A fermented milk high in bioactive peptides has a blood pressure–lowering effect in hypertensive subjects[1–3]

Leena Seppo, Tiina Jauhiainen, Tuija Poussa, and Riitta Korpela

ABSTRACT

Background: Angiotensin-converting enzyme (ACE; EC 3.4.15.1) plays a dual role in the regulation of hypertension: it catalyzes the production of the vasoconstrictor angiotensin II and it inactivates the vasodilator bradykinin. By inhibiting these processes, ACE inhibitors have antihypertensive effects. Peptides derived from milk proteins can have ACE-inhibiting properties and may thus be used as antihypertensive components.

Objective: We evaluated the long-term blood pressure–lowering effect of milk fermented by *Lactobacillus helveticus* LBK-16H in hypertensive subjects.

Design: In a randomized placebo-controlled study, 39 hypertensive patients received 150 mL/d of either *L. helveticus* LBK-16H fermented milk or a control product for 21 wk after a 2-wk run-in period. During the run-in period, the average baseline diastolic and systolic blood pressure values were 155 and 97 mm Hg, respectively, in the test product group and 152 and 96 mm Hg, respectively, in the control group. After the run-in period, blood pressure was measured at home on the same day every week with the use of an automatic blood pressure recorder.

Results: There was a mean difference of 6.7 ± 3.0 mm Hg in systolic blood pressure ($P = 0.030$) and of 3.6 ± 1.9 mm Hg ($P = 0.059$) in diastolic blood pressure between the test product and control groups. Demographic factors had no significant effect on the responses.

Conclusion: *L. helveticus* LBK-16H fermented milk containing bioactive peptides in normal daily use has a blood pressure–lowering effect in hypertensive subjects. *Am J Clin Nutr* 2003;77:326–30.

KEY WORDS Fermented milk, bioactive peptides, blood pressure, *Lactobacillus helveticus*, hypertension, valine-proline-proline, isoleusine-proline-proline, angiotensin-converting enzyme inhibitors

INTRODUCTION

Hypertension is a risk factor for cardiovascular diseases, including coronary heart disease, peripheral arterial disease, and stroke. The renin-angiotensin system is an important regulator of blood pressure. Therefore, drugs that inhibit the renin-angiotensin system, either by inhibiting angiotensin-converting enzyme (ACE; EC 3.4.15.1) or by blocking angiotensin (AT1) receptors, are widely used in the treatment of hypertension. ACE inhibitors have a dual effect on the renin-angiotensin system: they inhibit the production of the vasoconstrictor angiotensin II and they inhibit the degradation of the vasodilator bradykinin. In addition, ACE inhibitors have other beneficial effects in hypertensive patients, for example, in those with cardiac or renal insufficiency or diabetes.

Through fermentation, peptides that have an ACE-inhibiting and thus a blood pressure–lowering effect can be derived from milk proteins (1). Some of these peptides have also been found to have opioid receptor binding properties (2). A fermented milk product with the biologically active peptides valyl-prolyl-proline (Val-Pro-Pro) and isoleucyl-prolyl-proline (Ile-Pro-Pro) was shown to lower blood pressure in spontaneously hypertensive rats (3). It was suggested that small peptides are absorbed from the gastrointestinal tract without being decomposed by digestive enzymes (4). Two other peptides (Tyr-Pro and Lys-Val-Leu-Pro-Val-Pro-Gln) that were purified and characterized from fermented milk were also shown to have ACE-inhibitory activity in spontaneously hypertensive rats (5, 6). Nurminen et al (7) found that α-lactorphin (Tyr-Gly-Leu-Phe) also reduced blood pressure in normotensive and spontaneously hypertensive rats (7).

In a placebo-controlled study of hypertensive patients, sour milk fermented by *Lactobacillus helveticus* and *Saccharomyces cerevisiae* reduced systolic and diastolic blood pressure in an 8-wk intervention (8). We previously investigated the effects on blood pressure of a milk product fermented by *L. helveticus* LBK-16H, both in animal models (9) and in humans (10). In an 8-wk study (10), this milk product containing the bioactive tripeptides Val-Pro-Pro and Ile-Pro-Pro reduced blood pressure in mildly hypertensive subjects. In an unpublished placebo-controlled study, the same milk product also tended to lower systolic blood pressure in hypertensive subjects (J Tuomilehto, J Lindström, and J Hyyrynen, et al, unpublished observations, 2002). The aim of the present study was to evaluate the long-term blood pressure–lowering effect of *L. helveticus* LBK-16 H fermented milk, in normal daily use, in hypertensive subjects during a 21-wk intervention period.

[1] From the Valio, Ltd, Research Center, Helsinki (LS, TJ, and RK); Stat-Consulting, Tampere, Finland (TP); the Foundation for Nutrition Research, Helsinki (RK); and the Institute of Biomedicine, University of Helsinki (TJ and RK).

[2] The test milks were provided by Valio Ltd.

[3] Address reprint requests to R Korpela, Foundation for Nutrition Research, PO Box 30, FIN-00039 Helsinki, Finland. E-mail: riitta.korpela@valio.fi.

