.9% of the worldwide population, currently have high blood pressure, and over 500 million more will be diagnosed by 2025. Thus, hypertension is not a problem solely of western countries or United States citizens. Even in parts of Africa and Asia, high blood pressure is common and affects public health. Over the last two decades, hypertensive patients are estimated to have increased by 27%, and the death rate from hypertension is 60% in some regions of the world (Ostergren, 2007). According to the World Health Organization, 600 million people with high blood pressure are at risk of heart attacks and heart disease-related complications. Approximately 5 million premature deaths occur worldwide every year. Among people with hypertension, 90% is not aware of its problems and consequences. High blood pressure is a major leading cause of strokes and kidney failure, blindness and even dementia. Cardiovascular diseases (CVD) are a major health problem and the leading cause of death worldwide, both for women and men (Reiner, 2009). In Europe, CVD causes nearly half of all deaths (48%), and is the main causes of the disease burden (23% illness and death) (Allender et al., 2008). Millions of deaths occur from heart disease alone each year, and patients seldom notice symptoms until organs have already been damaged.

3. Influences on blood pressure

Excessive stress can temporarily or chronically increase blood pressure, which is a normal response, resulting from hormonal activation by the nervous system. In hypertension blood pressure is consistently high, even at rest. Blood pressure is necessary for circulation, providing oxygen for muscles, and removing carbon dioxide and other wastes. The human blood vessel system is over 60,000 miles long, sufficient to circle the globe more than two and half times. Blood travels this distance from the heart, which pumps blood approximately 60–75 times a minute, generating systolic blood pressure (SBP), and rest periods measured as diastolic blood pressure (DBP). Blood flows through arteries that branch from the heart and into small arteries called arterioles. After its trip throughout the body, blood returns to the heart. An adult human heart pumps 2000 gal per day throughout the body using heart pressure.

The arterioles act as the gatekeepers of blood flow. When the gatekeepers are opened, the blood flows freely and exerts little pressure on vessel walls. When vessels constrict, as angiotensin-II increases, the heart must pump harder, which can damage arteries and generate chronic pressure. More flex in arteries facilitates blood movement. Thus, the more flexible the inner wall of the artery, the more freely blood flows.

If the arteries are stretched from additional force, the inner walls can develop microscopic scar tissues of cholesterol and adipose material, which become trapped in the grooves, creating an atherosclerotic plaque. As plaques grow, the arteries become stiffer and narrower. Ultimately, the harder the heart has to pump, the more damage occurs in the artery inner walls, leading to heart attack and cardiovascular failure, and increases in SBP and DBP.

Since the heart does not always pump the same amount of blood at the same rate, maintaining normal blood pressure is a balance maintained by the nervous system and the kidneys. Fright or stress causes the nervous system to release hormones that increase heart rate, and arterioles constrict, causing blood to go quickly to muscles. This temporary increase in blood pressure is in response to a physiological need, with the kidneys responding to return blood pressure to normal levels. When blood pressure increases, the kidneys excrete salt and water for elimination through urine, to reduce blood pressure. The kidney can also excrete less salt and water to increase blood pressure. Thus, blood pressure is normalized by changes in

blood flow. In hypertension, control mechanisms fail, although the precise reasons are not known. The cause of primary hypertension is unknown in 85–90% of cases although lifestyle may support its development. Changes may take place in the heart, which pumps a large amount of blood, causing vessel constriction, and a building of pressure that is not reduced. Heredity plays a role in hypertension, the same as diet does, especially in people who are salt sensitive or have a family history of hypertension. Lifestyle, age, race and gender are also influences. Hypertension is twice as common in the obese as in people of normal weight. It is more common in African-Americans (NetWellness, 2010), obese Westerners, and people under constant stress or work, and in women over 70, as well as young women who use birth-control pills (American Heart Association, 2009). Generally, blood pressure can be affected by smoking, lack of exercise, stress, and excessive alcohol or salt intake. Since most people do not have symptoms, monthly blood pressure checks are recommended to monitor for hypertension.

4. Monitoring and controlling high blood pressure

Three strategies are suggested to monitor and control blood pressure:

a. Health monitoring with regular medical checkups, once in a month.
b. Cultural diffusion of information about food and nutritional care, and advice on healthy diets including functional foods, and warnings about alcohol, salty food and unhealthy daily lifestyle habits.
c. Twice-weekly physical regimens to increase relaxation and reduce stress, and exercise to reset the blood pressure.

5. Diet and functional foods

A lifestyle factor that increases the incidence of blood pressure is an unbalanced diet with high lipids and salt. The classic nutritional recommendations for preventing elderly-onset diseases are based on the proposed hypocholesterolemic activities of vegetable oils compared to animal fats. Dietary lipids can influence cardiac function via changes in membrane fatty acid composition, which usually reflect the fatty acid composition of the diet, although important metabolic alterations may also be a factor. Logistic regression analysis shows that visceral fat is strongly associated with hypertension, after adjustment for age, sex, ethnicity, site, height, smoking status, pack-years of smoking, alcohol consumption status, amount of alcohol consumption, and physical activity (Ding et al., 2004). The cardiac muscle appears to be more responsive than other organs to changes in diet fatty acid composition (Aguila & Mandarim-de-Lacerda, 2003), and other organs are highly sensitive to diet, for example, the stomach, the intestines, their absorptivity, and vessel membranes. Since the heart and vessels are the most sensitive to diet composition, they are more likely to be affected by hypertension.

Salt (sodium chloride) is an essential element that is normally controlled in animals by the kidneys. If salt content is too high, the kidneys eliminate it into urine. When salt intake levels are very high, the kidneys do not function well, resulting in salt in the bloodstream. Generally, salt attracts water, so high salt in the blood draws more water into the bloodstream, which increases blood volume, raising blood pressure. The extraordinary consistency in body fluids is achieved by elevating or lowering total body water to counteract changes in serum concentrations of sodium and its anions (Robertson, 1992). Based on these regulatory mechanisms, the commonly accepted hypothesis is that a high-sodium diet expands not only the intravascular fraction of extracellular volume, but also the total extracellular space (Walser, 1992), which consequently affect blood pressure.

The daily requirement for salt is 2500 mg, but most people take in approximately five times that amount, as shown by several nutritional studies. Consumers are increasingly interested in the health benefits of food, and have begun to look beyond the basic nutritional benefits, to disease prevention and health-enhancement by food, including novel functional foods and supplemental ingredients. Nutritional supplementation with antioxidant vitamins such as vitamins C or E, or with B vitamins such as vitamin B6, can lower blood pressure in persons with essential hypertension (Vasdev, Longerich, & Singal, 2002).

