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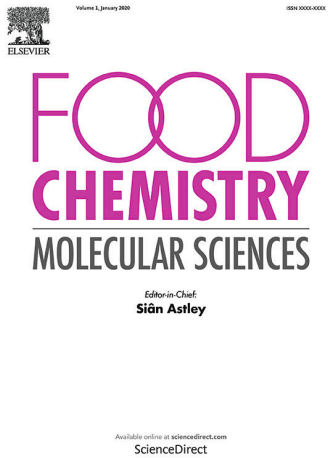

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Recent findings on the cellular and molecular mechanisms of action of novel food-derived antihypertensive peptides

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ABSTRACT

Hypertension impacts negatively on the quality of life of sufferers, and complications associated with uncontrolled hypertension are life-threatening. Hence, many research efforts are exploring the antihypertensive properties of bioactive peptides derived from food proteins using *in vitro* ACE-inhibitory assay, experimentally-induced and spontaneous hypertensive rats, normotensive and hypertensive human models. In this study, the cellular and molecular mechanisms of blood pressure-lowering properties of novel peptides reported in recent studies (2015-July 30, 2021) were discussed. In addition to common mechanisms such as the inhibition of angiotensin I-converting enzyme (ACE) and renin activities, recently recognized mechanisms through which bioactive peptides exert their antihypertensive properties including the induction of vasodilation via upregulation of cyclo-oxygenase (COX) and prostaglandin receptor and endothelial nitric oxide synthase expression and L-type Ca^{2+} channel blockade were presented. Similarly, emerging mechanisms of blood pressure-lowering by bioactive peptides such as modulation of inflammation (TNF-α, and other cytokines signaling), oxidative stress (Keap-1/Nrf2/ARE/HO-1 and related signaling pathways), PPAR-γ/caspase3/MAPK signaling pathways and inhibition of lipid accumulation were discussed. The review also highlighted factors that influence the antihypertensive properties of peptides such as method of hydrolysis (type and number of enzymes, and chemical used for hydrolysis, and microbial fermentation), and amino acid sequence and chain length of peptides.

Key words: Bioactive peptides; antihypertensive peptides; hypertension; ACE-inhibitory peptides; nutraceuticals; functional foods

Introduction

Hypertension is a medical condition in which an adult has systolic and diastolic blood pressure levels of 140/90 mmHg and above. The number of people living with hypertension (sustained high blood pressure) globally is worrisomely high (WHO, 2021). Despite many efforts in place to reduce this number, the statistics have remained almost the same between 2010 till date due to elevation in alcohol, tobacco and substance use and obesity, which are major risk factors to hypertension (Louca et al., 2020). Hypertension and its co-morbidities are among the leading cause of death accrued to noncommunicable diseases, and although effective, currently-available antihypertensives do not lower blood pressure in some hypertensive patients. In addition, the high cost and side effects such as high serum potassium level and hypotension associated with these drugs also contribute to poor adherence to treatment and increased risk to other chronic diseases associated with unmanaged hypertension such as organ failure and stroke (Leoncini et al., 2020). Hence, the continuous search for more agents that are safe and can effectively normalize blood pressure, which may be the hope of those who do not respond to the currently-available antihypertensives.

In traditional medicine, natural products derived from plants (Verma et al., 2021), and food proteins (Wang et al., 2021) are used in managing cases of hypertension. Plant extracts (especially those rich in polyphenolic compounds) and compounds isolated from them are generating major interest in reducing blood pressure in normotensive, experimentally-induced and spontaneous hypertensive rodents (Kim et al., 2020). Reports of clinical trials on the beneficial effects of dietary proteins on hypertension are accumulating. In POUNT Lost Trials, ingestion of dietary proteins was demonstrated to modify genetic susceptibility to hypertension by significantly reducing the risk of developing hypertension in cohorts receiving high protein diets compared to placebo (Sun et al., 2019). Similarly, a 5-year follow-up of over 13,000 middle-aged Korean men showed that participants who were placed on animal-based protein-rich diets were more susceptible to hypertension and other metabolic risks compared to participants receiving plant-based protein-rich diets (Chung et al., 2020). A similar result was

obtained in participants drawn from Iranian population that consumption of protein-based diets reduces the risk of hypertension (Mehrabani et al., 2017). Upon intestinal hydrolysis, peptides generated from these dietary proteins interact with receptors such as muscarinic and angiotensin II (Ang II) receptors to induce vasorelaxation; the peptides also modulate the renin-angiotensin signaling system (RAS), especially by inhibiting the activities of renin and ACE to lower blood pressure. In addition, some of these intervention agents modify risk factors and co-morbidities of hypertension such as oxidative stress, obesity and diabetes (Metchi Donfack et al., 2021). Furthermore, many dietary proteins reduce blood pressure by increasing nitric oxide availability and inhibiting the formation of advanced glycation end-products and insulin resistance (Ghatage et al., 2021). Among the natural products being screened as potential sources of antihypertensive agents, food proteins, their hydrolysates and peptides isolated from them with antihypertensive properties are dominating (Kaur et al., 2021; Oh et al., 2020). Generally, the exposure of unique side chains of amino acids in peptides encrypted in proteins during hydrolysis have been shown to increase their biological functionality. The hydrolysis of proteins derived from buffalo and cow milk with papain, pepsin and trypsin were shown to markedly enhance the ACE-inhibitory properties relative to intact proteins (Praveesh et al., 2011). In addition to enzymolysis, it is worthy of note that the release of peptides from proteins are also achieved using chemical hydrolysis and microbial fermentation (Aluko, 2015), discussed briefly later.

Previous review articles discussed antihypertensive protein hydrolysates and their peptides reported up to 2015 (Hernández-Ledesma et al., 2011; Martínez-Maqueda et al., 2012; Aluko, 2015). While the first two studies focused more on sources of the peptides, Aluko et al. further discussed methods of preparation of antihypertensive peptides (AHPs) isolated by 2015 and their mode of action, specifically the inhibition ACE and renin activities and blocking of interaction between the vasoconstrictor, Ang II and its receptors. Recently, the inhibitory properties of peptides isolated from proteins originating from Amaranth, fish and microalgae against ACE and renin activities were recently reviewed (Jiang et al., 2021; Nardo et al., 2020; Yathisha et al., 2018). It is worthy to mention that the methods of production, isolation, purification and quantification, and bioavailability of the antihypertensive peptides were discussed in previous reviews (Aluko, 2015; Jogi et al., 2021; Xue et al., 2021). Hence, only some unique steps in peptide isolation with special reference to recently adopted techniques to improve upon some of

the challenges associated with peptide isolation, identification and quantification were highlighted in this review. The review further discussed: (I) the ACE-inhibitory and blood pressure-lowering novel peptides isolated from protein hydrolysates of plant and animal origin investigated using *in vivo, in silico*, cell culture, animal and human clinical studies and reported in recent peer-reviewed articles (2015-July 30, 2021), (II) the cellular and molecular mechanisms of blood pressure-lowering potentials of these food protein hydrolysates and peptides due to the induction of vasodilation via upregulation of cyclo-oxygenase (COX) and prostaglandin receptor and endothelial nitric oxide synthase expression and L-type Ca^{2+} channel blockade, (III) how the method of preparation (type of microbes used for fermentation and type/number of enzymes used for enzymolysis), amino acid chain length and amino acid sequence influence antihypertensive properties of peptides and (IV) limitations of current research and future research directions. In addition to already demonstrated mechanisms by which food protein hydrolysates and peptides lower blood pressure, we also discussed other potential signaling pathways via which blood pressure can be regulated such as modulation of inflammation (TNF-α, and other cytokines signaling), oxidative stress (Keap-1/Nrf2/ARE/HO-1 and related signaling pathways), PPAR-γ/caspase3/MAPK signaling pathways and inhibition of lipid accumulation. This will encourage researchers to explore these signaling pathways as possible mechanisms of action of AHPs in future studies. Finally, this review aims to project the uniqueness of these novel AHPs mostly sourced from wastes and underutilized natural products such as bones and muscles of marine organisms, plant and animal wastes, and fermentation products of unique microorganisms as excellent candidates for functional food development.

Preparation of antihypertensive peptides from food proteins

Dietary proteins are first isolated from its source such as milk, egg, meat, snail, chicken, fish, soybean, rice, lupin, mung bean, and Amaranth. Proteins have been hydrolyzed into peptide units using a variety of ways by basically transferring the proteins into the active site of the proteases to hydrolyze their peptide bonds. Maximum hydrolytic efficiency is achieved by adjusting medium (water or buffer) to optimum temperature and optimum pH of the enzyme (Adjonu et al., 2013). Previously, the use of a single enzyme for protein hydrolysis is common but recently, a combination of two or more enzymes during protein hydrolysis is adopted to increase the yield of shorter chain peptides which are shown exert better bioavailability and bioactivity. Whereas in

multiple enzyme digestion method, two or more enzymes are used simultaneously (if they possess same optimal pH and temperature) or consecutively (Aluko, 2015). In many cases, the biological activity (such as antihypertensive activity) of the protein hydrolysates are assayed. This is followed by separation of the protein hydrolysates into fractions based on their molecular weight and the fractions with marked biological activities are selected for separation into their peptides. The peptides in the protein hydrolysate vary in chain length, hydrophobicity, net charge, and activity; these physicochemical properties inform the techniques needed to separate the peptides such as peptide purification, and amino acid sequence identification (Girgih et al., 2015). The most common technique, membrane ultracentrifugation, sort the peptides in protein hydrolysates based on their size/molecular weight/peptide chain length which could be from least to large and vice versa. Other improved techniques such as reverse-phase high-performance liquid chromatography (RP-HPLC) and Fast protein liquid chromatography are currently adopted to improve peptide yield and purity (Girgih et al., 2013; He et al., 2013; Franca-Oliveira et al., 2021). Recently, more advanced techniques such as matrix-assisted laser desorption ionization time-of-flight mass spectrophotometer are used for peptide purification while the amino acid sequence of the peptides are recognized using automated techniques such as peptide sequencer (He et al., 2021; Song et al., 2021). This is followed by the confirmation of the antihypertensive activity of the characterized peptide(s) using any of the in vitro assays, animal model and human subjects.

Figure 1: key steps in the preparation of antihypertensive peptides

Molecular mechanisms of action of food protein-derived antihypertensive peptides

Hypertension is a debilitating condition caused by irregularities with several pathophysiological factors and enzyme systems that play vital roles in maintaining homeostasis between the constriction and dilation of vascular systems. Some of these factors, in addition to RAS, are activities of the various isoform the endothelial nitric oxide synthase (eNOS), serum level of pro-inflammatory cytokines [such as interleukin (IL)-1β, IL-6, IL-8, IL-17, and IL-23, transforming growth factor beta (TGFβ), and tumor necrotic factor alpha (TNFα)], regulation of nuclear factor erythroid 2-like 2 (Nrf2) (Daiber et al., 2020), and possibly, regulation of COX-mediated

production of prostanoids and prostacyclins. Many studies have discovered some constitutive bioactive peptides that effectively help in reducing hypertension, by stimulating a balance between the constriction and dilation events of large blood vessels, especially during vascular injuries and blood clotting. This section provides a brief overview of the different pathways involved in blood pressure regulation and recently isolated antihypertensive peptides modulating these pathways.

Previously recognized mechanism of blood pressure lowering by bioactive peptides

Unregulated RAS activity results in elevated blood pressure, modulators of RAS activities such as ACE and renin inhibitors and Ang II receptor blockers are employed to lower blood pressure. These medications are one of the most effective strategies to manage high blood pressure, heart failure, renal failure, and the negative consequences of diabetes (Hanafi et al., 2018). Synthetic medicines such as captopril, enalapril, and lisinopril are being used to treat hypertension. These drugs inhibit the ability of ACE to convert Ang I to Ang II, the potent vasoconstrictor; therefore, inhibition of ACE would result in a decrease in blood pressure. However, undesirable side-effects such as angioedema, persistent dry coughs, and fetopathy are common with the use of these synthetic drugs (Hanafi et al., 2018). Unlike synthetic counterparts, natural ACE inhibitors are thought to be a safer option. A number of peptides having *in vitro* ACE inhibitory action have been demonstrated to impact blood pressure in spontaneously hypertensive rats (SHR) and humans in a beneficial way without adverse effects (Hanafi et al., 2018).

