Format for ANSWERING REVIEWERS

February 3rd, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 15932-edited).

Title: Antihypertensive Effects of Foods

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The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) **Comment:**

Data provided are mostly observational and the vast majority of interventional studies yielded results with no statistical significance. Moreover, the potential of clinical applications of such correlations remains far from proven. These caveats should be underlined by the authors

Response:

Thank you very much for your advice. As you indicated, we focused on the intervention trials with statistical significance, indicated in table2. Moreover, we additionally described the potential of clinical applications of functional foods in the revised manuscript, as follows:

Page4, L13- Page4, L19

~~In this paper, we review~~ functional foods having particularly beneficial effects on hypertension in animal experiments (Table1) and human clinical trials (**Table2**) are summarized in this paper. This manuscript is focusing on clinical findings than experimental ones. Moreover, we emphasize interventional studies yielded results with statistical significance. ~~They~~ Functional foods reduce the blood pressure by different mechanisms, such as rennin-angiotensin-aldosterone system (RAAS) inhibition, antioxidant effect, diuretic effect, NOS production- promoting effect. And there are also some foods with multiple mechanisms (**Fig1**)

Page11, L3- Page11, L9

We summarize the functional foods with antihypertensive effects from the evidences in clinical studies. In contradiction to these studies, there are several reports indicating opposite results and many interventional studies with no statistical significance. For example, Green tea consumption was inversely associated with mortality due to all causes and cardiovascular disease, [76] and there are a few reports described no effect of EPA on the blood pressure.[77, 78] So, the potential of clinical applications of functional foods remains undetermined. Randomized controlled trials are needed to establish the clinical applications of functional foods.

76) Kuriyama S, Shimazu T, Ohmori K, Kikuchi N, Nakaya N, Nishino Y, Tsubono Y, Tsuji I. Green tea consumption and mortality due to cardiovascular disease, cancer, and all causes in Japan: the Ohsaki study. *JAMA*. 2006; **296**:1255-65. [PMID: 16968850 DOI: 10.1001/jama.296.10.1255.]

77) **Erkkilä AT**, Schwab US, de Mello VD, Lappalainen T, Mussalo H, Lehto S, Kemi V, Lamberg-Allardt C, Uusitupa MI. Effects of fatty and lean fish intake on blood pressure in subjects with coronary heart disease using multiple medications. *Eur J Nutr.* 2008; **47**: 319-328 [PMID: 18665413 DOI: 10.1007/s00394-008-0728-5]

78) **Szabo de Edelenyi F**, Vergnaud AC, Ahluwalia N, Julia C, Hercberg S, Blacher J, Galan P. Effect of B-vitamins and n-3 PUFA supplementation for 5 years on blood pressure in patients with CVD. *Br J Nutr.* 2012; **107**: 921-927 [PMID: 21801476 DOI: 10.1017/S0007114511003692]

(2) **Comment:**

The manuscript should be shortened by focusing on clinical findings than experimental ones.

Response:

Thank you very much for your advice. As you suggested, we focused on clinical findings and shortened the manuscript, as follows:

Page4, L24-Page4, L27

~~In stroke-prone spontaneously hypertensive rats (SHRSP), a group that consumed black tea polyphenols or green tea polyphenols showed significant reductions of SBP and DBP compared to a control group during the daytime. Furthermore, because the amounts of polyphenols used in this experiment correspond to those in ~1 L of tea,~~ Using stroke-prone spontaneously hypertensive rats (SHRSP), treatment of black tea polyphenols or green tea polyphenols showed significant reductions of SBP and DBP. Moreover, several experiments indicated that the regular consumption of black and green tea may also provide some protection against hypertension in humans.[7)]

Page5, L25-Page5, L28

~~In spontaneously hypertensive rats (SHR), it has been reported that GABA has an antihypertensive effect due to the inhibition of noradrenaline release from sympathetic nerves.[17)]~~ Antihypertensive mechanisms of GABA have been considered as follow: an inhibitory effect on the sympathetic nervous system and peripheral sympathetic ganglia, a diuretic effect by the inhibition of anti-diuretic hormone secretion, and angiotensin converting enzyme (ACE) activity inhibition.[17, 18)]

Page6, L10-Page6, L11

The hypotensive effect of stevioside may be mediated by inhibiting Ca^{2+} influx into blood vessels and vasodilation.[24, 25)] ~~Furthermore, some experiments using isosteviol, a derivative of stevioside, and vascular smooth muscle cells reported a decrease in intracellular calcium concentrations by isosteviol mainly mediated by the selective opening of ATP-sensitive potassium channels and/or small conductance calcium-activated potassium channels.[25)]~~