Functional foods may be conventional foods or endogenous foods that are consumed as part of a usual diet. However, they contain bioactive compounds and nutraceutical ingredients, and are demonstrated to have physiological benefits that reduce the risk of chronic diseases and complications, beyond their basic nutritional functions. Functional foods available today include fermented dairy (yogurts) and soy products (Natto and Toufu) that have blood pressure-lowering effects, vegetables and olives that contain antioxidants, fresh fruits that have vitamins and minerals, and finally meat, because of the profound amounts of peptides and proteins that greatly reduce the risk of CLSRDs such as diabetes, osteoporosis, and high blood pressure. In our laboratory, we have been searching for novel bioactive peptides in meats from different animals (adult males of beef, pork and chicken). In the last 10 years, we have found several peptides that lower blood pressure *in vivo*. Therefore, we propose that meat contains valuable nutraceutical compounds that must be considered functional foods after digestion in the human stomach. The current review provides an in-depth focus on the nutrafunctional roles of novel bioactive peptides in meat.

6. Functional food approaches and hypertension

Coronary artery disease and hypertension are increasingly thought to develop from an association of hereditary and environmental factors. Diet and lifestyle may influence heritability of variant phenotypes that are dependent on the nutrient environment for expression (Vasdev et al., 2002). High blood pressure occurs when the walls of the larger arteries lose elasticity and become quite rigid, allowing less space for blood flow, increasing the fluid pressure. Smaller blood vessels become narrower with age, compounding the effects.

In normotensive people, some crucial peptides may be sufficient to maintain the normal activity of some metabolic enzymes, and normal blood pressure. Excessive intake of some peptides may not lower blood pressure, however, if metabolic enzymes are not defective. In essential hypertension, a dietary amount higher than the normal average may be needed to stimulate the activity of metabolic enzymes to achieve normal blood pressure (Vasdev et al., 2002). People with hypertension may need extra dietary supplementation of nutrients that, for them, are essential.

Currently, many known and as yet undiscovered nutraceutical compounds in animal products are thought to have positive effects on human health. Meat contains bioactive proteins and peptides with important roles in prevention of CLSRDs such as high blood pressure. In essential hypertension, a metabolic defect may occur in which glucose metabolism is altered because of insulin resistance, resulting in increased tissue levels of aldehydes and oxygen-free radicals, and hypertension. This metabolic defect can be corrected nutritionally by vitamin E, vitamin C, vitamin B6, (Sudesh et al., 2002) lipoic acid, or a diet rich in protein-containing cysteine (Vasdev et al., 2002). We attempted to categorize recently identified peptides with their amino acid sequences, and their functions. Table 1 shows the crucial peptides from meat and other foods, and their IC_{50} values against ACE inhibitors. The IC_{50} values are large enough to have the potential to lower blood pressure in animals. IC_{50} was defined as the concentration of protein or peptide required to inhibit 50% of ACE *in vivo*. The lower the IC_{50}, the greater the peptide ability to reduce blood pressure.

Table 1
IC_{50} of ACE inhibitory peptides derived from meat and seafood vertebrate specimens.

Peptide sequence	Activity and function	IC_{50}	Source		Reference
A5	Antihypertensive	83 μM	Sardine meat	N	Ukeda et al. (1992)
M6 (KRVIQY)	Antihypertensive (Pd)	20.3 μM	Porcine myosin B	N	Muguruma et al. (2009)
A5 (VKAGF)	Antihypertensive (I)	6.1 μM	Porcine actin	N	Muguruma et al. (2009)
ND	Antihypertensive (I)	3.9 mg/ml	Porcine meat	N	Ahhmed et al. (2009)
RMLGQTPTK	Antihypertensive (I)	34 μM	Porcine troponin	N	Katayama et al. (2004)
RMLGQTP	Antihypertensive (I)	503 μM	Porcine troponin	Y	Katayama et al. (2004)
RMLGQ	Antihypertensive (I)	358 μM	Porcine troponin	Y	Katayama et al. (2004)
RML	Antihypertensive (I)	1019 μM	Porcine troponin	Y	Katayama et al. (2004)
GQ	Antihypertensive (I)	5630 μM	Porcine troponin	Y	Katayama et al. (2004)
TP	Antihypertensive (I)	2071 μM	Porcine troponin	Y	Katayama et al. (2004)
TK	Antihypertensive (I)	1634 μM	Porcine troponin	Y	Katayama et al. (2004)
	Antihypertensive (I)	112 μg/ml	Porcine crude myosin	N	Katayama et al. (2003b)
VKKVLGNP	Antihypertensive (I)	28.5 μM	Porcine skeletal muscle	N	Katayama et al. (2007)
EKERERQ	Antihypertensive (I)	552.5 μM	Porcine skeletal muscle	N	Katayama et al. (2008)
KRQKYDI	Antihypertensive (I)	26.2 μM	Porcine skeletal muscle	N	Katayama et al. (2008)
IKPLNY and IVGRPRHQG	Antihypertensive (I)	43 and 2.4 μM	Muscle and Actin of bonito	N	Yokoyama et al. (1992)
DYGLYP and IWH	Antihypertensive (Pd)	62 and 6.9 μM	Muscle and Actin of bonito	N	Fujita and Yoshikawa (1999)
VLAQYK	Antihypertensive (I)	ND	Beef muscle	N	Jang & Lee (2005)
IW and LW	Antihypertensive (I)	4.7 and 17.4 μM	Salmon muscle	N	Ono et al. (2003)
YL and GWAP	Antihypertensive (I)	82 and 3.86 μM	Sardine muscle	N	Matsufuji et al. (1994)
PTHIKWGD	Antihypertensive (I)	ND	Tuna meat	N	Kohama et al. (1988)
LKA and FQKPKR	Antihypertensive (I)	8.5 and 14 μM	Chicken	N	Fujita et al. (2000)
LAP and IVGRPRHQG	Antihypertensive (I)	3.2 and 2.4 μM	Chicken	N	Fujita et al. (2000)
GFHI and DFHING	Antihypertensive (I)	117 and 64.3 μg/ml	Beef muscle	Y	Jang, Jo, Kang, & Lee (2008)
FHG and GLSDGEWQ	Antihypertensive (I)	52.9 and 50.5 μg/ml	Beef muscle	Y	Jang et al. (2008)
GDLGKTTTVSNWSPPKYKDTP	Antihypertensive (I)	11.28 μM	Tuna frame protein	N	Lee et al. (2010)
AHyp	Antihypertensive (I)	0.177 mM	Chicken collagen	N	Iwai et al. (2009)
ND	Antihypertensive (I)	1.2 and 0.81 mg/ml	Head and viscera of sardinelle	N	Bougatef et al. (2008)
VVYPWTQRF	Antihypertensive (I)	66 μmol/L	Oyster	N	Wang et al. (2008)
IW	Antihypertensive (I)	1.2 μM	Salmon	N	Enari et al. (2008)
VLAQYK	Antihypertensive (I)	ND	Beef muscle	Y	Jang and Lee (2005)

References are provided, but regardless of source, all peptides showed a high ACE inhibition.
ND: not detected; Y: yes; N: no; I: inhibitory type; Pd: pro-drug type.

