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Underlying anti-hypertensive mechanism of the *Mizuhopecten yessoensis* derived peptide NCW in spontaneously hypertensive rats via widely targeted kidney metabolomics

Wenjun Xue^{a,b}, Wenzhu Zhao^a, Sijia Wu^c, Zhipeng Yu^{a,*}

^a School of Food Science and Engineering, Hainan University, Haikou 570228, China

^b College of Food Science and Engineering, Bohai University, Jinzhou 121013, China

^c Lab of Nutrition and Functional Food, Jilin University, Changchun 130062, China

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ABSTRACT

The angiotensin-converting enzyme (ACE) inhibitory peptide NCW derived from *Mizuhopecten yessoensis* has been demonstrated to have significant *in vivo* anti-hypertensive effects, however, its anti-hypertensive mechanism is still not fully clarified. This study established a UPLC-Q-TRAP-MS/MS-based widely targeted kidney metabolomics approach to explore the changes of kidney metabolic profiles and to clarify the anti-hypertensive mechanism of peptide NCW in spontaneously hypertensive rats (SHRs). Multivariate statistical analysis indicated that the kidney metabolic profiles were clearly separated between the SHR-NCW and SHR-Untreated groups. A total of 85 metabolites were differentially regulated, and 16 metabolites were identified as potential kidney biomarkers, e.g., 3-hydroxybutyrate, malonic acid, deoxycytidine, and *L*-aspartic acid. The peptide NCW might regulate kidney metabolic disorder of SHRs to alleviate hypertension by suppressing inflammation and improving nitric oxide production under the regulation of linoleic acid metabolism, folate related pathways, synthesis and degradation of ketone bodies, pyrimidine metabolism, β -alanine metabolism, and retinal metabolism.

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1. Introduction

Food-derived bioactive peptides play vital physiological roles in human health and disease prevention for their safety, high activity, good absorption, and strong targeting ability^[1,2]. Especially, the application of angiotensin-converting enzyme (ACE) inhibitory peptides as dietary and nutritional supplements to prevent and improve hypertension have recently attracted great attention due to the rising prevalence and mortality of hypertension^[3].

ACE inhibitory peptides with *in vivo* anti-hypertensive effect have been identified from multiple food sources by using spontaneously hypertensive rats (SHRs), including peptides QIGLF, TNGIIR from egg white^[4,5], peptide LRW from pea^[6], peptide WGAP from rabbit meat^[7], and peptide LVLPGE from broccoli^[8]. However, the *in vivo* anti-hypertensive mechanism of most ACE inhibitory peptides has not been fully clarified, thereby greatly limiting their development and application^[9,10]. Therefore, it is necessary to further investigate thoroughly and provide a more comprehensive understanding of the *in vivo* anti-hypertensive mechanism of ACE inhibitory peptides for better preventing and improving hypertension.

Small molecule metabolites as the final products of gene expression are the basis for biological phenotypes, and metabolic dysfunction is a fundamental core mechanism of hypertension^[11]. Therefore, metabolomics appears to be a promising and effective

* Corresponding author at: School of Food Science and Engineering, Hainan University, Haikou 570228, China.

E-mail address: yuzhipeng20086@sina.com (Z.P. Yu)

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approach to reveal the underlying anti-hypertensive mechanism of ACE inhibitory peptides, which can detect the changes of a great number of metabolites, especially those metabolites that cannot be detected by traditional technologies and can achieve the qualification and quantification of the metabolites simultaneously^[12]. However, very limited research has been reported on the application of metabolomics in investigating the mechanism of ACE inhibitory peptides. The untargeted serum metabolomics analysis carried out by Yu et al. demonstrated that a total of 8 potential serum biomarkers were identified in SHR after intervention with peptide QIGLF and peptide QIGLF might lower blood pressure by improving endothelial dysfunction^[13]. Manoharan et al. found that the levels of serum metabolites associated with the renin-angiotensin system were significantly changed in SHR after administration with peptide GVR^[14]. It should be noted that the kidney is a key target organ for blood pressure regulation, hypertension, and anti-hypertensive treatment, which can provide highly selective metabolite information^[15,16]. Additionally, ACE inhibitory peptide can regulate kidney metabolic disorder to exert anti-hypertensive effects^[4,17].

Our previously published study has found that ACE inhibitory peptide Asn-Cys-Trp (NCW, IC₅₀ value = 35.5 μmol/L) derived from myosin of *Mizuhopecten yessoensis* has a significant anti-hypertensive effect in SHR after three weeks of oral administration^[18], which could significantly reduce systolic and diastolic blood pressure of SHR by (48.08 ± 3.84) mmHg and (48.92 ± 5.77) mmHg, respectively. However, the *in vivo* anti-hypertensive mechanism of peptide NCW has not been fully clarified, and how peptide NCW lowered blood pressure by improving kidney metabolic disorder also needs an in-depth investigation. Therefore, the primary objectives of this present study were to explore the influence of peptide NCW on kidney metabolic profiles of SHR, to identify the potential kidney biomarkers and corresponding metabolic pathways associated with the anti-hypertensive effect, and to clarify the underlying anti-hypertensive mechanism of peptide NCW by using a widely targeted kidney metabolomics approach (a new technology that integrates the advantages of untargeted and targeted metabolomics) combined with multivariate statistical analysis and bioinformatics analysis. The findings of this study will provide a novel insight into the development and application of ACE inhibitory peptides as dietary and nutritional supplements in the prevention and improvement of hypertension.

2. Materials and methods

2.1 Materials and reagents

Methanol and acetonitrile (UPLC grade) were provided by Merck (Darmstadt, Hesse, Germany). Formic acid (UPLC grade) was obtained from Aladdin (Shanghai, China). Standards of metabolites were provided by Sigma-Aldrich (Saint Louis, MO, USA) and BioBioPha Co., Ltd (Kunming, Yunnan, China). The peptide NCW was synthesized by Nanjing YuanPeptide Biotechnology Co., Ltd (Nanjing, Jiangsu, China) and was dissolved in 0.9% saline obtained from Shangdong Kelun Pharmaceutical Co., Ltd (Binzhou, Shangdong, China) before being administered to SHR. Rodent feed (Catalog number: LAD 1000 M) for SHR was supplied by Trophic Animal Feed High-Technology Co., Ltd (Nantong, Jiangsu, China).

2.2 In vivo animal experiments

Twelve SPF-grade male SHR (SCXK 2016-0006, tail SBP > 180 mmHg, 10 weeks, approximately 255 g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). All SHR were kept at standard conditions with a 12 h light / dark cycle, (22 ± 3) °C, (60 ± 5)% relative humidity, and were fed with feed and water *ad libitum*. After a week of adaption, the SHR were divided into two groups at random (*n* = 6 in each group): SHR-NCW group (peptide NCW at 80 mg/kg body weight) and SHR-Untreated group (saline alone). All SHR were carefully administered with the corresponding sample solution (5 mL/kg) at 8:00 am for consecutive 21 days. After the last administration, the SHR were fasted but fed with water for 24 h, and then were euthanized with ether. After confirming that the SHR were euthanized, the abdominal cavities of the SHR were opened along the midline of the lower abdomen. The kidney tissues were immediately separated on ice from SHR within 5 min, frozen with liquid nitrogen, and stored at –80 °C until further analysis. All effort was taken to minimize the pain of SHR. All animal experimental procedures were recognized by the Animal Research Ethics Committee of Jilin University (Approval Number: 201702003) and were strictly performed following the regulations set by the care and use of laboratory animals.

