Lactotripeptides Show No Effect on Human Blood Pressure: Results From a Double-Blind Randomized Controlled Trial

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Abstract—Milk-derived peptides with ACE-inhibiting properties may have antihypertensive effects in humans. We conducted a randomized double-blind placebo-controlled trial to examine the blood pressure lowering potential of 2 ACE-inhibiting lactotripeptides, ie, Isoleucine-Proline-Proline and Valine-Proline-Proline. We included 135 Dutch subjects with elevated systolic blood pressure who were otherwise healthy and who received no current antihypertensive treatment. After a 2-week run-in period on placebo, subjects randomly received a daily dose of 200 mL dairy drink with 14 mg lactotripeptides obtained by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis, or placebo for 8 weeks, followed by a 2-week wash-out. The primary outcome was 8-week change in office systolic blood pressure. Secondary outcomes were change in diastolic blood pressure, home blood pressure, 24-hour ambulatory blood pressure, plasma ACE-activity, and plasma angiotensin II. Blood pressure at baseline was on average 142/84 mm Hg. Lactotripeptides did not significantly change systolic blood pressure ($P=0.46$) or diastolic blood pressure ($P=0.31$) compared with placebo. The mean difference (95%-CI) in systolic blood pressure response between treatment and placebo was 2.8 mm Hg (−2.6;8.2) for concentrated fermented milk lactotripeptides, −0.5 mm Hg (−6.0;5.0) for enzymatic lactotripeptides, and 1.6 mm Hg (−3.9;6.9) for synthetic lactotripeptides. Treatment neither had a significant effect on secondary outcome measures. In conclusion, the present study does not support the hypothesis of a blood pressure lowering effect of the lactotripeptides Isoleucine-Proline-Proline and Valine-Proline-Proline. **(*Hypertension*. 2008;51:399-405.)**

Keywords: hypertension ■ ACE inhibition ■ dairy ■ bioactive peptides

There is growing evidence for a beneficial effect of dairy intake on blood pressure.[1] In the Dietary Approaches to Stop Hypertension (DASH) trial larger blood pressure reductions were achieved when low-fat dairy was added to a healthy fruit and vegetable diet.[2] Dairy food is rich in protein and calcium, which could have beneficial effects on blood pressure.[3–5] In addition, fermented milks and casein hydrolysates decreased blood pressure in a number of Japanese and Finnish trials in (mildly) hypertensive human subjects.[6–14] Studies of fermented milk showed reductions of 1.5 to 11.0 mm Hg for systolic blood pressure (SBP) and 0.5 to 6.8 mm Hg for diastolic blood pressure (DBP), compared with placebo.[6–8,10,12–14] The proposed underlying antihypertensive mechanism of these bioactive peptides is, at least partly, ACE-inhibition. Isoleucine-Proline-Proline (IPP) and Valine-Proline-Proline (VPP), 2 lactotripeptides (LTP), are considered the most promising ACE-inhibiting bioactive peptides. IPP and VPP can be derived from milk casein by the action of the proteolytic system of lactic acid bacteria (*Lactobacillus helveticus*). Recently, an enzymatic method using an *Aspergillus oryzae* protease has been developed to generate IPP and VPP from milk casein.[15] This casein hydrolysate decreased blood pressure in Japanese hypertensive subjects.[9,11] Both fermented and enzymatically hydrolyzed milk contain a large variety of peptides. Therefore, supplementation with chemically synthesized IPP and VPP may be more appropriate for studying specific effects of these peptides. An antihypertensive effect of chemically synthesized IPP and VPP has been demonstrated in spontaneously hypertensive rats,[16,17] but not yet in humans.

We performed a randomized double-blind placebo-controlled trial to examine whether daily intake of 14 mg LTP, obtained by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis, would influence blood pressure over an 8-week period in 135 Dutch subjects with elevated blood pressure who received no current antihypertensive treatment.

