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Commentary

Revisiting the mechanisms of ACE inhibitory peptides from food proteins

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ABSTRACT

Background: Angiotensin converting enzyme (ACE) is a key enzyme in the renin angiotensin system (RAS) responsible for conversion of angiotensin (Ang) I into Ang II, a vasoconstrictor leading to elevated blood pressure. ACE inhibitory (ACEi) peptides derived from food proteins have shown potential in the prevention and management of hypertension. Although most ACEi peptides were characterized based on *in vitro* ACEi activity, a relationship between ACE inhibition and physiological antihypertensive effect is not apparent, indicating the involvement of other mechanisms of action.

Scope and approach: This paper focuses on emerging antihypertensive mechanisms of ACEi peptides. As an alternate arm of the classic RAS, ACE2 cleaves Ang II into Ang (1–7) and thus counterbalances the harmful effects of Ang II. Endothelial dysfunction is now recognized as an early feature in the pathophysiology of metabolic syndrome and cardiovascular disorders including hypertension; endothelial dysfunction, vascular oxidative stress and inflammation are interplayed. Future perspectives on mechanistic study of ACEi peptides are forecasted.

Key findings and conclusions: Apart from classic ACE inhibition, emerging evidence suggests that food peptides can exert antihypertensive activity through upregulation of ACE2 (an ACE homologue that counterbalances the detrimental effect of elevated ACE), improvement of endothelial function, as well as reduced vascular oxidation and inflammation. Future research is expected to look into the effects of bioaccessibility, bioavailability, stability and reactivity of the peptides with food and gut matrices, as well as the gut microbiota, on blood pressure reduction.

1. Introduction

Bioactive peptides are specific fragments of food proteins that confer certain physiological benefits to human health. They are latent in intact food proteins but can be released during food processing such as fermentation, *in vitro* or *in vivo* enzymatic hydrolysis (Korhonen & Pihlanto, 2006; Udenigwe & Aluko, 2012). Among various types of bioactive peptides, angiotensin converting enzyme (ACE) inhibitory peptides have been most extensively studied for their potential in the prevention and management of hypertension, one of the well-defined risk factors for cardiovascular disease (FitzGerald, Murray, & Walsh, 2004; Miguel, López-Fandiño, Ramos, & Aleixandre, 2005). ACE is a key enzyme for blood pressure regulation through the renin-angiotensin system (RAS). Elevated ACE activity leads to increased formation of angiotensin (Ang) II, a potent vasoconstrictor that is responsible for the development of hypertension. Pharmaceutical drugs targeting ACE have proven successful in lowering high blood pressure; however, food-derived ACE inhibitors are believed to be safer than pharmaceutical drugs and to lack of some drug-associated adverse side effects such as cough and angioedema (Beltrami, Zingale, Carugo, & Cicardi, 2006).

ACE inhibition is often thought to play a central role in the mechanisms of blood pressure reduction *in vivo*, and most ACE

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inhibitory (ACEi) peptides were characterized based on *in vitro* ACE inhibition. A relationship between *in vitro* ACE inhibition and antihypertensive activity, however, is not apparent. For example, peptide KVLPVPN derived from β-casein showed significant blood pressure-lowering activity *in vivo*, despite its weak *in vitro* ACEi activity (FitzGerald et al., 2004), suggesting possible involvement of other mechanisms other than ACE inhibition. The pathophysiology of hypertension is complicated. Hypertension develops from a complex interaction of genetic, environmental and many other factors such as increased sympathetic nervous system activity, increased levels of long-term high sodium intake, inadequate dietary intake of potassium and calcium, elevated RAS activity, endothelial dysfunction, abnormalities in vessel resistance due to vascular inflammation, increased activity of vascular growth factors, and altered cellular ion channel (Foëx & Sear, 2004; Hall et al., 2012; Sarzani, Salvi, Dessi-Fulgheri, & Rappelli, 2008). The antihypertensive activity of YGLF (a peptide derived from bovine α-lactalbumin) is due to the stimulation of opioid receptors and nitric oxide (NO)-induced vasodilation function (Ijas et al., 2004). Moreover, milk lactoferrin-derived peptide RPYL was thought to function as an Ang II type 1 (AT_1) receptor antagonist (Fernandez-Musoles et al., 2013); Ang II acts mainly through AT_1 receptor causing vasoconstriction and hypertension. Antihypertensive activity of egg-derived peptide IRW also appears to be mediated through increased NO-mediated vasodilation, reduced vascular inflammation, and up-regulated expression of ACE2 (Majumder et al., 2013a, 2015a). ACEi peptides have also been shown to exhibit antihypertensive effects through mechanisms other than the classical circulatory RAS system. The objectives of this review are to discuss the newly emerging mechanisms of ACEi peptides and further considerations to enhance efforts towards the translation of antihypertensive peptides into functional food ingredients.