2.3 Extraction of kidney metabolites and preparation of quality control samples

Frozen kidney tissues were thawed on ice. Each kidney tissue was taken for 50 mg, and then homogenized 4 times (30 Hz, 0.5 min each time) using a MM400 high-speed vibrating ball mill (Retsch GmbH, Hanna, Germany). One mL cold methanol (70%) containing internal standard was added to the homogenized kidney tissue. Subsequently, the mixture was vigorously whirled for 5 min, placed on ice for 15 min, and centrifuged for 10 min (16 000 × *g*, 4 °C). The collected supernatant was placed at –20 °C for 12 h, and then centrifuged for 3 min (16 000 × *g*, 4 °C), so as to obtain high-purity kidney metabolites and prevent blocking the chromatographic column in the subsequent experiment. Finally, the supernatant (400 μL) was taken for ultrahigh pressure liquid chromatography triple-quadrupole linear ion-trap tandem mass spectrometry (UPLC-Q-TRAP-MS/MS).

Moreover, each kidney sample of the same volume (10 μL) from the SHR-NCW and SHR-Untreated groups was mixed and prepared as a quality control (QC) sample. Before kidney sample analysis, three QC samples were run to stabilize the instrument, and then a QC sample was run once after every 10 kidney samples during the whole run to monitor the stability and repeatability of the instrument.

2.4 UPLC-Q-TRAP-MS/MS analysis

Kidney metabolites were separated by a ExionLC™ AD UPLC system (AB SCIEX, Framingham, MA, USA). Two μL kidney sample was injected a Waters ACQUITY UPLC HSS T₃ C₁₈ column (1.8 μm, 2.1 mm × 100 mm) at a column temperature of 40 °C. The solvent system consisted of water containing 0.1% formic acid (mobile phase A) and acetonitrile containing 0.1% formic acid (mobile phase B). The detailed gradient elution parameters were presented in Table S1. The separated metabolites were alternatively connected

to a Triple QuadTM 6500+ Mass Spectrometer equipped with an electrospray ionization (ESI) source (AB SCIEX). The specific ESI source operation conditions were as follows: ion spray voltage, 5 500 V (positive) / -4 500 V (negative); ESI source temperature, 500 °C; ion source gas I, 55 psi; ion source gas II, 60 psi; curtain gas, 25 psi; collision-activated dissociation, high. Moreover, 10 and 100 µmol/L polypropylene glycol were used for instrument tuning and mass calibration in triple quadrupole (QQQ) and linear ion trap (LIT) modes, respectively.

2.5 Qualitative and quantitative analysis of kidney metabolites

Qualitative analysis of kidney metabolites was achieved by searching the internal database MWDB (Metware Biotechnology Co., Ltd. Wuhan, Hubei, China) and public databases (e.g., HMDB, MassBank, and METLIN) based on primary and secondary mass spectrometry data. Quantitative analysis of kidney metabolites was carried out by the multiple reaction monitoring (MRM) mode of the mass spectrometer. The mass spectrum peak of the same metabolite from different samples was integrated and corrected by MultiQuant software (version 3.0.3). The area of each mass spectrum peak represented the relative content of the corresponding metabolite.

2.6 Data processing and statistical analysis

MS data acquisition and analysis were performed using Analyst 1.6.3 software (AB SCIEX, version 1.6.3). And the data was log₂-transformed for statistical analysis to improve normality and was normalized. The normalized metabolic data was further processed by univariate statistical analysis [fold change (FC) analysis and student's *t*-test] and multivariate statistical analysis [principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA)]. Unsupervised PCA was performed by R package (<http://www.r-project.org>, version 3.5.0) to clarify the overall metabolic difference and variation degree between the kidney samples. Supervised OPLS-DA model was generated using MetaboAnalystR (version 1.0.1) and was validated using a permutation test (200 times). The variable importance projection (VIP) value from the OPLS-DA model was calculated for investigating the contribution of a kidney metabolite to the separation between the groups. And the VIP values were visualized in the form of the S plot.

2.7 Screening of differential kidney metabolites and bioinformatics analysis

The metabolite with VIP value > 1, *P*-value < 0.05, and FC value > 1.5 (up-regulated) or < 0.67 (down-regulated) was screened as a differential kidney metabolite, which were visualized in a volcano plot. The cluster heatmap analysis of differential kidney metabolites was performed using ComplexHeatmap software (version 2.2.0). The Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.genome.jp/kegg>) was used for annotation and enrichment analysis of the differential kidney metabolites to obtain specific metabolic pathways information. A differential kidney metabolite with metabolic pathways and closely related to the anti-

hypertensive effect of peptide NCW was identified as a potential kidney biomarker. Finally, a metabolic mechanism network of key potential kidney biomarkers and related metabolic pathways was constructed to clarify the anti-hypertensive mechanism of peptide NCW.

3. Results and discussion

3.1 Data quality assessment by quality control analysis

To obtain reliable and repeatable data, the total ion current (TIC) curves of three QC samples were overlapped and analyzed in the positive and negative ion modes, respectively. As shown in Fig. S1, the TIC curves of the QC samples were basically completely overlapped in the peak intensity and retention time, indicating that the mass spectrometer had high stability when detecting the same sample at different times. This excellent stability of the mass spectrometer provides an important guarantee for the reliability and repeatability of data^[19].

3.2 Multivariate statistical analysis of kidney metabolic profiles

A total amount of 613 kidney metabolites were detected in this study, and multivariate statistical analyses including PCA and OPLS-DA were performed based on these metabolites. PCA was utilized to explore the changes in the overall metabolic profiles by visualizing clusters and reducing the dimension of complex data^[13]. The PCA score plot (3D) showed a distinct separation between the SHR-NCW and SHR-Untreated groups (Fig. 1A), which indicated that peptide NCW greatly altered the kidney metabolic profiles of SHRs.

Subsequently, an OPLS-DA model was conducted for improving the classification performance and obtaining the differential kidney metabolites. Similarly, the SHR-NCW group was clearly separated from the SHR-Untreated group in the OPLS-DA score plot (Fig. 1B), indicating the kidney metabolites were significantly affected after treatment with peptide NCW for 3 weeks. R^2 and Q^2 values as the important parameters of the OPLS-DA model could measure the goodness of interpretation rate and prediction ability, respectively. The closer the two parameters are to 1, the more reliable and stable the OPLS-DA model would be^[20]. The R^2 and Q^2 values of the OPLS-DA model were higher than 0.7, suggesting this reliable model was effectively established, and had outstanding interpretation rate and prediction ability. Moreover, the result of the permutation test (200 times) showed that the *P*-value of R^2 was less than 0.005 and the *P*-value of Q^2 was less than 0.05 (Fig. 1C), indicating the establishing OPLS-DA model was optimal and not overfitting in the current study.

To investigate the contribution of kidney metabolites to intergroup separation, S plot analysis was carried out based on the VIP values calculated by the OPLS-DA model^[21]. In the S plot (Fig. 1D), red dots represented the metabolites with VIP values > 1, while green dots represented the metabolites with VIP values < 1. There were 259 metabolites with VIP values > 1, which were likely to be differential metabolites for anti-hypertensive effect of peptide NCW.