Subjects and Methods

Subjects

Men and women (35 to 70 years) with elevated SBP were recruited from the Dutch population in collaboration with general practitio-

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Table 1. Nutrient Composition per 100 g of Dairy Drinks (Daily Dose*)

Nutrient	Fermented LTP	Enzymatic LTP	Synthetic LTP	Placebo
Calorie, kcal	64 (128)	61 (122)	60 (120)	61 (122)
Protein, g	2.6 (5.2)	2.8 (5.6)	2.7 (5.4)	2.7 (5.4)
Carbohydrates, g	13.4 (26.8)	12.5 (25.0)	12.4 (26.8)	12.5 (25.0)
Fat, g	...	...	...	...
Calcium, mg	95 (190)	71 (142)	71 (142)	75 (150)
Sodium, mg	34 (68)	45 (90)	32 (64)	35 (70)
Potassium, mg	146 (292)	147 (294)	147 (294)	153 (306)
Magnesium, mg	13 (26)	9 (18)	9 (18)	9 (18)
Lactotripeptides				
IPP, mg	2.1 (4.2)	2.7 (5.4)	2.6 (5.2)	...
VPP, mg	2.9 (5.8)	2.5 (5.0)	2.5 (5.0)	...

*Subjects took 200 mL daily.

LTP indicates lactotripeptides (IPP and VPP); VPP, Valine-Proline-Proline; IPP, Isoleucine-Proline-Proline.

ners. During a screening phase 2 consecutive blood pressure measurements were performed at the study center with at least 48 hours in between. Subjects with elevated blood pressure, defined as SBP ≥140 mm Hg (mean of last 3 of 4 measurements, taken after 15-minute rest at 2-minute intervals) on both occasions, were invited for further screening. Subjects were excluded if they reported metabolic diseases, gastrointestinal disorders, cardiovascular disease, or renal disease. Plasma glucose, liver function (ALAT, ASAT, γ-GT), renal function (ureum and creatinine), and hematology (WBC, RBC, hemoglobin, hematocrit, platelet count) had to be normal based on the judgment of general practitioners, who evaluated these data for their own patients. Additional exclusion criteria were: antihypertensive medication less than 3 weeks before screening; body mass index >32 kg/m^2; weight loss (>10%) during 6 months before screening; cow milk allergy or lactose intolerance; pregnancy or lactation; blood donation; alcohol abuse; and participation in night shift work. The Medical Ethics Committee of Wageningen University approved the study and all subjects gave written informed consent. In addition, agreement was obtained from each subject's general practitioner. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Design and Randomization Procedure

We performed a randomized double-blind placebo-controlled trial with 4 parallel arms. Baseline measurements took place at the end of a 2-week run-in period during which subjects consumed the placebo product (ie, dairy drink without LTP) and followed study guidelines as described below. Two independent persons, not involved in the conduct of the study, randomly allocated eligible subjects to 1 of the 3 treatment groups or placebo by means of computer-generated randomization codes stratified by prestudy use of antihypertensive medication (yes versus no), baseline SBP (<150 mm Hg, 150 to 160 mm Hg, or >160 mm Hg), and gender. Subjects took one study drink each morning for an 8-week period. An additional blood pressure measurement was performed after a 2-week wash-out period.

Dairy Drinks

Test products were low-fat yoghurt drinks that were ready-to-drink and provided by Unilever Food & Health Research Institute, Vlaardingen, The Netherlands. Table 1 shows the nutritional composition of the products. The 3 treatment products contained IPP and VPP, which are lactotripeptides (LTP) generated by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis. Daily supply of dairy drink per subject was 200 mL, containing 4.2 to 5.4 mg IPP and 5.0 to 5.8 mg VPP, which equals 14 mg LTP equivalents in all active arms. ACE-inhibitory activity of the test products was analyzed according to Matsui et al.[18] The fermented and enzymatic LTP drinks had an ACE-inhibition of 50% using a concentration of 0.025% protein of the added active powder. The synthetic LTP drink had an ACE-inhibition of 45% using a 1000 times lower protein concentration. The placebo drink did not show any ACE inhibition. Additionally, all test products contained orange juice concentrate, sugars, flavors for taste, pectin for stability, and water. Test products were similar in appearance, color, taste and smell, and were provided in nontransparent white cups every 2 weeks. Two research assistants, not otherwise involved in the study, labeled and supplied the test products. Compliance was assessed by counting all empty and full cups that were returned by subjects. Research staff and subjects remained blinded toward the type of treatment during the study and data analysis.

