EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on
the substantiation of a health claim related to isoleucyl-prolyl-proline (IPP) and valyl-
prolyl-proline (VPP) and maintenance of normal blood pressure pursuant to Article
13(5) of Regulation (EC) No 1924/2006

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SCIENTIFIC OPINION

Scientific Opinion on the substantiation of a health claim related to isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) and maintenance of normal blood pressure pursuant to Article 13(5) of Regulation (EC) No 1924/2006

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA)²,³

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Following an application from Valio Ltd submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) and maintenance of normal blood pressure (BP). The tripeptides IPP and VPP are sufficiently characterised. Maintenance of normal blood pressure is a beneficial physiological effect. The applicant identified a total of 20 published intervention studies (in 19 papers; five papers were translated by the applicant into English from the Japanese original) and one unpublished study, as well as two published and one unpublished meta-analyses of randomised controlled trials (RCTs) as being pertinent to the claim. Thirteen of the RCTs provided, four of which were adequately powered to detect small between-group differences in systolic BP (SBP), did not observe an effect of IPP and VPP on SBP or diastolic BP. The interpretation of the results from seven out of the eight studies which reported an effect of IPP and VPP on office SBP was hampered by major methodological limitations. The animal studies did not provide additional information on the effect of IPP and VPP on BP in humans, and no convincing evidence for a mechanism by which IPP and VPP could exert the claimed effect at the proposed dose has been provided. The Panel concludes that a cause and effect relationship has not been established between the consumption of IPP and VPP and maintenance of normal blood pressure.

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KEY WORDS

Isoleucyl-prolyl-proline, IPP, valyl-prolyl-proline, VPP, blood pressure, health claims.

1 On request from the Competent Authority of Finland following an application by Valio Ltd, Question No EFSA-Q-2011-00121, adopted on 14 September 2011.
2 Panel members: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Lovik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hendrik van Loveren, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen. Two members of the Panel did not participate in the discussion on the subject referred to above because of potential conflicts of interest identified in accordance with the EFSA policy on declarations of interests. Correspondence: nda@efsa.europa.eu
3 Acknowledgement: The Panel wishes to thank the members of the Working Group on Claims: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Marina Heinonen, Hannu Korhonen, Martinus Lovik, Ambroise Martin, Hendrik van Loveren and Hans Verhagen for the preparatory work on this scientific opinion.

SUMMARY
Following an application from Valio Ltd submitted pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of Finland, the Panel on Dietetic Products, Nutrition and Allergies was asked to deliver an opinion on the scientific substantiation of a health claim related to isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) and maintenance of normal blood pressure.

The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence including a request for the protection of proprietary data.

The food constituent that is the subject of the health claim is the tripeptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP). The Panel considers that the tripeptides IPP and VPP are sufficiently characterised.

The claimed effect is “helps to maintain normal blood pressure”. The target population proposed by the applicant is mildly hypertensive subjects or subjects with an elevated risk of developing hypertension. The Panel considers that maintenance of normal blood pressure is a beneficial physiological effect.

The applicant identified a total of 20 published intervention studies (in 19 papers; five papers were translated by the applicant into English from the Japanese original) and one unpublished study, as well as two published and one unpublished meta-analyses of randomised controlled trials (RCTs) as being pertinent to the claim.

Because major differences in the quality of the studies selected for the meta-analyses were not taken into account in data analyses, the Panel considers that the meta-analyses do not provide information in addition to the individual studies considered pertinent to the claim by the applicant.

From the 21 human intervention studies provided by the applicant, eight reported an effect of IPP and VPP on office systolic blood pressure (SBP) of which seven, however, showed major methodological limitations related to treatment allocation, randomisation, blinding and statistical analyses. Of these trials, four also reported an effect of IPP and VPP on office diastolic blood pressure (DBP). The remaining 13 RCTs, of which four were designed to detect small (3-5 mm Hg) differences in SBP, did not observe an effect of IPP and VPP on SBP or DBP.

The applicant also provided 13 animal studies of which nine assessed the effects of IPP and VPP on BP in spontaneously hypertensive rats, in sodium loaded Goto-Kakasi rats or in transgenic rats expressing human genes of the renin-angiotensin system. The Panel considers that these animal studies do not provide additional information on the effect of IPP and VPP on BP in humans.

The applicant further provided ex vivo and in vitro studies related to the production of IPP and VPP from casein digestion, to the peptide transport by the intestinal epithelium and to the mechanisms of action, namely angiotensin converting enzymes (ACE 1 and ACE 2) inhibition. Besides ACE 1 inhibition, the applicant stated that the tripeptides IPP and VPP could have other effects on the renin-angiotensin-aldosterone system, improve endothelial function, and have anti-inflammatory properties, all of which could mediate a BP-lowering effect. The Panel considers that no convincing data in support of any of these proposed mechanisms at the proposed dose has been provided.

