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Evaluation of the Blood Pressure Lowering-Effect of the Lactotripeptides Valine-Proline-Proline and Isoleucine-Proline-Proline in Non-Hypertensive Japanese Subjects through a Meta-Analysis of Randomized-Controlled Studies

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Abstract

Several studies have reported the ability of the lactotripeptides valine-proline-proline (VPP) and isoleucine-proline-proline (IPP) to lower systolic blood pressure (SBP), including in Japanese populations. But the magnitude of the reported changes differs across trials. Conclusions from a previous meta-analysis in Japanese subjects suggest that this may be due at least partly to differences in subjects' blood pressure (BP) status. Therefore, we decided to resume this analysis, focusing only on non-hypertensive subjects and including newly-published eligible studies, in order to further evaluate the SBP-lowering effect of VPP/IPP and study the influence of the ingested dose, type of ingredient (enzymatic or fermented) and food product (drink or supplement). The systematic search of four databases (including two in Japanese) allowed to identify 11 relevant randomized-controlled trials (581 subjects), which were included in the meta-analysis. Results reported a significant decrease in SBP following VPP/IPP intake in non-hypertensive Japanese individuals, with an estimated effect-size of −3.44 mm Hg (95% CI, −4.53 to −2.34, $P < 0.0001$) as compared to placebo. There was no indication of heterogeneity or publication bias. Furthermore, the type of food product and ingredient did not influence the SBP-lowering effect, which was significant and

of same order of magnitude with either type of product and ingredient. Besides, the SBP-lowering effect remained significant when limiting to studies testing usual daily amounts of VPP/IPP (≤5 mg/d). This updated meta-analysis therefore confirms that VPP/IPP are effective in reducing SBP in non-hypertensive Japanese individuals, for amounts that may be ingested on a daily basis, and independently of the types of ingredient/food consumed. VPP/IPP-containing foods could therefore contribute to a better control of high-normal BP and/or to the maintenance of normal BP, and by such, may play a role in preventing high BP in individuals with normal or high-normal BP.

Keywords

Blood Pressure, IPP, VPP, Japanese, Meta-Analysis

1. Introduction

High blood pressure (BP) represents an important concern for population health, and may exert a significant burden on healthcare costs in many countries, including in Japan and other Asian countries [1]. Recommendations for the preliminary management of hypertension include modification of daily-life habits such as regular physical exercise and reduction of the consumption of salt or alcohol [2]. Simultaneously, clinical data from the literature suggest that some dairy-based peptides, such as the lactotripeptides valine-proline-proline (VPP) and isoleucine-proline-proline (IPP), can lower systolic blood pressure (SBP) [3] [4] [5] [6] [7]. These two peptides have initially been characterized as angiotensin-converting enzyme (ACE) inhibitors [8], and have been reported to display BP-lowering effects upon ingestion in animals, including in spontaneously hypertensive rats [9] [10] [11] [12]. Accordingly, it has been proposed that the SBP-lowering effect of the two peptides may be related to their ACE-inhibiting ability [13], although they may also act through the release of vasodilative elements [14] [15] including bradykinin [16], through the stimulation of Mas-receptors [16], or through an impact on the sympathetic nervous system [17] [18].

The ability of VPP/IPP to lower SBP following consumption has been reported in several previously-published meta-analyses, with effect-sizes of −2.9 mmHg (95% confidence interval [CI], −4.2 to −1.7) [19], −3.7 mmHg (95% CI, −6.7 to −1.8) [3] or −4.8 mmHg (95% CI:, −6.0 to −3.7) [5], in comparison to placebo. These meta-analyses have also indicated that the magnitude of the change would vary across trials, and some of this heterogeneity may be explained by the individuals' BP status [20] [21]. But few meta-analyses have specifically evaluated the impact of VPP/IPP on BP in subjects without overt hypertension. Furthermore, part of the heterogeneity may be due to ethnicity, with several meta-analyses reporting a larger efficacy in Asian individuals (from −5.5

mmHg to −6.9 mmHg) as compared with Europeans (from −1.2 mmHg to −1.4 mmHg) [3] [19], and no effect reported in some trials performed in Dutch subjects (e.g. [22]). Although not clearly elucidated, this ethnic difference in the efficacy of VPP/IPP may be due to variations in the pharmacokinetic response of VPP/IPP in Asian and Europeans individuals, or to environmental disparities such as different dietary habits [3] [19]. There have been numerous randomized-controlled studies in Asian individuals, especially in Japanese. However, several of them have only been available in Japanese journals which are not included in the major bibliographic databases, and most systematic reviews have not included searches for such journals [3] [4] [5]. In a meta-analysis released in 2015, which included all studies published until October 2014, including those from Japanese journals, we confirmed the ability of VPP/IPP to decrease SBP in Japanese individuals, as well as the significant impact of the individuals' BP status at baseline, with a stronger efficacy observed in hypertensive (HT) individuals (−8.4 mm Hg) as compared with non-hypertensive (non-HT) individuals (−3.4 mm Hg) [21]. Finally, VPP/IPP ingredients can be obtained by enzymatic hydrolysis or fermentation. Even though similar final products are obtained in either case, published findings indicate that the way of production may have an impact on the ingredient's ability to lower BP [19] [22]. Moreover, VPP/IPP can be included in different kinds of food products (e.g. drinks or dietary supplements), which may also influence the size of their SBP-reducing effect.

The aim of the current meta-analysis was to perform an update of our previous meta-analysis published in 2015, and to more specifically evaluate the magnitude of the SBP variation occurring following consumption of VPP/IPP in healthy non-HT Japanese subjects. For that purpose, we performed an update of the literature search in order to include any newly published eligible studies, and we focused on studies in non-HT Japanese subjects. Additional objectives of the current meta-analysis were to examine the impact of the consumed amount of VPP/IPP, as well as the impact of the form of VPP/IPP (enzymatic or fermented ingredient) and type of food product (drink or dietary supplement), on the SPB-lowering effect.

2. Methods

The methodology of the systematic review has been described in a protocol available in the PROSPERO international database (CRD42014014322). This study has been performed following the PRISMA guidelines [23]. The PROSPERO protocol, as well as the PRISMA worksheet, are provided as supplementary materials (see **Protocol S1** and **Checklist S1**, respectively).

2.1. Literature Searches

A systematic search of the following databases was performed up to May 22 2018: MEDLINE via PUBMED (internet link: http://www.ncbi.nlm.nih.gov/pubmed), Cochrane Central Register of Controlled

Trials (internet link: http://www.thecochranelibrary.com), J-STAGE (internet link: https://www.jstage.jst.go.jp/browse) and J Dream III (internet link: http://jdream3.com). The set of key-words was as follows: [lactotripeptide OR lactotripeptides OR "dairy peptide" OR "dairy peptides" OR ("Ile-Pro-Pro" AND "Val-Pro-Pro") OR ("Isoleucyl-prolyl-proline" AND "valyl-prolyl-proline") OR ("Valine-proline-proline" AND "isoleucine-proline-proline") OR ("IPP" and "VPP") OR "fermented milk" OR "milk fermented" OR "sour milk"] AND (hypertension OR "blood pressure"). The J-STAGE and J Dream III correspond to databases of Japanese journals. The words "Asian OR Japan OR Japanese" were included in the search queries for the two other databases. Articles written in all languages (without limitation) were searched for, and the lists of references of selected articles were also screened for any possibly relevant papers (hand search).

2.2. Screening Process

The screening of all retrieved papers was performed by three independent researchers (all with a doctoral degree), first on the basis of the reading of titles/abstracts, and secondly on the basis of full-texts for potentially relevant articles. Disagreements were addressed through discussion. We used professional English translations for articles published in Japanese. Inclusion criteria were as follows: randomized-controlled studies, with a single or double-blind design, in Japanese and non-HT (SBP lower than 140 mm Hg and diastolic blood pressure [DBP] lower than 90 mm Hg) individuals, who consumed VPP and IPP during more than 8 days, and which reported office SBP values at baseline and at least one additional time-point. Exclusion criteria were as follows: duplicate publications, reviews, non-human studies, non-randomized studies, studies with an open design, non-placebo controlled studies, trials where office SBP was not assessed, interventions in which VPP+IPP were not consumed, trials in non-Japanese subjects, in diseased individuals or exclusively in HT individuals, trials in which VPP and IPP were both tested separately, or in which VPP+IPP were consumed during a shorter period than 8 days. Finally, studies that presented results for non-HT and HT subjects together, without individualization of the results for non-HT subjects separately, were also excluded.

2.3. Data Collection

Data were extracted by two independent scientists through the use of a pre-defined worksheet, and any disagreements were solved by discussion. Additional data were requested through direct contacts of authors for nine articles, and appropriate answers were received in all cases. The information collected for all studies was as follows: 1) authors/date of publication, design, duration of the intervention, amount of VPP/IPP consumed daily, type of VPP/IPP ingredient (enzymatic or fermented) and type of food product (drink or dietary supplement) consumed; 2) individuals' details (mean age and BP status at baseline); 3)

primary outcome (variation in office SBP between baseline and last endpoint), secondary outcome (variation in office DBP between baseline and last endpoint), number of analyzed individuals, mean effect and corresponding variability measures (95% CI or standard deviation [SD]). We chose office BP measurements for our meta-analysis because this was the dimension of BP which was reported in all studies. When a study described results for HT and non-HT subjects, only data for non-HT subjects were extracted. If a study further categorized non-HT subjects as subjects with normal BP (referred to as normotensive [NT] subjects in this paper) or with high-normal BP (referred to as pre-hypertensive [PHT] subjects), BP changes were collected for each category of individuals separately. Daily doses of VPP/IPP were expressed in mg, as the sum of the VPP and IPP contents and also as "VPP equivalents"[1] [8] [24]. Finally, the Jadad-score was used to evaluate study quality [25].