Study Procedures

Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Body weight was measured without shoes and heavy clothing at each visit (Seck Bascule MT), and body mass index was computed (kg/m^2). At baseline, subjects filled out a 21-item food frequency questionnaire for intake of dairy products from which daily protein and calcium intake were computed using Dutch food composition tables (NEVO, The Hague, 2001).

Subjects recorded consumption of test products, deviations from the study protocol, episodes of illness or hospitalization, use of medication, and health complaints in a diary. Adverse events were coded by the study physician (EGS) according to the International Classification of Diseases (ICD10).

Subjects were asked to maintain their habitual dietary and lifestyle pattern during the study. In particular they were asked not to change intake of salt and salty foods, alcoholic beverages, and fermented products. Subjects were instructed to consume the test product daily at breakfast. At the day before a test day at the study center, subjects were asked to fast from 8 PM onwards. They were allowed to consume breakfast and test product on test days, but at a fixed point in time at least 2 hours before their appointment at the study center.

Blood Pressure Measurements

Blood pressure measurements were performed at the study center (office BP) and by subjects themselves at home (home BP). In 58 subjects (43%) we additionally obtained 24-hour ambulatory blood pressure readings (ABPM). Subjects remained blinded toward all blood pressure values until data analysis was completed. The primary outcome measure was 8-week change in SBP measured at the study center.

Trained staff measured blood pressures at the study center during screening, at baseline, after 4 weeks, after 8 weeks, and after the 2-week wash-out period. After a 15-minute rest, 4 measurements with 2-minute intervals were performed on the dominant arm in

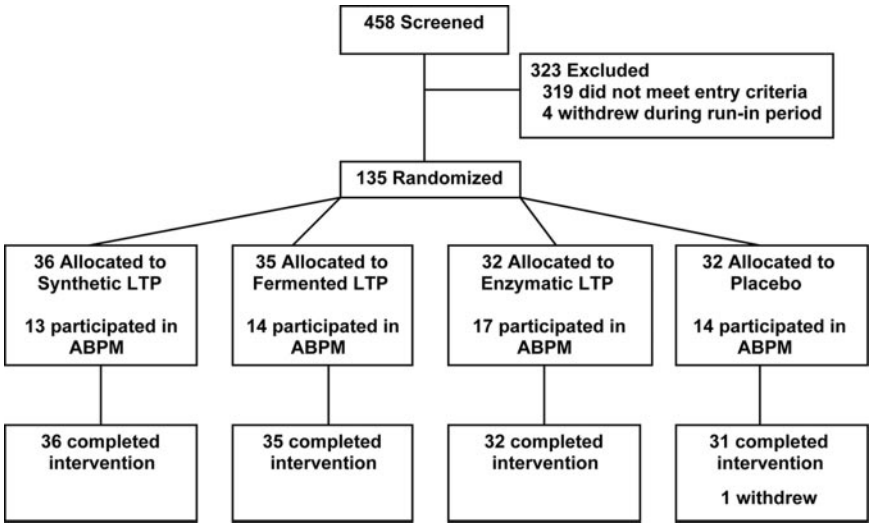

Figure 1. Flow chart of a double-blind, randomized, placebo-controlled trial of lactotripeptides and blood pressure in 135 Dutch subjects with untreated elevated blood pressure. LTP indicates lactotripeptides (IPP and VPP); ABPM, 24-hour ambulatory blood pressure measurement.

sitting position, using an automated blood pressure device with an appropriately sized cuff (Omron HEM-907). The first reading was discarded, and 3 subsequent readings were averaged. Blood pressure measurements were repeated after 1 day and values of both occasions were averaged. All blood pressure measurements were performed between 8 and 12 AM at a fixed time for each individual.

Subjects performed measurements at home on the same days as the study center visits according to the same standardized procedures. They measured blood pressure using a blinded automated device (Stabil-O-Graph) at 3 fixed time points: in the morning between awakening and breakfast, before lunch, and before bedtime. Readings were stored and downloaded afterward on a computer at the study center.

A group of 58 subjects volunteered for 24-hour ABPM at baseline, week 4, and week 8. Measurements were performed from 12 AM till 12 AM next day with 30-minute intervals during the day (7 AM to 11 PM) and 60-minute intervals during the night (11 PM to 7 AM), using a blinded automated device (SpaceLab type 90217). Readings were stored and downloaded afterward on a computer at the study center. Mean 24-hour, day (9 AM to 9 PM), and night (1 AM to 6 AM) values were calculated.