From plant-based proteins hydrolysates, a number of novel AHPs with ACE-inhibitory and blood pressure lowering properties have been isolated. For instance, EAQRLLF, PSLRSYLAE, PDRSIHGRQLAE, FITAFR and RGQVLS isolated from alcalase-hydrolyzed green soybean seed protein inhibited ACE activity by 94.19%, 99.31%, 92.92%, 101.51% and 90.40%, respectively (Hanafi et al., 2018). Other plant proteins derived novel peptide with ACE-inhibitory activity include LTFPGSAED from lupin seed in intestinal Caco-2 cells (IC_{50} =13.7 μM) and in renal HK-2 cells (IC_{50} =79.6 μM) (Lammi et al., 2020), QTDEYGNPPR, AGFAGDDAPR, IDESLR, IQDKEGIPPDQQR from black tea (IC_{50} values of 210.03, 178.91, 196.31 and 121.11 μmol/L respectively) (Lu et al., 2021), APKIEEV from defatted areca nut kernel globulin (IC_{50} = 550.41 mol/L) (Liu et al., 2021), ALAPE from *Pinctada imbricata fucata*

177 (IC_{50} = 167.5μM) (Liu et al., 2019) and IW form *Oncorhynchus gorbuscha* (IC_{50} = 1.2 μM)
178 (Abachi et al., 2019).

179 Similarly, animal protein-derived peptides have been shown to have antihypertensive effects via
180 inhibition of ACE and renin activities. A few examples of these include AEWLHDWKL and
181 MVPYPQR from camel milk (IC_{50} = 30 μM) (Soleymanzadeh et al., 2019), and IPP, LIVTQ,
182 IIAE and LVYPFP from whey/milk protein (IC_{50} = values of 1.23, 113, 128 and 97 μg/mL
183 respectively) (Chamata et al., 2020). Generally, these peptides inhibit ACE activity through the
184 formation of H-bonding with the enzyme's active site catalytic residues (Ala 354, Gln 281, His
185 513, Tyr 520, Lys 511, and Glu 162) (Yu et al., 2020). After demonstrating good ACE-inhibitory
186 activities *in vitro*, Yu et al. (2021) fed two pentapeptides, QIGLF and RVPSL to SHRs for four
187 weeks and recorded strong suppression of SBP. Molecular analysis demonstrated that the
188 peptides elicited their antihypertensive effects by competitively inhibiting ACE activity. Other
189 novel peptides with ACE-inhibitory effects recently isolated are presented in Table 1 while the
190 mechanism of action of the peptides targeting RAS is shown in Figure 2.

191 Table 1: Novel ACE-inhibitory peptides isolated recently from food proteins

Novel peptide	Protein source	Activity (IC_{50} value)	References
LY, LVS, YQ, APSY, and RGGY	Wheat gluten	0.31, 0.60, 2.00, 1.47 and 1.48 mmol/L, respectively	(Liu et al., 2021)
IIAPTPVPAAH	*Bellamya bengalensis* (gastropod snail) muscle meat	8.52 μg/mL	(Dey et al., 2021)
SFNLPILR and AFEDGFEWVSKF	Amaranth grains	2.50 and 1.47 mM, respectively	(Nardo et al., 2020)
IVDR, WYK and VASVI	*Paralichthys olivaceus (*Surimi) myofibrillar	46.90, 32.97 and 32.66 μM, respectively	(Oh et al., 2020)
EKVNELSK, MKP and LLYQEPVLGPVR	Casein hydrolysate	6.0, 0.43 and 5.0 μM, respectively	(Liu et al., 2019; Yuda et al., 2020)
IPP, IIAE, LVYPFP and LIVTQ	Whey/milk protein	1.23, 128, 97 and 113 μg/mL, respectively	(Chamata et al., 2020)
AVKILP, LSGPVKF, AVFQHNCQE, VGKPGARAPMY and QVGPLIGRYCG	Chicken foot	7.1, 80.9, 44.8, 29.7 and 11 μM, respectively	(Bravo et al., 2019)

AVQ and YPQ	Distilled spent grain	181 and 220 µM, respectively	(Wei et al., 2019)
TNLDWY, RADFY and RVFDGAV	*Ginkgo biloba* (Ginkgo) seeds	1.93, 1.35 and 1.01 mM, respectively	(Ma et al., 2019)
LSGYGP	*Oreochromis niloticus* Linnaeus (tilapia) skin gelatin	2.577 µmol/L	(Chen et al., 2020)
SSYYPFK	*Avena nuda* (Naked oat) globulin	91.82 µM	(Zheng et al., 2020)
WF and FASA	*Euphausia superba* (Antarctic krill)	0.32 and 0.15 mg/ml, respectively	(Zhao et al., 2019)
EAQRLLF, PSLRSYLAE, PDRSIHGRQLAE, FITAFR and RGQVLS,	*Glycine max* (L) Merr (Green soybean)	878, 532, 1552, 1342 and 993 µM respectively	(Hanafi et al., 2018)
VRP, LKY, VRY, KYKA, and LKYKA,	*Gallus gallus domesticus* (hen)	0.64, 0.81, 5.77, 2.87, and 0.034 µg/ml, respectively	(Fan & Wu, 2020)

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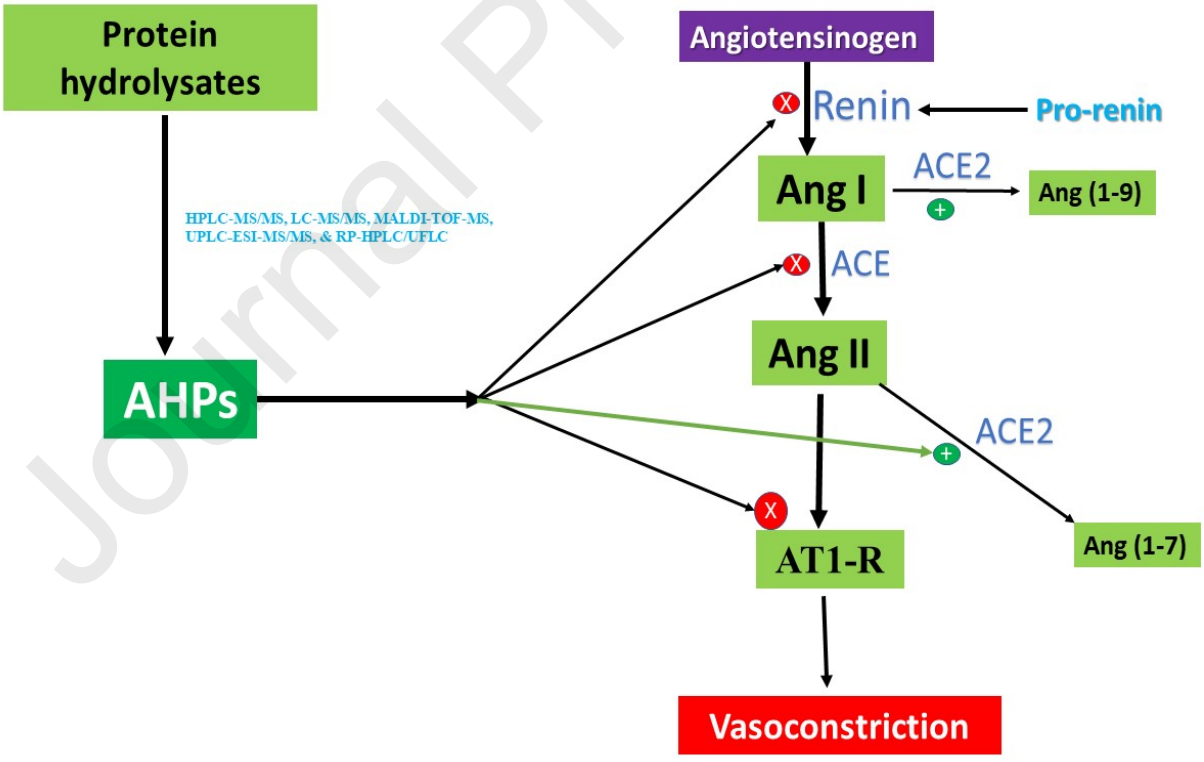

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Figure 2: Mechanism of action of antihypertensive peptides (AHPs) via modulation of renin-angiotensin system (RAS)

Recently recognized mechanisms of suppressing blood pressure by bioactive peptides

Up-regulation of angiotensin converting enzyme 2 (ACE2) gene expression and its enzyme activity: An additional mechanism of blood pressure-lowering properties of natural peptides is by up-regulation of gene expression and enzyme activation of ACE2, the enzyme that hydrolyzes the major vasoconstrictor of RAS, Ang II into its less active metabolite, angiotensin-(1-7). For example, IRW, an egg white-isolated peptide was shown to reduce both SBP and DBP in SHR model by enhancing ACE2 mRNA expression (Liao et al., 2016). Similarly, AKSLSDRFSY from pea protein hydrolysates, a biostable peptide which is resistant to pepsin was shown to upregulate the gene expression of ACE2 in cultured vascular smooth muscle cells (Liao et al., 2019). Upon hydrolysis with pancreatin, the two metabolites LSDRFS and SDRFSY identified, where also shown to upregulate the expression of ACE2 in a manner similar to the parent peptide, AKSLSDRFSY, suggesting that these metabolites may be playing major roles in the enhancement of ACE2 gene expression.

Modulation of PPAR-γ/caspase3/MAPK/eNOS signaling pathways: The eNOS is one of the isoforms of nitric oxide synthase (NOS) primarily located in the peri-nucleus, Golgi apparatus and caveolae of most endothelial cells (Li et al., 2015). The eNOS catalyzes the generation of NO from arginine, to help manage oxidative stress or damages caused by endogenously-and exogenously-generated reactive oxygen species (ROS). When released from the endothelial cells, NO causes an increase in the 3',5'-cyclic-guanosine monophosphate (cGMP), which activates cGMP-dependent kinase, to stimulate vasodilation (Li et al., 2015). Oxidative stress resulting from excessive ROS suppresses gene expression and enzyme activity of eNOS by uncoupling its bound cofactor vital for NO generation (Daiber et al., 2020). Hence, the Apo-eNOS, conversely produces superoxide anion rather than NO, which further worsen the oxidative damage on the cells, exacerbating endothelial dysfunction causing vascular constriction and cardiovascular diseases (Daiber et al., 2020). The activity of eNOS is also regulated by phosphorylation and dephosphorylation of specific amino acid residues in the enzyme. Phosphorylation of Ser-615, 633 and 1177 significantly activates the eNOS whereas phosphorylation of Thr495 inhibits it.

Studies have shown that eNOS from patients with cardiovascular diseases have reduced level of the phosphorylated catalytic serine residues and a reduced titer of the kinases known for phosphorylating eNOS (AMP-activated protein kinase (AMPK), protein kinase B (Akt), extracellular signal-regulated protein kinases (ERK-1/2), and calcium-calmodulin kinase II (CaMK-II) (Zippel et al., 2018). On the other hand, the transcription factor, peroxisome proliferator activated receptor (PPAR)-γ potentiates several physiological events including the suppression of oxidative stress, inflammation, and vasoconstriction, and expression of α-smooth muscle actin, RhoA, cleaved caspase-3 whereas action of eNOS and vasodilation were elevated (Stump et al., 2015). Furthermore, the suppression of Ang II-generated hypertension by pharmacological activation of PPAR-γ with its agonist, pioglitazone positions the PPAR-γ as a good target for blood pressure monitoring (Yu et al., 2015).