2.4. Statistical Analysis

The primary outcome for the meta-analysis was the mean difference between groups consuming VPP/IPP and groups consuming placebo in the variation in office SBP between baseline and last endpoint. The secondary outcome was office DBP reported in the same way. The mean pooled effect-size of VPP/IPP and its 95% CI were calculated by using fixed and random effects, with the REstricted Maximum Likelihood (REML) estimator [26] [27]. Since we assumed some heterogeneity from results of previous meta-analyses [3] [21], the random-effect model was defined as the main analysis. Trials were weighted according to the inverse of their variance. Heterogeneity across trials was estimated through tau^2, I^2, H^2, and Cochran's Q test statistics [28]. Publication bias was examined by means of a funnel plot (SE of effect plotted against estimate of effect-size for each trial) and through the Kendall's rank correlation test statistic (Kendall's tau) between the standardised effect-size and the SE values of the effect, according to Begg and Mazumdar [29].

We used adjusted meta-analyses, meta-regressions and sub-group analyses to examine the heterogeneity of the effect of VPP/IPP on SBP. More precisely, we studied the impact of the following parameters: type of individuals (NT or PHT, depending on their BP level at baseline), amount of VPP/IPP consumed daily, duration of VPP/IPP consumption, sort of VPP/IPP ingredient (enzymatic or fermented) and type of food product (drink or dietary supplement). Furthermore, the following sub-group analyses were performed: for NT and PHT individuals separately, within series which tested VPP/IPP amounts ≤ 5 mg/d (corresponding to amounts which can be ingested on a daily basis), and for each type of VPP/IPP ingredient and product separately. Finally, we also evaluated the impact of each study on the global findings by removing one study at a time, and

[1]VPP equivalents are calculated as the IPP content in mg multiplied by 1.7 and added to the VPP content in mg. The 1.7 correction factor is to take into account the difference in potency of the two tripeptides to inhibit ACE activity *in vitro*.

by computing influence statistics (with Cook distance, hat value and studentised residuals).

All analyses were planned in advance and detailed in a statistical analysis plan, with the exception of the sub-group analyses examining heterogeneity. The Meta for package [27] version 2.0 - 0 under R (The R Foundation for Statistical Computing 2017) version 3.4.2 was used for all statistical analyses.

3. Results

3.1. Characteristics of Selected Studies

Two hundred and seventy-seven articles were retrieved from the literature search in total (including 221 from the Japanese databases). Hand search allowed to identify two additional articles. By applying the inclusion and exclusion criteria described above, 268 papers were excluded and 11 studies were selected (**Figure 1**). All 11 included studies were available as full-texts in journals following a peer-review process; four have been published in international English periodicals [30] [31] [32] [33], and the others in Japanese periodicals [34]-[40]. Articles in Japanese were translated into English. All 11 selected trials involved Japanese and non-HT subjects, who were further categorized as NT or PHT subjects (no study only displayed data for NT and PHT individuals grouped together). Seventeen series, defined according to the type of individuals (NT or PHT) and amount of VPP/IPP, were considered from the 11 selected trials (**Table 1**). Indeed, there were six series involving NT subjects and 11 series involving PHT subjects. Six studies (nine series) used amounts of VPP/IPP ≤ 5 mg/d[2], which represent amounts that may possibly be ingested on a daily basis. For the five other trials (eight series), consumed amounts were greater than the "usual" amounts (from 9 to 17 mg/d[3]). This is related to the fact that the main objective of these five trials was to assess the security of VPP/IPP when consumed at higher amounts than the usual ones. The type of ingredient tested was fermented VPP/IPP in four studies (four series) and enzymatic VPP/IPP in the seven remaining studies (13 series). VPP/IPP were consumed as a dietary supplement (tablets) in seven studies (12 series) and as a drink in the four remaining studies (five series). In total, 581 individuals were included within the 17 analyzed series, corresponding to 581 treatment-periods (304 for VPP/IPP and 277 for placebo). All selected trials were randomized-controlled studies that had a parallel and double-blind design, except one (that was single-blind [32]) (**Table 1**). All needed information was extracted from the original papers or retrieved from authors, and the quality of the studies was considered as adequate (Jadad-score ≥ 4 for all trials but two that displayed a score of 3 [32] [36], see **Table 1**). This suggests that the risk of bias should have been marginal within studies. For the primary outcome (variation in office SBP between baseline and last

[2]For doses expressed both as the sum of the IPP and VPP contents or as "VPP equivalents" (as defined previously).

[3]For doses expressed as the sum of the IPP and VPP contents; for doses expressed as "VPP equivalents" (as defined previously), corresponding doses would be of 13 and 24 mg/d, respectively.

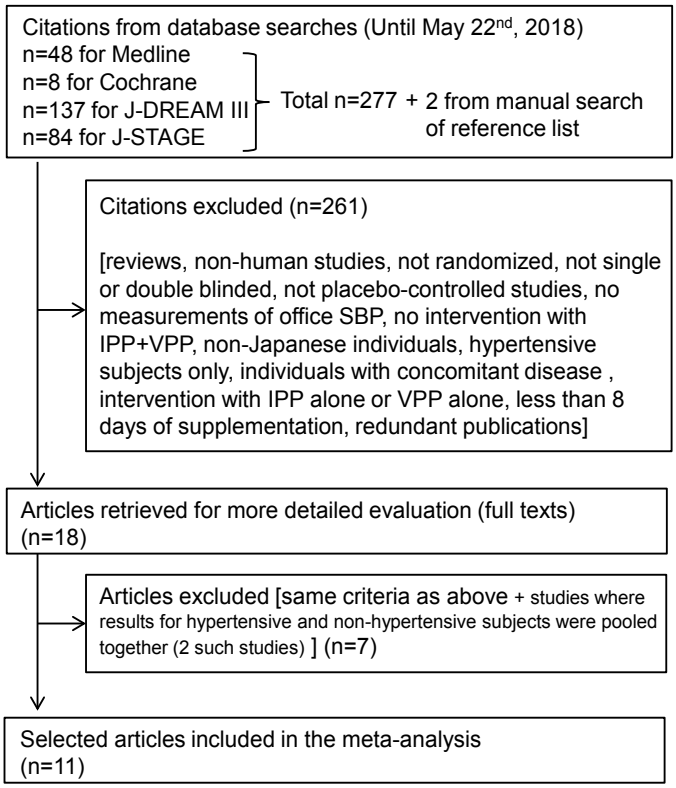

Figure 1. Flow diagram of study selection. The list of the 18 articles selected for full-text evaluation is available in **Table S1** (in supplementary material), which also describes the outcome of the selection process for each article (including justification for exclusion). IPP: isoleucine-proline-proline. SBP: systolicblood pressure. VPP: valine-proline-proline.

endpoint), the mean difference between VPP/IPP and placebo differed across studies from −5.8 (± 3.1 SE) mm Hg in favour of VPP/IPP to +0.6 (±5.4 SE) in favour of placebo (**Table 2** and **Figure 2**). Corresponding findings for DBP (secondary outcome) are described in supplementary material (**Table S2** and **Figure S1**).

3.2. Effect of VPP and IPP on Blood Pressure

The findings of the main meta-analysis (SBP variations at last endpoint) reported a statistically significant larger effect of VPP/IPP on SBP as compared with placebo in Japanese non-HT individuals, of a size of −3.44 mm Hg ($P < 0.0001$, 95% CI, −4.53 to −2.34; random and fixed-effect models) (**Figure 2**). The estimated effect-size was lower for DBP though still significant [−1.50 mm Hg (95% CI, −2.55 to −0.44, $P = 0.006$ with the random-effect model)] (**Figure S1**).

There was no significant heterogeneity between series for SBP ($I2 = 0.0\%$, $tau2 = 0.0$, $Q = 3.7$, $P = 1.00$) and for DBP ($I2 = 9.0\%$, $tau2 = 0.4$, $Q = 15.7$, $P = 0.47$). Nevertheless, heterogeneity was further explored, as described below, since this should provide interesting input regarding the possible parameters that may impact the results.

Table 1. Characteristics of the 11 studies included in the meta-analysis of randomized-controlled trials of the effect of valine-proline-proline and isoleucine-proline-proline on systolic blood pressure in non-hypertensive Japanese subjects[a].

		Intervention							Population		
Study #	Reference	Jadad score	Design	Ingredient type	Food product[b]	Duration (weeks)	VPP/IPP dose (mg/d)[c]	VPP/IPP dose in VPP eq. (mg/d)[d]	Type of subjects (BP status)	n	Mean age (y)
1	Aihara 2005 [30]	4	D	Fermented	Supplement	4	13	16	PHT	40	51.4
2	Ishida 2006 [34]	4	D	Enzymatic	Supplement	4	16	23	PHT	18	51.2
									NT	18	48.9
3	Ishida 2007 [35]	4	D	Enzymatic	Supplement	12	4	5	PHT	71	50.3
4	Ishida 2011 [31]	4	D	Enzymatic	Supplement	4	17	24	NT	16	44.2
									PHT	16	49.6
5	Itakura 2001 [36]	3	D	Fermented	Drink	8	3	3	NT	26	36.0
6	Mizuno 2005 [32]	3	S	Enzymatic	Supplement	6	4	5	PHT	24	42.8
							3	3	PHT	24	46.4
							2	2	PHT	24	45.0
7	Nakamura 2004 [38]	4	D	Fermented	Drink	12	4	5	PHT	106	38.5
8	Sano 2005 [33]	4	D	Enzymatic	Drink	12	3	4	PHT	104	49.0
9	Sano 2004 [39]	4	D	Enzymatic	Drink	4	9	13	NT	11	44.6
									PHT	16	45.5
10	Kajimoto 2001 [37]	4	D	Fermented	Supplement	2	12	16	NT	43	29.7
11	Uchida 2016 [40]	5	D	Enzymatic	Supplement	12	3	5	PHT	24	56.3
									NT	24	56.3

[a]Abbreviations: ACE: angiotensin-converting enzyme. BP: blood pressure. D: double blinded. IPP: isoleucine-proline-proline. NT: normotensive (subjects with normal BP). n: number of subjects analyzed. PHT: pre-hypertensive (subjects with high-normal BP). S: single-blinded. VPP: valine-proline-proline. Y: years. [b]Type of food product in which VPP/IPP has been added to, namely drink or dietary supplement ("supplement", as tablets). [c]IPP content in mg + VPP content in mg. [d]Dose expressed in "VPP equivalents", calculated as the IPP content in mg multiplied by 1.7 added to the VPP content in mg. The 1.7 correction factor is to take into account the difference in potency between the two tripeptides to inhibit ACE activity in vitro [8] [24].