In weighing the evidence, the Panel took into account that 13 of the human intervention studies provided, four of which were adequately powered to detect small between-group differences in SBP, did not observe an effect of IPP and VPP on SBP or DBP; that the interpretation of the results from seven out of the eight studies which reported an effect of IPP and VPP on office SBP was hampered by major methodological limitations; that the animal studies did not provide additional information on
the effect of IPP and VPP on BP in humans; and that there is no convincing evidence for a mechanism by which IPP and VPP could exert the claimed effect at the proposed dose.

The Panel concludes that a cause and effect relationship has not been established between the consumption of IPP and VPP and maintenance of normal blood pressure.
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BACKGROUND

Regulation (EC) No 1924/2006 harmonises the provisions that relate to nutrition and health claims, and establishes rules governing the Community authorisation of health claims made on foods. As a rule, health claims are prohibited unless they comply with the general and specific requirements of this Regulation, are authorised in accordance with this Regulation and are included in the lists of authorised claims provided for in Articles 13 and 14 thereof. In particular, Article 13(5) of this Regulation lays down provisions for the addition of claims (other than those referring to the reduction of disease risk and to children’s development and health) which are based on newly developed scientific evidence, or which include a request for the protection of proprietary data, to the Community list of permitted claims referred to in Article 13(3).

According to Article 18 of this Regulation, an application for inclusion in the Community list of permitted claims referred to in Article 13(3) shall be submitted by the applicant to the national competent authority of a Member State, which will make the application and any supplementary information supplied by the applicant available to the European Food Safety Authority (EFSA).

STEPS TAKEN BY EFSA:

- The application was received on 10/02/2011.
- The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence including a request for the protection of proprietary data.
- The scientific evaluation procedure started on 01/03/2011.
- On 13/05/2011, the NDA Panel agreed on a list of questions which requested the applicant to provide additional particulars to accompany the application and the clock was stopped on 19/05/2011 in compliance with Article 18(3) of Regulation (EC) No 1924/2006.
- On 06/06/2011, EFSA received the requested information as submitted by the applicant and the clock was restarted.
- During the meeting on 14/09/2011, the NDA Panel, having evaluated the data submitted, adopted an opinion on the scientific substantiation of a health claim related to isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) and maintenance of normal blood pressure.

TERMS OF REFERENCE

EFSA is requested to evaluate the scientific data submitted by the applicant in accordance with Article 16(3) of Regulation (EC) No 1924/2006. On the basis of that evaluation, EFSA will issue an opinion on the scientific substantiation of a health claim related to isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) and helps to maintain normal blood pressure.

EFSA DISCLAIMER

The present opinion does not constitute, and cannot be construed as, an authorisation to the marketing of isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP), a positive assessment of its safety, nor a decision on whether isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) is, or is not, classified as a foodstuff. It should be noted that such an assessment is not foreseen in the framework of Regulation (EC) No 1924/2006.

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It should also be highlighted that the scope, the proposed wording of the claim, and the conditions of use as proposed by the applicant may be subject to changes, pending the outcome of the authorisation procedure foreseen in Article 18(4) of Regulation (EC) No 1924/2006.
INFORMATION PROVIDED BY THE APPLICANT

Applicant’s name and address: Valio Ltd, P.O. Box 30, 00039 Valio, Finland.
The application includes a request for the protection of proprietary data in accordance with Article 21 of Regulation (EC) No 1924/2006.

Food/constituent as stated by the applicant

According to the applicant, the food constituents are the biologically active peptides, isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP), which are derived from milk proteins through fermentation or an enzymatic process. These peptides are used in low-fat milk products, which may contain fruit or berry juice as an ingredient and in fat spreads. Current commercially available products are fermented milk products with or without added sugar and fat spreads.

Health relationship as claimed by the applicant

According to the applicant, daily consumption of food products containing the biologically active peptides IPP and VPP reduces blood pressure in mildly hypertensive subjects and consequently are beneficial for cardiovascular health.

Wording of the health claim as proposed by the applicant

The applicant proposes the following wording: “Peptides IPP and VPP help to maintain normal blood pressure”

Specific conditions of use as proposed by the applicant

According to the applicant, the products are targeted at people with mildly elevated blood pressure levels or with an elevated risk of developing hypertension. The recommended daily intake of IPP and VPP is 5 mg/day.

ASSESSMENT

1. Characterisation of the food/constituent

The food constituent that is the subject of the health claim is the tripeptides isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP), which can be obtained through fermentation of milk by certain lactic acid bacteria, by enzymatic hydrolysis of casein or by chemical synthesis. All clinical studies presented have been conducted with either directly fermented milk, with powdered fermented milk, or with tripeptides obtained from enzymatically hydrolysed casein. IPP and VPP can be measured in foods by established methods.

The Panel considers that the food constituent, the tripeptides IPP and VPP, which is the subject of the health claim, is sufficiently characterised.

2. Relevance of the claimed effect to human health

The claimed effect is “helps to maintain normal blood pressure”. The target population proposed by the applicant is mildly hypertensive subjects or subjects with an elevated risk of developing hypertension.