Laboratory Determinations

Research nurses performed blood sampling at screening, baseline, and after 4 and 8 weeks of intervention. During screening, they used venipuncture. During the study, the nurses inserted a catheter into the antecubital vein, after which the subject rested for 20 minutes before blood was taken by a syringe. The collected blood was directly processed on ice and stored at −80°C. For determination of ACE-activity blood was collected in Li-heparin tubes. Plasma ACE activity was assessed in duplicate by use of a fluorescence assay (BiPharma, Fujirebio Diagnostics Inc), with an intraassay coefficient of variation of 3.8% and an interassay coefficient of variation of 5.8%. For the determination of angiotensin II blood was collected in chilled EDTA tubes containing ACE and renin inhibitors to prevent conversion. Before determination, plasma was treated with Sep-Pak C18 columns. Subsequently, plasma concentration of angiotensin II was assessed in duplicate by Radioimmunoassay (Penninsula Laboratories Inc Europe), with an intraassay coefficient of 4.6% and interassay coefficient of 7.7%. All samples of each subject were analyzed in one run. Plasma glucose, liver enzymes, indicators of renal function and hematology were assessed during screening and after 8 weeks using standard laboratory methods.

At baseline and after 8 weeks, 24-hour urine collections were obtained for determination of sodium and potassium excretion. Creatinine was measured to check completeness of urine collection. Urinary volume and collection times were used to calculate 24-hour excretion of sodium and potassium.

Statistical Analysis

Double-data entry was performed and discrepancies were solved. Treatment codes were broken after blind data-analysis. Data were analyzed according to the intention-to-treat principle.

Values reported in text and tables are means with standard errors (SE) or 95% confidence intervals (95% CI), unless stated otherwise. Response to treatment was defined as change in blood pressure from baseline. Blood pressure data were analyzed using analysis of variance (ANOVA). Subsequently, the Dunnett test was used to compare mean changes within different treatment groups with that of the control group. Data analysis was repeated after exclusion of noncompliant subjects who consumed less than 80% of the test products.

To examine whether dairy calcium or protein in the background diet would modify blood pressure response to intake of LTP (all 3 treatment groups combined), we performed a post-hoc analysis in 118 subjects who completed the food frequency questionnaire. For this purpose, blood pressure effects were reexamined after stratification for dairy calcium intake (below versus above the median of 726 mg per day) and for dairy protein intake (below versus above the median of 24 g per day).

Analyses were performed using SPSS 11.0.1 for Windows. Two-sided probability values below 0.05 were considered statistically significant. The present study had a power of 83% for detecting a 5 mm Hg difference in systolic blood pressure response, with $\alpha=0.05$ (2-sided).

Results

Subjects and Compliance

We enrolled 135 subjects in the trial. One subject dropped out after 3 weeks for personal reasons not related to the study. Figure 1 shows the number of subjects screened, excluded, and randomized. There were no relevant differences in subjects' characteristics, including blood pressure, among the groups (Table 2). Compliance was satisfactory, with only 4 subjects consuming less than 80% of the test products.

Blood Pressure

Figure 2 shows mean office SBP and DBP in the 4 groups throughout the study. There was no significant effect of LTP treatment on SBP ($P=0.46$). The mean difference (95% CI) in 8-week change between treatment and placebo was 2.8 mm Hg (−2.6;8.2) for concentrated fermented milk based LTP, −0.5 mm Hg (−6.0;5.0) for enzymatic LTP and 1.6 mm Hg (−3.9;6.9) for synthetic LTP (Table 3). LTP

Table 2. Baseline Characteristics of 135 Subjects With Untreated Elevated Blood Pressure, by Treatment