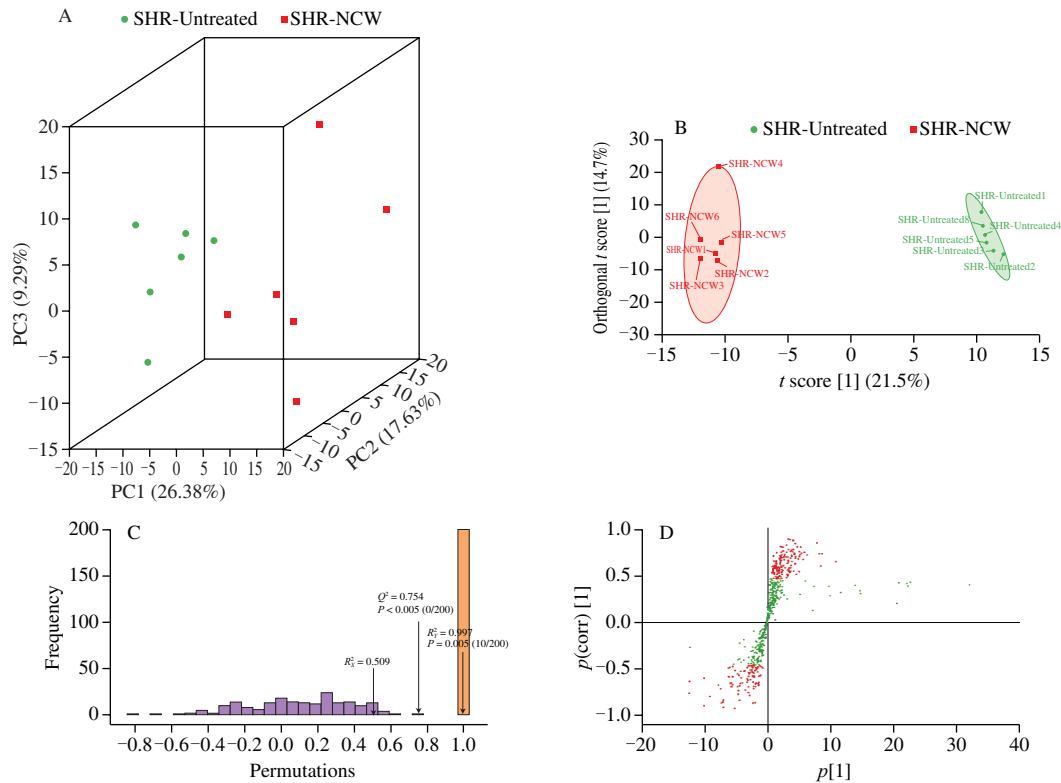


Fig. 1 Multivariate statistical analysis of kidney samples after intervention with peptide NCW between the SHR-NCW and SHR-Untreated groups, (A) PCA (3D) score plot; (B) OPLS-DA score plot; (C) permutation test plot of the OPLS-DA model; (D) S plot of the OPLS-DA model.

3.3 Identification and classification analysis of differential kidney metabolites

Combined with univariate statistical analysis, a total number of 85 metabolites were identified as differential kidney metabolites (Table S2). The difference of the expression levels of these differential kidney metabolites between the SHR-NCW and SHR-Untreated groups could be visualized by a volcano plot^[22]. In the volcano plot (Fig. 2A), 40 differential kidney metabolites represented by red dots were up-regulated ($FC > 1.5$), and 45 differential kidney metabolites represented by green dots were down-regulated ($FC < 0.67$) after treatment with peptide NCW in SHRs. In addition, the differential kidney metabolites were roughly divided into 10 categories (Fig. 2B), main concentrating on amino acid and its metabolites (16.47%), organic acid and its derivatives (16.47%), fatty acyls

(15.29%), and nucleotide and its metabolites (14.12%), indicating which played important roles for anti-hypertensive effect of peptide NCW. The change trends and number of differential kidney metabolites contained in each type of metabolite were shown in Fig. 2C. Moreover, the association and change law of differential metabolites could be quickly obtained by cluster heatmap analysis^[23]. The cluster heatmap showed that the SHR-NCW and SHR-Untreated groups were clearly divided into two different bands, and most differential kidney metabolites were well clustered (Fig. 2D).

3.4 KEGG pathway annotation and enrichment analysis of differential kidney metabolites

To better understand how the changes of differential kidney metabolites caused by peptide NCW intervention posed anti-

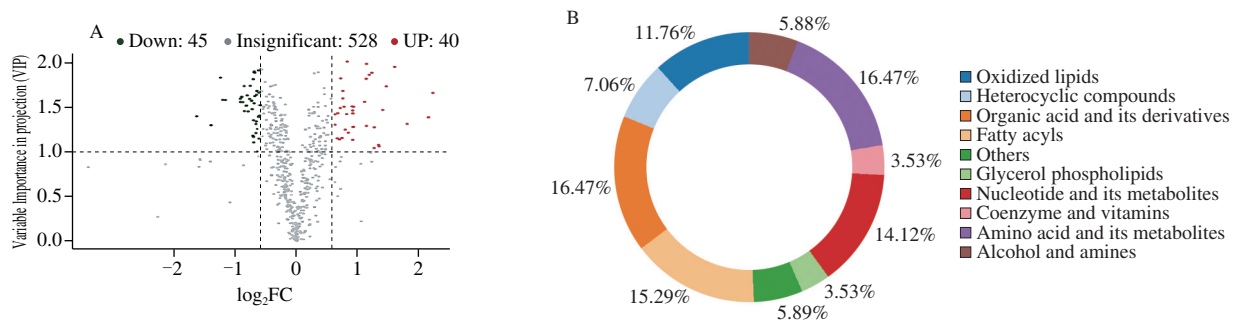


Fig. 2 Identification and classification analysis of differential kidney metabolites after intervention with peptide NCW between the SHR-NCW and SHR-Untreated groups, (A) volcano plot of the differential kidney metabolites, red dots: significantly up-regulated differential kidney metabolites, green dots: significantly down-regulated differential kidney metabolites, grey dots: insignificant metabolites; (B) types and proportions of the differential kidney metabolites; (C) change trends and number of metabolites of each type; (D) cluster heatmap of the differential kidney metabolites.

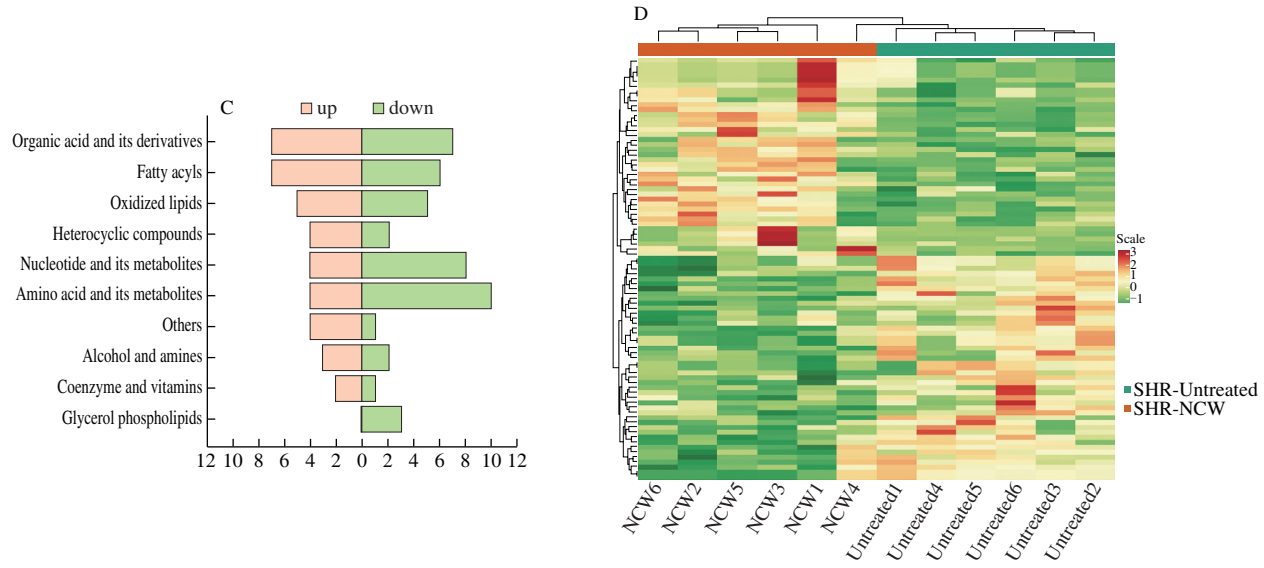


Fig. 2 (Continued)