Intragastric administration of alcalase/protease-hydrolyzed skate skin gelatin for 20 days by spontaneous hypertensive rats (SHRs) was shown to significantly reduce SBP (Ngo et al., 2015). The hydrolysate acted by activating PPAR-γ signaling, leading to the suppression of expression of endothelin-1, α-smooth muscle actin, RhoA, cleaved caspase 3, and MAPK whereas elevation in eNOS action in the lungs. Taken together, the mechanism of action of the hydrolysate is through PPAR-γ/caspase3/MAPK/eNOS signaling pathways. In addition, the potent ACE-inhibitory properties of two peptides isolated from the hydrolysate, LGPLGHQ and MVGSAPGVL (with IC_{50} values of 4.22 and 3.09 μM, respectively) suggest that inhibition of ACE may be an additional mechanism of the blood pressure-lowering effects of the skate skin gelatin hydrolysate. Hence, in addition to ACE inhibitory assay, researchers on AHPs should include the investigation of gene expression profiles of PPAR-γ, MAPK and eNOS in cultured cells to provide more details on the mechanism of action other than ACE inhibition. Low molecular weight peptides bearing proline at the terminal residues and in general, proline-rich peptides have been shown to have antioxidant properties, and are resistant against intestinal hydrolytic enzymes during transepithelial transport (Querobino et al., 2019). Considering the link between oxidative stress and hypertension, many antioxidant agents have been shown to have antihypertensive properties (Ikarashi et al., 2018). An egg white-derived tripeptide (IRW) that hinders oxidative stress, inflammation and migration of vascular smooth muscle cells induced by angiotensin II, was also reported to exhibit antihypertensive effects in SHRs via modulation of

endothelial function, suppression of vascular inflammation and enhancement of NO production (Majumder et al., 2015). Increased intracellular NO level in endothelium leads to vasodilation. The ability of IRW to halt angiotensin II-induced vascular smooth muscle cells migration was further shown to involve the suppression of matrix metallopeptidase-9 (MMP-9) gene expression and Ang I receptor-dependent inactivation of p38-MAPK signaling. Other tripeptides such as LKP, and IQW derived from egg white protein ovotransferrin were also shown to have blood pressure-lowering in SHRs and permeate intestinal epithelium via passive (TJ-mediated) and active (PepT1-mediated) transport routes (Xu et al., 2017).

Recent studies have revealed several bioactive peptides which manage hypertension and cardiovascular disease through the interaction and regulation of eNOS and its associative kinases, probably showing better biosafety and bioavailability profile than standard small molecule drugs (Cicero et al., 2017). A recent study conducted by Oh and colleagues investigated for bioactive peptides with antihypertensive and anti-inflammatory activities for from Olive flounder (*Paralichthys olivaceus*) (Oh et al., 2020). Three bioactive peptides, VASVI, IVDR, WYK were found to significantly increase the level of nitric oxide in the HUVECs cell line. More so, there was a significant improvement in the expression of eNOS and protein kinase B (Akt) (Oh et al., 2020). Similarly, another study on Antarctic krill (*Euphausia superba)* reported bioactive peptides (WF, YRK, and FQLFAS) with activities to improve the hypotensive marker– approximately 33% increase in NO and about 50 percent decrease in endothelin-1 (ET-1). Endothelin-1 on its own is a bioactive peptide first isolated from endothelial cells, with activities as vasoconstrictor, pro-inflammatory and proliferative agents. ET-1 is upregulated with a worsening oxidative state or increase in ROS and can serve as a marker for many cardiovascular conditions. Hence, decrease in ET-1 expression implied an improved cardiovascular state. Although many recent studies have investigated antihypertensive properties of protein-based peptides using the ACE/RAS system, the few recent studies on NO titre/eNOS expression for bioactive peptides are summarized in Table 2.

Table 2: Mechanisms of antihypertensive peptides other than ACE inhibition

Protein sources	**Bioactive peptide**	**Cell line/Animal Model**	**Activities**	**References**
Rapeseed and Captopril	CL and VAP	Rat	↑ 12.7% (NO) ↑ 74.1% (eNOS)	(Wang et al., 2021)

Antarctic krill (*Euphausia superba*)	WF, YRK, and FQLFAS	Human umbilical vein endothelial cells	↑ ≈33.3 % (NO) ↓ ≈50.0 % (ET-1)	(Zhao et al., 2019)
Rice Bran Protein hydrosylate	-	2K-1C hypertensive rats	↑ ≈37.5 % (NO) ↑ eNOS expression	(Boonla et al., 2015)
Olive flounder (*Paralichthys olivaceus*)	VASVI, IVDR and WYK	Human umbilical vein endothelial cells	↑ ≈10-20 % (NO) ↑ ≈ 500 - 900% eNOS expression ↑ ≈100 – 300% Akt expression	(Oh et al., 2020)
Mucuna pruriens seeds	Peptide fraction	Human blood	11.11 % ↓ platelet aggregation	(Herrera-Chalé et al., 2016)
Mucuna pruriens seeds	Peptide fraction	*In vitro* analysis	0.47 % ↓ cholesterol micellar solubility	(Herrera-Chalé et al., 2016)

Attenuation of insulin resistance via IP3K/Akt signaling pathway as a mechanism of antihypertensive property: The suppression of blood pressure by the peptides could have resulted from improvement in insulin sensitivity. Insulin resistance and by extension, type-2 diabetes have been shown to positively correlate with hypertension and other cardiovascular diseases (Rojas-Humpire et al., 2021). Interestingly, egg white protein hydrolysates have been demonstrated to reduce blood pressure in SHR models (Jahandideh et al., 2017) and enhance insulin recognition by its receptor in diet-induced insulin resistance in animal model by activating Akt signaling pathway (Jahandideh et al., 2019). This suggests that peptides in the hydrolysates could have upregulated gene expression and enzyme activity of phosphoinositide-dependent protein kinase 1 (IP3K), the enzyme that activates Akt by phosphorylation to enhance insulin sensitivity and the downstream modulation of metabolism that maintains energy and blood pressure homeostasis (Xing et al., 2019). In addition, the peptides in the hydrolysates could have acted by inducing vasodilation as mediated by activation of Akt to phosphorylate eNOS which produced NO, a potent vasodilator as illustrated in Figure 2.

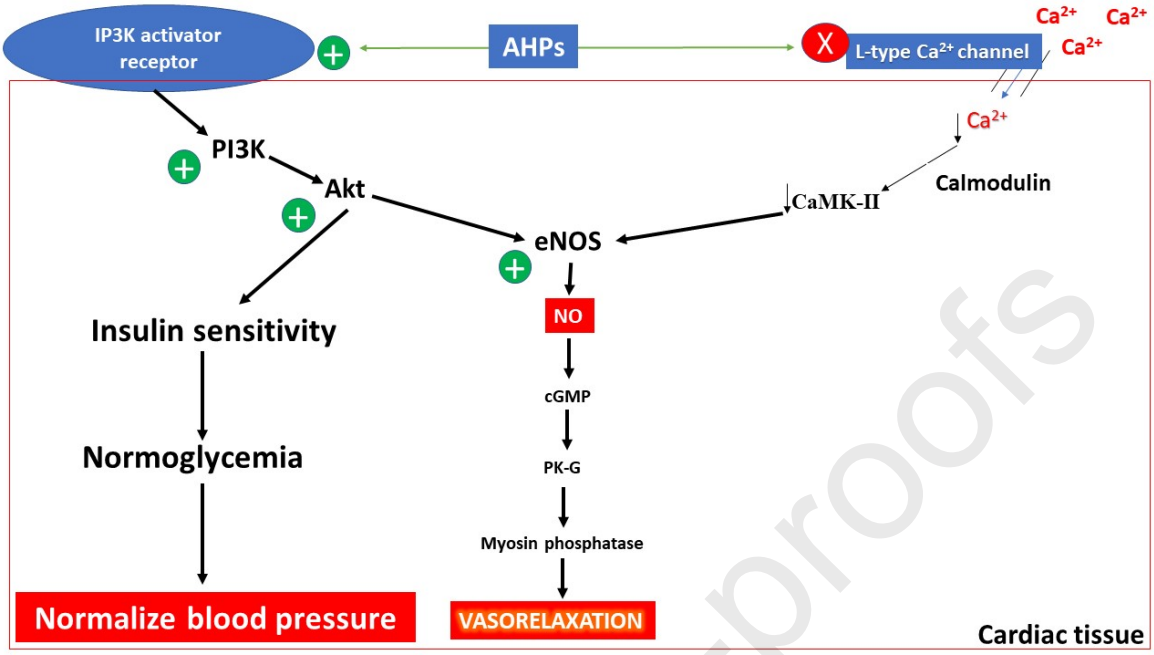

Figure 2: Mechanism of antihypertensive properties of peptides by attenuating insulin resistance via IP3K/Akt signaling pathway. AHPs is proposed to increase the gene expression and enzyme activity of phosphatidylinositiol-3-phosphate kinase (IP3K) through its activator- a membrane bound G-protein coupled subclass receptor. IP3K activates protein kinase B (Akt) by phosphorylation of Ser^{473} in its catalytic site while Akt activates endothelial nitric oxide synthase (eNOS) by phosphorylation of its catalytic residue (Ser^{1177} or Ser^{1179} depending on the specie). Similarly, the AHPs blocks L-type Ca^{2+} channel which increases intracellular concentration of Ca^{2+} that associates with calmodulin (Cd) to form Ca^{2+}-Cd complex which initiates contraction by depleting NO availability via inhibition of eNOS. Active eNOS synthesizes nitric oxide (NO) from L-arginine, and NO activates soluble guanylyl cyclase to convert guanosine triphosphate (GTP) to 5'-cylic guanosine monophosphate (cGMP). On binding to its site on protein kinase-G (PK-G), cGMP activates PK-G to phosphorylate and activate myosin phosphatase (myosin-P). Activate myosin-P dephosphorylates myosin and induce the relaxation of vascular endothelial smooth muscle, hence, reduction in blood pressure.

Emerging mechanisms of blood pressure-lowering by bioactive peptides

Considering the hypertension is a multifactorial disease, and that some peptides with *in vitro* ACE inhibitory effects are unable to lower blood pressure *in vivo*, while some peptides with low ACE inhibitory effects significantly lowered blood pressure, we propose here some possible molecular mechanisms through which peptides can mediate their antihypertensive properties.

Antioxidant-mediated antihypertensive properties: Apart from the eNOS regulation, the oxidant/antioxidant balance in the body system are also regulated by a transcription factor called the Nrf2 in association to its promoter bearing the antioxidant response element (ARE) (Pajares et al., 2016). Under normal physiological conditions, Nrf2 which is constitutively expressed in the cytoplasm is sequestered and repressed by the Kelch-like ECH-associated protein 1 (Keap-1). However, when the system is oxidatively-stressed, the Nrf2 from the cytoplasm is translocated to the nucleus, where it binds to the ARE of the gene coding for antioxidant proteins, activating a cascade of reaction which help to curb the oxidative pressure on the cells (Saha et al., 2020). Studies have shown with consensus evidence that decrease in Nrf2 activities invariably contributes to oxidative stress and cardiovascular diseases such as hypertension (Serafini et al., 2020; Zhan et al., 2021). Considering that some antioxidant peptides also exhibit antihypertensive properties, and that oxidative stress is implicated in hypertension (Griendling et al., 2021), the investigation of Keap-1/Nrf2 signaling pathway (the activation or upregulation the expression and translocation of nuclear Nrf2) and gene expression of antioxidant enzyme activities in peptide-treated SHR is recommended for future studies.

Anti-inflammatory-mediated antihypertensive properties: Inflammation is one common pathology for hypertension and cardiac problems and often result in damage of tissues within the body (Angeli et al., 2021). Inflammatory processes occur due to complex immune reactions involving the different cytokines (IL-1β, IL-6, IL-8, IL-17, IL-23, TGFβ, and TNFα) and other mediators. Several immune cells such as T lymphocytes, dendritic cells and macrophages express constitutive angiotensin 1 receptor (AT1R) (Zhang et al., 2014)**.** During vascular disturbance, angiotensin II binds to AT1R and activates the immune cell differentiation and pro-inflammatory cytokine production – especially IL-6, IFN-γ, and TNFα (Tanase et al., 2019). The pro-inflammatory cytokines IL-6, stimulates the activities of NAD(P)H oxidase, which consequently releases more ROS in the system causing the inhibition of reduced eNOS, endothelial damage, pro-thrombotic recruitment, hypertension and other cardiovascular diseases. Similarly, tumor necrosis factor (TNF)-α stimulates the production of ACE, which invariably also mediates inflammatory and cardiovascular disorder (Mahmudpour et al., 2020). When activated, Nrf2 bends to its nuclear receptor, ARE to upregulate the mRNA expression of its target genes, including heme oxygenase-1 (HO-1). This Nrf2-associated upregulation of HO-1

gene expression inhibits TNF-α-induced release of NF-κB and MCP-1, and other pro-inflammatory mediators (Da Costa et al., 2019) while increasing the secretion of anti-inflammatory cytokines (Ahmed et al., 2017). Based on this, we propose that future research should assess the effects of peptide treatment on Keap-1/Nrf2/ARE/HO-1/NF-κB signaling pathways by assessing the gene expression profiles of Keap-1, Nrf2, HO-1 and NF-κB, and pro-inflammatory cytokine levels in experimental models of hypertension.