7. Angiotensin-converting enzyme mechanism and blood pressure

ACE is a trans-membrane dipeptidyl peptidase. A soluble form of ACE in plasma is derived from the plasma membrane-bound form by proteolytic cleavage of the COOH-terminal domain. ACE occurs in two distinct isoforms: somatic and testicular, which are transcribed from a single gene from different initiation sites (Oscar, 2005). ACE degrades bradykinin and has the potential to cleave any peptide, including a potent vasodilator, and other vasoactive peptides such as angiotensin-I. It is a circulating enzyme that participates in the renin–angiotensin system by cutting two amino acids from the substrate angiotensin-I, in addition to liberating angiotensin-II from angiotensin-I and inactivating bradykinin (Ervin, 1990). Angiotensin-II constricts arteries, elevating blood pressure (Fig. 1). ACE inhibitors lower blood pressure by inhibiting the formation of angiotensin-II, thus relaxing the arteries, allowing more flexibility, which not only lowers blood pressure, but also improves the pumping efficiency of a failing heart, and improves SBP and DBP range and cardiac output in heart failure patients (Fig. 1).

As biologically active meat peptides are absorbed in the human body and begin to be active, depending on their type, they may competitively interact with ACE, blocking and inactivating the enzyme. ACE inactivation stabilizes angiotensin-I, which normalizes blood pressure. The peptides that inhibit ACE bind to the site in ACE that cleaves angiotensin. If competition is strong, ACE inhibition prevents arteries constriction.

8. Mechanism of ACE inhibitory peptides

The renin–angiotensin system is activated after loss of blood volume or a drop in blood pressure, after cells in the kidney release the enzyme renin. Generally, renin cleaves the inactive peptide angiotensinogen, converting it into angiotensin-I. Angiotensinogen is a glycoprotein that is synthesized and secreted into the bloodstream by the liver. Angiotensin-converting enzyme (also known as kinanase II) converts angiotensin-I into angiotensin-II at the principal site of action, which is the vascular epithelium, and then inactivates the vasodilator bradykinin. Angiotensin-II is the major product of the renin–angiotensin system and is considered a powerful vasoconstrictor. It splits off two amino acids from the 10-amino acid chain of angiotensin-I (Fig. 1). The resultant octapeptide, previously called hypertensin or angiotonin, constricts arterioles, causing a rise in both systolic and diastolic blood pressure. Hypertensin constricts the blood vessels and raises peripheral resistance, thereby restoring blood pressure. Production of angiotensin-II leads to conditions including high blood pressure, heart failure, heart attack, and diapetic nephropathy, and increases the secretion of aldosterone, leading to Na^+ reabsorption.

The competitiveness against ACE activity of different bioactive peptides is determined kinetically using a Burk plot. The mechanism of action of meat peptides in ACE inhibition is different from that of drugs. Generally, drugs indiscriminantly block ACE and interfere with its activity, while ACE inhibitory peptides act differently, by competing with ACE. Drugs work by directly blocking the action of ACE. However, ACE preferably reacts with the ACE inhibitory peptides instead of attacking angiotensin-I (Fig. 1). By inhibiting the formation of angiotensin-II, ACE inhibitory peptides relax the arterial walls and reduce fluid volume. Therefore, unlike other drug treatments, ACE inhibitory peptides actually improve heart function and increase blood and oxygen flow to the heart, liver, and kidneys.

9. Examining ACE inhibitory activity

9.1. ACE inhibitory activity assay

Previous assays were employed with slight improvements (Katayama et al., 2003a,b,c, 2004, 2007, 2008; Muguruma et al.,