Characteristic	Fermented LTP (n=35)	Enzymatic LTP (n=32)	Synthetic LTP (n=36)	Placebo (n=32)
Age, y	58.8 (9.1)	54.2 (8.8)	59.5 (8.2)	58.9 (10.3)
Sex, male/female	23/12	22/10	23/13	20/12
Blood pressure category, n (%)*				
Optimal	1 (3)	1 (3)	2 (6)	1 (3)
Normal	4 (11)	6 (19)	4 (11)	5 (26)
High normal	11 (31)	7 (22)	10 (28)	9 (28)
Hypertension				
Stage 1	17 (49)	14 (44)	16 (44)	14 (44)
Stage 2–4	2 (6)	4 (13)	4 (11)	3 (10)
Resting heart rate, beats/min	68.6 (8.6)	74.9 (7.5)	68.1 (8.0)	67.5 (7.5)
Pre-study use of antihypertensive medication (past 3 months), n (%)†	5 (14.3)	2 (6.3)	3 (8.3)	1 (3.1)
Body weight, kg	81.8 (12.3)	84.7 (11.1)	83.2 (12.2)	83.5 (13.5)
Body mass index, kg/m^2	26.9 (2.6)	26.8 (2.8)	27.0 (2.9)	26.8 (2.9)
Current cigarette smoking, n (%)†	3 (9)	3 (9)	5 (14)	3 (9)
Glucose, mmol/L†	6.0 (0.1)	5.8 (0.1)	6.0 (0.1)	6.0 (0.1)
Dairy protein intake, g/d†	24 (10)	25 (10)	26 (13)	29 (17)
Dairy calcium intake, mg/d†	738 (316)	807 (334)	811 (419)	926 (589)

LTP indicates lactotripeptides (IPP and VPP).

Values are mean (SD) or percentages.

*None of the subjects used antihypertensive medication at baseline. Blood pressure categories defined as follows: Optimal, SBP <120 mm Hg and DBP <80 mm Hg; Normal, 120–129 mm Hg for SBP or 80–84 mm Hg for DBP; High normal, 130–139 mm Hg for SBP or 85–89 mm Hg for DBP; Hypertension–stage 1 (mild), 140–159 mm Hg for SBP or 90–99 mm Hg for DBP; Hypertension–stage 2–4, ≥160 mm Hg for SBP or ≥110 mm Hg for DBP.

†Assessed during screening.

treatment did neither affect DBP (P=0.31, Table 3). Exclusion of 4 noncompliant subjects yielded similar results (data not shown).

Mean home blood pressure at baseline in the group as a whole was 153/95 mm Hg, ie, 149/94 mm Hg in the morning, 150/94 mm Hg at noon, and 158/98 mm Hg in the evening. Values were similar for treatment and placebo groups. LTP treatment did not significantly affect home blood pressure (all probability values >0.3). The mean difference (95% CI) in home SBP response between treatment and placebo was −0.9 mm Hg (−6.1;4.4) for concentrated fermented milk based LTP, −1.8 mm Hg (−7.2;3.6) for enzymatic LTP, and 0.6 mm Hg (−4.7;5.9) for synthetic LTP. Detailed data on home blood pressure responses throughout the day are tabulated in the online supplement to this article (please see Table S1 at http://hyper.ahajournals.org).

Mean ABPM in 58 subjects was 144/87 mm Hg at baseline, with higher values during the day (150/90 mm Hg) than the night (123/72 mm Hg). Changes in 24-hour SBP during intervention in the 3 active arms were not significantly different from placebo (all P>0.3): the mean difference (95% CI) in 24-hour SBP response (12 AM to 12 AM) was 4.6 mm Hg (−3.1;12.3) for concentrated fermented milk based LTP, 2.6 mm Hg (−4.7;9.9) for enzymatic LTP, and 4.2 mm Hg (−3.6;12.1) for synthetic LTP. Results were similar for daytime and nighttime SBP, and for different measures of DBP. Additional ambulatory 24-hour SBP curves for each group are shown in the online supplement to this paper (please see Figure S1 and S2 at http://hyper.ahajournals.org). Restricting the analysis to 46 compliant subjects (ie, subjects with over 70% readings and more than 20 hours on each occasion) yielded similar results.