2. Up-regulation of ACE2

Within the RAS, ACE is the key enzyme responsible for conversion of Ang I, an inactive decapeptide, into Ang II, a vasoconstrictive octapeptide (Zhuo, Ferrao, Zheng, & Li, 2013). Ang II mediates its biological function through binding with two G-protein-coupled receptors: AT_1 and Ang II type 2 (AT_2) receptors. As shown in Fig. 1, these receptors differ in their biological functions: AT_1 receptor is associated with vasoconstriction, inflammation, growth and fibrosis, while AT_2 receptor is associated with apoptosis and vasodilation. AT_1 receptor shares partial homology with AT_2 receptor in amino acid constitution and it is the dominant subtype after birth. Therefore, inhibition of ACE (reducing Ang II generation) and blocking AT_1 receptor (as receptor antagonists) are the widely used strategies for controlling blood pressure (Contreras et al., 2003; Donnelly, 1992). An alternate arm of the RAS, ACE2 is also involved in counterbalancing the harmful effects of Ang II (Fig. 1). First identified as a homologue of ACE (Donoghue et al., 2000), ACE2 cleaves the carboxyl-terminal phenylalanine of Ang II to form Ang (1–7), which can exert inhibitory effect on Ang II-induced vasoconstriction *via* binding to the Mas receptor (Santos et al., 2003; Vickers et al., 2002). Alternatively, ACE2 hydrolyzes Ang I to form Ang (1–9), which can be further converted by ACE to Ang (1–7). Nevertheless, the ACE2-Ang (1–7)-Mas pathway is more efficient in reducing the Ang II-induced effect than the ACE2-Ang (1–9) pathway (Vickers et al., 2002).

In spontaneously hypertensive rats (SHRs), increased ACE2 expression contributes to reduced blood pressure, attenuated perivascular fibrosis, decreased oxidative stress and inhibited cardiac remodeling (Díez-Freire et al., 2006; Keidar, Kaplan, & Gamliel-Lazarovich, 2007; Lo et al., 2013; Zhong et al., 2004). ACE2 knockout (ACE2KO) mice showed enhanced Ang II-induced fibrosis, superoxide production, inflammatory cytokine level and hypertrophic cardiomyopathy (Alghamri et al., 2013). Supplementation of human recombinant ACE2 (hrACE2) was found to blunt Ang II-mediated hypertrophic response and superoxide production in ACE2 knockout (ACE2KO) mice (Zhong et al., 2010), which further confirmed the significant roles of ACE2 in cardiovascular functions. Therefore, activation of ACE2 represents a potential target for blood pressure control (Kulemina & Ostrov, 2011; Prada et al., 2008). Interestingly, several established antihypertensive ACEi drugs such as enalapril and telmisartan also show concomitant upregulation of ACE2 expression (Yang et al., 2013; Zhong et al., 2011). Our transcriptomics study showed that mRNA expression of ACE2 in mesenteric artery of SHRs was significantly increased by oral administration of tripeptide IRW, which sheds light on the new mechanism of the ACEi peptide *in vivo* (Majumder et al., 2015a). Our recent research also showed that ACE2 expression in the aorta was enhanced by the peptide treatment (unpublished data). IRW is the first ACEi peptide reported to show ACE2 activation property; our ongoing research will determine if up-regulation/activation of ACE2 is a common feature of ACEi peptides.

3. Improvement of endothelial function

The vascular endothelium is a single layer of cells that lines the

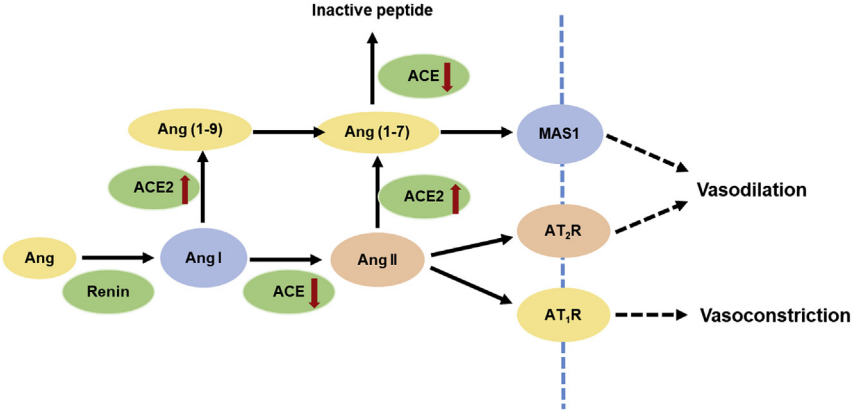

Fig. 1. Proposed role of tripeptide, IRW, from egg ovotransferrin on angiotensin converting enzyme 2 (ACE2) leading to enhanced vasodilation and inhibited vasoconstriction, and subsequently antihypertensive effect; Ang, angiotensin; AT_1R, Ang II type 1 receptor; AT_2R, Ang II type 2 receptor; MAS1, mas G-protein coupled receptor.