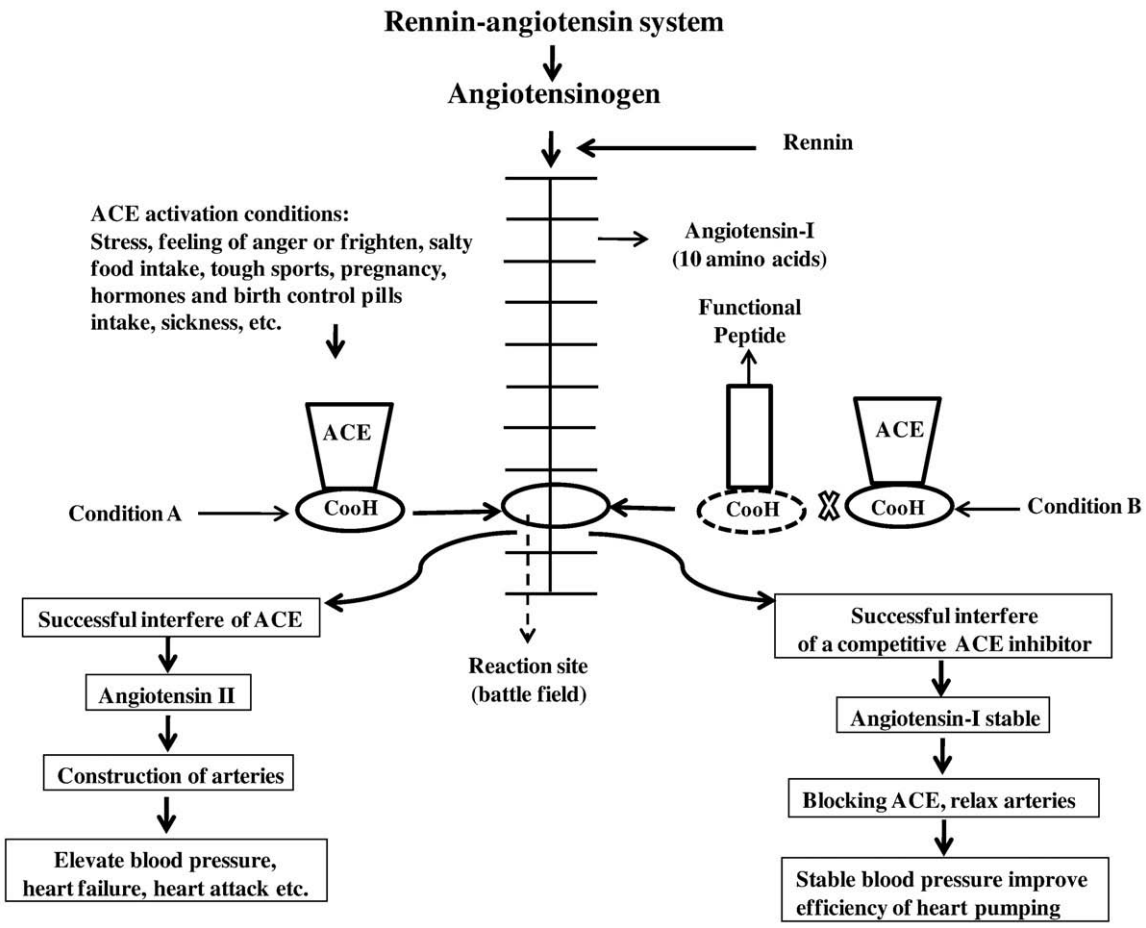

Fig. 1. Scheme showing the mechanism of ACE and competitive functional peptides in the renin–angiotensin system. Angiotensin-I is converted to angiotensin-II by ACE activity, while blood pressure increases with artery constriction.

2009). ACE inhibitory activity was measured according to the method of Cushman and Cheung (1971), in which liberation of hippuric acid from Hippuryl-L-Histidyl–Lucine (His-His-Leu) is stimulated by ACE (Cheung et al., 1980).

A filtered sample containing biologically active peptides (6 µl) was mixed with 50 µl of 7.6 mM His-His-Leu as substrate, in a reaction containing 100 mM sodium borate buffer (pH 8.3) and 608 mM NaCl, and pre-incubated at 37 °C for min. Reaction was initiated with 20 µl of ACE (60 MU/ml of rabbit lung) in 0.25 M sodium borate buffer (pH 8.3), followed by incubation 37 °C for 30 min. Reactions were terminated with 554 µl of 0.1 N HCl, except for blank samples, which were treated with the same amount of HCl before the pre-incubation. Hippuric acid liberated by ACE was extracted with 1.5 ml of ethyl acetate followed by vigorous shaking for 2 min. After centrifugation at 3000 rpm for 20 min, 1 ml ethyl acetate (upper layer) was collected and evaporated at 100 °C for 10 min. Hippuric acid was dissolved in 1 ml 1 M NaCl, and concentration determined by photometric instrument at 228 nm. The concentration of ACE inhibitors required to inhibit 50% ACE activity was defined as the IC_{50}. We found the IC_{50} of meat hydrolysate (from pork loin steaks) to be 27 times greater than the IC_{50} of undigested meat from the same muscle (Table 2) (Ahhmed, Kawahara, & Muguruma, 2009). This suggested that meat hydrolysate as a supplement, lowered blood pressure. ACE inhibitory activities can be generated by enzymatic hydrolysis of muscle proteins (Arihara et al., 2001). Meat hydrolysates may be considered a functional food because of this evidence for nutraceutical compound content.

Table 2
IC_{50} values of meat and meat hydrolysate derived from porcine (musculus biceps famoris).

	Protein concentration (mg/ml)	IC_{50}(mg/ml)
Meat	3.76	2000<
Meat hydrolysate	67.68	3.69

10. Evidence that meat proteins reduce hypertension

10.1. Criteria for novel functional meat peptides

Any functional foods must be evaluated and proven to be valid. Without proof of product safety, most consumers will hesitate to adapt new foods in their diet (Arihara, 2006). Hypertension, a chronic, debilitating, and life-affecting condition, is a major, worldwide problem. Hypertension causes many health problems including heart failure, which is currently the largest reason for use and cost of Medicare. Increasing heart failure prevalence is common to industrialized nations, and has begun to emerge in developing countries as well (Konstam & Greenberg, 2009). This is a challenging, yet exciting time for health care that includes great opportunities and pitfalls (Konstam & Greenberg, 2009). For the community of food suppliers and researchers, providing healthier food for patients with CLSRDs requires proof of concept, and policies that serve the complex needs of patients in the rapidly changing health care environment.

Meat and its derivatives may be considered functional foods since they contain numerous compounds thought to have physiological effects (Jiménez-Colmenero, Carballo, & Cofrades, 2001). The past two decades have seen great progress in hypertension through evidence-

based management, with functional food trials demonstrating the ability of several classes of nutrients and peptides to reduce risk and hospitalization rates for large subsets of the hypertensive population. Although more work is required to translate these findings into practice, and to improve clinical outcomes, important steps aid providers in delivering the best evidence-based care to heart failure patients (Konstam & Greenberg, 2009). An additional important step was the recent purification of numerous effective meat peptides with the ability to reduce blood pressure levels. Additional study of functional foods might lead to better nutraceutical therapy for minimizing health problems, and finding the most effective approaches for meeting the needs of all patients. A nutritional approach that gives nutritionists and patients the flexibility to make their own decisions has the potential to reduce risk factors. Medications often have side effects and food treatments and nutritional remedies do not always affect the health of animal models or humans. Therefore, and based on the remarkable results presented in the literature, we suggest that meat hydrolysates have potential applications as functional foods that could be used as nutraceuticals (Muguruma et al., 2009). We advocate transition from externally driven approaches and metrics to treatments that involve a functional food approach, where food scientists and providers are the decision-makers for patient treatment, and are incentivized to drive quality and efficiency across the overall population.

10.2. Results with peptides

The proteolytic action of pork muscle dipeptidyl peptidase (DPP) generates a number of dipeptides, some of which have ACE inhibitory activity (Sentandreu & Toldra, 2007). Most proteins contain bioactive sequences that are inactive until cleaved by some proteases. Active peptides fragments are released from native proteins only via proteolytic digestion, and once liberated, can act as regulatory compounds and inhibitory nutraceuticals (Arihara, 2006). Recently, bio-functional peptides that are antihypertensives, antioxidants, antimicrobials, and have antithrombotic and immunomodulatory activity have been isolated from food of animal origin. Among these, antihypertensive peptides that are ACE inhibitors are of particular interest for prevention and treatment of hypertension (Kobayashi, Yamauchi, Katsuda, Yamaji, & Katoh, 2008).