3.3. Heterogeneity and Sub-Group Meta-Analyses

Adjusted meta-analyses, meta-regressions and sub-group meta-analyses were used to further explore heterogeneity regarding the effect of VPP/IPP on SBP. The conclusions of these analyses are summarized below. First, the individuals' BP status at baseline was found to exert no significant impact (P = 0.93), and sub-group analyses showed that the size of the effect of VPP/IPP on SBP was similar in NT individuals [−3.54 mm Hg (95% CI, −6.07 to −1.01)] and PHT individuals [−3.41 mm Hg (95% CI, −4.63 to −2.20)], and remained significant in both groups (P = 0.006 and P < 0.0001, respectively) (**Figure 3**). Second, there was also no significant impact of the daily amount of VPP/IPP, independently of the way it was analyzed [*i.e.* as a continuous variable (P = 0.65) or as a categorical variable (≤ vs > 5 mg/d, *i.e.*, "usual" vs higher than usual amounts, respectively; P = 0.74]. Interestingly, in the sub-group analysis on the nine series in which usual daily amounts of VPP/IPP were consumed, the SBP-lowering effect

Table 2. Effect of valine-proline-proline and isoleucine-proline-proline on systolic blood pressure at final endpoint in non-hypertensive Japanese subjects[a].

Study #	Series #	Study reference	Type of subjects (BP status)	VPP/IPP dose (mg/d)[b]	VPP/IPP dose InVPP eq. (mg/d)[c]	Treated group			Placebo group			Effect-size	SE
						n	Change in SBP (mmHg)		n	Change in SBP (mmHg)		Mean difference between groups (mmHg)	
							Mean	SD		Mean	SD		
1	101	Aihara 2005 [30]	PHT	13	16	20	NA	NA	20	NA	NA	−3.2	2.8
2	201	Ishida 2006 [34]	PHT	16	23	9	−8.3	10.0	9	−3.9	9.6	−4.4	4.6
2	202	Ishida 2006 [34]	NT	16	23	9	−0.7	8.6	9	−1.3	13.6	0.6	5.4
3	301	Ishida 2007 [35]	PHT	4	5	35	−6.8	8.4	36	−3.2	7.5	−3.6	1.6
4	401	Ishida 2011 [31]	NT	17	24	8	−2.5	5.6	8	2.4	4.7	−4.9	2.6
4	402	Ishida 2011 [31]	PHT	17	24	8	−6.7	8.1	8	−1.9	4.8	−4.8	3.3
5	501	Itakura 2001 [36]	NT	3	3	13	−3.3	5.3	13	0.7	5.6	−4.0	2.1
6	601	Mizuno 2005 [32]	PHT	4	5	12	−2.8	5.8	12	0.3	7.4	−3.1	2.7
6	602	Mizuno 2005 [32]	PHT	3	3	12	−1.5	7.8	12	0.3	7.4	−1.8	3.1
6	603	Mizuno 2005 [32]	PHT	2	2	12	−1.5	10.4	12	0.3	7.4	−1.8	3.7
7	701	Nakamura 2004 [38]	PHT	4	5	53	−6.1	5.7	53	−2.1	8.4	−4.0	1.3
8	801	Sano 2005 [33]	PHT	3	4	52	−4.6	6.2	52	−1.7	6.2	−2.9	1.1
9	901	Sano 2004 [39]	NT	9	13	6	0.8	4.4	5	0.3	10.8	0.5	4.8
9	902	Sano 2004 [39]	PHT	9	13	8	−4.4	6.9	8	1.4	5.3	−5.8	3.1
10	1001	Kajimoto 2001 [37]	NT	12	16	21	−3.9	7.9	22	−0.9	11.2	−3.0	3.0
11	1101	Uchida 2016 [40]	PHT	3	5	11	−4.0	14.0	13	−2.2	16.5	−1.8	6.3
11	1102	Uchida 2016 [40]	NT	3	5	15	1.7	11.3	9	7.2	12.7	−5.5	5.0

[a]Abbreviations: ACE: angiotensin-converting enzyme. BP: blood pressure. IPP: isoleucine-proline-proline. n: number of subjects. NA: not available. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). SBP: systolic blood pressure. SD: standard deviation. SE: standard error. VPP: valine-proline-proline. [b]IPP content in mg + VPP content in mg. [c]Dose expressed in "VPP equivalents", calculated as the IPP content in mg multiplied by 1.7 added to the VPP content in mg. The 1.7 correction factor is to take into account the difference in potency betweenthe two tripeptides to inhibit ACE activity in vitro [8] [24].

was still significant (P < 0.0001), with a reduction of −3.34 mm Hg as compared to placebo (95%CI, −4.58 to −2.10) (**Figure 4**). Third, the form of VPP/IPP ingredient was shown to exert no significant impact (P = 0.65), and sub-group analyses reported that the effect on SBP was significant and of a similar magnitude with either type of ingredient. More precisely, the estimated effect-size was −3.80 mm Hg [(95% CI, −5.70 to −1.90), P < 0.0001] for fermented VPP/IPP, and −3.25 mm Hg [(95% CI, −4.59 to −1.92), P < 0.0001] for enzymatic VPP/IPP (**Figure 5**). The same overall conclusions were obtained when the analysis was restricted to series where subjects received usual amounts of VPP/IPP (*i.e.*, ≤5 mg/d). There was still no significant impact of the ingredient type (P = 0.46), with a pooled-effect of −4.00 mm Hg (95% CI, −6.15 to −1.85) for fermented

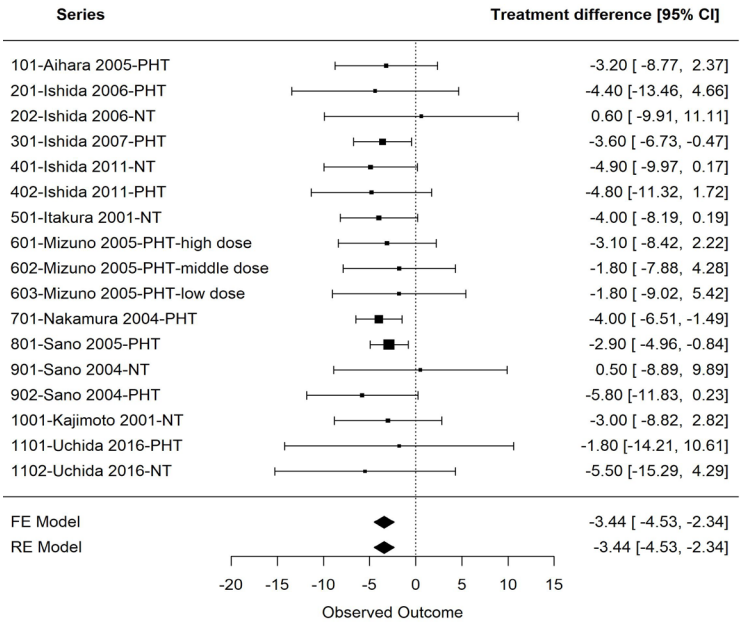

Figure 2. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline in the meta-analysis of 17 series of findings of its effect on systolic blood pressure in non-hypertensive Japanese subjects. BP: blood pressure. CI: confidence interval. FE: fixed effect. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). RE: random effect. SBP: systolic blood pressure. Series numbers are those indicated in Table 2.

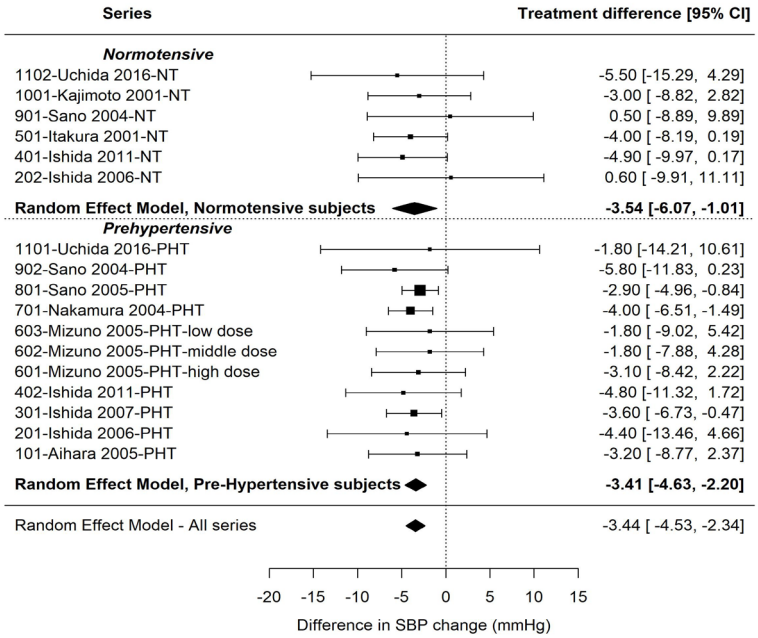

Figure 3. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline on systolic blood pressure in the sub-group analysis according to the baseline blood pressure status of the subject. BP: blood pressure. CI: confidence interval. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). SBP: systolic blood pressure. Series numbers are those indicated in Table 2.