inner surface of all blood vessels and the heart. In addition to function as a selective barrier to prevent the diffusion of macromolecules from the blood lumen to the intestinal space, the vascular endothelium is now considered as the biggest endocrine organ in the body (Münzel, Sinning, Post, Warnholtz, & Schulz, 2008; Schulz, Gori, & Münzel, 2011). Endothelial cells secrete various vasoactive agents such as the vasodilating NO, prostacyclin, and endothelium-derived hyperpolarizing factor (EDHF) as well as the vasoconstricting endothelin I, Ang II and thromboxane (Vanhoutte, 1989). An imbalance in the relative contribution of endothelium-derived relaxing and contracting factors is a feature of endothelial dysfunction (Zhao, Chen, Yao, & Chen, 2005). Endothelial dysfunction has been shown in the elderly, in patients with hypertension, diabetes, hypercholesterolemia, and smoking (Münzel et al., 2008; Schulz, Anter, & Keaney, 2004), and is an early feature in the pathophysiology of metabolic syndrome and several cardiovascular disorders such as atherosclerosis, hypertension and heart failure (Fichtlscherer, Breuer, & Zeiher. 2004; Halcox et al., 2002).

Improvement of endothelial function has been reported in studies of the blood pressure-lowering activity of ACEi peptides. The antihypertensive activity of ovotransferrin-derived IRW is due partially to its vasorelaxation activity. IRW exhibited relaxation in an isolated SHR mesenteric artery; pretreatment with *N*-nitro-L-arginine methyl ester (L-NAME), a endothelial nitric oxide synthase (eNOS) inhibitor, reduced IRW-mediated vasodilation, suggesting a key role of NO production (Majumder et al., 2013a). Indeed, eNOS expression was significantly enhanced in both the mesenteric artery and aorta, further supporting the role of NO in the vasorelaxation activity of IRW (Majumder et al., 2013a). The endothelium-dependent vasodilator activity of pentapeptide RADHP was also associated with enhanced NO formation as pretreatment with L-NAME inhibited vasodilation (Nakamura, Yamamoto, Sakai, & Takano, 1995; Miguel, Alvarez, López-Fandiño, Alonso, & Salaices, 2007). Milk casein hydrolysate containing tripeptides IPP and VPP was reported to improve the vascular endothelial function of subjects with stage I hypertension (Hirota et al., 2011). VPP and IPP were able to increase the mRNA expression of eNOS (Yamaguchi, Kawaguchi, & Yamamoto, 2009). Further study showed that both VPP and IPP induced endothelium-dependent relaxation in isolated aortic rings was related to enhanced production of vasodilating substances including NO (Hirota et al., 2011). Interestingly, vasorelaxation effect of IPP may be mediated through Mas receptor as the formation of $Ang_{1\text{-}7}$ was enhanced (Ehlers, Nurmi, Turpeinen, Korpela, & Vapaatalo, 2011); in contrast to Ang II, $Ang_{1\text{-}7}$ is vasodilating. Relaxation induced by α-lactorphin (YGLF) and β-lactorphin (YLLF), two opioid peptides derived from milk proteins α-lactalbumin and β-lactoglobulin, respectively, involved NO but not through EDHF or vasodilating prostanoids (Sipola, Finchenberg, Vapaatalo, Korhonen, & Nurminen, 2002; Yoshikawa, Tani, Yoshimura, & Chiba, 1986). The relaxation induced by casoxin D (YVPFPPF), an anti-opioid peptide derived from human casein, was not affected by L-NAME but was antagonized by a cyclooxygenase inhibitor; its relaxation was mediated by bradykinin B_1 receptor (Yoshikawa et al., 1994). Although NO might be involved in vasorelaxation, binding to neurokinin (NK_1) receptors was thought to be the mechanism of action of casomokinin L (YPFPPL) (Fujita et al., 1996). IQW and LKP are other two identified ACEi peptides from ovotransferrin; although relaxation in both peptide-treated groups was significantly decreased by L-NAME pretreatment, expression of eNOS was not affected (Majumder et al., 2015b), suggesting the restoration of NO-mediated vasorelaxation may have been achieved with the peptides by increasing NO bioavailability.

4. Antioxidative stress and anti-inflammation

Oxidative stress is a major cause of reduced endothelial NO bioavailability in hypertension, and inflammatory response is thought to play an important role in these processes (Blake & Ridker, 2001; Landmesser, Harrison, & Drexler, 2006). Reactive oxygen species (ROS), in particular superoxide radical, reacts rapidly with NO to form the highly reactive intermediate peroxynitrite, thus substantially limiting NO bioavailability and its protective roles (Cai & Harrison, 2000; Münzel et al., 2008). Peroxynitrite leads to tyrosine nitration of various proteins and, incidentally, tripeptides IQW and IRW but not LKP were found to reduce nitrotyrosine levels in the aorta of hypertensive rats (Majumder et al., 2015b). Since both the expression of eNOS and ROS scavenging activity were not affected, it remains to be determined if the vasorelaxation effect of LKP maybe through modulation of vascular reactivity at the level of EDHF or endothelin pathways (Jakala et al., 2009; Maes et al., 2004). Ovokinin, an octapeptide (FRADHPFL) derived from ovalbumin, exerted relaxation through prostaglandin I2 but not NO (Fujita, Usui, Kurahashi, & Yoshikawa, 1995).