Other Outcomes

Body weight did not change significantly during the study. Mean changes (±SD) were: 0.4±0.2 kg for concentrated fermented milk based LTP, 0.0±0.2 kg for enzymatic LTP, 0.1±0.2 kg for synthetic LTP, and 0.0±0.2 kg for placebo. In the group as a whole, baseline urinary 24-hour excretion of sodium, potassium, and creatinine (±SD) were 168.2±5.9 mmol (corresponding to 9.7 g NaCl per day), 91.1±2.6 mmol (3.5 g), and 13.9±4.5 mmol, respectively. The 8-week change in sodium was −17.7±15.0 mmol/L for concentrated fermented milk based LTP, −9.2±10.8 mmol/L for enzymatic LTP, −2.3±14.3 mmol/L for synthetic LTP, and 3.9±16.0 mmol/L for placebo. For potassium 8-week changes were −8.8±5.4, −0.2±5.8, −14.0±4.7, and −3.8±7.0 mmol/L, respectively. Changes in sodium and potassium did not significantly differ between treatment and placebo (all P>0.1).

Mean baseline ACE-activity (SE) was 30.0±0.7 U/L and remained constant during the study (Table 4). Baseline plasma angiotensin II concentration was 10.4±0.5 pmol/L and decreased slightly in the concentrated fermented milk based LTP group and in the enzymatic LTP group during intervention, but this was not significantly different from placebo (Table 4).

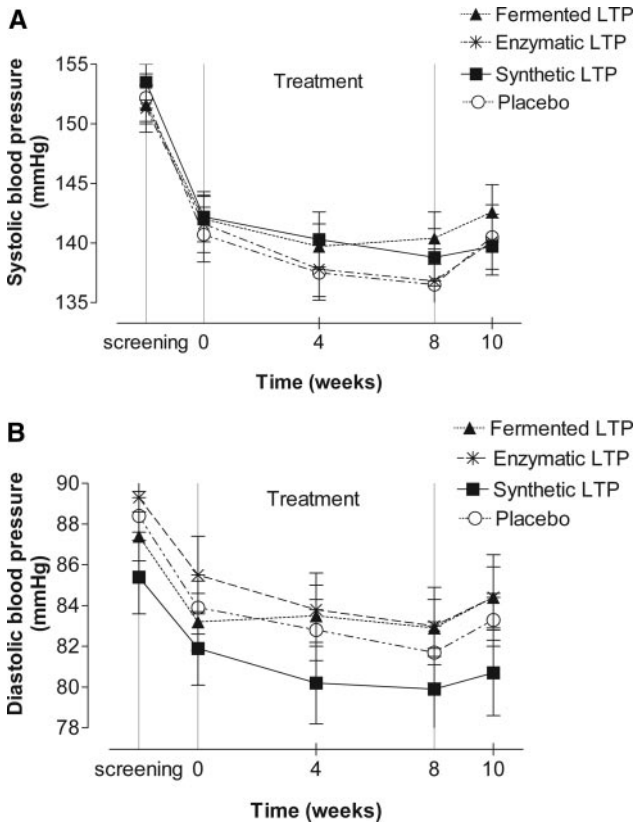

Figure 2. Mean values (SE) for office systolic and diastolic blood pressure during run-in, intervention, and wash-out in 135 randomized subjects in the 3 treatment groups and the placebo group. LTP indicates lactotripeptides (IPP and VPP).

Plasma glucose, liver enzymes, indicators of renal function, and hematology were similar in the 4 groups and did not change significantly during the study (data not shown). During the 8 weeks of treatment a few minor adverse events were recorded, but there were no differences according to treatment.

Post-Hoc Analysis

In 59 subjects with a dairy calcium intake <726 mg/d the change in blood pressure was −3.2/−1.6 mm Hg for all LTP treatments combined versus −5.8/−2.7 mm Hg for the placebo group. In 59 subjects with a higher calcium intake, values were −3.3/−1.7 mm Hg and −3.6/−1.8 mm Hg, respectively. In subjects with a low dairy protein intake (<24 g/d), corresponding values were −3.2/−1.3 mm Hg for LTP versus −6.8/−3.4 mm Hg for placebo, and in subjects with a higher dairy protein intake −3.3/−2.0 mm Hg and −2.6/−1.0 mm Hg, respectively. Probability values for all comparisons were >0.2.

Discussion

This trial showed no antihypertensive effect of the dairy peptides IPP and VPP that were generated by concentrating fermented milk, enzymatic hydrolysis, or chemical synthesis in 135 subjects with high normal and elevated blood pressure. Although SBP and DBP decreased during the study, this was not attributable to effects of IPP and VPP. The results were consistent for office, home, and ambulatory blood pressure.