Induction of vasodilation via upregulation of COX and prostaglandin receptor: The COX, an enzyme also known as prostaglandin endoperoxide synthase catalyzes the formation of prostaglandin H_2 (PGH_2) from arachidonic acids. The PGH_2 when acted upon different isozymes of synthases and isomerases yields the different prostanoids (PGE_2, PGI_2, PGF_{2a}, and thromboxane A_2 (TXA_2)) (Jang et al., 2020). The COX has two membrane-bound iso-enzymes, the COX-1 and COX-2. The cyclooxygenase 1 (COX-1) are constitutively expressed in most human tissues, whereas, COX-2 is triggered by inflammation and damage of the endothelial and vascular tissues (Faki & Er, 2020). Studies have thoroughly described the roles of COX and the different prostanoids in maintaining balance in the vascular system (Mitchell et al., 2021). The TXA_2 and prostacyclin (PGI_2), and to a lesser extent PGE_2 when either upregulated or downregulated effect vasodilation or vasoconstriction (Ozen & Norel, 2017). The platelet, normally recruited at site of injuries or inflammation expresses only COX-1, which catalyzes the formation of TXA_2. The TXA_2 in turn facilitates the aggregation of platelet (prothrombotic activities) leading to further constriction of the blood vessel and then hypertension (Mitchell et al., 2021). On the other hand, $PG1_2$ produced by the activities of COX-2 facilitates vasodilation with anti-thrombotic activities. Moreover, the PGE_2 at different conditions can act as a vasodilator and as well fostering vasoconstriction (Manual-Kollareth et al., 2020). A study exposed human blood to peptide fraction of *Mucuna pruriens* seed protein hydrolysates and observed 1.59-11.11% decrease platelet aggregation compared to control (human blood that was not treated with the protein hydrolysate fraction) (Herrera-Chalé et al., 2016). Interestingly, the protein hydrolysate fraction exhibited antioxidant effect, and inhibited cholesterol micellar solubility (0.24%-0.47%) and ACE activity (IC_{50} values range from 2.7 to 6.2 μg/mL). Taken together, the *in vitro* ant-platelet aggregatory, hypocholesterolemic and ACE inhibitory properties suggest that the peptides in the protein hydrolysate fraction may have good

antihypertensive properties when ingested intragastrically by hypertensive animal model. Further studies are warranted to isolate the specific peptide(s) and confirm their antihypertensive properties using *in vivo* model by examining the involvement of COX signaling pathway as proposed in Figure 3.

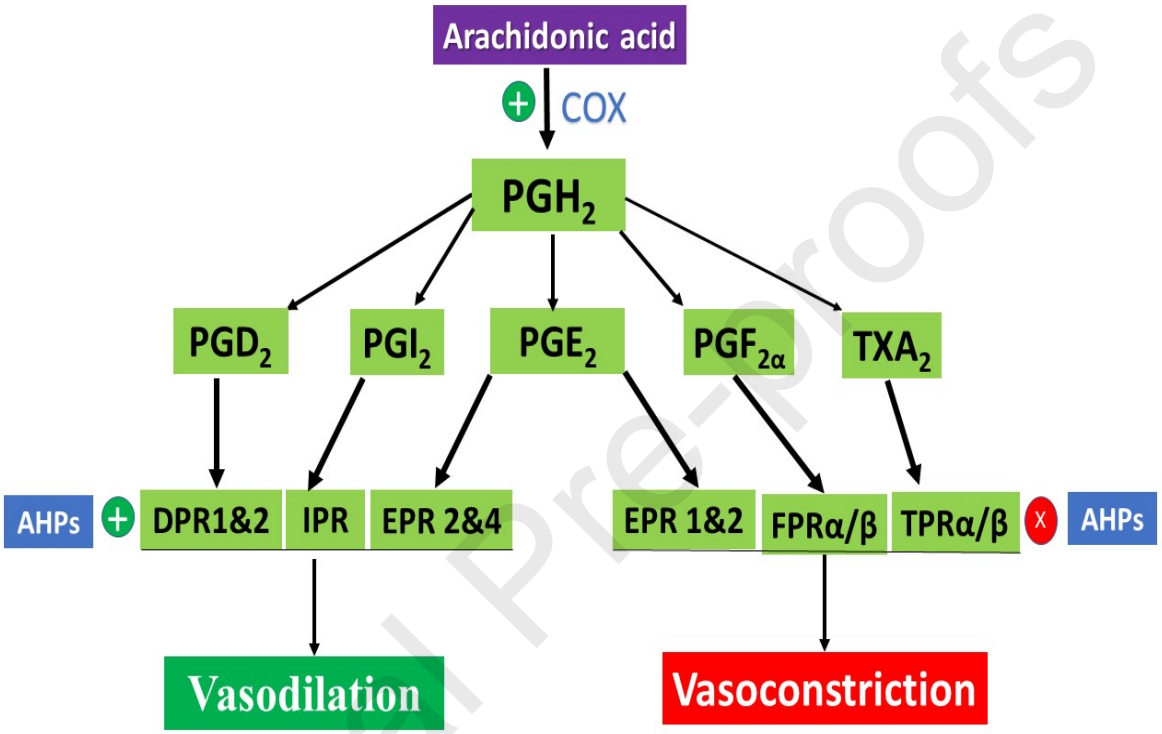

Figure 3: Proposed mechanism of antihypertensive properties of peptides via COX signaling pathway

Blockade of L-type Ca^{2+} channel and inhibition of lipid accumulation: The activation of L-type Ca^{2+} channels have been widely recognized to be involved in the pathogenesis of cardiovascular diseases including hypertension (Figure 2), making the specific channel blockers target drugs for managing hypertension and related cardiovascular diseases (Medvedev et al., 2021). Based on this, the effect of peptide treatment on experimentally-induced contractility of isolated aortic rings as well as that isolated from hypertensive animal models are recommended in future research.

In the same vein, excessive lipid accumulation has been recognized as a risk factor that plays a major role in the pathogenesis of hypertension (Ayoade et al., 2020). Hypolipidemic drugs such as statins that inhibit 3-hydroxy-3-methylglutaryl CoA reductase (HMGCoAR) - the key enzyme of cholesterol biosynthesis are used to prevent and manage hypertension (Ying Wang et al., 2020). A good number of peptides isolated from dietary products have been shown to reduce lipid production and accumulation. Notably, the exposure of cultured human hepatic cells with soybean and lupin-originated peptides such as LILPKHSDAD, LTFPGSAED and YDFYPSSTKDQQS demonstrated marked hypocholesterolemic properties by increasing SREBPs-1 and LDLR protein levels which are known to suppress the biosynthesis and accumulation of lipids whereas activating lipid breakdown via the activation of PI3K/Akt/MAPK pathways (Lammi et al., 2019, 2020; Zanoni et al., 2017). Based on this, the effects of these peptides on hypertensive rats should be investigated in future studies to clarify if the inhibition of lipid accumulation will translate into antihypertensive properties.

Factors that influence the antihypertensive properties and nutraceutical applications of food proteins and peptides for managing hypertension

Biostability is an important aspect of assessing the fitness of a potential drug for use in clinical disease management. Considering that several studies assessed the inhibition of ACE and renin activities *in vitro* as indices of antihypertensive properties, it is important that these biological activities reported in *in vitro* environment are confirmed using *in vivo* models such as animal and/or human cases of hypertension. This is because some ACE-inhibitory peptides were shown not to reduce blood pressure when orally ingested, partly due to their susceptibility to hydrolytic activities of intestinal proteases, and serum peptidases prior to reaching their target (Messina et al., 2021). For example, among five ACE-inhibitory peptides isolated from chicken foot protein hydrolysates (AVKILP, LSGPVKF, AVFQHNCQE, VGKPGARAPMY and QVGPLIGRYCG), only AVFQHNCQE and QVGPLIGRYCG significantly lowered blood pressure after 6 hours of oral ingestion by SHR at 10 mg/kg body weight (Bravo et al., 2019).

In addition, amino acid composition, sequence and chain length are among other factors that influence the antihypertensive properties of peptides. Protein hydrolysates containing proline-rich peptides have been reported to not only inhibit ACE activity *in vitro* but also lower blood

417 pressure *in vivo* (Chamata et al., 2020). The impact of proline could be attributed to conferment
418 of stability due to 'Keil rule' which states that the existence of proline and glutamic acid limits
419 the hydrolytic effects of some proteases such as trypsin on peptides (Udenigwe et al., 2021). This
420 may explain why short-chain peptides containing arginine, tryptophan, leucine, valine, histidine,
421 and phenylalanine from *Chlorella sorokiniana* and marine cobia skin protein hydrolysates were
422 reported to exhibit ACE-inhibitory and blood pressure lowering effects (Lin et al., 2019). To
423 further support this observation, several proline-rich peptides have been shown to permeate
424 through the intestinal membrane to elicit their biological response such as cholesterol-lowering
425 and blood pressure-lowering properties (Jiang et al., 2020).

426 Aside the physicochemical characteristics of peptides such as net charge, amino acid sequence
427 and the chain length, hydrophobicity and molecular weight (Karami & Akbari-adergani, 2019),
428 the interaction between AHPs and other components of food matrix used in delivering the
429 peptides, microbiota activities and mucin content of intestinal epithelium have been suggested to
430 influence their biostability, bioavailability, bioaccessibility and biological activities (Ozorio et
431 al., 2020). For example, interactions of certain peptides with micronutrient composition of food
432 matrix such as mineral elements have been demonstrated to influence their bioavailability and
433 permeability (Sun et al., 2020). Another factor that influences peptide biostability and
434 bioactivities is the presence of other peptides, some of which may be additive or
435 counterproductive. In a study, the transepithelial transport of lupin seed-originated peptide,
436 LTFPGSAED and its metabolite, LTFPG was reported to be increased in the presence of
437 YDFYPSSTKDQQS and LILPKHSDAD (Lammi et al., 2018). This observation was similar to
438 the report that the rate of transport of LILPKHSDAD which was also enhanced in the presence
439 of YDFYPSSTKDQQS and LTFPGSDAD (Lammi et al., 2021).

440 The antihypertensive properties of some peptides may have been, partly or totally a result of
441 hydrolytic metabolites of the peptides, and not only the intact ingested parent peptides. This
442 observation was demonstrated in a study where the ingestion of an ACE-inhibitory (IC_{50} value of
443 25.74 μM) and antioxidant peptide isolated from tilapia skin gelatin, LSGYGP by SHRs
444 suppressed both SBP and DBP (Tianrui et al., 2019). Further analysis showed that LSGYGP is
445 excellently permeable in Caco-2 cell monolayer with some metabolites such as SGYGP, LSGY,
446 GYGP, LSGYP, LSSGYGP, and LSLSGYGP observed after intestinal transport (Tianrui et al.,

2019). One implication of this observation is that the metabolites could have, in part, contributed to the antihypertensive activities recorded after oral consumption of the peptide. For more details on biostability, bioavailability, bioaccessibility and biological activities of bioactive peptides, consult previous reviews (Boegh & Nielsen, 2015; Sun et al., 2020; Sun & Udenigwe, 2020; Udenigwe et al., 2021; Wang & Li, 2018).

The method of preparation of peptides, including the enzymatic system used in hydrolysis, nature of chemicals used, pH and temperature influence their stability, bioavailability and functionality. This was demonstrated in a study where ultrasound treatment of watermelon seed and mung bean proteins prior to enzyme hydrolysis enhanced the hydrophobicity and release of peptides with terminal aromatic amino acids all of which influenced their stability, bioavailability, and bioactivity (such as radical scavenging and antihypertensive properties (Qichen Jiang et al., 2021; Wen et al., 2020; Xie et al., 2020). On the other hand, the use of procedures such as microwaves and other thermal techniques for peptide preparation have been shown to affect the functionality of the peptides, by changing the native physicochemical properties of the peptide which affects their stability and bioactivities (Hunsakul et al., 2021). Considering the better functionality of low molecular weight peptides, a combination of two or more hydrolyzing enzymes is likely to generate shorter chain peptides compared to using one enzyme (Aluko, 2015). For instance, the ACE-inhibitory activities of peptides generated from hard-to-cook bean protein hydrolysate generated with a combination of alcalase and flavourzyme were shown to be higher compared to the use of the enzymes separately (Ruiz-Ruiz et al., 2013). In another study, skimmed buffalo milk protein was hydrolyzed using papain, pepsin or trypsin, and it was observed that hydrolysate-generated with papain exhibited highest ACE-inhibitory and radical scavenging activities (Abdel-Hamid et al., 2017). Similarly, a study hydrolyzed spent hen muscle protein using Protex 26L, pepsin, and thermoase and compared the transepithelial transport and multifunctionality of the hydrolysates and using *in vitro* and *in vivo* models (Fan et al., 2020). The hydrolysates generated showed ACE inhibitory, antioxidant, and anti-inflammatory activities; however, only thermoase-generated hydrolysate (TGH) upregulated ACE2 gene expression. Additionally, it is only TGH that resisted hydrolysis in simulation study using Caco-2 cells. Notably, the enhanced ACE2 gene expression, antioxidant and anti-inflammatory activities post-absorption across the caco-2 monolayer suggests the involvement of

metabolites of the parent peptides. Furthermore, the intragastric ingestion of all the hydrolysates at 1 g/kg demonstrated that only TGH reduced blood pressure in SHR, indicating that the mechanisms of blood pressure lowering involves the upregulation of ACE2 and inhibition of ACE. Therefore, food protein scientists and nutraceutical developers are advised to adopt protein hydrolysis procedures with minimal effect on the native conformation of the peptide in order to conserve their functionality.