Inflammation is also implicated as an important precursor of endothelial dysfunction (Sattar, 2004). Many chronic diseases are characterized by uncontrolled inflammation (McEver, 1992); inflammatory processes enhance ROS formation, a well-characterized causation factor of endothelial dysfunction (Galle, Quaschning, Seibold, & Wanner, 2003). ROS activates the transcription factor nuclear factor (NF)-κB, leading to vascular inflammation. Endothelial dysfunction due to reduced NO bioavailability on the other hand promotes oxidative stress and inflammation (Schulz et al., 2004). Oxidative stress, inflammation and endothelial dysfunction are interrelated components of an etiological network that has been linked to the development of cardiovascular diseases (Lahera et al., 2007). Peptides IRW and IQW, but not LKP, showed anti-inflammatory effect in cultured endothelial cells and SHRs (Majumder, Chakrabarti, Davidge, & Wu, 2013b; Majumder et al., 2013a; Majumder et al., 2015b). Some other peptides, such as SSS, EEE and VPL (Ringseis, Gotze, & Eder, 2009), as well as VPP and IPP (Nakamura, Yamamoto, Sakai, & Takano, 2013), were reported to inhibit leukocyte-endothelial interactions. Recruitment of immune cells such as leukocyte into endothelial cells is a critical step for initiation of inflammation. Therefore, peptides inhibiting leukocyte recruitment activity may help maintain normal endothelial functions in an inflamed environment.

5. Considerations for future translational studies

The role of food peptides in blood pressure regulation is still a subject of ongoing debate considering the lack of consensus in their physiological antihypertensive effects in different human populations (Cicero, Gerocarni, Laghi, & Borghi, 2011; Fekete, Givens, & Lovegrove, 2013; Geleijnse & Engberink, 2010). It is clear however that, apart from ACE inhibition, food-derived peptides exert their blood pressure-lowering effects *via* mechanisms that target renin activity, endothelin system function, Ang receptors, calcium channels, arginine-nitric oxide pathway, vascular inflammation and oxidative stress, sympathetic nervous system, and vascular remodeling (Majumder & Wu, 2014; Udenigwe & Mohan, 2014). Although the peptides may not need to be absorbed as they can bind receptors in the gut to trigger signalling processes, many peptides need to be bioavailable in relevant amounts in the vascular system in order to exert their antihypertensive effects. Therefore, some important factors need to be considered to achieve health benefits with the food peptides in hypertensive human subjects:

1. The structural basis of the oral bioavailability and pharmacokinetics of ACEi and antihypertensive peptides needs to be clarified. Several food-derived ACEi peptides have been found in picomolar amounts in the blood of rats and humans after oral consumption (Vermeirssen, Van Camp, & Verstraete, 2004; Segura-Campos, Chel-Guerrero, Betancur-Ancona, & Hernandez-Escalante, 2011; Hernández-Ledesma, del Mar Contreras, & Recio, 2011; Sánchez-Rivera, Martínez-Maqueda, Cruz-Huerta, Miralles, & Recio, 2014). Using *in vitro*, *ex vivo* and *in situ* intestinal models, bioavailability of these peptides has been suggested to be facilitated mostly by intestinal peptide transporters, *trans*-cellular transport, endocytosis, or through paracellular passive diffusion (Quirós, Dávalos, Lasunción, Ramos, & Recio, 2008; Sánchez-Rivera et al., 2014; Vermeirssen et al., 2004). Based on these studies, it is thought that di- and tripeptides are likely to be transported into blood circulation *via* intestinal peptide transporter T1. However, the selectivity of the transporters for particular dietary peptides is still not clear. In other words, small-sized ACEi and potentially antihypertensive peptides may be less preferred for intestinal transport over other small dietary peptides that lack the bioactivity. Therefore, it is important to delineate the important peptide structural features that favour their interaction with intestinal peptide transporters, and relate these to the structural properties needed for blood pressure reduction. Furthermore, the discrepancies in blood pressure reduction during clinical trials can be investigated from the perspective of potential genetic variations in intestinal peptide transporters across different populations in the world.
2. Peptides are highly chemically reactive due to their amino, imino, carbonyl and sulfhydryl groups. Consequently, ACEi and antihypertensive peptides are susceptible to derivatization during food processing, which can lead to the formation of peptide analogues and other products. For instance, factors such as high water activity, temperature and pressure can lead to intramolecular cyclization of amino acid residues, backbone and sidechain modifications, condensation and cross-linking of peptides (Van Lancker, Adams, & De Kimpe, 2011). The identity of any newly formed peptide derivatives and other compounds during peptide-based functional food processing has yet to receive noticeable attention considering that these uncharacterized compounds may also be playing a role in regulating vascular health. For example, 2,5-diketopiperazines can be generated from peptide condensation and intramolecular cyclization under hyperthermal and hyperbaric conditions, and this class of compound has shown several bioactivities that include cardiovascular-related properties during drug development (Martins & Carvalho, 2007). Furthermore, Maillard reaction can occur when food peptides react with reducing sugars within the food matrix during processing (Mohan, Udechukwu, Rajendran, & Udenigwe, 2015). Other peptide reactions can also occur with carbonyl groups of lipid oxidation products. Were these reactions to occur with ACEi peptides, it is expected that important structural features relevant for bioactivity can be depleted, or new bioactive compounds can be formed. Future studies are needed to examine the formation of such potentially bioactive compounds from food peptides, their bioavailability relative to native food peptides, and whether they also contribute in promoting cardiovascular health.
3. Bioaccessibility of dietary peptides in the gut has been largely unexplored. It is well understood that *trans*-epithelial transport of several ACEi peptides can be achieved in cellular models, but the peptides still need to be accessible to peptide transporters in the gastrointestinal tract. For instance, peptides can chelate divalent metals in the gut due to their metal-binding properties, and this interaction can lead to the formation of less soluble and poorly absorbed peptide-metal chelates. Similar interactions can also occur with other components of food matrices thereby affecting the kinetics of release of the peptides. Therefore, work is needed to delineate the roles of both the dietary vehicle and gut matrices in enhancing or limiting peptide bioaccessibility for intestinal uptake.
4. Dietary peptides and their derivatives that are indigestible and not absorbable in the intestine can be transported to the lower gut where they can interact with and be metabolized by the gut microflora. Recently, the abundance of certain gut microbiota has been associated with increased blood pressure in different animal models of hypertension (Jose & Raj, 2015), and some of these microbiota act notably through Ang II-induced vascular dysfunction (Karbach et al., 2016). The effects of antihypertensive ACEi peptides and their chemical derivatives on the gut microbiota, and subsequent effect on blood pressure and vascular health are largely unexplored. This will shed light on the physiological mechanisms of the antihypertensive food peptides especially those that do not cross the intestinal barrier into blood circulation.