Blood pressure (BP) is the pressure (force per unit area) exerted by circulating blood on the walls of blood vessels. Elevated BP, by convention above 140 mm Hg (systolic) and/or 90 mm Hg (diastolic), may compromise the normal function of the arteries.
The Panel considers that maintenance of normal blood pressure is a beneficial physiological effect.

3. Scientific substantiation of the claimed effect

The applicant performed a literature search through PubMed to identify randomised, placebo-controlled, clinical trials (RCTs) and meta-analyses of RCTs published in English which assessed the effects of IPP and VPP at doses \( \leq 10 \text{ mg/day} \) on blood pressure in humans not taking BP-lowering medications. Additional studies were identified by hand searching. A total of 20 published intervention studies (in 19 papers; five papers were translated by the applicant into English from the Japanese original (Hirata et al., 2002; Kajimoto et al., 2001a; 2001b; 2002; Nakamura et al., 2004) and one unpublished study, as well as two published and one unpublished meta-analyses of RCTs were identified by the applicant as being pertinent to the claim.

The two published (Cicero et al., 2011; Xu et al., 2008) and one unpublished (Turpeinen et al., 2007, unpublished) meta-analyses of RCTs on the effects of IPP and VPP on BP in humans included nine, 18 and 20 studies, respectively. Because major differences in the quality of the studies selected for the meta-analyses were not taken into account in data analyses, the Panel considers that these meta-analyses do not provide information in addition to the individual studies considered pertinent to the claim by the applicant and described below for the scientific substantiation of the claim.

**Human intervention studies**

Five parallel, placebo-controlled intervention studies compared the effects of sour milk, liquid yoghurt or tablets containing IPP and VPP at doses of 3.74 to 4.26 mg/day to those of a placebo (artificially acidified milk or tablets assumed to be devoid of IPP and VPP) on BP in hypertensive subjects not on BP-lowering medications. The interventions lasted 8-12 weeks, were preceded by two-week run-in periods and followed by four-week post-intervention follow-ups. Office systolic BP (SBP) and diastolic BP (DBP) were measured at baseline and bi-weekly during the interventions and at follow-ups. The number of subjects enrolled was 36 in two studies (six drop-outs (Kajimoto et al., 2001a); four drop-outs (Hirata et al., 2002)), 70 (six drop-outs (Kajimoto et al., 2002)), 92 (11 drop-outs (Kajimoto et al., 2001b)) and 107 (one drop-out (Nakamura et al., 2004)), respectively. All statistical analyses were conducted on the results obtained in completers only. Analysis of variance (ANOVA) was used to assess treatment effects except in the study by Nakamura et al. (2004), where repeated-measures ANOVA (RM-ANOVA) was used. Between-group comparisons were primarily performed using multiple pair-wise comparisons (unpaired t-tests) at different time points to test the treatment effect. Compared to placebo, the treatment resulted in a statistically significant reduction in SBP ranging from -4 to -13 mm Hg. DBP did not change significantly (two studies) or significantly decreased by -4 to -8 mm Hg. The Panel notes that no information is provided in the publications about the method used to allocate treatments to the enrolled subjects and that randomisation is not mentioned. The applicant did not provide further documentation on this point following EFSA’s request. The Panel also notes that in four out of the five studies, the use of ANOVA did not properly take into account repeated measures, that statistical analyses were conducted in completers only, and that in all these studies multiple comparisons were carried out between groups without appropriate correction for multiple testing. The Panel notes that these studies report an effect of IPP and VPP on office SBP and that in three of these studies (Kajimoto et al., 2001a; 2001b; 2002) an effect of IPP and VPP on office DBP was also reported. However, the Panel considers that these five studies have major methodological limitations which hamper the interpretation of the results.

In a randomised, single-blind, placebo-controlled, parallel study (Mizuno et al., 2005), 131 subjects with high-normal BP or mild hypertension, not on anti-hypertensive medications, were divided into four groups to consume enzymatic casein hydrolysates in tablets providing 0, 1.8, 2.5 or 3.6 mg/day of IPP and VPP (32 subjects in the control group and 33 subjects per intervention group) for six weeks, after a two-week run-in period. Each study group included 20-21 subjects with mild hypertension and 12 subjects with high-normal BP. Office BP was measured during the run-in phase, at baseline, and at
1, 3 and 6 weeks of the intervention by a trained nurse. No drop-outs were reported in the study and analyses were conducted in the intention-to-treat (ITT) population. The effect of the intervention on BP was analysed by ANOVA. It is not reported in the publication how differences between groups were tested at different time points. A statistically significant difference in SBP was observed in the 3.6 mg/day dose group compared to control (exact figure not provided; \( p<0.001 \)) by ANOVA. No significant differences were observed for SBP between the intervention and control groups at doses of 1.8 and 2.5 mg/day, or for DBP at any dose. Changes in BP during the study were also analysed separately for subjects with high-normal and mildly elevated BP. The Panel notes that post-hoc analyses according to basal BP have not been justified in the publication (i.e. no \textit{a priori} stratification according to BP has been reported and interaction between treatment and baseline BP on the outcome measure has not been tested). The Panel also notes that no details were provided regarding the method used to allocate treatments to subjects, that nurses and investigators were not blinded to treatments, and that repeated measures were not properly taken into account. The Panel notes that this study reports an effect of IPP and VPP on office SBP at doses of 3.6 mg/day and no effect on DBP. However, the Panel considers that this study has major methodological limitations which hamper the interpretation of the results.