hypertensive effect in the SHRs, the KEGG pathway annotation and enrichment analysis was performed based on the KEGG database. A total of 41 differential kidney metabolites were annotated and distributed in 79 metabolic pathways. The top 20 KEGG metabolic pathways with the most significant enrichment were displayed in Fig. 3. Ultimately, comprehensively analysis of the *P*-values and rich factors of metabolic pathways, and the biological functions, VIP values, and FC values of the metabolites contained in these metabolic pathways, 8 metabolic pathways (linoleic acid metabolism, folate biosynthesis, one carbon pool by folate, antifolate resistance, synthesis and degradation of ketone bodies, pyrimidine metabolism, β -alanine metabolism, and retinal metabolism) were chosen as the important pathways closely associated with the anti-hypertensive effect of peptide NCW, and 16 differential kidney metabolites were identified as potential kidney biomarkers based on the results of KEGG pathway

enrichment analysis and related literature (Table 1). Additionally, the expression intensities of these potential kidney biomarkers were visualized in the form of violin plots (Fig. 4). Compared with the SHR-Untreated group, the levels of eicosapentaenoic acid (EPA), 15-oxo-eicosatetraenoic acid (15-oxo-ETE), 7,8-dihydrobiopterin (BH₂), 3-hydroxybutyrate, malonic acid, barbituric acid, deoxycytidine, thymine, 4-aceylaminobutyric acid, and 11-*cis*-retinol in the SHR-NCW group were significantly increased, whereas those of 9,10-dihydroxy-12Z-octadecenoic acid (9,10-DiHOME), 9-hydroperoxyoctadecadienoic acid (9-HpODE), 10-formyl-tetrahydrofolate (10-formyl-THF), uridine-5-monophosphate (UMP), *L*-aspartate, and spermidine were significantly decreased. The above potential kidney biomarkers alterations induced related metabolic pathways changes, thus improving metabolism disorders and lowering blood pressure in SHRs.

Table 1
Potential kidney biomarkers and related key pathways associated with the anti-hypertensive effect of peptide NCW.

No.	Potential biomarkers	VIP value	FC value	Trend	Classification	Related key pathways
1	9,10-DiHOME	1.609	0.541	↓	Oxidized lipids	Linoleic acid metabolism
2	9-HpODE	1.399	0.660	↓	Oxidized lipids	Linoleic acid metabolism
3	EPA	1.285	2.208	↑	Oxidized lipids	Biosynthesis of unsaturated fatty acids
4	15-Oxo-ETE	1.417	1.767	↑	Oxidized lipids	Arachidonic acid metabolism
5	7,8-Dihydrobiopterin	1.210	1.801	↑	Heterocyclic compounds	Folate biosynthesis
6	10-Formyl-THF	1.293	0.380	↓	Heterocyclic compounds	One carbon pool by folate; antifolate resistance
7	3-Hydroxybutyrate	1.856	2.280	↑	Organic acid and its derivatives	Synthesis and degradation of ketone bodies
8	Malonic acid	1.879	2.349	↑	Organic acid and its derivatives	Pyrimidine metabolism; β -Alanine metabolism
9	Barbituric acid	1.554	2.162	↑	Heterocyclic compounds	Pyrimidine metabolism
10	Deoxycytidine	1.485	1.645	↑	Nucleotide and its metabolites	Pyrimidine metabolism
11	Thymine	1.812	2.203	↑	Nucleotide and its metabolites	Pyrimidine metabolism
12	UMP	1.451	0.558	↓	Nucleotide and its metabolites	Pyrimidine metabolism
13	<i>L</i> -Aspartic acid	1.645	0.642	↓	Amino acid and its metabolites	β -Alanine metabolism
14	Spermidine	1.824	0.423	↓	Alcohol and amines	β -Alanine metabolism
15	4-Acetamidobutyric acid	1.501	1.909	↑	Organic acid and its derivatives	Arginine and proline metabolism
16	11- <i>cis</i> -retinol	1.382	4.449	↑	Coenzyme and vitamins	Retinal metabolism

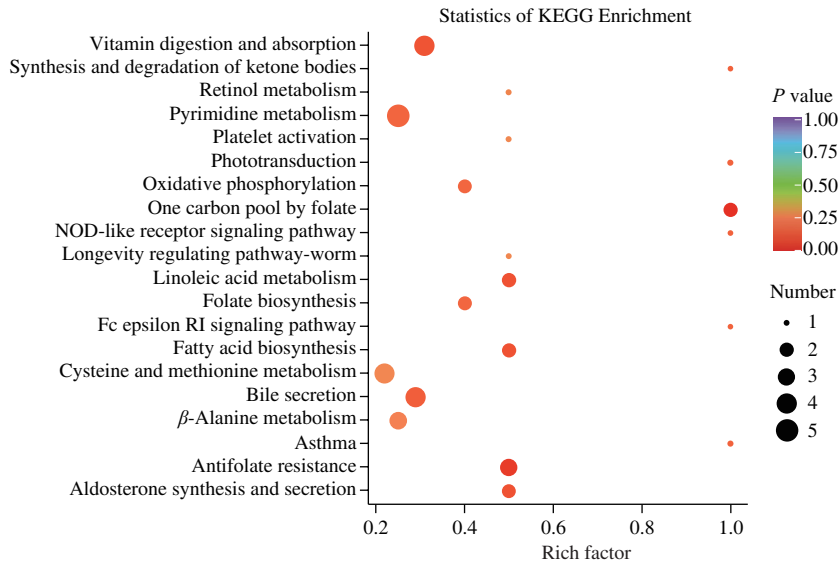


Fig. 3 The top 20 KEGG metabolic pathways with the most significant enrichment. The size of the dot represents the number of differential kidney metabolites contained in a metabolic pathway. The color of the dot represents the *P*-value of a metabolic pathway, and the redder a dot is, the more the metabolic pathway is significant.