Although a flood of ACEi peptides has been published in the past two decades, recent evidence of alternative pathways of action and the aforementioned considerations provide possible ways of clarifying discrepancies in clinical vascular health outcomes of hypertensive subjects that received the food peptides across different populations. These insights will enhance efforts towards the translation of knowledge of antihypertensive peptides from laboratory to practical applications as functional food ingredients.

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References

Alghamri, M. S., Weir, N. M., Anstadt, M. P., Elased, K. M., Gurley, S. B., & Morris, M. (2013). Enhanced angiotensin II-induced cardiac and aortic remodeling in ACE2 knockout mice. *Journal of Cardiovascular Pharmcology and Therapeutics, 18*, 138–151.

Beltrami, L., Zingale, L. C., Carugo, S., & Cicardi, M. (2006). Angiotensin-converting enzyme inhibitor-related angioedema: How to deal with it. *Expert Opinion on Drug Safety, 5*, 643–649.

Blake, G. J., & Ridker, P. M. (2001). Novel clinical markers of vascular wall inflammation. *Circulation Research, 89*, 763–771.

Cai, H., & Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases: The role of oxidant stress. *Circulation Research, 87*, 840–844.

Cicero, A. F. G., Gerocarni, B., Laghi, L., & Borghi, C. (2011). Blood pressure lowering effect of lactotripeptides assumed as functional foods: A meta-analysis of current available clinical trials. *Journal of Human Hypertension, 25*, 425–436.

Contreras, F., de la Parte, M. A., Cabrera, J., Ospino, N., Israili, Z. H., & Velasco, M. (2003). Role of angiotensin II AT_1 receptor blockers in the treatment of arterial hypertension. *American Journal of Therapeutics, 10*, 401–408.

Díez-Freire, C., Vázquez, J., Correa de Adjounian, M. F., Ferrari, M. F. R., Yuan, L., Silver, X., et al. (2006). ACE2 gene transfer attenuates hypertension-linked pathophysiological changes in the SHR. *Physiological Genomics, 27*, 12–19.

Donnelly, R. (1992). Angiotensin-converting enzyme-inhibitors and insulin sensitivity-metabolic effects in hypertension, diabetes, and heart-failure. *Journal of Cardiovascular Pharmcology, 20*, S38–S44.

Donoghue, M., Hsieh, F., Baronas, E., Godbout, K., Gosselin, M., Stagliano, N., et al. (2000). A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circulation Research, 87*, e1–e9.

Ehlers, P. I., Nurmi, L., Turpeinen, A. M., Korpela, R., & Vapaatalo, H. (2011). Casein-derived tripeptide Ile-Pro-Pro improves angiotensin-(1-7)- and bradykinin-induced rat mesenteric artery relaxation. *Life Sciences, 88*, 206–211.

Fekete, A. A., Givens, D. I., & Lovegrove, J. A. (2013). The impact of milk proteins and peptides on blood pressure and vascular function: A review of evidence from human intervention studies. *Nutrition Research Reviews, 26*, 177–190.