In a randomised, double-blind, placebo-controlled, parallel, pilot study (Seppo et al., 2002), 17 mildly hypertensive subjects not on pharmacological treatment for hypertension consumed either sour milk containing IPP and VPP (5.25 mg/day) or a control milk not containing IPP and VPP for eight weeks, after a run-in period of four weeks and followed by a four-week follow-up at the end of the intervention. All subjects completed the study. Power calculations were not performed. Office BP was measured weekly during the study by a nurse which was blinded to the intervention. Changes in BP were calculated in absolute values and as percent changes from baseline within groups at every visit. Between-group differences were tested at different time-points using t-test for independent samples. At the end of the eight-week intervention period, the treatment difference for SBP and for DBP was statistically significant. However, the Panel notes that repeated measures were not taken into account in the analysis and that adjustments for multiple comparisons were not performed. The Panel also notes the small sample size of the study. The Panel notes that this study reports an effect of IPP and VPP on office SBP and DBP. However, the Panel considers that this study has major methodological limitations which hamper the interpretation of the results.

In a randomised, double-blind, placebo-controlled, parallel study (Sano et al., 2005), 150 men and women not on anti-hypertensive medications were randomised to consume a concentrated beverage mixture of vegetable and fruit juices containing an enzymatic casein hydrolysate with IPP and VPP (3.07 mg/day) or the same mixture without IPP and VPP for 12 weeks. Office BP was measured at baseline and every two weeks during the study. A sample size of 48 subjects (24 per group) was calculated to detect a 5 mm Hg difference in SBP between groups with \( \beta=0.20 \) and \( \alpha=0.05 \). A total of 144 subjects completed the study and entered data analysis (\( n=72 \) per group). The effect of treatment on BP was analysed using RM-ANOVA. A statistically significant decrease was observed in the intervention group compared to placebo (\( p<0.001 \)) by RM-ANOVA for SBP, but not for DBP. The Panel notes that the mean difference between the \textit{verum} and the placebo groups with respect to SBP at all time points was below the 5 mm Hg difference in SBP pre-defined for power calculations. The Panel also notes that, in this study, investigators over-recruited the calculated sample size by three times, and that reasons for over-recruitment have not been reported in the publication. The Panel considers that this study shows a statistically significant effect of IPP and VPP on office SBP and no effect on DBP.

The Panel notes that eight RCTs (Hirata et al., 2002; Kajimoto et al., 2001a; 2001b; 2002; Mizuno et al., 2005; Nakamura et al., 2004; Sano et al., 2005; Seppo et al., 2002) reported an effect of IPP and VPP on office SBP, and that four of these studies also reported an effect of IPP and VPP on office DBP (Kajimoto et al., 2001a; 2001b; 2002; Seppo et al., 2002). However, the Panel considers that seven of the RCTs have major methodological limitations which hamper the interpretation of the results.
In a randomised, double-blind, placebo-controlled, parallel study (Mizushima et al., 2004) 46 mildly hypertensive males not on anti-hypertensive medications consumed sour milk containing IPP and VPP (3.15 mg/day) or an artificially acidified control milk (devoid of IPP and VPP) for four weeks. Office BP was measured at baseline and at weeks two and four. A sample size of 62 subjects (31 per group) was calculated to detect a 5 mm Hg difference in SBP between groups with a power of 80 % and α=0.05. A total of 42 subjects (22 in the intervention group and 20 in the control group) completed the study. Changes in BP between groups during the four-week intervention were not statistically significant by RM-ANOVA. The Panel considers that this study does not show an effect of IPP and VPP on SBP or DBP. However, the Panel notes that this study may have been underpowered with respect to BP outcomes.

In the randomised, placebo-controlled, parallel intervention study by Hata et al. (1996), 36 hypertensive subjects (32 on stable blood pressure-lowering medications) consumed sour milk containing IPP and VPP (2.6 mg/day) or a control milk (artificially acidified milk devoid of IPP and VPP) for eight weeks, after a four-week run-in period. Office BP was measured at baseline and at four and eight weeks. Thirty subjects completed the study (17 in the intervention group and 13 in the control group). Data analysis was conducted in completers only. Changes in BP between groups were assessed at different time points using pair-wise comparisons (unpaired t tests). The Panel notes that no adjustment for multiple comparisons was made and that possible confounding effects of anti-hypertensive medications were not taken into account. No significant differences in anti-hypertensive medication use were observed between groups. No significant differences in office SBP or DBP between groups were observed during the study. The Panel considers that this study does not show an effect of IPP and VPP on office SBP or DBP.