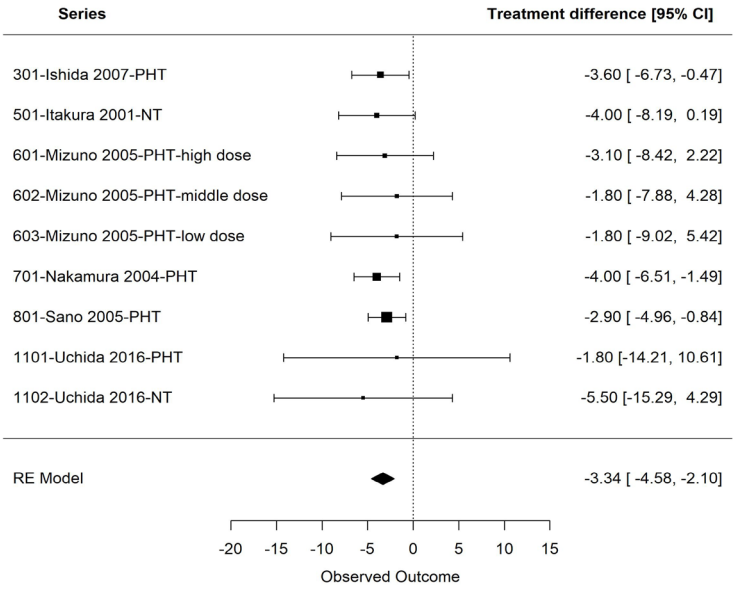

Figure 4. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline (VPP/IPP) on systolic blood pressure in the sub-group analysis within the nine series that tested usual daily doses of VPP/IPP (*i.e.*, ≤5 mg/d). BP: blood pressure. CI: confidence interval. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). RE: random effect. SBP: systolic blood pressure. Series numbers are those indicated in Table 2.

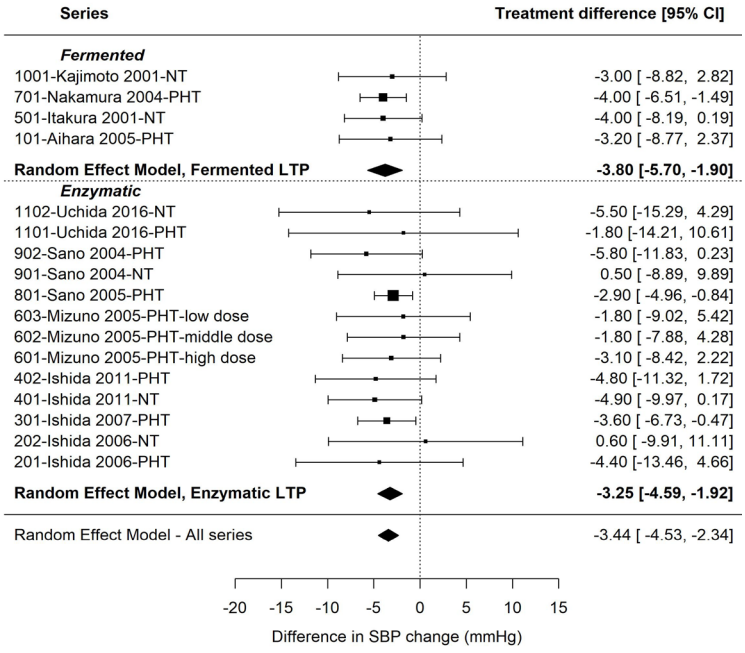

Figure 5. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline (VPP/IPP) on systolic blood pressure in the sub-group analysis according to the type of VPP/IPP ingredient. BP: blood pressure. CI: confidence interval. LTP: lactotripeptides (VPP/IPP). NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). SBP: systolic blood pressure. Series numbers are those indicated in Table 2.

VPP/IPP and of −3.01 mm Hg (95% CI, −4.53 to −1.49) for enzymatic VPP/IPP (Figure S2). The VPP/IPP-induced change in SBP also remained significant with either type of ingredient (P = 0.0003 and P < 0.0001 for fermented and enzymatic VPP/IPP, respectively). Fourth, and similarly, no significant impact of the food product type was observed (P = 0.94), the effect of VPP/IPP on SBP being of the same magnitude when consumed as a drink [−3.47 mm Hg (95% CI, −4.90 to −2.04)] or as a dietary supplement [−3.39 mm Hg (95% CI, −5.09 to −1.69) (Figure 6). Interestingly, the change in SBP induced by VPP/IPP was significant with either type of products (P < 0.0001 in both cases). The same conclusions were obtained if the analysis was restricted to series where subjects received usual amounts of VPP/IPP (*i.e.*, ≤5 mg/d), with no significant influence of the type of food (P = 0.83), and a pooled-effect of −3.43 mm Hg with drink (95% CI, −4.92 to −1.94) and of −3.14 mm Hg with dietary supplement (95% CI, −5.37 to −0.90) (Figure S3). And the SBP reduction induced by VPP/IPP remained significant with either type of product (P < 0.0001 and P = 0.006 for drink and dietary supplement, respectively). Finally, the duration of the VPP/IPP ingestion was not shown to exert any significant impact on the effect of VPP/IPP on SBP (P = 0.96).

3.4. Analysis of the Influence of Individual Series and Publication Bias

The impact of omitting one series at a time and the computation of influence diagnostics found that no single series exerted a strong-enough impact on the results that may have generated bias in the observed conclusions. Indeed, VPP/IPP always exerted a significant effect (P < 0.0001) on SBP, independently on which series was omitted. Two series of PHT subjects were shown to exert the strongest influence on the results, namely series #701 and #801, due to their larger sample sizes (106 and 104 patients, respectively, see Table 2). Nevertheless, their results were consistent with those of other studies and with the overall estimate of the effect from the meta-analysis. Therefore, they did not introduce any heterogeneity, and the results remained consistent with the overall analysis when either of these two series was excluded from the analysis.

Finally, the funnel plot did not show any indication of asymmetry (Figure 7), and the Kendall's Tau statistic was not significant (Kendall's Tau = 0.21, P = 0.27). This suggests that there was no publication bias for SBP in the 11 trials included in our meta-analysis. The same was reported for DBP (Figure S4).

4. Discussion

Conclusions from the meta-analysis of the 11 randomized-controlled studies identified with data in non-HT Japanese subjects indicated that VPP/IPP ingestion produced a significant decrease in SBP in this population, when compared with placebo-control. The effect-size was estimated at −3.44 mm Hg (95% CI, −4.53 to −2.34, P < 0.0001) and there was no indication of heterogeneity or publication bias. A smaller but significant effect was also observed on DBP

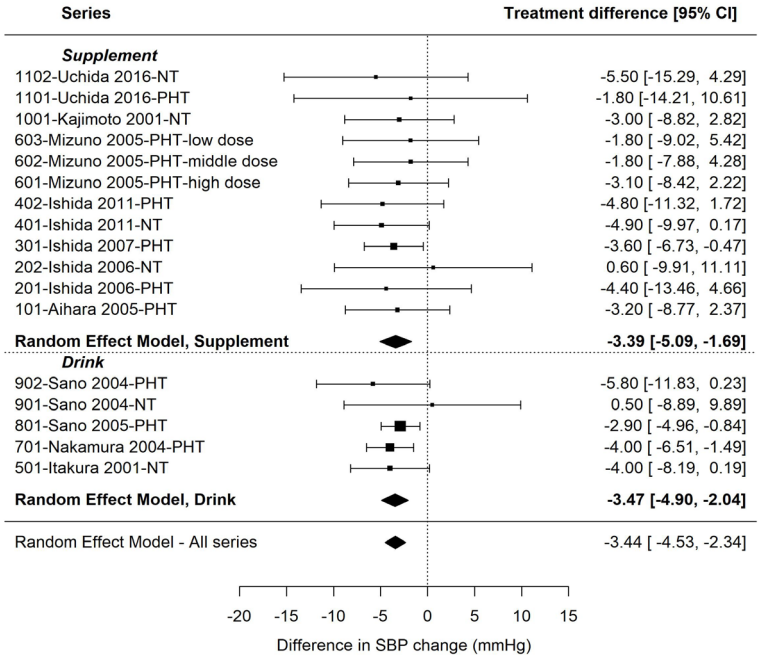

Figure 6. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline (VPP/IPP) on systolic blood pressure in the sub-group analysis according to the type of food product. BP: blood pressure. CI: confidence interval. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). SBP: systolic blood pressure. Series numbers are those indicated in Table 2.

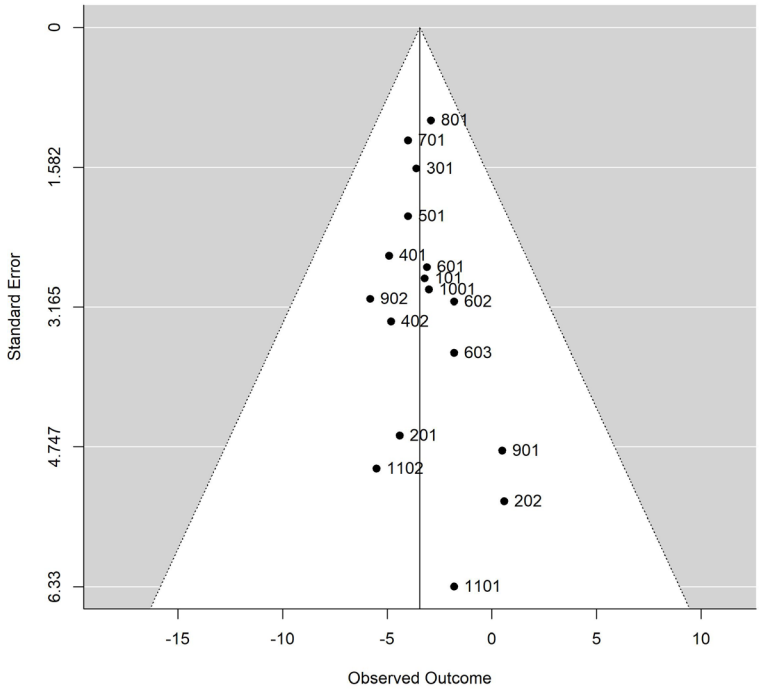

Figure 7. Funnel plot used to explore the potential for publication bias in the meta-analysis of 17 series for the effect of valine-proline-proline/isoleucine-proline-proline on systolic blood pressure in non-hypertensive Japanese subjects. Series numbers are those indicated in Table 2.