Fernandez-Musoles, R., Castello-Ruiz, M., Arce, C., Manzanares, P., Ivorra, M. D., &

Salom, J. B. (2013). Antihypertensive mechanism of lactoferrin-derived peptides: Angiotensin receptor blocking effect. *Journal of Agricultural and Food Chemistry, 62*, 173–181.
Fichtlscherer, S., Breuer, S., & Zeiher, A. M. (2004). Prognostic value of systemic endothelial dysfunction in patients with acute coronary syndromes: Further evidence for the existence of the "vulnerable" patient. *Circulation, 110*, 1926–1932.
FitzGerald, R. J., Murray, B. A., & Walsh, D. J. (2004). Hypotensive peptides from milk proteins. *The Journal of Nutrition, 134*, 980S–988S.
Foëx, P., & Sear, J. (2004). Hypertension: Pathophysiology and treatment. *Continuing Education in Anaesthesia, Critical Care & Pain, 4*, 71–75.
Fujita, H., Suganuma, H., Usui, H., Kurahashi, K., Nakagiri, R., Sasaki, R., et al. (1996). Vasorelaxation by Casomokinin L, a derivative of h-Casomorphin and Casoxin D, is mediated by NK1 receptor. *Peptides, 17*, 635–639.
Fujita, H., Usui, H., Kurahashi, K., & Yoshikawa, M. (1995). Isolation and characterization of ovokinin, a bradykinin B1 agonist peptide derived from ovalbumin. *Peptides, 16*, 785–790.
Galle, J., Quaschning, T., Seibold, s., & Wanner, C. (2003). Endothelial dysfunction and inflammation: What is the link? *Kidney International, 84*, S45–S49.
Geleijnse, J. M., & Engberink, M. F. (2010). Lactopeptides and human blood pressure. *Current Opinion in Lipidology, 21*, 58–63.
Halcox, J. P., Schenke, W. H., Zalos, G., Mincemoyer, R., Prasad, A., Waclawiw, M. A., et al. (2002). Prognostic value of coronary vascular endothelial dysfunction. *Circulation, 106*, 653–658.
Hall, J. E., Granger, J. P., do Carmo, J. M., da Silva, A. A., Dubinion, J., George, E., et al. (2012). Hypertension: Physiology and pathophysiology. *Journal of Comparative Physiology, 2*, 2393–2442.
Hernández-Ledesma, B., del Mar Contreras, M., & Recio, I. (2011). Antihypertensive peptides: Production, bioavailability and incorporation into foods. *Advances in Colloid and Interface Science, 165*, 23–35.
Hirota, T., Nonaka, A., Matsushita, A., Uchida, N., Ohki, K., Asakura, M., et al. (2011). Milk casein-derived tripeptides, VPP and IPP induced NO production in cultured endothelial cells and endothelium-dependent relaxation of isolated aortic rings. *Heart and Vessels, 26*, 549–556.
Ijas, H., Collin, M., Finckenberg, P., Pihlanto-Leppälä, A., Korhonen, H., Korpla, R., et al. (2004). Antihypertensive opioid-like milk peptide-lactorphin: Lack of effect on behavioral tests in mice. *International Dairy Journal, 14*, 201–205.
Jakala, P., Pere, E., Lehtinen, R., Turpeinen, A., Korpela, R., & Vapaatalo, H. (2009). Cardiovascular activity of milk casein-derived tripeptides and plant sterols in spontaneously hypertensive rats. *Journal of Physiology Pharmacology, 60*, 11–20.
Jose, P. A., & Raj, D. (2015). Gut microbiota in hypertension. *Current Opinion in Nephrology and Hypertension, 24*, 403–409.
Karbach, S. H., Schönfelder, T., Brandão, I., Wilms, E., Hörmann, N., Jäckel, S., et al. (2016). Gut microbiota promote angiotensin II–induced arterial hypertension and vascular dysfunction. *Journal of the American Heart Association, 5*. e003698.
Keidar, S., Kaplan, M., & Gamliel-Lazarovich, A. (2007). ACE2 of the heart: from angiotensin I to angiotensin (1-7). *Cardiovascular Research, 73*, 463–469.
Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal, 16*, 945–960.
Kulemina, L. V., & Ostrov, D. (2011). Prediction of off-target effects on angiotensin converting enzyme 2. *Journal of Biomolecular Screening, 16*, 878–885.
Lahera, V., Goicoechea, M., de Vinuesa, S. G., Miana, M., de las Heras, N., Cachofeiro, V., et al. (2007). Endothelial dysfunction, oxidative stress and inflammation in atherosclerosis: Beneficial effects of statins. *Current Medicinal Chemistry, 14*, 243–248.
Landmesser, U., Harrison, D. G., & Drexler, H. (2006). Oxidant stress - a major cause of reduced endothelial nitric oxide availability in cardiovascular disease. *European Journal of Clinical Pharmacology, 62*, 13–19.