Seppo et al. (2003) reported on a double-blind, placebo-controlled, parallel study in which 42 hypertensive subjects were randomised to consume a fermented milk containing IPP and VPP (5.25 mg/day) or a control milk (heat treated fermented milk without IPP and VPP) designed to mimic the sensory attributes of the test product for 21 weeks after a run-in period of two weeks. Nine subjects in the test group and seven subjects in the control group were taking blood-pressure lowering medications. Home BP was measured at baseline and weekly on the same day using an automatic BP recorder. Thirty-six subjects completed the study. RM-ANOVA was used to assess the effect of the intervention on BP. Areas under the BP curve (AUC) were also compared between groups using t tests for independent samples. The Panel notes that no information is provided in the publication about the treatment of missing data and that the possible confounding effects of antihypertensive medications were not taken into account. ITT analysis did not indicate any significant difference in either SBP or DBP between groups. A significant decrease in SBP (-6.7 mm Hg), but not in DBP, was observed in the intervention group compared to placebo in the per protocol (PP) analysis (p=0.03). AUC for BP values did not significantly differ between groups. The Panel notes that the results are inconsistent and considers that this study does not show an effect of IPP and VPP on SBP or DBP.

In a randomised, double-blind, placebo-controlled, parallel study (Turpeinen et al., 2009), 62 hypertensive and hypercholesterolaemic subjects not on antihypertensive or cholesterol-lowering medications consumed 20 g/day of a vegetable oil fat spread providing 4.2 mg/day IPP and VPP and 2 g/day plant sterols or the same amount of a placebo fat spread for 10 weeks after a run-in period of two weeks. A total of 58 subjects completed the study. BP was measured twice a week at home by the study subjects using an automated sphygmomanometer. At baseline and at week 10 of the study, office BP was measured using the same technique prior to calculating central SBP (cSBP) using pulse-wave analysis in order to evaluate aortic stiffness. Power calculations and the primary outcome of the study were not reported. Analyses were performed in the ITT population with the last observation carried forward. ANCOVA with baseline values as covariate was applied to assess BP changes between groups. Home BP values at baseline and at the end of the intervention period were used. There was a significant decrease in home SBP in the intervention group as compared to placebo (-6 mm Hg), whereas no significant differences between groups were observed for DBP. However, the Panel notes that the statistical analysis did not appropriately take into account repeated measures of
home BP and that intermediate home BP measurements were not considered in the analysis. No significant differences between groups were observed for cSBP. The Panel notes that results for office BP measurements were not reported. The Panel considers that this study does not show an effect of IPP and VPP on SBP or DBP.

In a randomised, double-blind, placebo-controlled, parallel study (Turpeinen et al., unpublished), 101 hypertensive subjects, not on antihypertensive or cholesterol-lowering medications, consumed 20 g/day of a vegetable oil fat spread providing 4.2 mg/day IPP and VPP and 2 g/day plant sterols or the same amount of a placebo fat spread for 10 weeks. Home BP was measured three times per week, office BP at an undisclosed rate and 24 h ambulatory BP at baseline and at the end of the intervention period on a subset of 51 subjects. A sample of 45 subjects per group was calculated to be needed to observe a 5 mm Hg difference between groups in office SBP. The authors state that home BP was the primary outcome of the study. However, the Panel notes that data on office SBP was used for power calculations. Analyses were performed in the ITT population with the last observation carried forward. ANCOVA with baseline values as covariate was applied to assess BP changes between groups. Results for between-group comparisons using baseline and week-10 home, office and 24 h BP values are provided. A statistically significant reduction in home SBP (-3.6 mm Hg) was observed in the intervention group compared to placebo at 10 weeks, whereas no significant differences between groups were observed for home DBP. However, the Panel notes that intermediate home BP measures were not considered and that the statistical analysis did not appropriately take into account repeated measures of home BP. No differences in office SBP or DBP or 24 h ambulatory SBP or DBP (the latter measured in a subset of 51 participants) were observed between groups. The Panel considers that this study does not show an effect of IPP and VPP on SBP or DBP.

In a randomised, double-blind, placebo-controlled study (Tuomilehto et al., 2004), 60 hypertensive volunteers not on anti-hypertensive medication consumed either sour milk containing IPP and VPP (4.8 to 5.1 mg/day) or a control milk not containing IPP and VPP (i.e. traditionally fermented milk) for 8-10 weeks (phase I). An “interim analysis” revealed that SBP and DBP decreased in all study subjects regardless of the study group to which they had been assigned. After a three to four week wash-out period, it was proposed to the subjects (n=58) who had completed this first study to carry on for another five to seven weeks while inverting treatments (placebo in place of verum and vice versa). Out of the 40 subjects who agreed to participate in this second phase of the study (phase II), 39 completed it. The Panel notes that only phase I of this study complies with basic requirements for subjects’ enrolment and treatment allocation and that the combination of phases I and II cannot be considered as an appropriate cross-over. Office BP was measured at weeks 3, 6, 8, 9 and 10 (phase I). RM-ANOVA was used to assess the effect of the intervention on BP. There was no significant effect of treatment on SBP or DBP. The Panel considers that this study does not show an effect of IPP and VPP on office SBP or DBP.