Limitations of current research and future research directions

Several studies have been conducted with the aim of uncovering the antihypertensive properties of food protein-based peptides. However, the majority of the studies explored the inhibitory effects of the peptides against ACE and renin activities *in vitro*, while quite a few studies assessed the effects of protein hydrolysates and their peptides on Ang II receptors. In general, a greater number of research efforts in discovering clinically-effective food protein-derived peptides for hypertension focused on RAS. Additionally, among the *in vivo* using experimentally-induced hypertensive and SHR rats, suppression of SBP and/or DBP were recorded without uncovering the molecular mechanisms such as analysis of expression profile of genes that regulate molecular pathways implicated in hypertension.

Future research should not only confirm if all the ACE and renin-inhibitory peptides can lower blood pressure when ingested by normal and experimental models of hypertension, and if active, the molecular mechanism of blood pressure-lowering should be investigated. In addition, the use of multiple enzymes for protein hydrolysis during peptide preparation have been shown to generate low molecular weight peptides which are more biostable and easily permeate the intestinal membrane compared to high molecular weight peptides. Despite the strong scientific evidence on a number of food-based peptides with antihypertensive potentials, quite a few clinical trials on the application of food protein-derived peptides for managing hypertension have been recently reported. Worthy of mention among the bioactive proteins and their peptides under clinical trial are Amaranth hydrolysate-enriched beverages (Valdez-Meza et al., 2019) that was shown to suppress blood pressure after three hours of ingestion. The blood pressure lowering activity was maintained till nine hours post-ingestion, agreeing with the report that Amaranth-derived AHPs are biostable (Espinosa-Hernández et al., 2019). More clinical trials are

encouraged especially for peptides sourced from marine organisms, and seeds of soybean and lupin whose multifunctional properties in relation to hypertension have been well characterized.

Finally, the current knowledge on structural-activity relationship of peptides in relation to antihypertensive properties are not well-defined. Mechanistic studies are needed to clarify the structural requirements needed for a peptide to exert antihypertensive properties, especially after oral ingestion. This may involve the use of sequential hydrolysis to determine the specific amino acids and/or amino acid sequence requirements. Additionally, chemical modification of functional group(s) of amino acids in AHPs may underscore the specific functional group(s) involved in antihypertensive activities of the peptides.

Conclusions

Hypertension has continued to remain a great burden to global health, and a "silent killer". Hence, serious research efforts are ongoing to improve the current available strategies for the prevention and management of the diseases. In this study, we have discussed molecular mechanisms of antihypertensive properties of protein hydrolysates and their peptides other than RAS, including induction of vasodilation via upregulation of expression of eNOS, COX and prostaglandin receptor genes. We also have proposed some emerging mechanisms through which these bioactive peptides may have exerted their antihypertensive properties such as modulation of inflammation signaling, Keap-1/Nrf-2 and related antioxidative signaling, and PPAR-γ/caspase3/MAPK signaling pathways, blockade of L-type Ca^{2+} channel and inhibition of lipid accumulation. We have also briefly discussed factors that influence the biostability, transepithelial transport, bioavailability and activities of AHPs. It is our hope that this review has thrown more light into the current understanding of how newly isolated novel peptides lower blood pressure and by extension, position these AHPs as promising candidates for functional food development for hypertension.

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References

535 Abachi, S., Bazinet, L., & Beaulieu, L. (2019). Antihypertensive and Angiotensin-I-Converting
536 Enzyme (ACE)-Inhibitory Peptides from Fish as Potential Cardioprotective Compounds.
537 *Marine Drugs*, *17*(11). https://doi.org/10.3390/MD17110613

538 Abdel-Hamid, M., Otte, J., De Gobba, C., Osman, A., & Hamad, E. (2017). Angiotensin I-
539 converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived
540 from enzymatic hydrolysis of buffalo milk proteins. *International Dairy Journal*, *66*, 91–98.
541 https://doi.org/10.1016/J.IDAIRYJ.2016.11.006

542 Adinkrah, E., Bazargan, M., Wisseh, C., & Assari, S. (2020). *Adherence to Hypertension*
543 *Medications and Lifestyle Recommendations among Underserved African American*
544 *Middle-Aged and Older Adults*. 1–15.

545 Adjonu, R., Doran, G., Torley, P., & Agboola, S. (2013). Screening of whey protein isolate
546 hydrolysates for their dual functionality: Influence of heat pre-treatment and enzyme
547 specificity. *Food Chemistry*, *136*(3–4), 1435–1443.
548 https://doi.org/10.1016/J.FOODCHEM.2012.09.053

549 Ahmed, S. M. U., Luo, L., Namani, A., Wang, X. J., & Tang, X. (2017). Nrf2 signaling pathway:
550 Pivotal roles in inflammation. *Biochimica et Biophysica Acta - Molecular Basis of Disease*,
551 *1863*(2), 585–597. https://doi.org/10.1016/j.bbadis.2016.11.005

552 Aluko, R. (2015). Antihypertensive peptides from food proteins. *Annual Review of Food Science*
553 *and Technology*, *6*, 235–262. https://doi.org/10.1146/ANNUREV-FOOD-022814-015520

554 Angeli, F., Reboldi, G., & Verdecchia, P. (2021). The Link Between Inflammation and
555 Hypertension: Unmasking Mediators. *American Journal of Hypertension*, *34*(7), 683–685.
556 https://doi.org/10.1093/AJH/HPAB034

557 Ayoade, O. G., Umoh, I., & Amadi, C. (2020). Dyslipidemia and Associated Risk Factors among
558 Nigerians with Hypertension. *Dubai Medical Journal*, *3*(4), 155–161.
559 https://doi.org/10.1159/000509570

560 Boegh, M., & Nielsen, H. M. (2015). Mucus as a barrier to drug delivery - Understanding and
561 mimicking the barrier properties. *Basic and Clinical Pharmacology and Toxicology*, *116*(3),
562 179–186. https://doi.org/10.1111/bcpt.12342

563 Boonla, O., Kukongviriyapan, U., Pakdeechote, P., Kukongviriyapan, V., Pannangpetch, P., &
564 Thawornchinsombut, S. (2015). Peptides-derived from Thai rice bran improves endothelial
565 function in 2K-1C renovascular hypertensive rats. *Nutrients*, *7*(7), 5783–5799.
566 https://doi.org/10.3390/nu7075252

567 Bravo, F. I., Mas-Capdevila, A., Margalef, M., Arola-Arnal, A., & Muguerza, B. (2019). Novel
568 Antihypertensive Peptides Derived from Chicken Foot Proteins. *Molecular Nutrition &*
569 *Food Research*, *63*(12), 1801176. https://doi.org/10.1002/MNFR.201801176

570 Chamata, Y., Watson, K. A., & Jauregi, P. (2020). Whey-derived peptides interactions with ace

by molecular docking as a potential predictive tool of natural ace inhibitors. *International Journal of Molecular Sciences*, *21*(3), 1–14. https://doi.org/10.3390/ijms21030864

Chen, J., Ryu, B., Zhang, Y. Y., Liang, P., Li, C., Zhou, C., Yang, P., Hong, P., & Qian, Z. J. (2020). Comparison of an angiotensin-I-converting enzyme inhibitory peptide from tilapia (Oreochromis niloticus) with captopril: inhibition kinetics, in vivo effect, simulated gastrointestinal digestion and a molecular docking study. *Journal of the Science of Food and Agriculture*, *100*(1), 315–324. https://doi.org/10.1002/jsfa.10041

Chung, S., Chung, M. ., Choi, H. ., Park, J. ., Hwang, J. ., & Joung, H. (2020). Animal Protein Intake Is Positively Associated with Metabolic Syndrome Risk Factors in Middle-Aged Korean Men. *Nutrients*, *12*(11), 1–15. https://doi.org/10.3390/NU12113415

Cicero, A. F. G., Fogacci, F., & Colletti, A. (2017). Potential role of bioactive peptides in prevention and treatment of chronic diseases: a narrative review. *British Journal of Pharmacology*, *174*(11), 1378–1394. https://doi.org/10.1111/bph.13608

Da Costa, R. M., Rodrigues, D., Pereira, C. A., Silva, J. F., Alves, J. V., Lobato, N. S., & Tostes, R. C. (2019). Nrf2 as a potential mediator of cardiovascular risk in metabolic diseases. *Frontiers in Pharmacology*, *10*(APR), 1–12. https://doi.org/10.3389/fphar.2019.00382

Daiber, A., Steven, S., Vujacic-Mirski, K., Kalinovic, S., Oelze, M., Lisa, F. Di, & Münzel, T. (2020). Regulation of Vascular Function and Inflammation via Cross Talk of Reactive Oxygen and Nitrogen Species from Mitochondria or NADPH Oxidase—Implications for Diabetes Progression. *International Journal of Molecular Sciences 2020, Vol. 21, Page 3405*, *21*(10), 3405. https://doi.org/10.3390/IJMS21103405

Dey, T. K., Chatterjee, R., Mandal, R. S., Roychoudhury, A., Paul, D., Roy, S., Pateiro, M., Das, A. K., Lorenzo, J. M., & Dhar, P. (2021). ACE Inhibitory Peptides from Bellamya bengalensis Protein Hydrolysates: In Vitro and In Silico Molecular Assessment. *Processes 2021, Vol. 9, Page 1316*, *9*(8), 1316. https://doi.org/10.3390/PR9081316

Faki, Y., & Er, A. (2020). *Open Access Rambam Maimonides Medical Journal Different Chemical Structures and Physiological/Pathological Roles of Cyclooxygenases*. https://doi.org/10.5041/RMMJ.10426

Fan, H., & Wu, J. (2020). Purification and identification of novel ACE inhibitory and ACE2 upregulating peptides from spent hen muscle proteins. *Food Chemistry*, *345*, 128867. https://doi.org/10.1016/j.foodchem.2020.128867

Fan, H., Yu, W., Liao, W., & Wu, J. (2020). Spent hen protein hydrolysate with good gastrointestinal stability and permeability in caco-2 cells shows antihypertensive activity in SHR. *Foods*, *9*(10). https://doi.org/10.3390/foods9101384

Franca-Oliveira, G., Fornari, T., & Hernández-Ledesma, B. (2021). A review on the extraction and processing of natural source-derived proteins through eco-innovative approaches. *Processes*, *9*(9). https://doi.org/10.3390/pr9091626

608 Gazme, B., Boachie, R. T., Tsopmo, A., & Udenigwe, C. C. (2019). Occurrence, properties and
609 biological significance of pyroglutamyl peptides derived from different food sources. *Food*
610 *Science and Human Wellness*, *8*(3), 268–274. https://doi.org/10.1016/J.FSHW.2019.05.002

611 Ghatage, T., Goyal, S. ., Dhar, A., & Bhat, A. (2021). Novel therapeutics for the treatment of
612 hypertension and its associated complications: peptide- and nonpeptide-based strategies.
613 *Hypertension Research : Official Journal of the Japanese Society of Hypertension*, *44*(7),
614 740–755. https://doi.org/10.1038/S41440-021-00643-Z

615 Girgih, A. T., Nwachukwu, I. D., Hasan, F., Fagbemi, T. N., Gill, T., & Aluko, R. E. (2015).
616 Kinetics of the inhibition of renin and angiotensin I-converting enzyme by cod (Gadus
617 morhua) protein hydrolysates and their antihypertensive effects in spontaneously
618 hypertensive rats. *Food and Nutrition Research*, *59*, 29788.
619 https://doi.org/10.3402/fnr.v59.29788