Strengths of this study are its randomized double-blind placebo-controlled design. In addition, compliance was excellent and only 1 subject dropped out. Data were analyzed according to the intention-to-treat principle. Restricting the analysis to compliant subjects yielded similar results. Baseline characteristics, including initial levels of blood pressure, were similar among the 4 groups. Subjects were also able to maintain their habitual level of exercise and dietary pattern during the study, as confirmed by their unchanged body weight and urinary excretion of sodium and potassium.

From most trials in humans it was concluded that IPP and VPP are effective in lowering blood pressure.[6–14] In the majority of studies milk fermented with *Lactobacillus helveticus* was examined in subjects with high normal blood pressure or mild hypertension. The effect on blood pressure, either office, home, or ABPM, ranged from −1.5 to

Table 3. Office Blood Pressure During Intervention in 135 Subjects With Untreated Elevated Blood Pressure, by Treatment

Outcome	Fermented LTP (n=35)	Enzymatic LTP (n=32)	Synthetic LTP (n=36)	Placebo (n=32)
Systolic BP, mm Hg				
Baseline	142.0 (1.9)	141.6 (2.4)	142.2 (2.1)	140.7 (2.3)
Week 8*	140.4 (2.2)	136.8 (2.7)	138.8 (2.4)	136.5 (2.3)
Change*	−1.6 (1.5)	−4.8 (1.8)	−2.8 (1.7)	−4.3 (1.3)
Change attributed to treatment, mean difference (95% CI)	2.8 (−2.6;8.2)	−0.5 (−6.0;5.0)	1.6 (−3.9;6.9)	
	P=0.47	*P*=0.99	*P*=0.83	
Diastolic BP, mm Hg				
Baseline	83.2 (1.4)	85.5 (1.9)	81.9 (1.8)	83.9 (1.3)
Week 8*	82.9 (1.4)	83.0 (1.9)	79.9 (2.0)	81.7 (1.5)
Change*	−0.3 (1.0)	−2.4 (0.9)	−2.4 (1.1)	−2.4 (0.9)
Change attributed to treatment, mean difference (95% CI)	2.2 (−1.2;5.5)	0.01 (−3.4;3.5)	−0.01 (−3.4;3.4)	
	P=0.30	*P*=1.00	*P*=1.00	

LTP indicates lactoptripeptides (IPP and VPP). Values are mean (SE); 2-sided *P* values for the difference from placebo (Dunnett test).
*Missing for 2 subjects (1 synthetic LTP, 1 placebo).

Table 4. Plasma ACE-Activity and Angiotensin II Concentration in 135 Subjects With Untreated Elevated Blood Pressure, by Treatment

Outcome	Fermented LTP (n=35)	Enzymatic LTP (n=32)	Synthetic LTP (n=36)	Placebo (n=32)
ACE-activity, U/L				
Baseline*	30.5 (1.5)	29.1 (1.2)	30.9 (1.4)	29.2 (1.1)
Week 8†	30.6 (1.5)	29.4 (1.1)	30.7 (1.4)	28.7 (1.1)
Change†	0.1 (0.5)	0.3 (0.4)	−0.1 (0.4)	−0.3 (0.5)
Change attributed to treatment, mean difference (95% CI)	0.4 (−1.2;2.0)	0.6 (−1.0;2.2)	0.2 (−1.4;1.8)	
	P=0.86	*P*=0.65	*P*=0.98	
Angiotensin II, pmol/L				
Baseline‡	12.2 (1.0)	10.2 (1.0)	9.5 (0.7)	9.8 (1.0)
Week 8§	9.6 (0.8)	8.9 (0.6)	9.1 (0.5)	8.8 (0.6)
Change‖	−3.4 (0.9)	−1.6 (0.8)	−0.3 (0.7)	−0.5 (1.0)
Change attributed to treatment, mean difference (95% CI)	−2.9 (−5.8;0.1)	−1.1 (−4.2;2.0)	0.2 (−2.7;3.1)	
	P=0.06	*P*=0.73	*P*=1.00	

LTP indicates lactoptripeptides (IPP and VPP). Values are mean (SE); 2-sided *P* values for the difference from placebo (Dunnett test).