In a randomised, double-blind, placebo-controlled, cross-over trial, 42 participants consumed a fermented milk to which IPP and VPP (8.7 mg/day) were added and a placebo milk drink (no VPP or IPP added) for four weeks each, with a four week wash-out period in between (Van der Zander et al., 2008a). Office BP was measured at the beginning and end of each period. Sample size was calculated with changes in SBP as primary outcome (42 subjects were needed to observe a 5 mm Hg difference in SBP with an 80% power and \( \alpha=0.05 \)). ANOVA including treatment, intervention period, treatment order and subject was used on an ITT basis to assess the effect of the intervention on BP. The Panel notes that repeated measures were not appropriately taken into account in the data analysis. There was no significant treatment effect on either SBP or DBP. A post-hoc sub-group analysis was conducted on the basis of baseline SBP (SBP>130 mm Hg and SBP≤130 mm Hg). The Panel notes that no interaction analysis between SBP values at baseline and SBP changes during the intervention was performed to justify post-hoc analyses, and that the number of subjects with SBP>130 mm Hg (and with SBP≤130 mm Hg) has not been reported. The Panel considers that this study does not show an effect of IPP and VPP on office SBP or DBP.
In a multicentre, randomised, double-blind, placebo-controlled parallel trial, 245 hypertensive subjects not on anti-hypertensive medications consumed a yoghurt drink enriched with casein hydrolysate and providing IPP and VPP (10.2 mg/day) or a placebo yoghurt drink for eight weeks (Van der Zander et al., 2008b). Sample size was calculated with changes in office SBP as primary outcome (135 subjects per group were needed to observe a 3 mm Hg difference in SBP with a 90% power and α=0.05). A total of 136 subjects were randomised to the intervention and 139 to the control group. Office BP was measured for two to three hours on two to three consecutive days at baseline and at four and eight weeks of the intervention. ANCOVA including treatment, subject and baseline BP was used on an ITT and PP (in compliant subjects) basis to assess the effect of the intervention on BP. All BP measures were considered in the analysis. Compliance was 98% in both groups. A total of 134 subjects in the intervention and 137 in the control group completed the study. No significant differences in SBP or DBP were observed between groups either in the ITT or in the PP analysis. The Panel notes that this study powered to detect small (3 mm Hg) differences in SBP does not show an effect of IPP and VPP on office SBP or DBP.

In a double-blind, parallel, placebo-controlled trial (Engberink et al., 2008), 135 subjects with elevated SBP were randomly assigned to one of the following four groups: yoghurt drinks devoid of IPP and VPP (placebo) or yoghurt drinks providing 9.2-11.2 mg/day of IPP and VPP obtained from either fermented milk, enzymatic hydrolysis, or chemical synthesis. The intervention lasted eight weeks. A total of 134 subjects completed the intervention. Office and home BP were measured at baseline and weeks four and eight of the study. 24 h ambulatory BP measurements were obtained in a sub-group of 58 subjects at the same time points. ANOVA was used on an ITT basis to assess the effect of the intervention on BP. The Panel notes that repeated measures were not appropriately taken into account by ANOVA. Data analysis was repeated after exclusion of non-compliant subjects who consumed <80% of the test products. Consumption of IPP and VPP did not affect SBP or DBP compared with placebo regardless of the method by which IPP and VPP were obtained, of the methods used to measure BP, and of the level of compliance. The Panel considers that this study does not show an effect of IPP and VPP on office, home, or 24 h ambulatory SBP or DBP.

In a randomised, double-blind, parallel-group, dose-response intervention (De Leeuw et al., 2009), 185 hypertensive subjects received either a placebo or one of three yoghurt drinks providing IPP and VPP at a daily dose of 2.3, 4.6 or 9 mg for eight weeks, after a run-in period of two weeks and followed by a post-intervention follow-up period of two weeks. The primary outcome of the study was changes in SBP. A sample size of 40 per group was calculated to detect a change in SBP of 5 mm Hg with a power of 80% and α=0.05 (one sided). Office and home BP were measured every two weeks, and 24 h BP measures were obtained at baseline and at week eight. One hundred and sixty-six subjects completed the study and entered data analysis. The effects of treatment over time were assessed by RM-ANOVA. The Panel notes that it is unclear from the publication whether statistical analyses were performed on an ITT or PP basis. Office BP significantly decreased in all four groups (including placebo) during the study. When the results of office BP measurements over eight weeks were corrected for the placebo response, no significant differences in SBP or DBP were observed between groups. The percentages of subjects who showed a fall in SBP>3 mm Hg or who attained an SBP<140 mm Hg in each intervention group were calculated and analysed post-hoc. The Panel notes that the post-hoc response rate analysis was not pre-planned and considers that no conclusions can be drawn from this analysis for the scientific substantiation of the claim. There were no significant differences in home or 24 h ambulatory BP between groups throughout the study. The Panel considers that this study powered to detect differences of 5 mm Hg in SBP does not show an effect of IPP and VPP on office, home or 24 h ambulatory SBP or DBP.