[−1.50 mm Hg (95% CI, −2.55 to −0.44), P = 0.006]. Interestingly, the impact of VPP/IPP on SBP was significant and of the same order of magnitude in subjects with normal BP (NT: −3.54 mm Hg) as in those with high-normal BP (PHT: −3.41 mm Hg). Results from this updated meta-analysis therefore confirm results from our previous meta-analysis published in 2015 [21], with a larger number of non-HT subjects included.

Furthermore, results from adjusted meta-analyses and sub-group analyses showed that the form of VPP/IPP ingredient (fermented or enzymatic) and the type of food product (drink or dietary supplement) did not influence significantly the SBP-lowering effect of VPP/IPP, which was significant and of the same order of magnitude with either type of ingredient and product. These findings, obtained in non-HT subjects, therefore do not support data from the literature suggesting that the mode of production of VPP/IPP (by fermentation or enzymatic hydrolysis) may influence the efficacy of the lactotripeptides [19] [22]. Finally, the SBP-lowering effect of VPP/IPP observed in non-HT subjects remained significant in a sub-analysis that considered only studies in which usual daily amounts (≤5 mg/d) of VPP/IPP were ingested. Results from this updated meta-analysis therefore further support the ability of VPP/IPP to decrease SBP in non-HT Japanese subjects at amounts likely to be consumed on a daily basis, and independently of the types of ingredient and food product consumed.

In Japan, the lactotripeptides VPP/IPP have been permitted as functional components of products for Foods for Specified Health Uses (FOSHU) since 1997, under the health claim "suitable for a person with high-normal blood pressure" and for a labeled daily dose of 3.4 mg of VPP/IPP, expressed in "VPP equivalents". "VPP equivalents" was chosen to express the VPP/IPP dose because it was considered as easier to understand by consumers. Results from this meta-analysis support the VPP/IPP health claim for FOSHU under the above-mentioned wording and daily dose.

Significant relationships between SBP and risk of cardiovascular diseases (CVD) and between SBP and mortality have been reported in epidemiological studies performed in Asian individuals, including Japanese. For CVD, available data suggest that reducing SBP until 115 mm Hg can exert a possible beneficial impact [41] [42]. Thus, the reduction in SBP induced by the consumption of VPP/IPP and reported here in non-HT individuals, although modest (−3.44 mm Hg), may be of interest at the scale of the population, especially regarding CVD prevention. In addition, some advocated and effective preventive strategies, such as the reduction of sodium dietary intakes, induce decreases in SBP of a same extent [43] [44]. Conclusions of this updated meta-analysis, in which results from all existing trials carried out in Japanese subjects have been combined, therefore ascertain that VPP/IPP ingestion can be beneficial in order to help in maintaining a normal SBP or improving SBP regulation in Japanese subjects without overt hypertension.

Finally, it should be mentioned that few of the studies included in this meta-analysis have evaluated other outcomes beside office BP. For instance, no trial

measured twenty-four-hour ambulatory BP, and flow-mediated dilation (FMD) was investigated in one study only [40]. Findings from this study showed a significant increase in FMD following VPP/IPP intake when compared with control, therefore suggesting that VPP/IPP may exert a beneficial impact on vascular endothelial function in healthy non-HT subjects [40].

Our meta-analysis displays several strengths. First, two Japanese databases were used for the literature search, which allowed to retrieve eligible trials written in Japanese and which are not included in the MEDLINE and Cochrane databases. This strategy permitted to identify more studies than the previous meta-analyses with results in Japanese subjects (e.g., [3]). This also allowed to evaluate more accurately the magnitude of the impact of VPP/IPP on SBP in Japanese individuals. Second, we focused on results obtained in non-HT subjects, which allowed us to characterize the extent of the variation in SBP induced by VPP/IPP in healthy subjects without overt hypertension. Besides, robustness of our meta-analysis is supported by the fact that we did not find any indication of publication bias and that no individual study was shown to exert a high impact on the global findings. Moreover, all included trials were randomized-controlled studies published in peer-reviewed journals, all but one with a double-blind design. This lowered the risk of bias, which was further limited through the fact that every data had been extracted from the original papers or retrieved from authors.

Nevertheless, this meta-analysis displays a few weaknesses that should be mentioned since they may have influenced the analysis of the data. Despite a reasonable number of included trials and individuals in the overall analysis, the small dataset of some of the sub-group analyses should lead to consider their conclusions with some caution. For instance, in the sub-analyses on the impact of the type of food product and ingredient within series in which usual amounts of VPP/IPP were consumed, only two series were available for fermented VPP/IPP at doses ≤ 5 mg/d. Besides, the number of subjects in individual study groups was low for some included studies. This is related to the fact that we purposely chose to not apply any exclusion criteria on the basis of the number of subjects included in the eligible studies for our meta-analysis. This was done in the objective to exploit the totality of the data available in the literature, which allowed us to evaluate the effect of VPP/IPP on BP more accurately. Finally, although there was no indication of any publication bias, some eligible trials may nevertheless be unpublished.

Therefore, conclusions from this updated meta-analysis confirm that the lactotripeptides VPP and IPP can decrease office SBP in a significant way in healthy Japanese individuals without overt hypertension, with a magnitude which was statistically and clinically significant, and which may lower the risk of CVD at the population level [45]. Moreover, the SBP decrease was reported for amounts of VPP/IPP that can potentially be consumed on a regular daily basis, and independently of the types of ingredient (enzymatic or fermented) and food product (drink or dietary supplement) consumed. These findings suggest that the com-

bination of the lactotripeptides VPP and IPP may be effective in preventing hypertension in healthy Japanese populations exhibiting normal or high-normal BP. In these subjects, VPP/IPP-containing foods could contribute to a better control of high-normal BP level and/or to the maintenance of a normal BP. However, further studies are needed to better dimension the clinical efficacy of the milk-derived VPP and IPP peptides in this population.

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Conflicts of Interest

Veronique Braesco, Aurelie Chanson-Rolle, François Aubin, Ryuji Takeda, and Yasuhiro Saito have received fees from Asahi Group Holdings for performing the systematic review.

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Supplementary Material

Protocol S1: Protocol of the systematic review as published on the PROSPERO register.

Influence of the lactotripeptides Isoleucine-Proline-Proline and Valine-Proline-Proline on blood pressure in Asian subjects: a systematic review and meta-analysis of randomized controlled trials (update)

Aurelie Chanson-Rolle, Veronique Braesco, François Aubin, Ryuji Takeda, Yasuhiro Saito

Citation

Aurelie Chanson-Rolle, Veronique Braesco, François Aubin, Ryuji Takeda, Yasuhiro Saito. Influence of the lactotripeptides Isoleucine-Proline-Proline and Valine-Proline-Proline on blood pressure in Asian subjects: a systematic review and meta-analysis of randomized controlled trials (update). PROSPERO 2014 CRD42014014322 Available from:

http://www.crd.york.ac.uk/PROSPERO/display_record.php?ID=CRD42014014322

Review question

Do the lactotripeptides Isoleucine-Proline-Proline and Valine-Proline-Proline reduce systolic blood pressure in Asian subjects? What is the influence of the ingested dose of the lactotripeptides Isoleucine-Proline-Proline and Valine-Proline-Proline on the effect on blood pressure?

Searches

The following bibliographic databases had been searched: MEDLINE (via PubMed), Cochrane Central Register of Controlled Trials (via the Cochrane Library), J-STAGE (developed by Japan Science and Technology Agency - JST), J Dream III (originally developed by JST and maintained until version II, although transferred to G-search Ltd from version III, Apr 2013). J-STAGE and J Dream III are bibliographic databases for publications written in Japanese language. MEDLINE and Cochrane were searched until September 17th 2014. J-STAGE was searched until October 1st 2014 and J Dream III was searched until September 25th 2014. For the update of the sytematic review, all four databases were searched until May 22nd 2018.

No restrictions (vs language or publication period) were applied to the search. The following keywords were searched: (lactotripeptide* OR "Dairy peptide*" OR ("Ile-Pro-Pro" AND "Val-Pro-Pro") OR ("Isoleucyl-prolyl-proline" AND "valyl-prolyl-proline") OR ("Valine-proline-proline" AND "isoleucine-proline-proline") OR ("IPP" and "VPP") OR "Fermented milk" OR "milk fermented" or "sour milk")

AND

(hypertension OR "blood pressure")

AND

(japan OR japanese OR asian)

The key words "japan OR japanese OR asian" were not used for the search on

the Japanese databases. Paper screening and selection will be performed independently by two team members and discrepancies will be resolved by discussions. All retrieved studies will be screened by the two collaborators on the basis of the reading of titles and abstracts to select studies to be assessed further, and all potentially relevant studies will be further considered by reading the full texts.