620 Girgih, A. T., Udenigwe, C. C., & Aluko, R. E. (2013). *Reverse-phase HPLC Separation of*
621 *Hemp Seed (Cannabis sativa L .) Protein Hydrolysate Produced Peptide Fractions with*
622 *Enhanced Antioxidant Capacity*. https://doi.org/10.1007/s11130-013-0340-6

623 Griendling, K. K., Camargo, L. L., Rios, F. J., Alves-Lopes, R., Montezano, A. C., & Touyz, R.
624 M. (2021). Oxidative Stress and Hypertension. *Circulation Research*, 993–1020.
625 https://doi.org/10.1161/CIRCRESAHA.121.318063

626 Guo, H., Hao, Y., Richel, A., Everaert, N., Chen, Y., Liu, M., Yang, X., & Ren, G. (2020).
627 Antihypertensive effect of quinoa protein under simulated gastrointestinal digestion and
628 peptide characterization. *Journal of the Science of Food and Agriculture*, *100*(15), 5569–
629 5576. https://doi.org/10.1002/JSFA.10609

630 Hanafi, M. A., Hashim, S. N., Chay, S. Y., Ebrahimpour, A., Zarei, M., Muhammad, K., Abdul-
631 Hamid, A., & Saari, N. (2018). High angiotensin-I converting enzyme (ACE) inhibitory
632 activity of Alcalase-digested green soybean (Glycine max) hydrolysates. *Food Research*
633 *International*, *106*(September 2017), 589–597.
634 https://doi.org/10.1016/j.foodres.2018.01.030

635 Hanari, M. A., Hashim, S. N., Chay, S. Y., Ebrahimpour, A., Zarei, M., Mohammed, K., Abdul-
636 Hamid, A., & Saari, N. (2018). High angiotensin-I converting enzyme (ACE) inhibitory
637 activity of Alcalase- digested green soybean (Glycine max) hydrolysates. *Food Research*
638 *International*, *106*(January), 589–597. https://doi.org/10.1016/j.foodres.2018.01.030

639 He, R., Alashi, A., Malomo, S. A., Girgih, A. T., Chao, D., Ju, X., & Aluko, R. E. (2013).
640 Antihypertensive and free radical scavenging properties of enzymatic rapeseed protein
641 hydrolysates. *Food Chemistry*, *141*(1), 153–159.
642 https://doi.org/10.1016/J.FOODCHEM.2013.02.087

643 He, Z., Liu, G., Qiao, Z., Cao, Y., & Song, M. (2021). Novel Angiotensin-I Converting Enzyme
644 Inhibitory Peptides Isolated From Rice Wine Lees: Purification, Characterization, and
645 Structure-Activity Relationship. *Frontiers in Nutrition*, *8*, 646.

https://doi.org/10.3389/FNUT.2021.746113/BIBTEX

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*(1), 23–35. https://doi.org/10.1016/J.CIS.2010.11.001

Herrera-Chalé, F., Ruiz-Ruiz, J. C., Betancur-Ancona, D., & Segura-Campos, M. R. (2016). Potential therapeutic applications of mucuna pruriens peptide fractions purified by high-performance liquid chromatography as angiotensin-converting enzyme inhibitors, antioxidants, antithrombotic and hypocholesterolemic agents. *Journal of Medicinal Food*, *19*(2), 187–195. https://doi.org/10.1089/jmf.2015.0098

Hunsakul, K., Laokuldilok, T., Prinyawiwatkul, W., & Utama-ang, N. (2021). Effects of thermal processing on antioxidant activities, amino acid composition and protein molecular weight distributions of jasmine rice bran protein hydrolysate. *International Journal of Food Science & Technology*, *56*(7), 3289–3298. https://doi.org/10.1111/IJFS.15028

Ikarashi, N., Toda, T., Hatakeyama, Y., Kusunoki, Y., Kon, R., Mizukami, N., M, K., S, O., & K, S. (2018). Anti-Hypertensive Effects of Acacia Polyphenol in Spontaneously Hypertensive Rats. *International Journal of Molecular Sciences*, *19*(3). https://doi.org/10.3390/IJMS19030700

Jahandideh, F., Chakrabarti, S., Davidge, S., & Wu, J. (2017). Egg white hydrolysate shows insulin mimetic and sensitizing effects in 3T3-F442A pre-adipocytes. *PloS One*, *12*(10). https://doi.org/10.1371/JOURNAL.PONE.0185653

Jahandideh, F., De Campos Zani, S. C., Son, M., Proctor, S. D., Davidge, S. T., Chan, C. B., & Wu, J. (2019). Egg white hydrolysate enhances insulin sensitivity in high-fat diet-induced insulin-resistant rats via Akt activation. *British Journal of Nutrition*, *122*(1), 14–24. https://doi.org/10.1017/S0007114519000837

Jang, Y., Kim, M., & Hwang, S. W. (2020). Molecular mechanisms underlying the actions of arachidonic acid-derived prostaglandins on peripheral nociception. *Journal of Neuroinflammation 2020 17:1*, *17*(1), 1–27. https://doi.org/10.1186/S12974-020-1703-1

Jiang, Q, Chen, Q., Zhang, T., Liu, M., Duan, S., & Sun, X. (2021). The Antihypertensive Effects and Potential Molecular Mechanism of Microalgal Angiotensin I-Converting Enzyme Inhibitor-Like Peptides: A Mini Review. *International Journal of Molecular Sciences*, *22*(8). https://doi.org/10.3390/IJMS22084068

Jiang, Qichen, Chen, Q., Zhang, T., Liu, M., Duan, S., & Sun, X. (2021). The antihypertensive effects and potential molecular mechanism of microalgal angiotensin i-converting enzyme inhibitor-like peptides: A mini review. *International Journal of Molecular Sciences*, *22*(8). https://doi.org/10.3390/ijms22084068

Jiang, X., Pan, D., Zhang, T., Liu, C., Zhang, J., Su, M., Wu, Z., Zeng, X., Sun, Y., & Guo, Y. (2020). Novel milk casein–derived peptides decrease cholesterol micellar solubility and

683 cholesterol intestinal absorption in Caco-2 cells. *Journal of Dairy Science*, *103*(5), 3924–
684 3936. https://doi.org/10.3168/JDS.2019-17586

685 Jogi, N., Yathisha, U. G., Bhat, I., & Mamatha, B. S. (2021). Antihypertensive activity of orally
686 consumed ACE-I inhibitory peptides. *Https://Doi.Org/10.1080/10408398.2021.1938508*.
687 https://doi.org/10.1080/10408398.2021.1938508

688 Johnson, M. T., Gudlur, A., Zhang, X., Xin, P., Emrich, S. M., Yoast, R. E., Courjaret, R.,
689 Nwokonko, R. M., Li, W., Hempel, N., Machaca, K., Gill, D. L., Hogan, P. G., & Trebak,
690 M. (2020). L-type Ca2+ channel blockers promote vascular remodeling through activation
691 of STIM proteins. *Proceedings of the National Academy of Sciences of the United States of*
692 *America*, *117*(29), 17369–17380. https://doi.org/10.1073/pnas.2007598117

693 Karami, Z., & Akbari-adergani, B. (2019). Bioactive food derived peptides: a review on
694 correlation between structure of bioactive peptides and their functional properties. *Journal*
695 *of Food Science and Technology*, *56*(2), 535. https://doi.org/10.1007/S13197-018-3549-4

696 Kaur, A., Kehinde, B., Sharma, P., Sharma, D., & Kaur, S. (2021). Recently isolated food-
697 derived antihypertensive hydrolysates and peptides: A review. *Food Chemistry*, *346*.
698 https://doi.org/10.1016/J.FOODCHEM.2020.128719

699 Kim, K., Hwang, E., Kim, M., Park, J., & Kim, D. (2020). Antihypertensive Effects of
700 Polyphenolic Extract from Korean Red Pine (Pinus densiflora Sieb. et Zucc.) Bark in
701 Spontaneously Hypertensive Rats. *Antioxidants (Basel, Switzerland)*, *9*(4).
702 https://doi.org/10.3390/ANTIOX9040333

703 Lammi, C., Aiello, G., Bollati, C., Li, J., Bartolomei, M., Ranaldi, G., Ferruzza, S., Fassi, E. M.
704 A., Grazioso, G., Sambuy, Y., & Arnoldi, A. (2021). Trans-epithelial transport, metabolism
705 and biological activity assessment of the multi-target lupin peptide lilpkhsdad (P5) and its
706 metabolite lpkhsdad (p5-met). *Nutrients*, *13*(3), 1–17. https://doi.org/10.3390/nu13030863

707 Lammi, C., Aiello, G., Dellafiora, L., Bollati, C., Boschin, G., Ranaldi, G., Ferruzza, S., Sambuy,
708 Y., Galaverna, G., & Arnoldi, A. (2020). Assessment of the Multifunctional Behavior of
709 Lupin Peptide P7 and Its Metabolite Using an Integrated Strategy. *Journal of Agricultural*
710 *and Food Chemistry*, *68*(46), 13179–13188. https://doi.org/10.1021/acs.jafc.0c00130

711 Lammi, C., Bollati, C., Lecca, D., Abbracchio, M. P., & Arnoldi, A. (2019). Lupin Peptide T9
712 (GQEQSHQDEGVIVR) Modulates the Mutant PCSK9D374Y Pathway: in vitro
713 Characterization of its Dual Hypocholesterolemic Behavior. *Nutrients 2019, Vol. 11, Page*
714 *1665*, *11*(7), 1665. https://doi.org/10.3390/NU11071665

715 Lammi, C., Zanoni, C., Arnoldi, A., & Aiello, G. (2018). YDFYPSSTKDQQS (P3), a peptide
716 from lupin protein, absorbed by Caco-2 cells, modulates cholesterol metabolism in HepG2
717 cells via SREBP-1 activation. *Journal of Food Biochemistry*, *42*(3), 1–8.
718 https://doi.org/10.1111/jfbc.12524

719 Lammi, C., Zanoni, C., Calabresi, L., & Arnoldi, A. (2016). Lupin protein exerts cholesterol-

lowering effects targeting PCSK9: From clinical evidences to elucidation of the in vitro molecular mechanism using HepG2 cells. *Journal of Functional Foods*, *23*, 230–240. https://doi.org/10.1016/j.jff.2016.02.042

Lammi, C., Zanoni, C., Ferruzza, S., Ranaldi, G., Sambuy, Y., & Arnoldi, A. (2016). Hypocholesterolaemic activity of lupin peptides: Investigation on the crosstalk between human enterocytes and hepatocytes using a co-culture system including Caco-2 and HepG2 cells. *Nutrients*, *8*(7). https://doi.org/10.3390/nu8070437

Leoncini, G., Viazzi, F., S, D. C., G, R., P, F., & R, P. (2020). Blood pressure reduction and RAAS inhibition in diabetic kidney disease: therapeutic potentials and limitations. *Journal of Nephrology*, *33*(5), 949–963. https://doi.org/10.1007/S40620-020-00803-3

Li, Q., Yon, J.-Y., & Cai, H. (2015). Mechanisms and Consequences of eNOS Dysfunction in Hypertension. *Journal of Hypertension*, *33*(6), 1128. https://doi.org/10.1097/HJH.0000000000000587

Liao, W., Chakrabarti, S., Davidge, S. T., & Wu, J. (2016). Modulatory Effects of Egg White Ovotransferrin-Derived Tripeptide IRW (Ile-Arg-Trp) on Vascular Smooth Muscle Cells against Angiotensin II Stimulation. *Journal of Agricultural and Food Chemistry*, *64*(39), 7342–7347. https://doi.org/10.1021/ACS.JAFC.6B03513

Liao, W., Fan, H., Liu, P., & Wu, J. (2019). Identification of angiotensin converting enzyme 2 (ACE2) up-regulating peptides from pea protein hydrolysate. *Journal of Functional Foods*, *60*, 103395. https://doi.org/10.1016/J.JFF.2019.05.051

Lin, Y., Chen, C., Tsai, J., & Chen, G. (2019). Preparation and Identification of Novel Antihypertensive Peptides from the In Vitro Gastrointestinal Digestion of Marine Cobia Skin Hydrolysates. *Nutrients*, *11*(6). https://doi.org/10.3390/NU11061351

Lin, Y. H., Chen, G. W., Yeh, C. H., Song, H., & Tsai, J. S. (2018). Purification and identification of angiotensin I-Converting enzyme inhibitory peptides and the antihypertensive effect of chlorella sorokiniana protein hydrolysates. *Nutrients*, *10*(10). https://doi.org/10.3390/nu10101397

Liu, P., Lan, X., Yaseen, M., Wu, S., Feng, X., Zhou, L., Sun, J., Liao, A., Liao, D., & Sun, L. (2019). Purification, Characterization and Evaluation of Inhibitory Mechanism of ACE Inhibitory Peptides from Pearl Oyster (Pinctada fucata martensii) Meat Protein Hydrolysate. *Marine Drugs*, *17*(8). https://doi.org/10.3390/md17080463

Liu, W. Y., Zhang, J. T., Miyakawa, T., Li, G. M., Gu, R. Z., & Tanokura, M. (2021). Antioxidant properties and inhibition of angiotensin-converting enzyme by highly active peptides from wheat gluten. *Scientific Reports*, *11*(1). https://doi.org/10.1038/S41598-021-84820-7

Liu, X., Li, G., Wang, H., Qin, N., Guo, L., Wang, X., & Shen, S. (2021). Areca Nut Kernel Globulin Hydrolysates. *Molecules*, *26*, 3308.