Van Mierlo et al. (2009) reported the results of two multicentre, randomised, placebo-controlled, cross-over studies, each consisting of two four week intervention periods separated by a four week wash-out period. The sample size calculation indicated that for each cross-over study, 48 subjects should be sufficient to detect a 3 mm Hg difference in SBP with a power of 90% and a one-sided α=0.05. In study 1, 69 subjects received a yoghurt drink to which casein hydrolysate had been added
in order to provide 10.2 mg/day IPP and VPP or the same yoghurt drink without casein hydrolysate (placebo). Sixty-four subjects completed the study. In study 2, 93 subjects received a yoghurt drink to which casein hydrolysate (in order to provide 4.6 mg/day IPP and VPP) and potassium (350 mg/day) had been added, or the same yoghurt drink without added VPP, IPP or potassium (placebo). Ninety-one subjects completed this study. Subjects had high-normal BP or grade I hypertension and were not pharmacologically treated for hypertension. The order of the intervention was randomly assigned according to daytime ambulatory BP at baseline. BP was assessed before and after each intervention period both at the office and with a 24 h BP ambulatory monitor. ANCOVA was applied on the ITT population to evaluate the effect of the intervention on BP measures. Selection of subjects who entered the PP analysis and all data analyses were performed before unblinding data. No significant differences between periods in either study were observed for mean 24 h SBP or DBP. Office BP decreased over the course of both studies, but differences between intervention and placebo periods were not significant. The Panel considers that these two studies powered to detect small (3 mm Hg) differences in SBP do not show an effect of IPP and VPP on office or 24 h ambulatory SBP or DBP.

In a randomised, double-blind, placebo-controlled, parallel study (Jauhiainen et al., 2010a), 94 hypertensive volunteers, not on anti-hypertensive medication, received either a fermented milk containing IPP and VPP or a control fermented milk for two consecutive periods of 12 weeks, after a four week run-in period and followed by a four week follow-up after completion of the intervention phase. The intervention arm consumed 5 mg/day IPP and VPP for the first 12 weeks. The dose was increased up to 50 mg/day IPP and VPP for the subsequent 12 weeks. Eighty-nine subjects completed the study. The primary outcomes of the study were arterial stiffness and endothelial function. 24 h ambulatory BP was measured at baseline and at weeks 12 and 24 in both groups. Office BP was measured using an oscillometric technique at an undisclosed number of times during the study. Data analysis was carried out on an ITT basis, using ANCOVA with baseline BP as covariate. The Panel notes that no power calculation is provided. No significant differences between groups were found in either office or 24 h SBP or DBP at the end of the first intervention period (12 weeks). SBP and DBP significantly decreased in both groups from baseline to week 24, but there were no significant differences between groups in BP values. The Panel considers that this study does not show an effect of IPP and VPP on office or 24 h ambulatory SBP or DBP.

The Panel notes that 13 RCTs (De Leeuw et al., 2009; Engberink et al., 2008; Hata et al., 1996; Jauhiainen et al., 2010a; Mizushima et al., 2004; Seppo et al., 2003; Tuomilehto et al., 2004; Turpeinen et al., 2009; Turpeinen et al., unpublished; Van der Zander et al., 2008a; 2008b; Van Mierlo et al., 2009 two studies), of which four were designed to detect small (3-5 mm Hg) differences in SBP (De Leeuw et al., 2009; Van der Zander et al., 2008b; Van Mierlo et al., 2009 two studies), did not observe an effect of IPP and VPP on SBP or DBP.

The Panel notes that seven RCTs with major methodological limitations (Hirata et al., 2002; Kajimoto et al., 2001a; 2001b; 2002; Mizuno et al., 2005; Nakamura et al., 2004; Seppo et al., 2002) which hamper the interpretation of the results and one additional RCT (Sano et al., 2005) showed an effect of IPP and VPP on office SBP. Four of these studies also reported an effect of IPP and VPP on office DBP (Kajimoto et al., 2001a; 2001b; 2002; Seppo et al., 2002). However, the Panel also notes that 13 RCTs (De Leeuw et al., 2009; Engberink et al., 2008; Hata et al., 1996; Jauhiainen et al., 2010a; Mizushima et al., 2004; Seppo et al., 2003; Tuomilehto et al., 2004; Turpeinen et al., 2009; Turpeinen et al., unpublished; Van der Zander et al., 2008a; 2008b; Van Mierlo et al., 2009 two studies), of which four were designed to detect small (3-5 mm Hg) differences in SBP (De Leeuw et al., 2009; Van der Zander et al., 2008b; Van Mierlo et al., 2009 two studies), did not observe an effect of IPP and VPP on SBP or DBP.