Types of study to be included

Inclusion criteria: randomized controlled trials, single blinded or double blinded, involving the consumption of Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP) by Asian adult subjects for longer than 8 days, with office systolic blood pressure measurements at baseline and one/more time points. Exclusion criteria: reviews (general or systematic reviews, meta-analyses), letters, opinion papers, view points, etc…, non-human studies, studies which are not intervention trials, intervention trials which are not randomized-controlled trials, randomized-controlled trials with an open design, studies in which no measure of SBP was reported, studies in which there was no intervention with IPP+VPP (studies testing IPP alone or VPP alone will be excluded), studies where IPP/VPP intake period is less than 8 days

Condition or domain being studied

Hypertension is a major determinant of health and is likely to have an effect on medical economics worldwide, including in Japan (Nakamura *et al* 2014). Hypertension can be prevented with lifestyle measures, such as physical activity, maintaining a normal body weight, and adopting a healthy diet. Besides this, several randomized trials and meta-analyses have shown that some peptides derived from milk proteins, such as isoleucine-proline-proline (IPP) and valine-proline-proline (VPP), decrease systolic blood pressure (SBP). However, the size of the effect varies among studies, and it seems to be stronger in Asian subjects than in European subjects (Cicero *et al.* 2011). Several randomized trials have been performed in Japanese subjects. No sufficient meta-analysis has been performed to specifically assess the size of the effect in Asian subjects, especially in Japanese subjects, because many studies have been written in Japanese language and because the literature has not been searched for publications in Japanese language.

Participants/population

Inclusion: Asian adults (subjects older than 20 yo). Exclusion: studies in diseased subjects, studies in hypertensive patients receiving blood pressure lowering drugs, studies in children (younger than 20 yo)

Intervention(s), exposure(s)

Intervention to be reviewed will be consumption of the lactotripeptides Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP) during more than 8 days

Comparator(s)/control

Comparison will be made with placebo

Context

Excluded studies: studies with missing information (e.g., change from baseline

to endpoint in office SBP) for which no appropriate answer is received from the authors and for which the missing information cannot be calculated/extrapolated from the publication; redundant studies

Primary outcome(s)

Change from baseline to endpoint in systolic blood pressure.

Secondary outcome(s)

Change from baseline to endpoint in diastolic blood pressure.

Data extraction (selection and coding)

For studies that fulfill the inclusion criteria, two review team members will independently extract relevant information from the full texts of the selected studies, using a standardized, pre-piloted form. Extracted information:

1) General information: authors, article title, year of publication and references.

2) Study characteristics: country, design, randomization, blinding, duration and dose of IPP/VPP administration, method used to measure blood pressure (e.g., office measurement).

3) Characteristics of participants: age, blood pressure status (normotensive, prehypertensive, hypertensive).

4) Outcomes: primary outcome measure, secondary outcome measure, number of subjects for which study data were analyzed, mean effect and variability measures (SD or SEM or 95% CI). Delicate issues will be resolved through discussions. Missing data will be requested from study authors.

Risk of bias (quality) assessment

Two team members will be involved in study selection and data extraction, and disagreements will be resolved through discussions. Estimation of the quality of individual studies will be assessed by using the Jadad score. Publication bias will be assessed by means of a funnel plot and asymmetry test (Kendall's tau).

Strategy for data synthesis

Preplanned analyses will be described in a statistical analysis plan. However, the planned general approach for data synthesis will be as follows: The outcome measure will be mean difference between groups receiving IPP/VPP and those receiving placebo in the change from baseline to endpoint office SBP (primary outcome). The mean pooled effect of IPP/VPP and its 95% confidence interval will be calculated by using both fixed and random-effect models. Between study heterogeneity will be explored. The random-effect model meta-analysis will be considered as the primary analysis, on the basis of evidence of heterogeneity between treatment effects in the literature and previous demonstration of significant heterogeneity (Cicero *et al* 2011).

Analysis of subgroups or subsets

If relevant and if the necessary data are available, we will conduct exploratory analyses to study the influence of relevant covariates. Meta-regressions and sub-group analyses will be used to this end. Potential covariates will be chosen among: blood pressure status at baseline (normotensive, pre-hypertensive or

hypertensive), age, dose of IPP+VPP, duration of IPP+VPP administration.

Contact details for further information

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Organisational affiliation of the review

None

Review team members and their organisational affiliations

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Anticipated or actual start date

11 September 2014

Anticipated completion date

31 August 2018

Funding sources/sponsors

The review was initially sponsored by Calpis Co., Ltd. (11-10, 5-chome, Fuchinobe, Chuo-ku, Sagamiharashi, Kanagawa 252-0206, Japan). Since Calpis has been acquired by Asahi Group Holdings, Ltd, the review is now sponsored by Asahi Group Holdings, Ltd (23-1, Azumabashi, 1-chome, Sumida-ku, Tokyo 130-8602, Japan).

Conflicts of interest

Veronique Braesco, Aurelie Chanson-Rolle, François Aubin, Ryuji Takeda and Yasuhiro Saito have received fees from Asahi Group Holdings for performing the systematic review.

Language

English, Japanese

Country

France, Japan

Stage of review

Review_Ongoing

Details of final report/publication(s)

http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0142235

Subject index terms status

Subject indexing assigned by CRD

Subject index terms

Asian Continental Ancestry Group; Blood Pressure; Humans; Oligopeptides

Date of registration in PROSPERO

17 October 2014

Date of publication of this version

02 August 2018

Details of any existing review of the same topic by the same authors

This systematic review is an update of the systematic review which was published on the PROSPERO register under the following title "Influence of the lactotripeptides Isoleucine-Proline-Proline and Valine-Proline-Proline on blood pressure in Asian subjects: a systematic review and meta-analysis of randomized controlled trials" and under registration number CRD42014014322.

Stage of review at time of this submission

Stage	Started	Completed
Preliminary searches	Yes	Yes
Piloting of the study selection process	Yes	Yes
Formal screening of search results against eligibility criteria	Yes	Yes
Data extraction	Yes	No
Risk of bias (quality) assessment	Yes	No
Data analysis	Yes	No

Versions

17 October 2014

30 October 2014

12 July 2018

02 August 2018

PROSPERO

This information has been provided by the named contact for this review. CRD has accepted this information in good faith and registered the review in PROSPERO. CRD bears no responsibility or liability for the content of this registration record, any associated files or external websites.

Checklist S1. PRISMA checklist for the reporting of meta-analyses of randomized controlled trials.

Section/topic	#	Checklist item	Reported on section
		TITLE	
Title	1	Identify the report as a systematic review, meta-analysis, or both.	Title page
		ABSTRACT	
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	Abstract
		INTRODUCTION	
Rationale	3	Describe the rationale for the review in the context of what is already known.	Introduction
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	Introduction
		METHODS	
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	Methods (beginning of the section)
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	Methods (paragraph on study selection)
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	Methods (paragraph on data sources and searches)
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Methods (paragraph on data sources and searches)
Study selection	9	State the process for selecting studies (*i.e.*, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	Methods (paragraph on study selection)
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	Methods (paragraph on data extraction)
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	Methods (paragraph on data extraction)
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	Methods (paragraph on data extraction)
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	Methods (paragraph on statistical analysis)
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	Methods (paragraph on statistical analysis)
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Methods (paragraph on statistical analysis)
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	Methods (paragraph on statistical analysis)
		RESULTS	
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Results (paragraph on characteristics of included studies) and **Figure 1**

Continued

Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	Results (paragraph on characteristics of included studies) and **Table 1**
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Results (paragraph on characteristics of included studies) and **Table 1**
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: a) simple summary data for each intervention group b) effect estimates and confidence intervals, ideally with a forest plot.	Results (paragraph on characteristics of included studies), **Table 2**, **Figure 2**, **Table S2**, **Figure S1**
Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency	Results (paragraph on effect of IPP/VPP on blood pressure)
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Results (paragraph on influence of individual studies and publication bias), **Figure 7** and **Figure S4**
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Results (paragraph on exploration of heterogeneity & subgroup meta-analyses), **Figures 3-6**, **Figures S2** and **Figures S3**
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	Discussion
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	Discussion
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	Discussion
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	Paragraph on acknowledgments

Table S1. List of the 18 articles selected for full text evaluation. The outcome of the selection process is indicated for each article (with justification for exclusion).