Page7, L14-Page7, L17

~~Deoxycorticosterone acetate (DOCA)-salt hypertensive rats fed a diet containing 0.1 or 1% sesamin showed significantly suppressed DOCA-salt-induced hypertension, and reduced aortic superoxide (O_2^-)~~

~~production, NADPH oxidase activity, and subunit mRNA expression. An antihypertensive drug combination (triple therapy: reserpine, hydralazine, and hydrochlorothiazide) also similarly suppressed the development of DOCA-salt-induced hypertension, but did not suppress O_2^- production.[35,36] Sesamin metabolites exhibits radical scavenging activities and led to endothelium-dependent vasorelaxation. Sesamin metabolite-induced vasorelaxations were markedly attenuated by pretreatment with a nitric oxide synthase (NOS) inhibitor, NG-nitro-L-arginine (NOARG). The antihypertensive effects of sesamin feeding were not observed in chronically NOARG-treated rats nor in DOCA-salt-treated endothelial NOS-deficient mice. These findings~~ Several animal experiments suggest that the hypotensive action of sesamin is involved in the vasodilating effect caused by NOS production enhancement and oxidative stress reduction in blood vessels due to the antioxidant effect.[35, 36, 37)]

EPA

~~It has reported that SHR orally given fish oil at 1.5 g/kg/day for 13 weeks showed a decrease in blood pressure.[39)]~~

Page7, L29-Page7, L32

~~When SHR and normotensive rats were orally administrated 30 to 300 mg/kg/day of EPA for 8 weeks, the EPA significantly decreased the development of hypertension in SHR dose-dependently. Eight-week treatment with 30, 100, and 300 mg/kg of EPA reduced the mean SBP by 23, 29, and 32 mmHg, respectively, compared to untreated rats. Hypotensive effects of EPA progressed slowly and were reversible after the termination of treatment. However, daily administration of EPA to normotensive rats did not affect SBP.[42)]~~ Many animal experiments indicate that daily administration of EPA significantly decreased the development of hypertension in SHR dose dependently, although it did not affect to BP in normotensive rats.[41)]

Page8, L19-Page8, L26

~~5/6 nephrectomized rats treated with S-allylcysteine (200 mg/kg ip) and aged garlic extract (1.2 mL/kg ip) administrated every other day for 30 days showed increased SOD activity, reduced SBP, and improved markers of renal damage such as serum creatinine, BUN concentrations, proteinuria, and the degree of glomerulosclerosis and tubulointerstitial damage.[51)] Two kidney one clip hypertensive rats given 50 mg/day of aqueous extract of garlic orally for 4 weeks showed significantly decreased SBP and an ACE activity in the serum and various tissues such as the kidney, heart, aorta, and lung.[50)]~~ Antihypertensive effects of galic were reported in many studies using hypertensive rat models. The antihypertensive mechanism of garlic is assumed to involve ACE inhibitory effect,[47)] antioxidant effect, [48)]~~the scavenging of different reactive oxygen or nitrogen species,[52)]~~ activation of NO formation,[49)] and reduction in the synthesis of vasoconstrictor prostanoids.[50)] ~~On the other hand,~~ Although SHR fed diets containing either aged garlic extract (AGE) or raw garlic (RG) powder for 10 weeks showed a reduction of SBP from 4 weeks, ~~However~~ harmful effects were observed in the RG group, including a decrease in erythrocytes, an increase in reticulocytes, and the generation of a papilloma in the forestomach.

Page9, L7-Page9, L11

~~N^G-nitro-L-arginine methyl ester (L-NAME, NOS inhibitor) induced hypertensive rats and SHRSP rats took dried onion at 5% in their diets showed significantly decreased SBP from 1 to 4 weeks. Dietary onion decreased the thiobarbituric acid reactive substances (TBARS) in the plasma of these hypertensive rats. Also, onion increased nitrate/nitrite excreted in urine and NOS activity in the kidneys in SHRSP. These results suggest that the increased NO caused by the greater NOS activity, and additionally by the~~

~~increased saving of NO by the antioxidative activity of onion, may be one of the causes of the antihypertensive effect of onion.[63] Moreover,~~ There are ~~some~~ several reports that quercetin shows an antihypertensive effect through the antioxidant activity, [59] inhibition of ACE activity[60] and Ca^{2+} influx.[61] It has been considered that these results show synergistic antihypertensive effects ~~other than antioxidant activity. Rats which consumed quercetin-supplemented chow (1.5 g quercetin/kg chow) for 7 days before abdominal aortic constriction (AAC) showed the attenuation of carotid arterial blood pressure and cardiac hypertrophy versus AAC rats treated with vehicle. The level of aortic endothelial dysfunction was also similar between vehicle and quercetin treated groups, in spite of reduced aortic thickening in quercetin group. Importantly, quercetin treated rats did not show any deleterious changes in myocardial function.~~ Animal experiment using abdominal aortic constriction rat indicated ~~It has been suggested~~ that quercetin is also useful for preventing cardiovascular disease.[62]