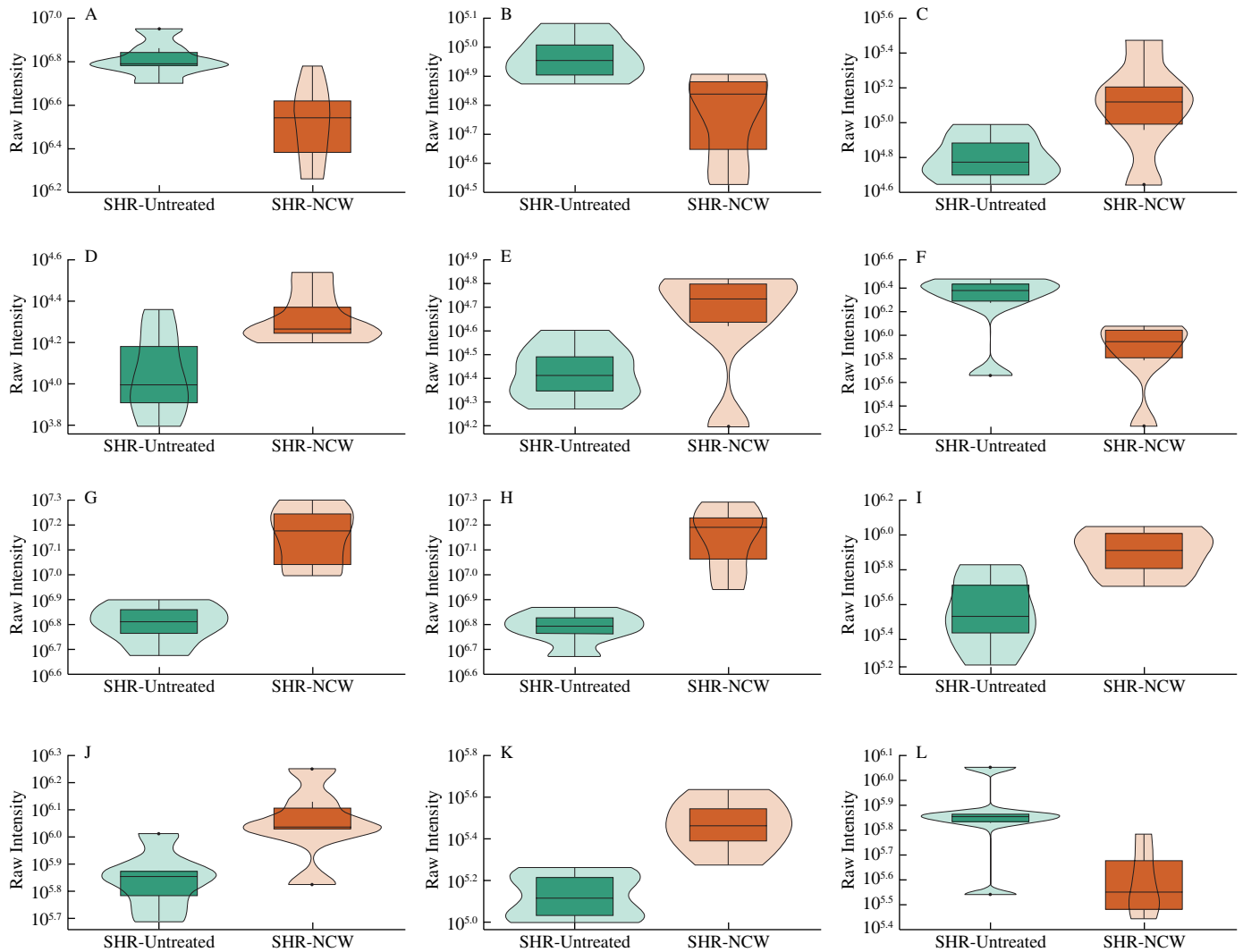


Fig. 4 Violin plots of the potential kidney biomarkers. The abscissa represents the grouping, and the ordinate represents the expression intensities (that is original peak areas) of potential kidney biomarkers. The box in the middle represents the four-digit score range, and the thin black line extending from it represents the 95% confidence interval. The black horizontal line in the middle represents the median of the data, and the external shape represents the distribution density of the data.

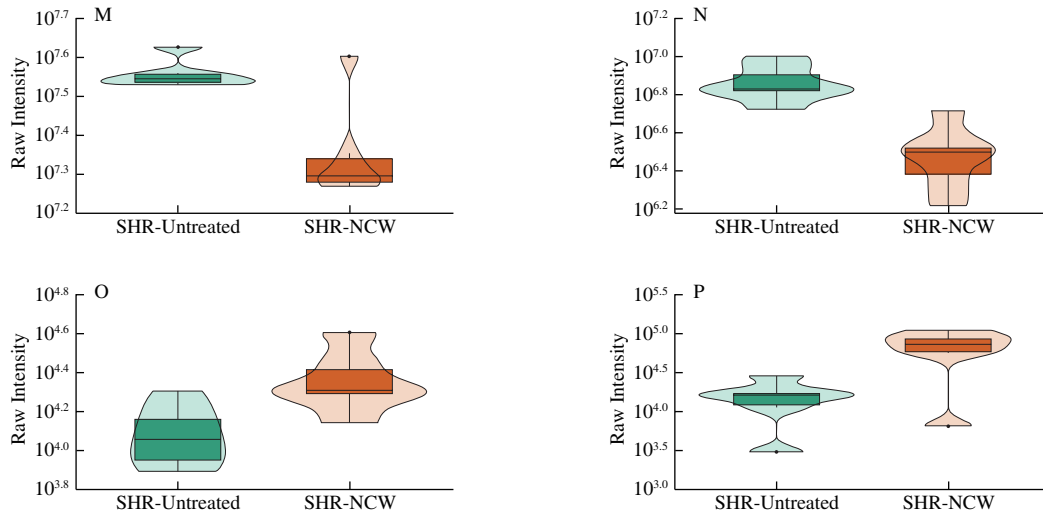


Fig. 4 (Continued)

3.5 Construction and analysis of the metabolic mechanism network of potential kidney biomarkers and related metabolic pathways

To intuitively understand the anti-hypertensive mechanism of peptide NCW, a metabolic mechanism network was constructed based on these potential kidney biomarkers and related metabolic pathways (Fig. 5).

As a ω -6 polyunsaturated fatty acid (PUFA), linoleic acid has an important effect on cell signal transduction and gene expression regulation, and its oxidation products are widely used as important homeostatic regulators of inflammation, vasodilation, and other physiological processes^[24]. 9, 10-DiHOME and 9-HpODE, which were involved in linoleic acid metabolism, were decreased by 0.541-fold and 0.66-fold in the SHR-NCW group, respectively.