Lo, J., Patel, V. B., Wang, Z., Levasseur, J., Kaufman, S., Penninger, J. M., et al. (2013). Angiotensin-converting enzyme 2 antagonizes angiotensin II-induced pressor response and NADPH oxidase activation in Wistar–Kyoto rats and spontaneously hypertensive rats. *Experimental Physiology, 98*, 109–122.
Maes, W., Van Camp, J., Vermeirssen, V., Hemeryck, M., Ketelslegers, J. M., Schrezenmeir, J., et al. (2004). Influence of the lactokinin Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) on the release of endothelin-1 by endothelial cells. *Regulatory Peptides, 118*, 105–109.
Majumder, K., Chakrabarti, S., Davidge, S. T., & Wu, J. (2013b). Structure and activity study of egg protein ovotransferrin derived peptides (IRW and IQW) on endothelial inflammatory response and oxidative stress. *Journal of Agricultural and Food Chemistry, 61*, 2120–2129.
Majumder, K., Chakrabarti, S., Morton, J., Sareh, P., Kaufman, S., Davidge, S. T., et al. (2013a). Egg-derived tri-peptide IRW exerts antihypertensive effects in spontaneously hypertensive rats. *PLoS One, 8*, e82829.
Majumder, K., Chakrabarti, S., Morton, J., Sareh, P., Kaufman, S., Davidge, S. T., et al. (2015b). Egg-derived ACE-inhibitory peptides IQW and LKP reduce blood pressure in spontaneously hypertensive rats. *Journal of Functional Foods, 13*, 50–60.
Majumder, K., Liang, G., Chen, Y., Guan, L. L., Davidge, S. T., & Wu, J. (2015a). Egg ovotransferrin derived ACE inhibitory peptide IRWincreases ACE 2 but decreases pro-inflammatory genes expression in mesenteric artery of spontaneously hypertensive rats. *Molecular Nutrition and Food Research, 59*, 1735–1744.
Majumder, K., & Wu, J. (2014). Molecular targets of antihypertensive peptides: Understanding the mechanisms of action based on the pathophysiology of hypertension. *International Journal of Molecular Sciences, 16*(1), 256–283.
Martins, M. B., & Carvalho, I. (2007). Diketopiperazines: Biological activity and synthesis. *Tetrahedron, 63*, 9923–9932.
McEver, R. P. (1992). Leukocyte-endothelial cell interactions. *Current Opinion in Cell Biology, 4*, 840–849.
Miguel, M., Alvarez, Y., López-Fandiño, R., Alonso, M. J., & Salaices, M. (2007). Vasodilator effects of peptides derived from egg white proteins. *Regulatory Peptides, 140*, 131–135.
Miguel, M., López-Fandiño, R., Ramos, M., & Aleixandre, A. (2005). Short-term effect of egg white hydrolysate products on the arterial blood pressure of hypertensive rats. *British Journal of Nutrition, 94*, 731–737.
Mohan, A., Udechukwu, M. C., Rajendran, S. R., & Udenigwe, C. C. (2015). Modification of peptide functionality during enzymatic hydrolysis of whey proteins. *RSC Advances, 5*, 97400–97407.
Münzel, T., Sinning, C., Post, F., Warnholtz, A., & Schulz, E. (2008). Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. *Annals of Medicine, 40*, 180–196.
Nakamura, T., Hirota, T., Mizushima, K., Ohki, K., Naito, Y., Yamamoto, N., et al. (2013). Milk-derived peptides, Val-Pro-Pro and Ile-Pro-Pro, attenuate atherosclerosis development in apolipoprotein E-deficient mice: A preliminary study. *Journal of Medicinal Food, 16*, 396–403.
Nakamura, Y., Yamamoto, N., Sakai, K., & Takano, T. (1995). Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-Converting enzyme. *Journal of Dairy Science, 78*, 1253–1257.
Prada, J. A. H., Ferreira, A. J., Katovich, M. J., Shenoy, V., Qi, Y., Santos, R. A. S., et al. (2008). Structure-based identification of small molecule angiotensin converting enzyme 2 activators as novel antihypertensive agents. *Hypertension, 51*, 1312–1317.
Quirós, A., Dávalos, A., Lasunción, M. A., Ramos, M., & Recio, I. (2008). Bioavailability of the antihypertensive peptide LHLPLP: Transepithelial flux of HLPLP. *International Dairy Journal, 18*, 279–286.
Ringseis, R., Gotze, V., & Eder, K. (2009). Tripeptides from dietary proteins inhibit TNF alpha-induced monocyte adhesion to human aortic endothelial cells. *Regulatory Peptides, 154*, 91–96.
Sánchez-Rivera, L., Martínez-Maqueda, D., Cruz-Huerta, E., Miralles, B., & Recio, I. (2014). Peptidomics for discovery, bioavailability and monitoring of dairy bioactive peptides. *Food Research International, 63*, 170–181.
Santos, R. A. S., Silva, A. C. S., Maric, C., Silva, D. M. R., Machado, R. P., Buhr, I., et al. (2003). Angiotensin-(1-7) is an endogenous ligand for thr G protein-coupled receptor Mas. *Proceedings of the National Academy of Sciences, 100*, 8258–8263.