Page9, L26-Page9, L27

~~The oral administration of PPH, that contained < 3 kDa peptides, isolated by membrane ultrafiltration from the thermolysin digest of pea protein isolate (PPI), to SHR at doses of 100 and 200 mg/kg body weight led to a lowering of the hourly SBP, with a maximum reduction of 19 mmHg at 4 h. In contrast, orally administered unhydrolyzed PPI exhibited no blood pressure-reducing effect in SHR, suggesting that thermolysin hydrolysis may have been responsible for releasing bioactive peptides from the native protein.[69] Futhermore, the oral administration of PPH to Han:SPRD-cy rats (a model of chronic kidney disease) over an 8-week period led to 29- and 25-mmHg reductions in SBP and DBP, respectively. The PPH-fed rats had lower plasma levels of angiotensin II, but there was no effect on plasma activity or renal mRNA levels of ACE. However, the renal expression of renin mRNA levels was reduced by approximately 50% in the PPH-fed rats. PPH also showed weak *in vitro* activities against renin and ACE with inhibitory activities of 17 and 19%, respectively, at a 1 mg/mL test concentration, suggesting that~~ PPH shows antihypertensive effects by influencing the renin-angiotensin system in rat model [65].

~~Nicotianamine~~

~~Nicotianamine, abundant in soybeans and pumpkin, is an intermediate of Munegi acid produced in plants and low molecular weight compounds from natural origins involved in metal transport. This nicotianamine showed ACE inhibitory activity in experiments *in vitro*. From the results, it is considered that foods rich in nicotianamine may have antihypertensive effects.[71] In studies with SHR, a single oral dose of nicotianamine (0.9, 4.5, and 9.0 mg/kg), from soybean broth, decreased the blood pressure after 1 hour. Also, the long-term oral administration of nicotianamine (0.9 and 4.5 mg/kg) decreased the blood pressure for 4 weeks, while that of nicotianamine (9.0 mg/kg) was decreased for the full 8 week feeding period.[72] Furthermore, asparagus exhibits marked ACE inhibitory activity in spite of its low content of nicotianamine compared to soybeans and pumpkin. It was found that 2 hydroxy nicotianamine, contained in asparagus, act as the active compound. SHR fed a diet with 5% asparagus for 10 weeks showed significantly decreased SBP, urinary protein excretion/creatinine excretion, and ACE activity in the kidney. Creatinine clearance was significantly higher in the asparagus group compared with a placebo group.[73] Buckwheat, a plant of the family Polygonaceae, is also high in 2-hydroxy-nicotianamine. Noodles and buckwheat mash using buckwheat flour are expected to have antihypertensive effects.~~

~~Peptides derived from egg white proteins~~

~~Eggs have been extensively studied regarding their biological activity in addition to nutritional contents. In particular, it has been reported that peptides derived from egg white protein hydrolysis show marked ACE inhibitory activity[74] and antioxidant activity.[75] These facts are surprising and notable. RVPSL (Arg-Val-Pro-Ser-Leu), a peptide contained in egg white,[76] has a strong antihypertensive effect similarly to captopril, an ACE inhibitor. SHR orally administrated 50 mg/kg/day of RVPSL for 4 weeks showed a significant decrease in SBP, along with a group of SHR 10 mg/kg/day of captopril. Humoral factors with a vasopressor effect, such as the serum Ang II, renin, and aldosterone concentrations, of the treatment group were reduced. The mRNA levels of renin, ACE, and AT1 receptor in the kidney also decreased significantly. Thus, it is suggested that RVPSL shows renoprotective and antihypertensive effects through the down-regulation of these humoral factors by inhibiting the RAAS in the same way as ACE inhibitors.[77] Since this peptide is very heat-stable, the daily ingestion of egg white may exert an antihypertensive effect even if it is cooked. However, human clinical trials using peptides derived from egg white proteins have not been performed. The results of the clinical trials are expected to come out in the near future.~~

Page10, L6

Besides the foods introduced in this paper, grains, vegetables, fruits, milk, cheese, meat, chicken, wine, mushrooms, lactic acid bacteria, nicotianamine and egg are various food sources with potential antihypertensive effects.

(3) **Comment:**

Figure 1 may be ommitted.

Response:

Thank you very much for your advice. According to your suggestion, we deleted Fig1 in revised manuscript.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Hypertension*

Sincerely yours,

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