*Missing for 2 subjects (1 synthetic LTP, 1 placebo).

†Missing for 4 subjects (1 synthetic LTP, 3 placebo).

‡Missing for 3 subjects (1 enzymatic LTP, 1 synthetic LTP, 1 placebo); values below detection limit for 10 subjects (2 fermented LTP, 3 enzymatic LTP, 3 synthetic LTP, 2 placebo).

§Missing for 5 subjects (2 synthetic LTP, 3 placebo); values below detection limit for 11 subjects (5 fermented LTP, 3 enzymatic LTP, 2 synthetic LTP, 1 placebo).

‖Missing for 6 subjects (1 enzymatic LTP, 2 synthetic LTP, 3 placebo); values below detection limit for 16 subjects (5 fermented LTP, 5 enzymatic LTP, 3 synthetic LTP, 3 placebo).

−11.0 mm Hg for SBP and from −0.5 to −6.8 mm Hg for DBP compared with placebo.[6–8,10,12–14] Also, IPP and VPP obtained by enzymatic hydrolysis of casein, using *Aspergillus oryzae* protease, have been shown to lower blood pressure in mild hypertensives.[9,11] We considered several possible explanations for the absence of an antihypertensive effect of IPP and VPP in our study. Previous studies that did show an effect had a duration of 4 to 21 weeks and daily doses of IPP and VPP between 2.5 and 52.5 mg. Hence, we conclude that our intervention of 8 weeks with a dose of 14 mg LTP equivalents, containing 4.2 to 5.4 mg IPP and 5.0 to 5.8 mg VPP, should have been sufficient for detecting any effect on blood pressure, if present. Also, the contrast in ACE-inhibitory capacity between the treatment drinks and the placebo should have been sufficient in our study. Secondly, LTP may exert their effect only in subjects with clinically established hypertension. Ninety percent of our subjects had a blood pressure in the high-normal or mildly hypertensive range, after repeated measurements. Some studies that were positive for LTP had higher blood pressure levels at baseline.[7,12,14] It should also be noted that in most studies, despite randomization, initial blood pressure was higher in the intervention than in the control group.[7,8,10,12,14] As a result, blood pressure effects attributed to IPP and VPP may have been overestimated. Thirdly, we considered the role of background diet. Our Dutch study population already had a high habitual intake of dairy foods (520 g per day) and calcium from dairy (820 milligram per day). Previous studies were almost exclusively performed in Japanese and Finnish subjects, with different dietary patterns. In a post-hoc analysis we examined whether a low or high habitual intake of dairy protein and calcium could modify blood pressure response to LTP, but this was not the case. Finally, because our subjects were asked to maintain their regular lifestyle or dietary pattern during the study, we cannot exclude a possible beneficial effect of LTP in combination with favorable lifestyle or dietary changes, although we consider this unlikely.

The putative antihypertensive effect of dairy peptides has been related to ACE inhibition,[17,19–21] but we found no consistent changes in plasma ACE activity or angiotensin II. The potency of ACE inhibition of most food-derived peptides in vitro ranges from 2 to 1000 μmol/L, which is over 1000 times weaker than antihypertensive drugs.[22] Such effects in vivo may not have been detectable in our study because of large inter- and intraindividual variation. We did not examine other mechanisms of antihypertensive action, such as a direct relaxation of vascular muscle cells or opioid or antioxidant activities.[23]

In conclusion, the present randomized double-blind controlled trial provides no evidence for a blood pressure lowering effect of the lactotripeptides IPP and VPP in human subjects with untreated elevated blood pressure.

Perspectives

There is growing interest in the role of low-fat dairy for the prevention of hypertension. A new line of research in this field focuses on bioactive peptides derived from milk proteins (eg, IPP and VPP) that could inhibit the angiotensin-converting enzyme (ACE). Previous studies identified IPP and VPP as promising food ingredients for blood pressure control in mildly hypertensive subjects.[6–14] The present double-blind randomized controlled trial in 135 Dutch sub-

jects with elevated blood pressure, however, provides no evidence for an antihypertensive effect of IPP and VPP. More well-conducted trials in different populations are needed to see whether supplementation with dairy peptides could have a place in the treatment of mild hypertension.

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