Louca, P., Mompeo, O., Leeming, E., Berry, S., Mangino, M., Spector, T., S, P., & C, M. (2020). Dietary Influence on Systolic and Diastolic Blood Pressure in the TwinsUK Cohort. *Nutrients*, *12*(7), 1–12. https://doi.org/10.3390/NU12072130

Lu, Y., Wang, Y., Huang, D., Bian, Z., Lu, P., Fan, D., & Wang, X. (2021). Inhibitory mechanism of angiotensin-converting enzyme inhibitory peptides from black tea. *Journal of Zhejiang University-SCIENCE B (Biomedical & Biotechnology)*, *22*(7), 575–589.

Ma, F., Wang, H., Wei, C., Thakur, K., Wei, Z., & Jiang, L. (2019). Three Novel ACE Inhibitory Peptides Isolated From Ginkgo biloba Seeds: Purification, Inhibitory Kinetic and Mechanism. *Frontiers in Pharmacology*, *9*, 1579. https://doi.org/10.3389/FPHAR.2018.01579

Mahmudpour, M., Roozbeh, J., Keshavarz, M., Farrokhi, S., & Nabipour, I. (2020). COVID-19 cytokine storm: The anger of inflammation. *Cytokine*, *133*, 155151. https://doi.org/10.1016/J.CYTO.2020.155151

Majumder, K., Liang, G., Chen, Y., Guan, L., Davidge, S., & Wu, J. (2015). Egg ovotransferrin-derived ACE inhibitory peptide IRW increases ACE2 but decreases proinflammatory genes expression in mesenteric artery of spontaneously hypertensive rats. *Molecular Nutrition & Food Research*, *59*(9), 1735–1744. https://doi.org/10.1002/MNFR.201500050

Manual-Kollareth, D. J., Chang, C. L., Zirpoli, H., & Deckelbaum, R. J. (2020). Molecular mechanisms underlying effects of n−3 and n−6 fatty acids in cardiovascular diseases. *Lipid Signaling and Metabolism*, 427–453. https://doi.org/10.1016/B978-0-12-819404-1.00021-X

Martínez-Maqueda, D., Miralles, B., Recio, I., & Hernández-Ledesma, B. (2012). Antihypertensive peptides from food proteins: A review. *Food and Function*, *3*(4), 350–361. https://doi.org/10.1039/C2FO10192K

Mas-Capdevila, A., Pons, Z., Aleixandre, A., Bravo, F. I., & Muguerza, B. (2018). Dose-Related Antihypertensive Properties and the Corresponding Mechanisms of a Chicken Foot Hydrolysate in Hypertensive Rats. *Nutrients 2018, Vol. 10, Page 1295*, *10*(9), 1295. https://doi.org/10.3390/NU10091295

Medvedev, R. Y., Sanchez-Alonso, J. L., Mansfield, C. A., Judina, A., Francis, A. J., Pagiatakis, C., Trayanova, N., Glukhov, A. V., Miragoli, M., Faggian, G., & Gorelik, J. (2021). Local hyperactivation of L-type Ca2+ channels increases spontaneous Ca2+ release activity and cellular hypertrophy in right ventricular myocytes from heart failure rats. *Scientific Reports*, *11*(1), 1–9. https://doi.org/10.1038/s41598-021-84275-w

Mehrabani, S., Asemi, M., Najafian, J., Sajjadi, F., Maghroun, M., & Mohammadifard, N. (2017). *Association of Animal and Plant Proteins Intake with Hypertension in Iranian Adult Population : Isfahan Healthy Heart Program*. https://doi.org/10.4103/2277-9175.213877

Messina, C. M., Manuguerra, S., Arena, R., Renda, G., Ficano, G., Randazzo, M., Fricano, S., Sadok, S., & Santulli, A. (2021). In Vitro Bioactivity of Astaxanthin and Peptides from

794 Hydrolisates of Shrimp (Parapenaeus longirostris) By-Products: From the Extraction
795 Process to Biological Effect Evaluation, as Pilot Actions for the Strategy "From Waste to
796 Profit." *Marine Drugs*, *19*(4), 1–18. https://doi.org/10.3390/md19040216

797 Metchi Donfack, M. ., Atsamo, A. ., Temdié Guemmogne, R. ., Kenfack Ngouateu, O. .,
798 Dongmo, A., & Dimo, T. (2021). Antihypertensive Effects of the Vitex cienkowskii
799 (Verbenaceae) Stem-Bark Extract on L-NAME-Induced Hypertensive Rats. *Evidence-*
800 *Based Complementary and Alternative Medicine : ECAM*, *2021*.
801 https://doi.org/10.1155/2021/6668919

802 Mitchell, J. A., Kirkby, N. S., Ahmetaj-Shala, B., Armstrong, P. C., Crescente, M., Ferreira, P.,
803 Lopes Pires, M. E., Vaja, R., & Warner, T. D. (2021). Cyclooxygenases and the
804 cardiovascular system. *Pharmacology and Therapeutics*, *217*.
805 https://doi.org/10.1016/J.PHARMTHERA.2020.107624

806 Mudgil, P., Baby, B., Ngoh, Y. Y., Kamal, H., Vijayan, R., Gan, C. Y., & Maqsood, S. (2019).
807 Molecular binding mechanism and identification of novel anti-hypertensive and anti-
808 inflammatory bioactive peptides from camel milk protein hydrolysates. *Lwt*, *112*(January),
809 108193. https://doi.org/10.1016/j.lwt.2019.05.091

810 Nardo, A. E., Suárez, S., Quiroga, A. V., & Añón, M. C. (2020). Amaranth as a Source of
811 Antihypertensive Peptides. *Frontiers in Plant Science*, *11*(September), 1–15.
812 https://doi.org/10.3389/fpls.2020.578631

813 Ngo, D. H., Kang, K. H., Ryu, B., Vo, T. S., Jung, W. K., Byun, H. G., & Kim, S. K. (2015).
814 Angiotensin-I converting enzyme inhibitory peptides from antihypertensive skate
815 (Okamejei kenojei) skin gelatin hydrolysate in spontaneously hypertensive rats. *Food*
816 *Chemistry*, *174*, 37–43. https://doi.org/10.1016/j.foodchem.2014.11.013

817 Oh, J.-Y., Je, J.-G., Lee, H.-G., Kim, E.-A., Kang, S. I., Lee, J.-S., & Jeon, Y.-J. (2020). Anti-
818 Hypertensive Activity of Novel Peptides Identified from Olive Flounder (Paralichthys
819 olivaceus) Surimi. *Foods*, *9*(5). https://doi.org/10.3390/FOODS9050647

820 Oh, J. Y., Je, J. G., Lee, H. G., Kim, E. A., Kang, S. I., Lee, J. S., & Jeon, Y. J. (2020). Anti-
821 hypertensive activity of novel peptides identified from olive flounder (paralichthys
822 olivaceus) surimi. *Foods*, *9*(5). https://doi.org/10.3390/foods9050647

823 Ozen, G., & Norel, X. (2017). Prostanoids in the pathophysiology of human coronary artery.
824 *Prostaglandins and Other Lipid Mediators*, *133*, 20–28.
825 https://doi.org/10.1016/J.PROSTAGLANDINS.2017.03.003

826 Ozorio, L., Mellinger-Silva, C., Cabral, L. M. C., Jardin, J., Boudry, G., & Dupont, D. (2020).
827 The influence of peptidases in intestinal brush border membranes on the absorption of
828 oligopeptides from whey protein hydrolysate: An ex vivo study using an ussing chamber.
829 *Foods*, *9*(10). https://doi.org/10.3390/foods9101415

830 Pajares, M., Jiménez-Moreno, N., García-Yagüe, A., Escoll, M., de Ceballos, M., Van Leuven,

F., Rábano, A., Yamamoto, M., Rojo, A., & Cuadrado, A. (2016). Transcription factor NFE2L2/NRF2 is a regulator of macroautophagy genes. *Autophagy*, *12*(10), 1902–1916. https://doi.org/10.1080/15548627.2016.1208889

Praveesh, B. V., Angayarkanni, J., & Palaniswamy, M. (2011). Antihypertensive and anticancer effect of cow milk fermented by Lactobacillus plantarum and Lactobacillus casei. *International Journal of Pharmacy and Pharmaceutical Sciences*, *3*(SUPPL. 5), 452–456.

Querobino, S., Costa, M., & Alberto-Silva, C. (2019). Protective effects of distinct proline-rich oligopeptides from B. jararaca snake venom against oxidative stress-induced neurotoxicity. *Toxicon : Official Journal of the International Society on Toxinology*, *167*, 29–37. https://doi.org/10.1016/J.TOXICON.2019.06.012

Quesada, O., Claggett, B., Rodriguez, F., Cai, J., Moncrieft, A. E., Garcia, K., Rivera, M. D. R., Hanna, D. B., Daviglus, M. L., Talavera, G. A., Merz, C. N. B., Solomon, S. D., Cheng, S., & Bello, N. A. (2021). Associations of Insulin Resistance With Systolic and Diastolic Blood Pressure: A Study From the HCHS/SOL. *Hypertension*, *78*(3), 716–725. https://doi.org/10.1161/HYPERTENSIONAHA.120.16905

Rojas-Humpire, R., Olarte-Durand, M., Medina-Ramirez, S., Gutierrez-Ajalcriña, R., Canaza, J. F., & Huancahuire-Vega, S. (2021). Insulin resistance indexes as biomarkers of lifetime cardiovascular risk among adults from Peru. *Journal of Nutrition and Metabolism*, *2021*. https://doi.org/10.1155/2021/6633700

Ruiz-Ruiz, J., Dávila-Ortíz, G., Chel-Guerrero, L., & Betancur-Ancona, D. (2013). Angiotensin I-converting enzyme inhibitory and antioxidant peptide fractions from hard-to-cook bean enzymatic hydrolysates. *Journal of Food Biochemistry*, *37*(1), 26–35. https://doi.org/10.1111/J.1745-4514.2011.00594.X

Saha, S., Buttari, B., Panieri, E., Profumo, E., & Saso, L. (2020). An Overview of Nrf2 Signaling Pathway and Its Role in Inflammation. *Molecules 2020, Vol. 25, Page 5474*, *25*(22), 5474. https://doi.org/10.3390/MOLECULES25225474

Serafini, M. M., Catanzaro, M., Fagiani, F., Simoni, E., Caporaso, R., Dacrema, M., Romanoni, I., Govoni, S., Racchi, M., Daglia, M., Rosini, M., & Lanni, C. (2020). Modulation of Keap1/Nrf2/ARE signaling pathway by curcuma- And garlic-derived hybrids. *Frontiers in Pharmacology*, *10*(January), 1–12. https://doi.org/10.3389/fphar.2019.01597

Soleymanzadeh, N., Mirdamadi, S., Mirzaei, M., & Kianirad, M. (2019). Novel β-casein derived antioxidant and ACE-inhibitory active peptide from camel milk fermented by Leuconostoc lactis PTCC1899: Identification and molecular docking. *International Dairy Journal*, *97*, 201–208. https://doi.org/10.1016/j.idairyj.2019.05.012