Exclusion criteria	Authors	Title	Ref	Database
Included	Aihara K., Kajimoto O., Hirata H., Takahashi R., Nakamura Y.	Effect of powdered fermented milk with Lactobacillus helveticus on subjects with high-normal blood pressure or mild hypertension.	Journal of the American College of Nutrition 2005; 24(4):257-265.	Medline/ Cochrane
7	Hirota T., Ohki K., Kawagishi R., Kajimoto Y., Mizuno S., Nakamura Y. and Kitakaze M.	Casein hydrolysate containing the antihypertensive tripeptides Val-Pro-Pro and Ile-Pro-Pro improves vascular endothelial function independent of blood pressure-lowering effects: contribution of the inhibitory action of angiotensin-converting enzyme.	Hypertension Research 2007; 30(6): 489-496.	Medline/ Cochrane
Included	Ishida Y., Aihara K., Sagitani A., Kaneko K., Mizutani J., Nakamura K., *et al.*	[Safety Evaluation of Excessive Intake of the Tablet Containing "Lactotripeptides (VPP, IPP)" in Subjects with Normal Blood Pressure to Mild Hypertension].	Japanese Pharmacology & Therapeutics 2006; 34:1107-1117. Japanese	J-DREAM III/ J-STAGE
Included	Ishida Y., Sagitani A., Kaneko K., Nakamura Y., Mizutani J., Masuda O.	[Antihypertensive Effects of the Tablet Containing "Lactotripeptide (VPP, IPP)" in Subjects with High Normal Blood Pressure to Mild Hypertension].	Japanese Pharmacology & Therapeutics 2007; 35(12):1249-1260. Japanese	J-DREAM III/ J-STAGE
Included	Ishida Y., Shibata Y., Fukuhara I., Yano Y., Takehara I., Kaneko K.	Effect of an excess intake of casein hydrolysate containing Val-Pro-Pro and Ile-Pro-Pro in subjects with normal blood pressure, high-normal blood pressure, or mild hypertension.	Bioscience, Biotechnology, and Biochemistry 2011; 75(3):427-433.	Medline/ Cochrane
Included	Itakura H., Ikemoto S., Terada S., Kondo K.	[The Effect of Sour Milk on Blood Pressure in Untreated Hypertensive and Normotensive Subjects].	Journal of Japanese Society of Clinical Nutrition 2001; 23(3):26-31. Japanese	J-DREAM III/ J-STAGE
Included	Kajimoto O., Aihara K., Hirata H., Takahashi R., Nakamura Y.	[Safety evaluation of excessive intake of the tablet containing "Lactotripeptides (VPP, IPP)" on healthy volunteers].	Journal of Nutritional Food 2001; 4(4):37-46. Japanese	J-DREAM III/ J-STAGE
12	Kawase M., Hashimoto H., Hosoda M., Morita H. and Hosono A.	Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure.	Journal of Dairy Science 2000; 83(2): 255-263.	Medline/ Cochrane
Included	Mizuno S., Matsuura K., Gotou T., Nishimura S., Kajimoto O., Yabune M., *et al.*	Antihypertensive effect of casein hydrolysate in a placebo-controlled study in subjects with high-normal blood pressure and mild hypertension.	The British Journal of Nutrition 2005; 94(1):84-91.	Medline/ Cochrane
14	Mizushima S., Ohshige K., Watanabe J., Kimura M., Kadowaki T., Nakamura Y., *et al.*	Randomized controlled trial of sour milk on blood pressure in borderline hypertensive men.	American Journal of Hypertension 2004; 17(8):701-706.	Medline/ Cochrane
Included	Nakamura Y., Kajimoto O., Kaneko K., Aihara K., Mizutani J., Ikeda N., *et al.*	[Effects of the liquid yogurts containing "lactotripeptide (VPP, IPP)" on high-normal blood pressure].	Journal of Nutritional Food 2004; 7(1):123-137. Japanese	J-DREAM III/ J-STAGE
Included	Sano J., Higuchi T., Aihara K., Mizuno S., Kajimoto O., Nakagawa S., *et al.*	[Safety evaluation of excessive intake of drink containing "lactotripeptides (VPP, IPP)" in subjects with normal blood pressure to mild hypertension].	Journal of Nutritional Food. 2004; 7(4):17-30. Japanese	J-DREAM III/ J-STAGE

Continued

Included	Sano J., Ohki K., Higuchi T., Aihara K., Mizuno S., Kajimoto O., *et al.*	Effect of casein hydrolysate, prepared with protease derived from Aspergillus oryzae, on subjects with high-normal blood pressure or mild hypertension.	Journal of Medicinal Food. 2005; 8(4):423-430.	Medline/ Cochrane
4	Yamasue K., Morikawa N., Mizushima S. and Tochikubo O.	The blood pressure lowering effect of lactotripeptides and salt intake in 24-h ambulatory blood pressure measurements.	Clinical and Experimental Hypertension 2010; 32(4): 214-220.	Medline/ Cochrane
7	Yasuda K., Aihara K., Komazaki K., Mochii M. and Nakamura Y.	[Effect of large high intake of tablets containing "lactotripeptides (VPP, IPP)" on blood pressure, pulse rate and clinical parameters in healthy volunteers].	Journal of Nutritional Food 2001; 4(3): 63-72. Japanese	J-DREAM III/ J-STAGE
13	Yoshizawa M., Maeda S., Miyaki A., Misono M., Choi Y., Shimojo N., Ajisaka R. and Tanaka H.	Additive beneficial effects of lactotripeptides and aerobic exercise on arterial compliance in postmenopausal women.	American Journal of Physiology Heart and Circulatory Physiology 2009; 297(5): H1899-1903.	Medline/ Cochrane
14	Yoshizawa M., Maeda S., Miyaki A., Misono M., Choi Y., Shimojo N., *et al.*	Additive beneficial effects of lactotripeptides intake with regular exercise on endothelium-dependent dilatation in postmenopausal women.	American Journal of Hypertension 2010; 23(4):368-372.	Medline/ Cochrane
Included	Uchida, N., Ohsawa, K., Nakamura, T., Ohki, K., Amagai, S., Nakagawa, K., *et al.*	[Effect of Tablets Containing Lactotripeptides (Vpp, Ipp) on Vascular Endothelial Function in Healthy Subjects: A Randomized, Double-Blind, Placebo-Controlled Trial].	Japanese Pharmacology & Therapeutics 2016; 44(7):1025-1034. Japanese.	J-DREAM III/J-STAGE

1: Reviews (general or systematic reviews, meta-analyses, letters, opinion papers, view points, etc… included data presented at a meeting). 2: Non-human studies. 3: Studies which are not intervention trials. 4: Uncontrolled intervention trials. 5: Intervention trials which are not randomized-controlled trials. 6: Randomized-controlled trials with an open design. 7: Studies where VPP/IPP intake period is less than 8 days. 8: Studies on subjects with disease (including hypertensive patients when they are receiving blood pressure-lowering drugs). 9: Studies on children (less than 20 years of age). 10: Studies on non-Asian subjects. 11: Studies in which no measure of SBP was reported. 12: Studies in which there was no intervention with VPP + IPP (studies testing VPP alone or IPP alone were excluded). 13: Redundant studies. 14: Studies that presented results for non-hypertensive and hypertensive subjects together, without individualization of results on non-hypertensive subjects only.

Table S2. Effect of valine-proline-proline and isoleucine-proline-proline on diastolic blood pressure at final endpoint in non-hypertensive Japanese subjects. Abbreviations: ACE: angiotensin-converting enzyme. BP: blood pressure. DBP: diastolic blood pressure. IPP: isoleucine-proline-proline. n: number of subjects. NA: not available. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). SD: standard deviation. SE: standard error. VPP: valine-proline-proline.

Study #	Series #	Study reference	Type of subjects (BP status)	VPP/IPP dose (mg/d)[a]	VPP/IPP dose In VPP eq. (mg/d)[b]	Treated group			Placebo group			Effect-size	
						n	Change in SBP (mmHg)		n	Change in SBP (mmHg)		Mean difference between groups (mmHg)	SE
							Mean	SD		Mean	SD		
1	101	Aihara 2005 [30]	PHT	13	16	20	NA	NA	20	NA	NA	−5.0	2.4
2	201	Ishida 2006 [34]	PHT	16	23	9	−5.8	5.3	9	−0.3	12.4	−5.5	4.5
2	202	Ishida 2006 [34]	NT	16	23	9	2.6	8.8	9	1.7	6.3	0.9	3.6
3	301	Ishida 2007 [35]	PHT	4	5	35	−1.3	6.8	36	−2.8	7.0	1.5	1.6
4	401	Ishida 2011 [31]	NT	17	24	8	−0.1	5.3	8	0.4	4.4	−0.5	2.4
4	402	Ishida 2011 [31]	PHT	17	24	8	−4.1	6.0	8	1.1	4.8	−5.2	2.7
5	501	Itakura 2001 [36]	NT	3	3	13	−2.5	4.0	13	0.4	4.9	−2.9	1.8
6	601	Mizuno 2005 [32]	PHT	4	5	12	−1.3	6.4	12	−0.9	4.5	−0.4	2.3
6	602	Mizuno 2005 [32]	PHT	3	3	12	−2.1	6.7	12	−0.9	4.5	−1.2	2.3
6	603	Mizuno 2005 [32]	PHT	2	2	12	−0.9	6.1	12	−0.9	4.5	0.0	2.2
7	701	Nakamura 2004 [38]	PHT	4	5	53	−3.8	6.3	53	−0.3	7.2	−3.5	1.3
8	801	Sano 2005 [33]	PHT	3	4	52	−2.4	5.0	52	−1.4	5.2	−1.0	1.0
9	901	Sano 2004 [39]	NT	9	13	6	−1.7	5.5	5	−4.3	4.3	2.6	3.0
9	902	Sano 2004 [39]	PHT	9	13	8	−3.5	6.1	8	−1.3	4.9	−2.2	2.8
10	1001	Kajimoto 2001 [37]	NT	12	16	21	−3.0	8.2	22	−1.0	8.3	−2.0	2.5
11	1101	Uchida 2016 [40]	PHT	3	5	11	−2.2	7.2	13	−3.5	7.7	1.3	3.1
11	1102	Uchida 2016 [40]	NT	3	5	15	2.9	7.5	9	6.3	7.9	−3.4	3.2

[a]IPP content in mg + VPP content in mg. [b]Dose expressed in "VPP equivalents", calculated as the IPP content in mg multiplied by 1.7 added to the VPP content in mg. The 1.7 correction factor is to take into account the difference in potency between the two tripeptides to inhibit ACE activity in vitro [8] [24].