**Animal studies**

The applicant also provided 13 animal studies, nine of which assessed the effect of administration of IPP and VPP given for 3 to 12 weeks on BP in spontaneously hypertensive rats, in sodium loaded
Goto-Kakisaki rats or in transgenic rats expressing human genes of the renin-angiotensin system (Ehlers et al., 2011; Jakala et al., 2009a; 2009b; 2010; Jauhiainen et al., 2005b; 2010b; Nakamura et al., 1995; Sipola et al., 2001; 2002). The Panel notes that, in several instances, BP values obtained in animals fed IPP and VPP were not significantly different from BP values obtained in the control groups (Jakala et al., 2009a; Jauhiainen et al., 2005b; 2010b), that substantial differences between experimental and control groups (e.g. in nutrient intake, particularly sodium and calcium, (Jauhiainen et al., 2005b) or in body weight gain (Sipola et al., 2002)) may have confounded the results in some of the studies, and that the doses of IPP and VPP used in these studies (from about 3 to 14 mg/kg body weight/day) depart from those proposed by the applicant (>50 times higher). The Panel considers that these animal studies do not provide additional information on the effect of IPP and VPP on BP in humans.

**Mechanistic studies**

The applicant further provided *ex vivo* and *in vitro* studies related to the production of IPP and VPP from casein digestion, to the peptide transport by the intestinal epithelium and to the mechanisms of action, namely angiotensin converting enzyme (ACE 1 and ACE 2) inhibition. To this respect, IPP and VPP IC₅₀ are in the micromolar range for ACE 1 and in the millimolar range for ACE 2 (Lehtinen et al., 2010; Luhtala et al., 2009). These values compare with an IPP Cₘₓ reaching 0.5-0.9 nmol/l in humans (VPP concentration remaining stable or below the limit of detection) after an oral load of 11 to 40 mg of both peptides (Foltz et al., 2007; Wuerzner et al., 2009). The concentrations of IPP and VPP required to inhibit ACE 1 and 2 by 50 % *in vitro* appear to be several orders of magnitude higher than the plasma maximal concentration that was achieved after an oral dose two to eight times higher than that proposed by the applicant. Thus, the plausibility of an effect on BP in humans through ACE inhibition with a daily dose of 5 mg IPP and VPP is not apparent. Besides ACE 1 inhibition, the applicant states that the tripeptides IPP and VPP could have other effects on the renin-angiotensin-aldosterone system, improve endothelial function, and have anti-inflammatory properties, all of which could mediate a BP-lowering effect. The Panel considers that no convincing data in support of any of these proposed mechanisms at the proposed dose has been provided.

In weighing the evidence, the Panel took into account that 13 of the human intervention studies provided, four of which were adequately powered to detect small between-group differences in SBP, did not observe an effect of IPP and VPP on SBP or DBP; that the interpretation of the results from seven out of the eight studies which reported an effect of IPP and VPP on office SBP was hampered by major methodological limitations; that the animal studies did not provide additional information on the effect of IPP and VPP on BP in humans; and that there is no convincing evidence for a mechanism by which IPP and VPP could exert the claimed effect at the proposed dose.

The Panel concludes that a cause and effect relationship has not been established between the consumption of IPP and VPP and maintenance of normal blood pressure.

**CONCLUSIONS**

On the basis of the data presented, the Panel concludes that:

- The food constituent, the tripeptides IPP and VPP, which is the subject of the health claim, is sufficiently characterised.

- The claimed effect is “helps to maintain normal blood pressure”. The target population proposed by the applicant is mildly hypertensive subjects or subjects with an elevated risk of developing hypertension. Maintenance of normal blood pressure is a beneficial physiological effect.
A cause and effect relationship has not been established between the consumption of IPP and VPP and maintenance of normal blood pressure.

**DOCUMENTATION PROVIDED TO EFSA**

Health claim application on isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) and maintenance of normal blood pressure pursuant to Article 13(5) of Regulation (EC) No 1924/2006 (Claim serial No: 00295_FI). March 2011. Submitted by Valio Ltd.

**REFERENCES**


Isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP) and maintenance of normal blood pressure


Isoleucyl-prolyl-proline (IPP) and valyl-prolyl-proline (VPP)
and maintenance of normal blood pressure


**Glossary and Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>ACE</td>
<td>Angiotensin converting enzyme</td>
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<tr>
<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AUC</td>
<td>Area under the curve</td>
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<td>BP</td>
<td>Blood pressure</td>
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<td>DBP</td>
<td>Diastolic blood pressure</td>
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<tr>
<td>IPP</td>
<td>Isoleucyl-prolyl-proline</td>
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<tr>
<td>ITT</td>
<td>Intention-to-treat</td>
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<tr>
<td>PP</td>
<td>Per protocol</td>
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<tr>
<td>RCT</td>
<td>Randomised controlled trial</td>
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<td>SBP</td>
<td>Systolic blood pressure</td>
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<td>VPP</td>
<td>Valyl-prolyl-proline</td>
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