Sarzani, R., Salvi, F., Dessi-Fulgheri, P., & Rappelli, A. (2008). Renin-angiotensin system, natriuretic peptides, obesity, metabolic syndrome, and hypertension: An integrated view in humans. *Journal of Hypertension, 26*, 831–843.
Sattar, N. (2004). Inflammation and endothelial dysfunction: Intimate companions in the pathogenesis of vascular disease? *Clinical Science, 106*, 443–445.
Schulz, E., Anter, E., & Keaney, J. F., Jr. (2004). Oxidative stress, antioxidants, and endothelial function. *Current Medicinal Chemistry, 11*, 1093–1104.
Schulz, E., Gori, T., & Münzel, T. (2011). Oxidative stress and endothelial dysfunction in hypertension. *Hypertension Research, 34*, 665–673.
Segura-Campos, M., Chel-Guerrero, L., Betancur-Ancona, D., & Hernandez-Escalante, V. M. (2011). Bioavailability of bioactive peptides. *Food Reviews International, 27*, 213–226.
Sipola, M., Finchenberg, P., Vapaatalo, H., Korhonen, H., & Nurminen, M. L. (2002). α-lactorphin and β-lactorphin improve arterial function in spontaneously hypertensive rats. *Life Sciences, 71*, 1245–1253.
Udenigwe, C. C., & Aluko, R. E. (2012). Food protein-derived bioactive peptides: Production, processing, and potential health benefits. *Journal of Food Science, 77*, R11–R24.
Udenigwe, C. C., & Mohan, A. (2014). Mechanisms of food protein-derived antihypertensive peptides other than ACE inhibition. *Journal of Functional Foods, 8*, 45–52.
Van Lancker, F., Adams, A., & De Kimpe, N. (2011). Chemical modifications of peptides and their impact on food properties. *Chemical Reviews, 111*, 7876–7903.
Vanhoutte, P. M. (1989). Endothelium and control of vascular function: State of the art lecture. *Hypertension, 13*, 658–667.
Vermeirssen, V., Van Camp, J., & Verstraete, W. (2004). Bioavailability of angiotensin I converting enzyme inhibitory peptides. *British Journal of Nutrition, 92*, 357–366.
Vickers, C., Hales, P., Kaushik, V., Dick, L., Gavin, J., Tang, J., et al. (2002). Hydrolysis of biological peptides by human angiotensin-converting enzyme related carboxypeptidase. *The Journal of Biological Chemistry, 277*, 14838–14843.
Yamaguchi, N., Kawaguchi, K., & Yamamoto, N. (2009). Study of the mechanism of antihypertensive peptides VPP and IPP in spontaneously hypertensive rats by DNA microarray analysis. *European Journal Pharmacology, 620*, 71–77.
Yang, Z., Yu, X., Cheng, L., Miao, L., Li, H., Han, L., et al. (2013). Effects of enalapril on the expression of cardiac angiotensin-converting enzyme and angiotensin-converting enzyme 2 in spontaneously hypertensive rats. *Archives of Cardiovascular Diseases, 106*, 196–201.
Yoshikawa, M., Tani, F., Shiota, A., Suganuma, H., Usui, H., Kurahashi, K., et al. (1994). Casoxin D, an opioid antagonist/ileum-contracting/vasorelaxing peptide derived from human αs1-casein. In V. Brantl, & H. Teschemacher (Eds.), *β-Casomorphins and related peptides: Recent developments. (pp.43–48)*. Germany: VCH-Weinheim.
Yoshikawa, M., Tani, F., Yoshimura, T., & Chiba, H. (1986). Opioid peptides from milk proteins. *Agricultural and Biological Chemistry, 50*, 2419–2421.
Zhao, R. Z., Chen, X., Yao, Q., & Chen, C. (2005). TNF-alpha induces interleukin-8 and endothelin-1 expression in human endothelial cells with different redox

pathways. *Biochemical and Biophysical Research Communications, 327*, 985–992.
Zhong, J., Basu, R., Guo, D., Chow, F. L., Byrns, S., Schuster, M., et al. (2010). Angiotensin-converting enzyme 2 suppresses pathological hypertrophy, myocardial fibrosis, and cardiac dysfunction. *Circulation, 122*, 717–728.
Zhong, J., Huang, D., Yang, Y., Li, Y., Liu, G., Song, H., et al. (2004). Upregulation of angiotensin-converting enzyme 2 by all-trans retinoic acids in spontaneously hypertensive rats. *Hypertension, 44*, 907–912.
Zhong, J., Ye, J., Jin, H., Yu, X., Yu, H., Zhu, D., et al. (2011). Telmisartan attenuates aortic hypertrophy in hypertensive rats by the modulation of ACE2 and profilin-1 expression. *Regulatory Peptides, 166*, 90–97.
Zhuo, J. L., Ferrao, F. M., Zheng, Y., & Li, X. C. (2013). New frontiers in the intrarenal renin-angiotensin system: A critical review of classical and new paradigms. *Frontiers in Endocrinology, 4*, 166.