In the last 10 years, many researchers worldwide have paid considerable attention to the use of certain food constituents including meats to prevent the action of ACE in elevating blood pressure. Some physiological functions of food constituents have been elucidated, and it is important to use such food constituents for the maintenance and promotion of health (Muguruma et al., 2009). Since many purified meat peptides have nutrafunctional roles, we have analyzed meat peptides from local beef, pork and chicken. In a previous publication, it is suggested that ACE inhibitory peptides were generated not only from proteins such as myosin and actin but also from regulatory proteins such as tropomyosin and troponin (Katayama et al., 2003a). Crucial peptides were obtained from muscle protein hydrolysates of porcine crude myosin (Katayama et al., 2003b), skeletal muscle protein (Katayama et al., 2003a), troponin C (Katayama et al., 2003c; Katayama et al., 2004), skeletal muscle myosin (Katayama et al., 2007), skeletal muscle troponin (Katayama et al., 2008) and myosin B (Muguruma et al., 2009). All purified peptides affected blood pressure in spontaneously hypertensive rats. The *in vitro* IC_{50} of meat peptides was very low, which means that even consumption of small amounts could lead to 50% inhibition of ACE activity. Table 1 shows some of the peptides from beef, pork, chicken, sardine, tuna and salmon. Other studies obtained ACE inhibitory peptides from marine products, and meat samples including beef (Jang and Lee, 2005; Jang et al., 2007, 2008), chicken collagen (Iwai et al., 2009), tuna (Lee, Qian, & Kim, 2010), fugu muscle (Nagai et al., 2008), salmon (Enari, Takahashi, Kawarasaki, Tada, &

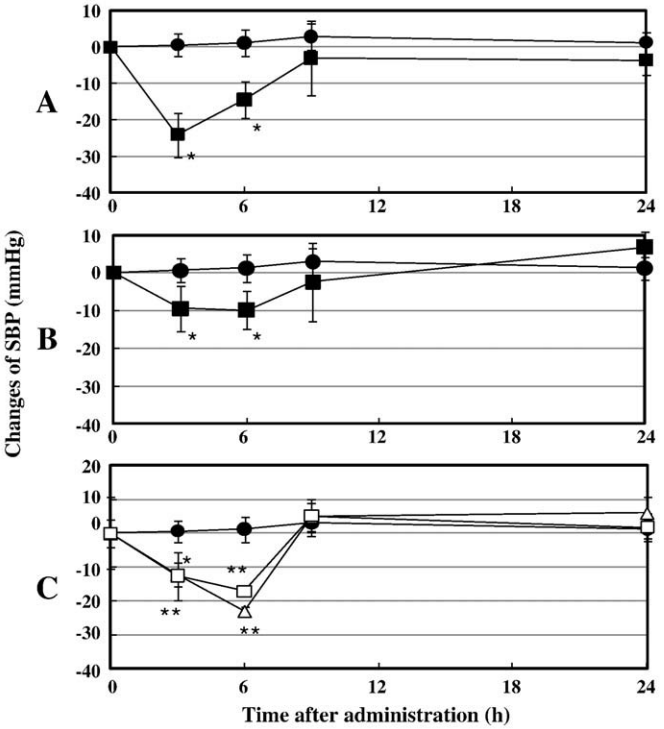

Fig. 2. Effects of a single oral administration of meat-derived peptides on SHR. Changes in systolic blood pressure (SBP) from time zero expressed as means, with vertical bars representing standard deviations. Treatments were control (labeled circles; distilled water), VKKVLGNP (squares) in A, and KRQKYDI (squares) in B, and KRVIQY (squares) and VKAGF (triangles) in C. Peptides were administrated at 10 mg/kg body weight. Findings were published in A (Katayama et al., 2007); B (Katayama et al., 2008); C (Muguruma et al., 2009). Significant difference from the control ($^{*}p<0.05$ and $^{**}p<0.01$).

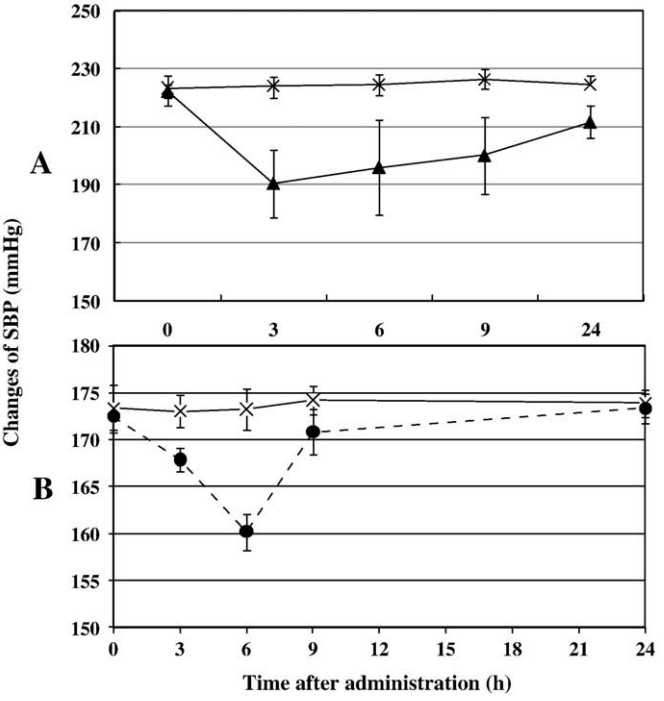

Fig. 3. Effects of a single oral administration of meat-derived peptides and meat hydrolysate on SHR. Changes in systolic blood pressure (SBP) from time zero expressed as means, with vertical bars representing standard deviations. Treatments were control (multiplication sign ×; distilled water) in both graphs, and nanopeptide RMLGQTPTK (triangles) in A, and meat hydrolysate (labeled circles) in B. Samples were administrated at 10 mg/kg body weight. Nanopeptide was isolated by our formal student as in Katayama et al. (2004) and meat hydrolysates were as in Ahhmed et al. (2009).

Tatsuta, 2008), sardine (Otani et al., 2009), chicken bone (Nakade et al., 2008), oyster (Wang et al., 2008), sardinelle by-products (Bougatef et al., 2008), porcine skeletal muscle (Arihara et al., 2001), and chicken (Fujita et al., 2000). All these studies aimed to identify novel angiotensin I-converting enzyme (ACE) inhibitory peptides from different meat species. Generally, those studies suggest that peptides of animal origin may serve several physiological purposes. Based on their remarkable antihypertensive activities *in vivo* and *in vitro*, results suggest that meat bioactive peptide especially skeletal muscle may have potential applications as functional food, which could be used as nutraceutical compounds. In a previous study, we reported that mixing 5% meat hydrolysate from the porcine musculus biceps famoris with the normal diet of rats resulted in clear positive effects on common lifestyle-related diseases (Ahhmed et al., 2009). Changes in the SBP of SHRs after oral administration of peptides or meat hydrolysates are in Fig. 2. After peptide administration, SBP decreased significantly, indicating that the meat peptides had a strong blood pressure-reducing effect. Controlling blood pressure in this way is useful and beneficial.