Song, Y., Yu, J., Song, J., Wang, S., Cao, T., Liu, Z., Gao, X., & Wei, Y. (2021). The antihypertensive effect and mechanisms of bioactive peptides from Ruditapes philippinarum fermented with Bacillus natto in spontaneously hypertensive rats. *Journal of Functional Foods*, *79*, 104411. https://doi.org/10.1016/J.JFF.2021.104411

869 Stump, M., Mukohda, M., Hu, C., & Sigmund, C. (2015). PPARγ Regulation in Hypertension
870 and Metabolic Syndrome. *Current Hypertension Reports*, *17*(12).
871 https://doi.org/10.1007/S11906-015-0601-X

872 Sun, D., Zhou, T., Li, X., Heianza, Y., Liang, Z., Bray, G., Sacks, F., & Qi, L. (2019). Genetic
873 Susceptibility, Dietary Protein Intake, and Changes of Blood Pressure: The POUNDS Lost
874 Trial. *Hypertension (Dallas, Tex. : 1979)*, *74*(6), 1460–1467.
875 https://doi.org/10.1161/HYPERTENSIONAHA.119.13510

876 Sun, X., Acquah, C., Aluko, R. E., & Udenigwe, C. C. (2020). Considering food matrix and
877 gastrointestinal effects in enhancing bioactive peptide absorption and bioavailability.
878 *Journal of Functional Foods*, *64*(October 2019), 103680.
879 https://doi.org/10.1016/j.jff.2019.103680

880 Sun, X., & Udenigwe, C. C. (2020). Chemistry and Biofunctional Significance of Bioactive
881 Peptide Interactions with Food and Gut Components. *Journal of Agricultural and Food*
882 *Chemistry*, *68*(46), 12972–12977. https://doi.org/10.1021/acs.jafc.9b07559

883 Tanase, D. M., Gosav, E. M., Radu, S., Ouatu, A., Rezus, C., Ciocoiu, M., Costea, C. F., &
884 Floria, M. (2019). Arterial Hypertension and Interleukins: Potential Therapeutic Target or
885 Future Diagnostic Marker? *International Journal of Hypertension*, *2019*.
886 https://doi.org/10.1155/2019/3159283

887 Tianrui, Z., Bingtong, L., Ling, Y., Liping, S., & Yongliang, Z. (2019). ACE inhibitory activity
888 in vitro and antihypertensive effect in vivo of LSGYGP and its transepithelial transport by
889 Caco-2 cell monolayer. *Journal of Functional Foods*, *61*(January), 103488.
890 https://doi.org/10.1016/j.jff.2019.103488

891 Touyz, R. M., Rios, F. J., Alves-Lopes, R., Neves, K. B., Camargo, L. L., & Montezano, A. C.
892 (2020). Oxidative Stress: A Unifying Paradigm in Hypertension. *Canadian Journal of*
893 *Cardiology*, *36*(5), 659–670. https://doi.org/10.1016/J.CJCA.2020.02.081

894 Tu, M., Wang, C., Chen, C., Zhang, R., Liu, H., Lu, W., Jiang, L., & Du, M. (2018).
895 Identification of a novel ACE-inhibitory peptide from casein and evaluation of the
896 inhibitory mechanisms. *Food Chemistry*, *256*(October 2017), 98–104.
897 https://doi.org/10.1016/j.foodchem.2018.02.107

898 Udenigwe, C. C., Abioye, R. O., Okagu, I. U., & Obeme-Nmom, J. I. (2021). Bioaccessibility of
899 bioactive peptides: recent advances and perspectives. *Current Opinion in Food Science*, *39*,
900 182–189. https://doi.org/10.1016/J.COFS.2021.03.005

901 Valdez-Meza, E. E., Raymundo, A., Figueroa-Salcido, O. G., Ramírez-Torres, G. I., Fradinho,
902 P., Oliveira, S., De Sousa, I., Suárez-Jiménez, M., Cárdenas-Torres, F. I., Islas-Rubio, A.
903 R., Rodríguez-Olibarría, G., Ontiveros, N., & Cabrera-Chávez, F. (2019). Pasta enrichment
904 with an amaranth hydrolysate affects the overall acceptability while maintaining
905 antihypertensive properties. *Foods*, *8*(8), 1–10. https://doi.org/10.3390/foods8080282

Verma, T., Sinha, M., Bansal, N., Yadav, S. ., Shah, K., & Chauhan, N. . (2021). Plants Used as Antihypertensive. *Natural Products and Bioprospecting*, *11*(2), 155–184. https://doi.org/10.1007/S13659-020-00281-X

Wang, B., & Li, B. (2018). Charge and hydrophobicity of casein peptides influence transepithelial transport and bioavailability. *Food Chemistry*, *245*(September), 646–652. https://doi.org/10.1016/j.foodchem.2017.09.032

Wang, Y, Li, Y., Ruan, S., Lu, F., Tian, W., & Ma, H. (2021). Antihypertensive effect of rapeseed peptides and their potential in improving the effectiveness of captopril. *Journal of the Science of Food and Agriculture*, *101*(7), 3049–3055. https://doi.org/10.1002/JSFA.10939

Wang, Ying, Jiang, L., Feng, S.-J., Tang, X.-Y., & Kuang, Z.-M. (2020). Effect of Combined Statin and Antihypertensive Therapy in Patients with Hypertension: A Systematic Review and Meta-Analysis. *Cardiology*, *145*(12), 802–812. https://doi.org/10.1159/000508280

Wei, D., Fan, W., & Xu, Y. (2019). In vitro production and identification of angiotensin converting enzyme (ACE) inhibitory peptides derived from distilled spent grain prolamin isolate. *Foods*, *8*(9). https://doi.org/10.3390/foods8090390

Wen, C., Zhang, J., Zhou, J., Feng, Y., Duan, Y., Zhang, H., & Ma, H. (2020). Slit divergent ultrasound pretreatment assisted watermelon seed protein enzymolysis and the antioxidant activity of its hydrolysates in vitro and in vivo. *Food Chemistry*, *328*, 127135. https://doi.org/10.1016/j.foodchem.2020.127135

Xie, J., Ye, H., Du, M., Yu, Q., Chen, Y., & Shen, M. (2020). Mung bean protein hydrolysates protect mouse liver cell line Nctc-1469 cell from hydrogen peroxide-induced cell injury. *Foods*, *9*(1). https://doi.org/10.3390/foods9010014

Xing, Y., Zhang, J., Wei, H., Zhang, H., Guan, Y., Wang, X., & Tong, X. (2019). Reduction of the PI3K/Akt related signaling activities in skeletal muscle tissues involves insulin resistance in intrauterine growth restriction rats with catch-up growth. *PLOS ONE*, *14*(5), e0216665. https://doi.org/10.1371/JOURNAL.PONE.0216665

Xu, Q., Fan, H., Yu, W., Hong, H., & Wu, J. (2017). Transport Study of Egg-Derived Antihypertensive Peptides (LKP and IQW) Using Caco-2 and HT29 Coculture Monolayers. *Journal of Agricultural and Food Chemistry*, *65*(34), 7406–7414. https://doi.org/10.1021/acs.jafc.7b02176

Xue, L., Yin, R., Howell, K., & Zhang, P. (2021). Activity and bioavailability of food protein-derived angiotensin-I-converting enzyme–inhibitory peptides. *Comprehensive Reviews in Food Science and Food Safety*, *20*(2), 1150–1187. https://doi.org/10.1111/1541-4337.12711

Yathisha, U. G., Ishani, B., Iddya, K., & Mamatha, B. S. (2018). Antihypertensive activity of fish protein hydrolysates and its peptides. *Https://Doi.Org/10.1080/10408398.2018.1452182*, *59*(15), 2363–2374.

943 https://doi.org/10.1080/10408398.2018.1452182

944 Yu, X., Su, Q., Shen, T., Chen, Q., Wang, Y., & Jia, W. (2020). Antioxidant peptides from sepia
945 esculenta hydrolyzate attenuate oxidative stress and fat accumulation in caenorhabditis
946 elegans. *Marine Drugs*, *18*(10). https://doi.org/10.3390/md18100490

947 Yu, Y., Xue, B., Wei, S., Zhang, Z., Beltz, T., Guo, F., AK, J., & RB, F. (2015). Activation of
948 central PPAR-γ attenuates angiotensin II-induced hypertension. *Hypertension (Dallas,*
949 *Tex. : 1979)*, *66*(2), 403–411. https://doi.org/10.1161/HYPERTENSIONAHA.115.05726

950 Yu, Z., Wang, L., Wu, S., Zhao, W., Ding, L., & Liu, J. (2021). In vivo anti-hypertensive effect
951 of peptides from egg white and its molecular mechanism with ACE. *International Journal*
952 *of Food Science and Technology*, *56*(2), 1030–1039. https://doi.org/10.1111/ijfs.14756

953 Yuda, N., Tanaka, M., Yamauchi, K., Abe, F., Kakiuchi, I., Kiyosawa, K., Miyasaka, M.,
954 Sakane, N., & Nakamura, M. (2020). Effect of the casein-derived peptide met-lys-pro on
955 cognitive function in community-dwelling adults without dementia: A randomized, double-
956 blind, placebo-controlled trial. *Clinical Interventions in Aging*, *15*, 743–754.
957 https://doi.org/10.2147/CIA.S253116

958 Zanoni, C., Aiello, G., Arnoldi, A., & Lammi, C. (2017). Investigations on the
959 hypocholesterolaemic activity of LILPKHSDAD and LTFPGSAED, two peptides from
960 lupin β-conglutin: Focus on LDLR and PCSK9 pathways. *Journal of Functional Foods*, *32*,
961 1–8. https://doi.org/10.1016/j.jff.2017.02.009

962 Zhan, X., Li, J., & Zhou, T. (2021). Targeting Nrf2-Mediated Oxidative Stress Response
963 Signaling Pathways as New Therapeutic Strategy for Pituitary Adenomas. *Frontiers in*
964 *Pharmacology*, *12*. https://doi.org/10.3389/FPHAR.2021.565748

965 Zhang, J., Patel, M. B., Griffiths, R., Dolber, P. C., Ruiz, P., Sparks, M. A., Stegbauer, J., Jin, H.,
966 Gomez, J. A., Buckley, A. F., Lefler, W. S., Chen, D., & Crowley, S. D. (2014). Type 1
967 angiotensin receptors on macrophages ameliorate IL-1 receptor–mediated kidney fibrosis.
968 *The Journal of Clinical Investigation*, *124*(5), 2198. https://doi.org/10.1172/JCI61368

969 Zhang, R., Chen, J., Mao, X., Qi, P., & Zhang, X. (2019). Separation and Lipid Inhibition Effects
970 of a Novel Decapeptide from Chlorella pyenoidose. *Molecules*, *24*(19), 1–16.
971 https://doi.org/10.3390/molecules24193527

972 Zhao, Y. Q., Zhang, L., Tao, J., Chi, C. F., & Wang, B. (2019). Eight antihypertensive peptides
973 from the protein hydrolysate of Antarctic krill (Euphausia superba): Isolation, identification,
974 and activity evaluation on human umbilical vein endothelial cells (HUVECs). *Food*
975 *Research International*, *121*(January), 197–204.
976 https://doi.org/10.1016/j.foodres.2019.03.035

977 Zheng, Y., Wang, X., Zhuang, Y., Li, Y., Shi, P., Tian, H., Li, X., & Chen, X. (2020). Isolation
978 of novel ACE-inhibitory peptide from naked oat globulin hydrolysates in silico approach:
979 Molecular docking, in vivo antihypertension and effects on renin and intracellular

endothelin-1. *Journal of Food Science*, *85*(4), 1328–1337. https://doi.org/10.1111/1750-3841.15115

Zhou, B., Perel, P., Mensah, G., & Ezzati, M. (2021). Global epidemiology, health burden and effective interventions for elevated blood pressure and hypertension. *Nature Reviews. Cardiology*. https://doi.org/10.1038/S41569-021-00559-8

Zippel, N., Loot, A. E., Stingl, H., Randriamboavonjy, V., Fleming, I., & Fisslthaler, B. (2018). Endothelial AMP-Activated Kinase α1 Phosphorylates eNOS on Thr495 and Decreases Endothelial NO Formation. *International Journal of Molecular Sciences*, *19*(9). https://doi.org/10.3390/IJMS19092753

Highlights

- Hypertension has remained a silent-killer
- Novel peptides recently isolated from food proteins
- Molecular mechanism of blood pressure-lowering: renin and ACE-inhibition, and beyond
- Proposed molecular mechanisms for future research
- Novel peptides are excellent candidates for nutraceutical development