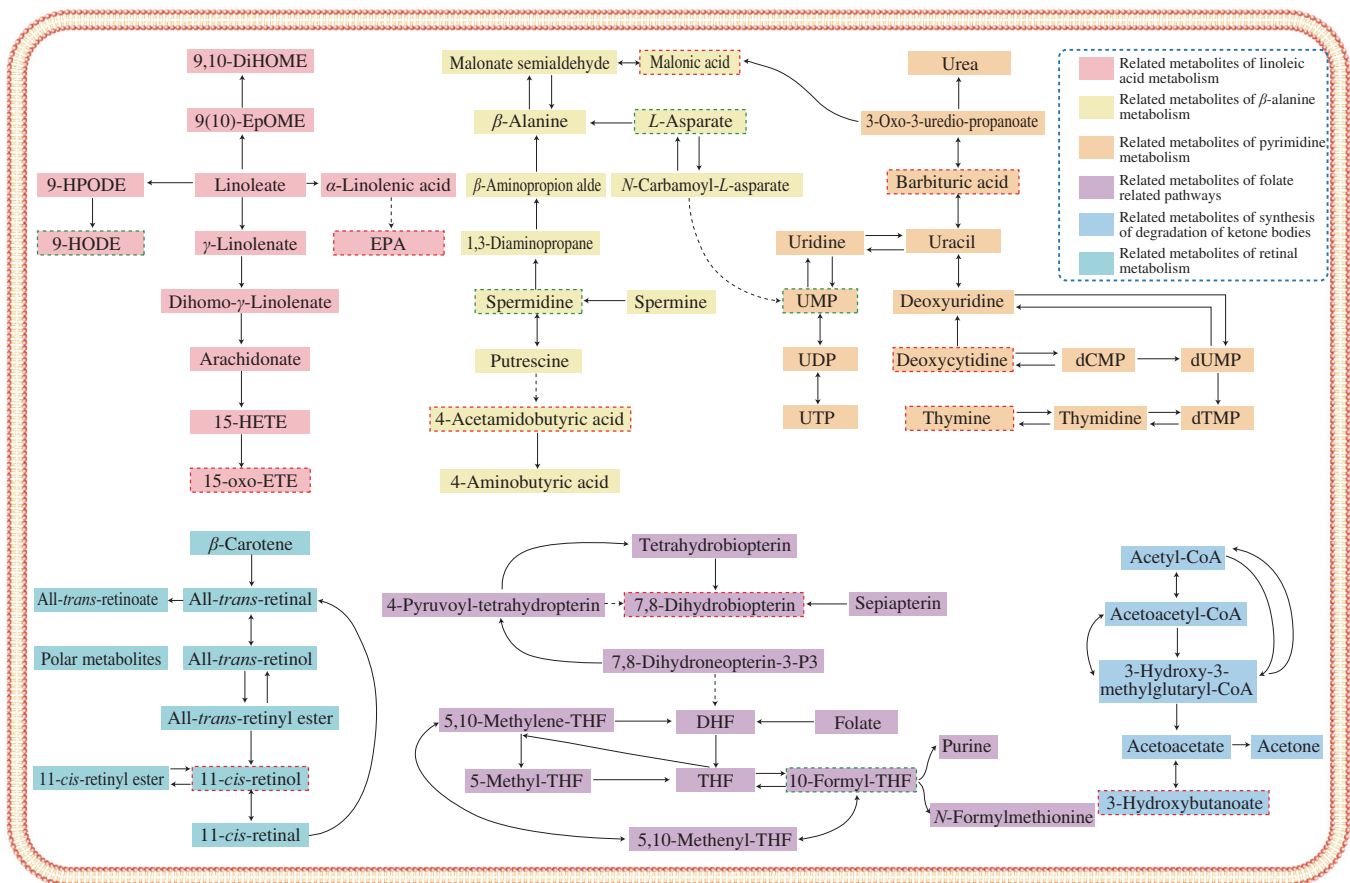


Fig. 5 Metabolic mechanism network of the potential kidney biomarkers and related metabolic pathways associated with the anti-hypertensive effect of peptide NCW. Red dashed box: up-regulated potential kidney biomarker; green dashed box: down-regulated potential kidney biomarker. Solid arrow: direct reaction; dashed arrow: indirect reaction; bidirectional arrow: reversible reaction.

9,10-DiHOME is a leukotoxin derivative of linoleic acid diol produced by inflammatory leukocytes, and has cytotoxicity and tissue toxicity. Mitochondrial dysfunction, increased oxidative stress, and apoptosis are the main toxicity characteristics of 9, 10-DiHOME^[25]. It has been reported that high concentrations of 9,10-DiHOME and 9,10-EpOME can activate AP-1 and NF- κ B transcription factors, both of which mediate inflammation^[26]. However, 9-HpODE is extremely unstable and further decomposed to form 9-hydroxyoctadecaenoic acid (9-HODE) *in vivo*^[27]. 9-HODE has pro-inflammatory properties and can promote the apoptosis of monocytes and macrophages^[28]. However, EPA and 15-oxo-EETE as the important related metabolites of linoleic acid metabolism, were significantly increased by 2.208-fold and 1.767-fold after intervention with peptide NCW. EPA is an important ω -3 PUFA produced from α -linolenic acid by desaturase and elongation enzyme. EPA is believed to improve impaired endothelial function and increase endothelium-dependent vasodilation, mainly by activating endothelial nitric oxide synthase (eNOS) and promoting nitric oxide (NO, an endothelial derived relaxing factor) production in endothelial cells^[29]. In addition, 15-oxo-EETE is formed from 15-hydroxyprostaglandin dehydrogenase-mediated oxidation of 15-HETE. And 15-oxo-EETE is a vital anti-inflammatory mediator, which can activate anti-inflammatory Nrf2 signaling and downregulate pro-inflammatory cytokine^[30]. Combining of all the above analysis, peptide NCW might regulate the related metabolites of linoleic acid metabolism in the kidney of SHR to inhibit inflammation and apoptosis, and improve NO production.

The role of folate related pathways in maintaining homeostasis, material metabolism, and energy metabolism has been gradually underscored. Compared with the SHR-Untreated group, the level of BH₂ in folate biosynthesis was increased by 1.801-fold in the SHR-NCW group. BH₂ is reduced to tetrahydrobiopterin (BH₄) by dihydrofolate reductase, and BH₄ is an important NOS cofactor^[31]. BH₄ can stabilize and couple the dimer structure of eNOS, and catalyze the conversion of *L*-arginine to *L*-guanidine and NO. BH₄ deficiency can lead to eNOS uncoupling, resulting in reduced NO synthesis, increased peroxide synthesis, and vascular endothelial damage^[32]. In this study, the change of BH₂ might increase the production of BH₄ to attenuate hypertension by mediating the eNOS uncoupling and improving the production of NO in the kidney of SHR. In addition, one carbon metabolism is necessary for the biosynthesis required for cell proliferation and vital for redox balance^[33]. 10-Formyl-THF as a pivotal precursor of purine and N-formylmethionine synthesis, was decreased by 0.38-fold in the pathways of one carbon pool by folate and antifolate resistance after treatment with peptide NCW. Reportedly, if an accumulation of 10-formyl-THF occurs in endothelial cells, this accumulation might influence the adjacent pathways of folate metabolism, and then affect the activity of eNOS^[34].

3-Hydroxybutyrate, which was involved in synthesis and degradation of ketone bodies, was increased by 2.28-fold in the SHR-NCW group. As is well known, 3-hydroxybutyrate belongs to the ketone body family produced mainly by the liver, and has the abilities of anti-inflammatory, anti-oxidative, and preventing oxidative stress^[35]. And 3-hydroxybutyrate is a vital inhibitor of the over activation of the Nlrp3 inflammasome, which has a vital role in kidney injury and contributes to aggravating inflammation^[36]. Nutritional supplement

with the 3-hydroxybutyrate precursor 1,3-butanediol can attenuate hypertension and suppress kidney injury by inhibiting the renal Nlrp3 inflammasome formation in salt-sensitive hypertension^[37]. Moreover, a recent study showed that 3-hydroxybutyrate was an autophagy-dependent vasodilator^[38]. The above analysis suggested that peptide NCW might attenuate hypertension by increasing the level of 3-hydroxybutyrate to inhibit inflammation in the kidney of SHR.

Pyrimidines are the basic constituents of DNA and RNA, and pyrimidine metabolism has been reported to be closely associated with the development of hypertension and its complications^[39]. Compared with the SHR-Untreated group, malonic acid, barbituric acid, deoxycytidine, and thymine, which were involved in pyrimidine metabolism, were increased in the SHR-NCW group by 2.349-fold, 2.162-fold, 1.645-fold, and 2.203-fold, respectively. And the level of UMP was decreased by 0.558-fold. Malonic acid can reduce the production of mitochondrial ROS in Ang II-induced hypertension, which contributes to improving oxidative stress and endothelial dysfunction^[36]. However, the anti-hypertensive mechanism of barbituric acid (a condensation metabolite of malonic acid and urea) is still not clear. Deoxycytidine is the main nucleoside of DNA, thymine is the pyrimidine base, and UMP is the uracil nucleotide, which participate in almost all biochemical reactions^[40]. This suggested that the anti-hypertensive mechanism of peptide NCW might be related to the regulation of deoxycytidine, thymine, and UMP levels in pyrimidine metabolism. Importantly, pyrimidine metabolism and β -alanine metabolism were well connected by malonic acid. In β -alanine metabolism, the levels of *L*-aspartate and spermidine were decreased by 0.642-fold and 0.423-fold in the kidney of SHR after intervention with peptide NCW. Spermidine was converted to 4-aceylaminobutyric acid by complex multi-step reaction. 4-Aceylaminobutyric acid as the precursor of 4-aminobutyric acid (GABA) was increased by 1.909-fold in the SHR-NCW group. Reportedly, GABA inhibits the release of norepinephrine from sympathetic nerve fibers by acting on presynaptic GABA_B receptors, thereby suppressing the increase of blood pressure in SHR^[41]. In short, pyrimidine metabolism and β -alanine metabolism have complex multifaceted effect in hypertension, the anti-hypertensive effect of peptide NCW in this study might be the result of comprehensive action of many active substances in pyrimidine metabolism and β -alanine metabolism.