Fig. 3-A shows the effects of an orally administered nonapeptide at 10 mg per kg, on the blood pressure of SHR. This peptide was from porcine troponin C, and showed a significant effect, especially after 3 h, when blood pressure decreased by 35 mm Hg. This nanopeptide is unique, because it stabilized blood pressure, which remained low from 3 to 9 h after administration. At 24 h, compared to the water control it slightly decreased blood pressure, so this peptide is considered to have the ability to maintain blood pressure in SHR. The stability of this peptide coincided with its IC_{50} (RMLGQTPTK ~ TK) (Table 1). Increased ACE cleavage of the peptide increased competetiveness, so this peptide inhibited ACE activity, and inhibited angitensin-I conversion to angiotensin-II. The longer amino acid peptide appeared to have stronger peptide activities. Thus, ACE is inactivated as the active site of the enzyme is filled with particular bioactive peptides with long amino acid sequences. Other large decreases in blood pressure (35 mm Hg) depend on the morphology of peptides and their amino acid sequence. Ahhmed et al. (2009) showed that after administration of a meat hydrolysate, SBP decreased by 6 mm Hg at 3 h, and 13 mm Hg at 6 h, so meat hydrolysate has a considerable blood pressure-lowering effect (Fig. 3-B).

Angiotensin-II concentration was measured in rats given meat or meat hydrolysate, and results showed that the meat hydrolysate had a considerable effect on angiotensin-II concentration after a 2-week diet containing 5% meat hydrolysate. The values decreased significantly ($p<0.01$) compared to a group that was fed a meat diet only (Fig. 4).

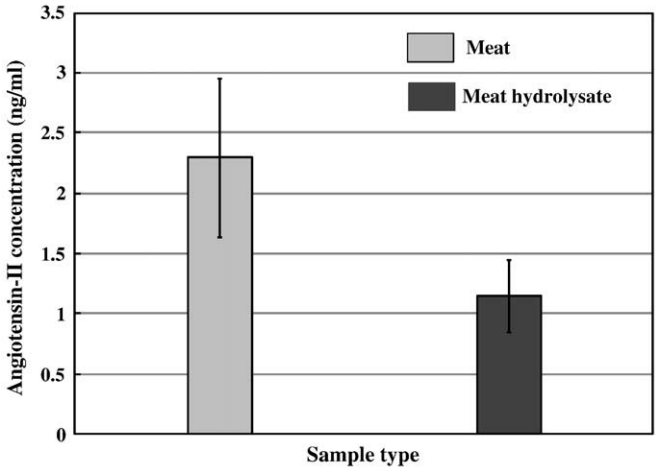

Fig. 4. Changes in angiotension II concentration in HSR rats fed meat or meat hydrolysates.

This suggested that the meat hydrolysate may prevent the conversion of ACE to angiotensin-II through a competitive peptide (Fig. 1). Angiogenesis involves oligopeptides that regulate blood pressure through vasoconstriction and fluid homeostasis via the renin–angiotensin system. It is also associated with atherosclerosis, and is an aggravating factor for hypertension. Therefore, suppressing increases in angiotensin levels is important, and might be achieved by activation of peptides from the native proteins of meat products.

Administration of meat hydrolysate led to blood pressure-lowering effects similar to those observed in previous reports on products of animal origin. We monitored blood pressure and found that it could be reduced in both the short- and long-term by feeding rats a diet containing meat hydrolysate. The meat hydrolysate also contained a peptide that reduced blood sugar. The HbA1c, which measures blood sugar, in both groups of rats increased (data not shown). However, the test group, which was fed a diet containing 5% meat hydrolysate, showed better blood sugar results than the control group for blood sugar (Ahhmed et al., 2009). HbA1c percentage generally correlates with diabetes, and diabetic people often suffer from high blood pressure. We also examined blood sugar after a single oral administration of glucose, using standard oral syringes. The values of the test group were lower than the group that was fed a meat diet only. These findings could help in reducing the blood pressure of diabetics and suggest that meat peptides both reduce blood pressure and decrease diabetes. Thus, meat hydrolysates contain peptides that could be useful in preventing, or at least reducing, lifestyle-related diseases. Jang and Lee (2005) reported that chromatographic techniques on beef hydrolysates suggested the possibility of producing functional fresh meat products.

In hypertension, as in other progressive CLSRDs with high mortality rates, the functional food approach offers major advantages over approaches with prevention tools, inhibiting rather than reducing disease, and providing palliative strategies and programs for medical and emotional care for the terminally ill. To determine whether a peptide is a long-term ACE inhibitor requires further investigation. Most studies carried out on the blood pressure-lowering effects of meat hydrolysates examined their actvity as inhibitors, and particularly for short periods. This effect differs from pills, which often, if not always, have side effects on heart efficiency. This review aimed to illustrate the physiological functions of meat proteins, particularly the potential ACE-inhibiting peptides derived from meat and marine products.

11. Conclusions

For healthcare reform initiatives to succeed, they must do more than mandate universal coverage, but must also validate nutritional therapies that utilize nutrients of animal origin, such as peptides, that prevent the occurrence of common diseases.

The transition of disease prevention from chemically manufactured medication to natural, biologically active nutrients of either plant or animal origin will not be easy or straightforward, but patients will benefit from a focused effort. In our opinion, the nutritional therapy approach and the use of nutraceuticals from meat is ideal for continual healthcare for patient populations. Decreasing CLSRDs in general is our best hope for obtaining the desired achievements for hypertensive and normotensive populations. The results of our research and many other studies provide a body of evidence that meat possesses a variety of proteins and a large number of peptides that affect CLSRDs. We propose that the more we maintain meat consumption at the recommended daily allowance, the better healthcare we can provide. However, excessive consumption of meat, rather than regular consumption, may lead to undesired results that affect the mechanisms of the human body, such as increasing uric acid, which strains the kidneys, causes dyspepsia and contributes to obesity.

As a result, maximum reduction in blood pressure occurred at 3–6 h after oral administration of meat hydrolysate, this extract may be an important functional food. Moreover, meat hydrolysate also reduced glucose level, as observed by the decrease in blood sugar level over time. These results indicate that enzymatic digestion of meat may generate active peptides with antihypertensive and antidiabetic activities. Meat hydrolysate may also contain other constituents that allow it to be used as a functional food and nutraceutical. The collective data from animal experiments indicate that fresh meat products should be considered to contain compounds that have temporal, immunopharmacological effects.