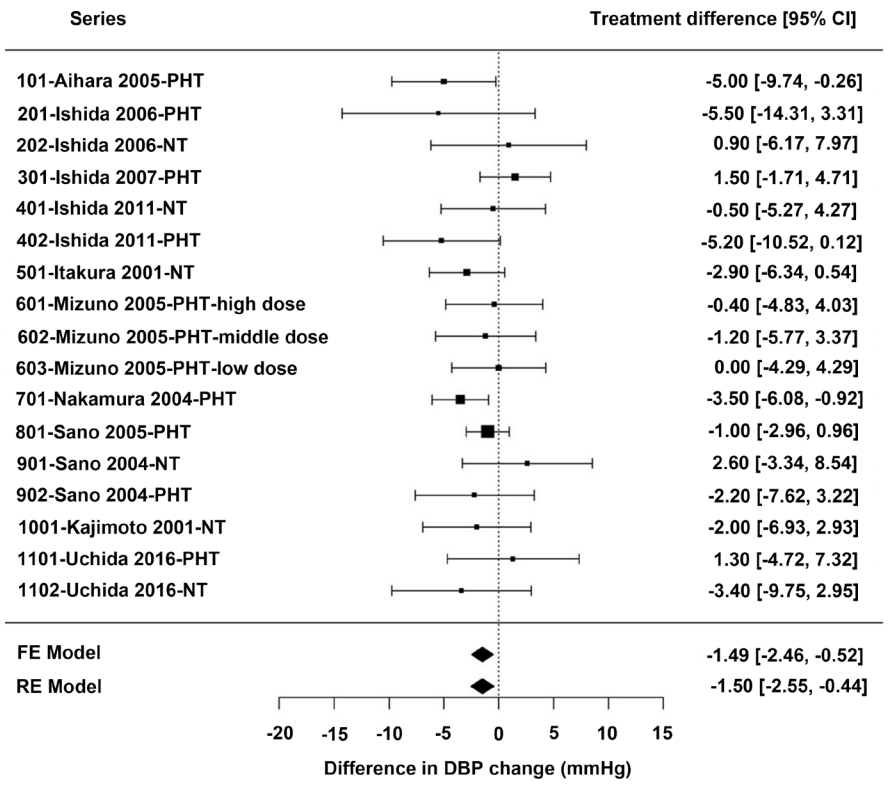

Figure S1. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline in the meta-analysis of 17 series of findings of its effect on diastolic blood pressure in non-hypertensive Japanese subjects. BP: blood pressure. CI: confidence interval. DBP: diastolic blood pressure. FE: fixed effect. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). RE: random effect. Series numbers are those indicated in **Table S2**.

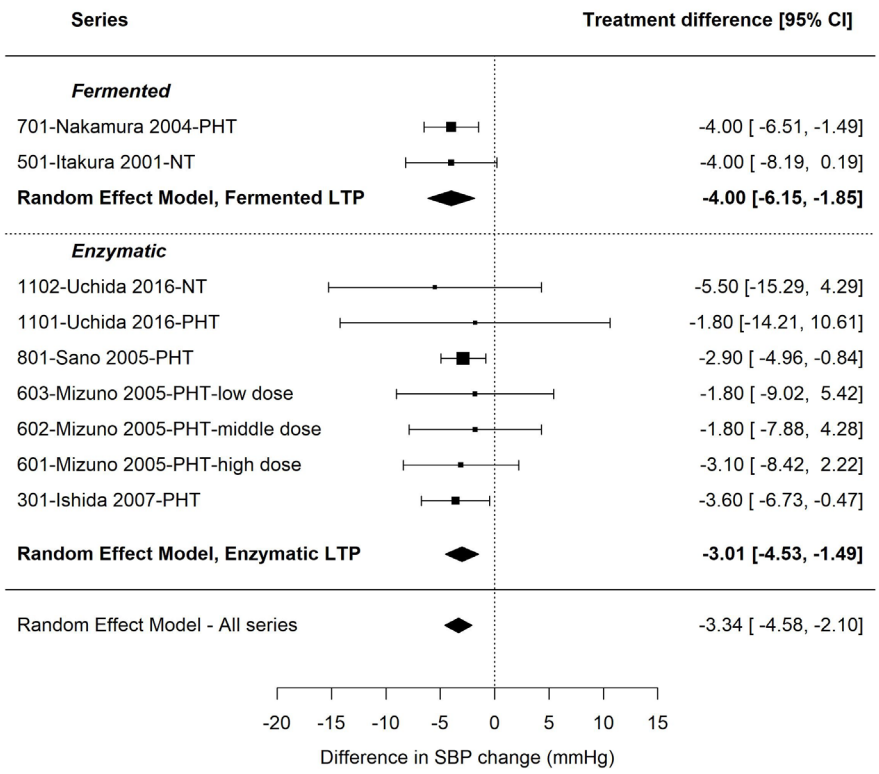

Figure S2. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline (VPP/IPP) on systolic blood pressure in the sub-group analysis according to the type of VPP/IPP ingredient within the nine series that tested usual daily doses of VPP/IPP (i.e., ≤5 mg/d). BP: blood pressure. CI: confidence interval. LTP: lactotripeptides (VPP/IPP). NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). SBP: systolic blood pressure. Series numbers are those indicated in Table 2.

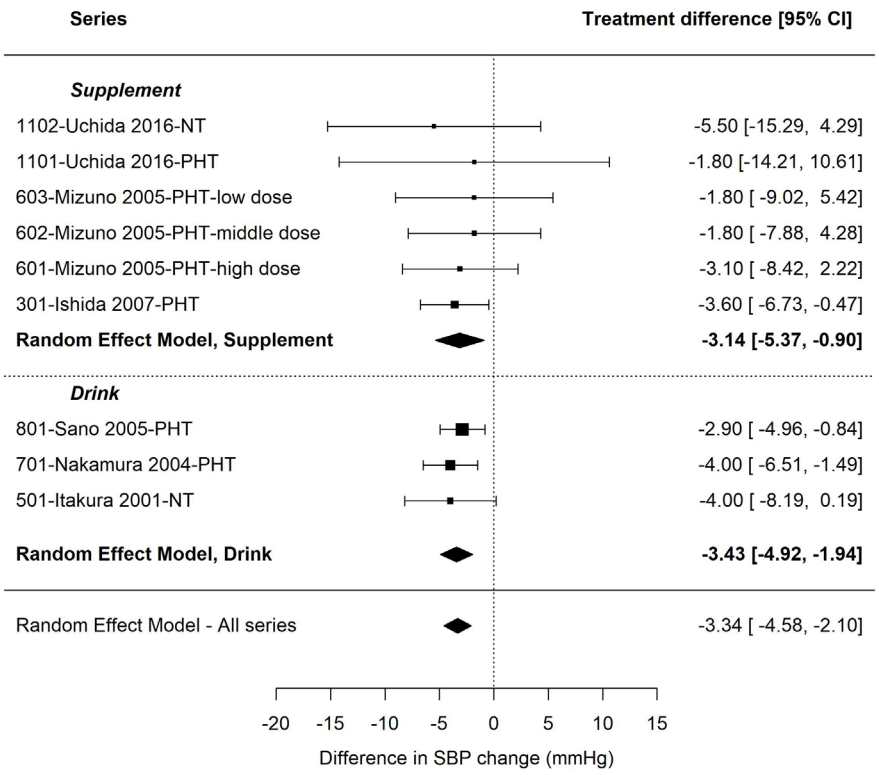

Figure S3. Forest plot of treatment effects of valine-proline-proline/isoleucine-proline-proline (VPP/IPP) on systolic blood pressure in the sub-group analysis according to the type of food product within the nine series that tested usual daily doses of VPP/IPP (*i.e.*, ≤5 mg/d). BP: blood pressure. CI: confidence interval. NT: normotensive (subjects with normal BP). PHT: pre-hypertensive (subjects with high-normal BP). SBP: systolic blood pressure. Series numbers are those indicated in **Table 2**.

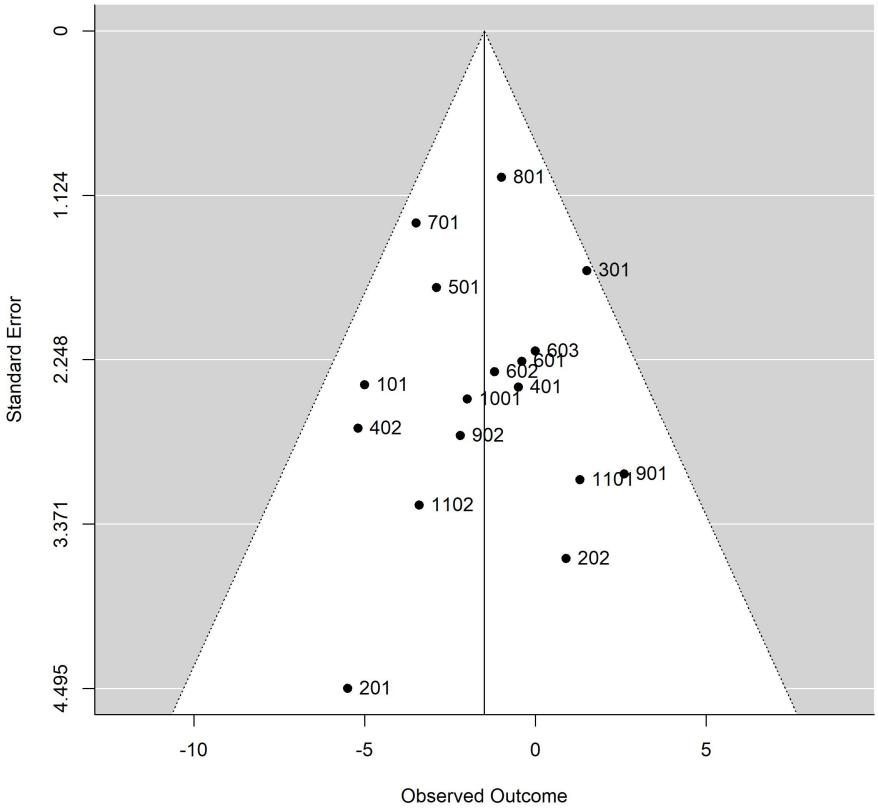

Figure S4. Funnel plot used to explore the potential for publication bias in the meta-analysis of 17 series for the effect of valine-proline-proline/isoleucine-proline-proline on diastolic blood pressure in non-hypertensive Japanese subjects. Series numbers are those indicated in **Table S2**.