Retinol (vitamin A) is a vital regulator of cell proliferation, differentiation, apoptosis, immune function, and visual system. The kidney is a target organ for retinol action^[42]. 11-*cis*-retinol was involved in retinol metabolism, which was increased by 1.645-fold after intervention with peptide NCW. Emerging findings indicated that retinol seemed to have a vital inhibitory effect on inflammation and might improve endothelial function by regulating NO related pathway^[43]. This suggested that retinol might play an important role in lowering blood pressure, and peptide NCW intervention increased the expression level of 11-*cis*-retinol, which contributes to inhibiting inflammation and regulating NO production in the kidney of SHR.

4. Conclusion

In summary, the widely targeted kidney metabolomics analysis indicated kidney metabolites disorder caused by hypertension was

partially alleviated by peptide NCW intervention. Peptide NCW might exert anti-hypertensive effect by suppressing inflammation and improving NO production in the kidney of SHR under the regulation of linoleic acid metabolism, folate related pathways, synthesis and degradation of ketone bodies, pyrimidine metabolism, β -alanine metabolism, and retinal metabolism. This study will provide an important clue and insight for the anti-hypertensive mechanism of peptide NCW, and provide a theoretical basis for the development and application of ACE inhibitory peptides in the prevention and improvement of hypertension. In future studies, the relationship between the changes in kidney metabolites and the underlying anti-hypertensive mechanism will be further validated by target metabolomic studies, cell experiments, and other biological technologies.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2022.9250041>.

References

- [1] J.O. Onuh, R.E. Aluko, Metabolomics as a tool to study the mechanism of action of bioactive protein hydrolysates and peptides: a review of current literature, *Trends Food Sci. Technol.* 91 (2019) 625-633. <https://doi.org/10.1016/j.tifs.2019.08.002>.
- [2] H.Z. Ji, W.Z. Zhao, Z.P. Yu, et al., Mechanism of interactions between egg protein-derived tri-peptides and cellular membrane by molecular dynamic simulation and isothermal titration calorimetry, *Int. J. Food Sci. Technol.* 57 (7) (2022) 4163-4174. <https://doi.org/10.1111/ijfs.15736>.
- [3] J.P. Wu, W. Liao, C.C. Udenigwe, Revisiting the mechanisms of ACE inhibitory peptides from food proteins, *Trends Food Sci. Technol.* 69 (2017) 214-219. <https://doi.org/10.1016/j.tifs.2017.07.011>.
- [4] Z.P. Yu, H. Guo, D. Shiuan, et al., Interaction mechanism of egg white-derived ACE inhibitory peptide TNGIIR with ACE and its effect on the expression of ACE and AT1 receptor, *Food Sci. Hum. Wellness* 9 (1) (2020) 52-57. <https://doi.org/10.1016/j.fshw.2019.12.009>.
- [5] Z.P. Yu, L. Wang, S.J. Wu, et al., *In vivo* anti-hypertensive effect of peptides from egg white and its molecular mechanism with ACE, *Int. J. Food Sci. Technol.* 56 (2) (2021) 1030-1039. <https://doi.org/10.1111/ijfs.14756>.
- [6] X. Wang, K.S. Bhullar, H.B. Fan, et al., Regulatory effects of a pea-derived peptide Leu-Arg-Trp (LRW) on dysfunction of rat aortic vascular smooth muscle cells against angiotensin II stimulation, *J. Agric. Food Chem.* 68 (13) (2020) 3947-3953. <https://doi.org/10.1021/acs.jafc.0c00028>.
- [7] J.B. Chen, X.D. Yu, W.X. Huang, et al., A novel angiotensin-converting enzyme inhibitory peptide from rabbit meat protein hydrolysate: identification, molecular mechanism, and antihypertensive effect *in vivo*, *Food Funct.* 12 (23) (2021) 12077-12086. <https://doi.org/10.1039/d1fo02830h>.
- [8] J.Y. Pei, Y. Hua, T.Y. Zhou, et al., Transport, *in vivo* antihypertensive effect, and pharmacokinetics of an angiotensin-converting enzyme (ACE) inhibitory peptide IVLPGE, *J. Agric. Food Chem.* 69 (7) (2021) 2149-2156. <https://doi.org/10.1021/acs.jafc.0c07048>.
- [9] A. Ganguly, K. Sharma, K. Majumder, Food-derived bioactive peptides and their role in ameliorating hypertension and associated cardiovascular diseases, *Adv. Food Nutr. Res.* 89 (2019) 165-207. <https://doi.org/10.1016/bs.afnr.2019.04.001>.
- [10] H.Z. Ji, W.Z. Zhao, Z.P. Yu, et al., Interaction mechanism of three egg protein derived ACE inhibitory tri-peptides and DPPC membrane using FS, FTIR, and DSC studies, *Food Chem.: X.* 15 (2022) 100366. <https://doi.org/10.1016/j.fochx.2022.100366>.
- [11] J.O. Onuh, H.Y. Qiu, Metabolic profiling and metabolites fingerprints in human hypertension: discovery and potential, *Metabolites* 11 (10) (2021) 687. <https://doi.org/10.3390/metabo11100687>.
- [12] J. Fu, L.L. Zhang, W. Li, et al., Application of metabolomics for revealing the interventional effects of functional foods on metabolic diseases, *Food Chem.* 367 (2022) 130697. <https://doi.org/10.1016/j.foodchem.2021.130697>.
- [13] Z.P. Yu, W.J. Xue, M.J. Zhou, et al., Potential antihypertensive mechanisms of the egg white-derived peptide QIGLF in spontaneously hypertensive rats revealed using untargeted serum metabolomics, *J. Agric. Food Chem.* 69 (40) (2021) 12063-12071. <https://doi.org/10.1021/acs.jafc.1c05599>.
- [14] S. Manoharan, A.S. Shuib, N. Abdullah, et al., An angiotensin-I-converting enzyme inhibitory tripeptide ameliorates hypertension on spontaneously hypertensive rats, *Process Biochem.* 69 (2018) 224-232. <https://doi.org/10.1016/j.procbio.2018.03.014>.
- [15] J.M. Eales, X. Jiang, X.G. Xu, et al., Uncovering genetic mechanisms of hypertension through multi-omic analysis of the kidney, *Nat. Genet.* 53 (5) (2021) 630-637. <https://doi.org/10.1038/s41588-021-00835-w>.
- [16] S.B. Nikolic, J.E. Sharman, M.J. Adams, et al., Metabolomics in hypertension, *J. Hypertens.* 32 (6) (2014) 1159-1169. <https://doi.org/10.1097/HJH.000000000000168>.
- [17] Z.P. Yu, M.J. Zhou, J.B. Liu, et al., Underlying antihypertensive mechanism of egg white-derived peptide QIGLF using renal metabolomics analysis, *Food Res. Int.* 157 (2022) 111457. <https://doi.org/10.1016/j.foodres.2022.111457>.
- [18] S.J. Wu, W.Z. Zhao, Z.P. Yu, et al., Antihypertensive effect and underlying mechanism of tripeptide NCW on spontaneously hypertensive rats using metabolomics analysis, *Food Funct.* 13 (4) (2022) 1808-1821. <https://doi.org/10.1039/d1fo03924e>.
- [19] J.Q. Xiao, C.Q. Gu, S. He, et al., Widely targeted metabolomics analysis reveals new biomarkers and mechanistic insights on chestnut (*Castanea mollissima* Bl.) calcification process, *Food Res. Int.* 141 (2021) 110128. <https://doi.org/10.1016/j.foodres.2021.110128>.
- [20] H.F. Zhang, Q. Lu, R. Liu, Widely targeted metabolomics analysis reveals the effect of fermentation on the chemical composition of bee pollen, *Food Chem.* 375 (2022) 131908. <https://doi.org/10.1016/j.foodchem.2021.131908>.
- [21] Y.H. Yang, J. Qian, B.W. Li, et al., Metabolomics based on ¹H-NMR reveal the regulatory mechanisms of dietary methionine restriction on splenic metabolic dysfunction in obese mice, *Foods* 10 (10) (2021) 2439. <https://doi.org/10.3390/foods10102439>.
- [22] Z.P. Ai, Y. Zhang, X.Y. Li, et al., Widely targeted metabolomics analysis to Reveal Transformation Mechanism of *Cistanche deserticola* active compounds during steaming and drying processes, *Front. Nutr.* 8 (2021) 742511. <https://doi.org/10.3389/fnut.2021.742511>.
- [23] J. Yi, X.Y. Wang, K.X. Song, et al., Integrated metabolomics and mechanism to reveal the protective effect of kaempferol on pulmonary arterial hypertension, *J. Pharm. Biomed. Anal.* 212 (2022) 114662. <https://doi.org/10.1016/j.jpba.2022.114662>.
- [24] K. Hildreth, S.D. Kodani, B.D. Hammock, et al., Cytochrome P450-derived linoleic acid metabolites EpOMEs and DiHOMEs: a review of recent studies, *J. Nutr. Biochem.* 86 (2020) 108484. <https://doi.org/10.1016/j.jnutbio.2020.108484>.
- [25] D.C. Nieman, J. Scherr, B.B. Luo, et al., Influence of pistachios on performance and exercise-induced inflammation, oxidative stress, immune dysfunction, and metabolite shifts in cyclists: a randomized, crossover trial, *PLoS ONE.* 9 (11) (2014) e113725. <https://doi.org/10.1371/journal.pone.0113725>.