References

Ahhmed, M. A., Kawahara, S., & Muguruma, M. (2009). Transglutaminase improves the textural and structural properties of chicken skeletal, smooth, and cardiac muscles. *Proceeding of the 55th International Congress of Meat Science and Technology. (PE 4.26), 16th–21st August, 2009. Copenhagen, Denmark.*

Aguila, B. M., & Mandarim-de-Lacerda, A. C. (2003). Heart and blood pressure adaptations in Wistar rats fed with different high-fat diets for 18 months. *Nutrition*, *19*, 347—352.

Allender, S., Scarborough, P., Peto, V., Rayner, M., Leal, J., Luengo- Fernendaz, R., et al. (2008). European cardiovascular disease statistics edition of 2008. *Brussels: European Heart Network.*

American Heart Association (2009). Heart Disease and Stroke Statistics — 2009 update: A report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*, *119*, e21—e181.

Anand, P., Kunnumakara, B. A., Sundaram, C., Harikumar, H. K., Tharakan, T. S., Lai, S. O., Sung, B., & Aggarwal, B. B. (2008). Cancer is a preventable disease that requires major lifestyle changes. *Pharmaceutical Research*, *25*, 2097—2116.

Arihara, K. (2006). Strategies for designing novel functional meat products. *Meat Science*, *74*, 219—229.

Arihara, K., Nakashima, Y., Mukai, T., Ishikawa, T., & Itoh, M. (2001). Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Science*, *57*, 319—324.

Bougatef, Al, Nedjar-Arroume, N., Ravallec-Ple, R., Leroy, Y., Guillochon, D., Barkia, A., et al. (2008). Angiotensin I-converting enzyme (ACE) inhibitory activities of sardinelle (*Sardinella aurita*) by-products protein hydrolysates obtained by treatment with microbial and visceral fish serine proteases. *Food Chemistry*, *111*, 350—356.

Cheung, H. S., Wang, F. L., Ondetti, M. A., Sabo, E. F., & Cushman, D. W. (1980). Binding of peptide substrate and inhibitors of angiotensin-converting enzyme. *Journal of Biological Chemistry*, *255*, 401—407.

Cushman, D. W., & Cheung, H. S. (1971). Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology*, *20*, 1637—1648.

Descalzo, A. M., & Sancho, A. M. (2008). A review of natural antioxidants and their effects on oxidative status, odor and quality of fresh beef produced in Argentina. *Meat Science*, *79*, 423—436.

Ding, J., Visser, M., Kritchevsky, B. S., Nevitt, M., Newman, A., Kim, S., et al. (2004). The association of regional fat depots with hypertension in older persons of white and African American ethnicity. *American Journal of Hypertension*, *17*, 971—976.

Enari, H., Takahashi, Y., Kawarasaki, M., Tada, M., & Tatsuta, K. (2008). Identification of angiotensin I-converting enzyme inhibitory peptides derived from salmon muscle and their antihypertensive effect. *Fisheries Science*, *74*, 911—920.

Ervin, G. E. (1990). Angiotensin I converting enzyme and the changes in our concepts through the years. *Journal of the American heart association*, *116*, 363—370.

Fujita, H., Yokoyama, K., & Yoshikawa, M. (2000). Classification and antihypertensive activity of angiotensin I-converting enzyme inhibitory peptides derived from food proteins. *Journal of Food Science*, *65*, 564—569.

Fujita, H., & Yoshikawa, M. (1999). LKPNM: A prodrug type ACE inhibitory peptide derived from fish protein. *Immunopharmacology*, *44*, 123—127.

Iwai, K., Zhang, Y., Kouguchi, T., Saiga-Egusa, A., Shimizu, M., Ohomori, T., et al. (2009). Blood concentration of food-derived peptides following oral intake of chicken collagen hydrolysate and its angiotensin-converting enzyme inhibitory activity in healthy volunteers. *Nippon Shokuhin Kagaku Kogaku Kaishi*, *56*, 326—330.

Jang, A., & Lee, M. (2005). Purification and identification of angiotensin converting enzyme inhibitory peptides from beef hydrolysates. *Meat Science*, *69*, 653—661.

Jang, A., Jo, C., Kang, K. S., & Lee, M. (2008). Antimicrobial and human cancer cell cytotoxic effect on synthetic angiotensin-converting enzyme (ACE) inhibitory peptides. *Food Chemistry*, *107*, 327—336.

Jang, A., Jo, C., & Lee, M. (2007). Storage stability of the synthetic angiotensin converting enzyme (ACE) inhibitory peptides separated from beef sarcoplasmic protein extract at different pH, temperature, and gastric digestion. *Food Science and Biotechnology*, *16*, 572—575.

Jiménez-Colmenero, F., Carballo, J., & Cofrades, S. (2001). Healthier meat and meat products: Their role as functional foods. *Meat Science*, *59*, 5—13.

Katayama, K., Fuchu, H., Sakata, A., Kawahara, S., Yamauchi, K., Muguruma, M., et al. (2003a). Angiotensin I-converting enzyme inhibitory activities of porcine skeletal muscle proteins following enzyme digestion. *Asian-Australian Journal of Animal Science*, *16*, 417—424.

Katayama, K., Fuchu, H., Sugiyama, M., Kawahara, S., Yamauchi, K., Kawamura, Y., & Muguruma, M. (2003b). Peptic hydrolysate of porcine crude myosin has many active fractions inhibiting angiotensin-I converting enzyme. *Asian-Australian Journal of Animal Science*, *16*, 1348—1389.

Katayama, K., Tomatsu, M., Fuchu, H., Sugiyama, M., Kawahara, S., Yamauchi, K., Kawamura, Y., & Muguruma, M. (2003c). Purification and characterization of an angiotensin I-converting enzyme inhibitory peptide derived from porcine troponin C. *Animal Science Journal*, *74*, 53—58.

Katayama, K., Tomatsu, M., Kawahara, S., Yamauchi, K., Fuchu, H., Kodama, Y., Kawamura, Y., & Muguruma, M. (2004). Inhibitory profile of nanopeptide derived from porcine troponin C against angiotensin I-converting enzyme. *Journal of Agricultural and Food Chemistry*, *52*, 771—775.

Katayama, K., Jamhari, Mori, T., Kawahara, S., Miake, K., Kodama, Y., Sugiyama, M., Kawamura, Y., Nakayama, T., Maruyama, M., & Muguruma, M. (2007). Angiotensin-I converting enzyme inhibitory peptide derived from porcine skeletal muscle myosin and its antihypertensive activity in spontaneously hypertensive rats. *Journal of Food Science*, *72*, S702—S706.

Katayama, K., Anggraeni, E. H., Mori, T., Ahhmed, M. A., Kawahara, S., Sugiyama, M., Nakayama, T., Maruyama, M., & Muguruma, M. (2008). Porcine skeletal muscle troponin is a good source of peptides with angiotensin-I activity and antihypertensive effects in spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry*, *56*, 355—360.

Kawamura, Y., Takane, T., Satake, M., & Sugimoto, T. (1992). Physiologically active peptide motif. In proteins: Peptide inhibitor of ACE from the hydrolysates of antarctic krill muscle protein. *Japan Agriculture Research Quarterly*, *26*, 210—213.

Konstam, M. A., & Greenberg, B. (2009). Transforming health care through the medical home: The example of heart failure. *Journal of Cardiac Failure*, *15*, 736—739.

Kobayashi, Y., Yamauchi, T., Katsuda, T., Yamaji, H., & Katoh, S. (2008). Angiotensin-I converting enzyme (ACE) inhibitory mechanism of tripeptides containing aromatic residues. *Journal of Bioscience and Bioengineering*, *106*, 310—312.

Kohama, Y., Matsumoto, S., Oka, H., Teramoto, T., Okabe, M., & Mimura, Y. (1988). Isolation of angiotensin-converting enzyme-inhibitor from tuna muscle. *Biochemical Biophysical Research Communication*, *155*, 332—337.

Korhonen, H. (2009). Milk-derived bioactive peptides: From science to applications. *Journal of Functional Foods*, *1*, 177—187.

Lee, S. H., Qian, Z. J., & Kim, S. K. (2010). A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. *Food Chemistry*, *118*, 96—102.

Matsufuji, H., Matsui, T., Seki, E., Osajima, K., Nakashima, M., & Osajima, Y. (1994). Angiotensin I-converting enzyme inhibitory peptides in an alkaline protease hydrolysate derived from sardine muscle. *Bioscience Biotechnology Biochemistry*, *58*, 2244—2245.

MNT "Medical News Today". (2010). *Global high blood pressure situation growing dire, but does not have to be*. "States University of New York Health report". (http://www.medicalnewstoday.com/articles/71331.php).

Muguruma, M., Ahhmed, M. A., Katayama, K., Kawahara, S., Maruyama, M., & Nakamura, T. (2009). Identification of pro-drug type inhibitory peptide sourced from porcine myosin B: Evaluation of its antihypertensive effects in vivo. *Food Chemistry*, *114*, 516—522.

Murray, C. J., & Lopez, A. D. (1997). Global mortality, disability, and the contribution of risk factors: Global Burden of Disease Study. *Lancet*, *349*, 1436—1442.

Nagai, T., Suzuki, N., Tanue, Y., Kai, N., Nakazawa, Y., & Nagashima, T. (2008). Angiotensin I-converting enzyme inhibitory activities of enzymatic hydrolysates from lees as by-product produced during sake making using fugu muscle and fin. *Journal of Food, Agriculture and Environment*, *6*, 19—22.

Nakade, K., Kamishima, R., Inoue, Y., Ahhmed, A., Kawahara, S., Nakayama, T., et al. (2008). Identification of an antihypertensive peptide derived from chicken bone extract. *Animal Science Journal*, *79*, 710—715.

NetWellness (2010). High blood pressure (hypertension) and African Americans http://www.netwellness.org/healthtopics/aahealth/hypertension.cfm.

Ono, S., Hosokawa, m., Miyashita, K., & Takahashi, K. (2003). Isolation of angiotensin I-converting enzyme inhibitory effect derived from hydrolyaste of upstream chum salmon muscle. *Journal of Food Science*, *68*, 1611—1614.

Ostergren, J. (2007). Blood pressure worldwide statistics. A report published in: (http://salaswildthoughts.blogspot.com/2007/05/blood-pressure-worldwide-statistics.html.

Oscar, A. C. (2005). Novel mechanism of action of ACE and its inhibitors. *American Journal of Physiology Heart and Circulatory Physiology*, *289*, H1796—H1797.

Otani, L., Ninomiya, T., Murakami, M., Osajima, K., Kato, H., & Murakami, T. (2009). Sardine peptides with angiotensin I-converting enzyme inhibitory activity improves glucose tolerance in stroke-porne spontaneously hypertensive rats. *Biosciences Biotechnology Biochemistry*, *73*, 2203—2209.

Pihlanto, A. (2006). Antioxidative peptides derived from milk proteins. *International Dairy Research*, *16*, 1306—1314.

Reiner, Z. (2009). How to improve cardiovascular diseases prevention in Europe? *Nutrition, Metabolism & Cardiovascular Diseases*, *19*, 451—454.

Robertson, G. L. (1992). Regulation of vasopressin secretion. In D. W. Seldin & G. Giebisch (Eds.), *The Kidney: Physiology and Pathophysiology* (pp. 1595—1613)., 2nd ed New York: Raven.

Sudesh, V., Longerich, L., & Singal, P. (2002). Nutrition and hypertension. *Nutrition Research*, *22*, 111—123.

Sentandreu, A. M., & Toldra, F. (2007). Evaluation of ACE inhibitory activity of dipeptides generated by the action of porcine muscle dipeptidyl peptidases. *Food Chemistry*, *102*, 511—515.

Ukeda, H., Matsuda, H., Osajima, K., Matsufuji, H., Matsui, T., & Osajima, Y. (1992). Peptides from peptic hydrolysate of heated sardine meat that inhibit angiotensin- I converting enzyme. *Nippon Nogei Kagaku Kaishi*, *66*, 25—29.

Vasdev, S., Longerich, L., & Singal, P. (2002). Nutrition and hypertension. *Nutrition Research*, *22*, 111−123.

Vercruysse, L., Camp, V. J., & Smagghe, G. (2005). ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscles protein: A review. *Journal of Agricultural and Food Chemistry*, *53*, 8106−8115.

Wang, J., Hu, J., Cui, J., Bai, X., Du, Y., Miyaguchi, Y., et al. (2008). Purification and identification of a ACE inhibitory peptide from oyster proteins hydrolysate and the antihypertensive effect of hydrolysate in spontaneously hypertensive rats. *Food Chemistry*, *111*, 302−308.

Walser, M. (1992). Phenomenological analysis of electrolyte homeostasis. In D. W. Seldin & G. Giebisch (Eds.), *In The Kidney: Physiology and Pathophysiology* (pp. 31−44)., 2nd ed New York: Revan.

Yokoyama, K., Chiba, H., & Yoshikawa, M. (1992). Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. *Bioscience, Biotechnology, and Biochemistry*, *56*, 1541−1545.