- [26] S. Viswanathan, B.D. Hammock, J.W. Newman, et al., Involvement of CYP 2C9 in mediating the proinflammatory effects of linoleic acid in vascular endothelial cells, *J. Am. Coll. Nutr.* 22 (6) (2003) 502-510. <https://doi.org/10.1080/07315724.2003.10719328>.
- [27] E. Niki, Lipid peroxidation: physiological levels and dual biological effects, *Free Radic. Biol. Med.* 47 (5) (2009) 469-484. <https://doi.org/10.1016/j.freeradbiomed.2009.05.032>.
- [28] V.N. Vangaveti, V.M. Shashidhar, C. Rush, et al., Hydroxyoctadecadienoic acids regulate apoptosis in human THP-1 cells in a PPAR γ -dependent manner, *Lipids* 49 (12) (2014) 1181-1192. <https://doi.org/10.1007/s11745-014-3954-z>.
- [29] C.I. Bercea, G.S. Cottrell, F. Tamagnini, et al., Omega-3 polyunsaturated fatty acids and hypertension: a review of vasodilatory mechanisms of docosahexaenoic acid and eicosapentaenoic acid, *Br. J. Pharmacol.* 178 (4) (2021) 860-877. <https://doi.org/10.1111/bph.15336>.
- [30] G.H. Ma, B. Pan, S.F. Ren, et al., 15-oxoicosatetraenoic acid mediates monocyte adhesion to endothelial cell, *Lipids Health Dis.* 16 (1) (2017) 1-9. <https://doi.org/10.1186/s12944-017-0518-2>.
- [31] K. Chalupsky, D. Kračun, I. Kanchev, et al., Folic acid promotes recycling of tetrahydrobiopterin and protects against hypoxia-induced pulmonary hypertension by recoupling endothelial nitric oxide synthase, *Antioxid. Redox Signal.* 23 (14) (2015) 1076-1091. <https://doi.org/10.1089/ars.2015.6329>.
- [32] M.F. Yuyun, L.L. Ng, G.A. Ng, Endothelial dysfunction, endothelial nitric oxide bioavailability, tetrahydrobiopterin, and 5-methyltetrahydrofolate in cardiovascular disease. Where are we with therapy? *Microvasc. Res.* 119 (2018) 7-12. <https://doi.org/10.1016/j.mvr.2018.03.012>.
- [33] W.L. Xu, S.A. Comhair, R.Y. Chen, et al., Integrative proteomics and phosphoproteomics in pulmonary arterial hypertension, *Sci. Rep.* 9 (2019) 18623. <https://doi.org/10.1038/s41598-019-55053-6>.
- [34] E. McAuley, H. McNulty, C. Hughes, et al., Riboflavin status, MTHFR genotype and blood pressure: current evidence and implications for personalised nutrition, *Proc. Nutr. Soc.* 75 (3) (2016) 405-414. <https://doi.org/10.1017/S0029665116000197>.
- [35] T. Shimazu, M.D. Hirschey, J. Newman, et al., Suppression of oxidative stress by β -hydroxybutyrate, an endogenous histone deacetylase inhibitor, *Science* 339 (6116) (2013) 211-214. <https://doi.org/10.1126/science.1227166>.
- [36] X.F. Chen, X.Q. Chen, X.Q. Tang, Short-chain fatty acid, acylation and cardiovascular diseases, *Clin. Sci.* 134 (6) (2020) 657-676. <https://doi.org/10.1042/CS20200128>.
- [37] S. Chakraborty, S. Galla, X. Cheng, et al., Salt-responsive metabolite, β -hydroxybutyrate, attenuates hypertension, *Cell Rep.* 25 (3) (2018) 677-689. <https://doi.org/10.1016/j.celrep.2018.09.058>.
- [38] C.G. McCarthy, S. Chakraborty, G. Singh, et al., Ketone body β -hydroxybutyrate is an autophagy-dependent vasodilator, *JCI insight.* 6 (20) (2021) e149037. <https://doi.org/10.1172/jci.insight.149037>.
- [39] Y. Cheng, D.M. Sun, B.J. Zhu, et al., Integrative metabolic and proteomic profiling of the brainstem in spontaneously hypertensive rats, *J. Proteome Res.* 19 (10) (2020) 4114-4124. <https://doi.org/10.1021/acs.jproteome.0c00585>.
- [40] D.X. Wang, Q. Wang, R.H. Chen, et al., Exploring the effects of *Gastrodia elata* Blume on the treatment of cerebral ischemia-reperfusion injury using UPLC-Q/TOF-MS-based plasma metabolomics, *Food Funct.* 10 (11) (2019) 7204-7215. <https://doi.org/10.1039/C9FO01729A>.
- [41] K. Hayakawa, M. Kimura, K. Kamata, Mechanism underlying γ -aminobutyric acid-induced antihypertensive effect in spontaneously hypertensive rats, *Eur. J. Pharmacol.* 438 (1-2) (2002) 107-113. <https://doi.org/>
- [42] P.V. Bhat, D.C. Manolescu, Role of vitamin A in determining nephron mass and possible relationship to hypertension, *J. Nutr.* 138 (8) (2008) 1407-1410. [https://doi.org/10.1016/S0014-2999\(02\)01294-3](https://doi.org/10.1016/S0014-2999(02)01294-3).
- [43] Y.R. Yu, H. Zhang, Y. Song, et al., Plasma retinol and the risk of first stroke in hypertensive adults: a nested case-control study, *Am. J. Clin. Nutr.* 109 (2) (2019) 449-456. <https://doi.org/10.1093/ajcn/nqy320>.