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TABLE 1
Demographic characteristics of the subjects[1]

	Test product group (n = 10 M, 12 F)	Control group (n = 9 M, 8 F)
Age (y)	50.9 ± 6.9 (33.7–61.7)[2]	47.9 ± 6.9 (30.2–61.4)
Weight (kg)	85.6 ± 16.3 (67.5–125.0)	77.6 ± 17.1 (48.0–118.0)
Users of antihypertensive medication (n)	9	7
Smokers (n)	2	2
Blood pressure at the clinic visits (mm Hg)[3]		
Systolic	155 ± 13 (133–176)	152 ± 13 (137–176)
Diastolic	97 ± 6 (86–108)	96 ± 6 (88–108)

[1] There were no significant differences between groups.
[2] $\bar{x}$ ± SD; range in parentheses.
[3] Weeks −2 and −1 of the run-in period.

TABLE 2
Nutritional composition of the test and control products[1]

	Test product	Control product
Energy (kJ/100 g)	265	147
Carbohydrate (g/100 g)	14	4.3
Fat (g/100 g)	0.07	0.4
Protein (g/100 g)	2.8	3.7
Calcium (mg/100 g)	200	130
Val-Pro-Pro (mg/100 g)	2	—
Ile-Pro-Pro (mg/100 g)	1.5	—

[1] Val-Pro-Pro, valyl-prolyl-proline; Ile-Pro-Pro, isoleucyl-prolyl-proline.

SUBJECTS AND METHODS

Subjects

Forty-two hypertensive volunteers with mean systolic blood pressure ≥ 140 mm Hg, mean diastolic blood pressure ≥ 90 mm Hg, or both taken during 2 ambulatory visits with an interval of 5–14 d were included in the study. The demographic data of the subjects are presented in **Table 1**.

Design

The study was conducted according to the Helsinki declaration and good clinical practice. The subjects were randomly allocated to 2 groups to receive a daily dose of 150 mL of either the test product or a control drink that was similar but did not contain the 2 peptides (Ile-Pro-Pro and Val-Pro-Pro). The subjects received the products for 21 wk after a 2-wk run-in period. Separate randomization lists were used for those who were or were not taking antihypertensive medication. Blood pressure and heart rate were measured from the left arm with the use of the same fully automatic blood pressure recorder with preset inflation (Omron M4; Omron Matsusaka Co Ltd, Matsusaka, Japan), with the subject sitting after resting for 10 min. At the second visit, the subjects were taught how to measure their own blood pressure. They then measured their blood pressure at home on the same weekday, in the morning, 1 h after waking. The subjects were advised to avoid eating, smoking, exercising, and taking antihypertensive medication before measuring their blood pressure.

During the 21-wk study period, either the test or the control product was taken as a 150-mL dose in the morning on the measurement days after the subjects had measured their blood pressure. The researchers and the subjects were blinded as to which group the subjects were in. The subjects were questioned by means of a pretested postal questionnaire about their daily use of the study products; they were also asked whether they had noticed any symptoms.

Three of the subjects withdrew from the study during the first week and were excluded from the statistical calculations. Thirty-nine subjects (19 male, 20 female) aged between 30.2 and 61.7 y were included in the statistical analyses (Table 1). Three subjects withdrew from the study after 14–20 wk for the following reasons: their blood pressure medication dose had been lowered, they were feeling ill, or they did not have enough time for the study. Their blood pressure values are included in the statistical analyses. Two subjects who withdrew were from the control group and 4 were from the test product group.

Test and control products

The test milk Evolus was produced by Valio Ltd (Helsinki) from heat-treated, low-lactose, skim milk inoculated with a single-strain of *L. helveticus* (LBK 16 H strain) under aseptic conditions. The milk was fermented for 18–20 h in optimal growth conditions to reach a high proteolytic activity at 37 °C until a final pH of 4.0–4.2. The milk solid content was increased to standardize the content of the Val-Pro-Pro (2.0 mg/100 g product) and Ile-Pro-Pro (1.5 mg/100 g product) peptides. The peptide contents of the fermented milk products and feed were determined by the modified method of Masuda et al (4), in which the peptide fraction was collected by gel filtration chromatography (Superdex Peptide HR 10/30; Amersham Pharmacia Biotech, Bucks, United Kingdom) and analyzed by reversed-phase HPLC at 214 nm (Novapak C18; Waters Alliance HPLC, Milford, MA). A blueberry concentrate was mixed with the fermented milk, and the mixture was poured into 1-L blank cartons. The nutritional composition of the product is shown in **Table 2**.

The control milk was produced from heat-treated, low-lactose, skim milk fermented by a normal fermentation process with a mesofilic *Lactococcus sp.* mixed culture at optimal temperature (30 °C) under optimal growth conditions until a final pH of 4.0–4.5. Blueberry concentrate, sugar, and flavorings were added to make the sensory attributes of the control product as similar to those of the test product as possible. The control milk was poured into 1-L blank cartons identical to the test product package.

Statistical methods

Baseline systolic and diastolic blood pressure were defined as the mean of the values measured in the first 3 wk (weeks −2, −1, and 0), of the 2 first ambulatory measurements, and of 1 home measurement. In addition to the weekly blood pressure values, the values were combined (mean of weeks 1–4, 5–8, 9–12, 13–16, and 17–21). The absolute changes in weekly and monthly blood pressure from baseline were calculated. Area under the curve (AUC) statistics were estimated by using the trapezoidal rule to summarize the weekly within-subject changes in blood pressure from baseline. The changes from baseline and the AUC values were compared between the groups with use of the *t* test for independent samples. Treatment differences are given with 95% CIs. Analysis of variance for repeated measurements was used *1*) to compare the groups with respect to the changes in blood pressures after 1, 2, 3, 4, and 5 mo of treatment; *2*) to test the period effect;

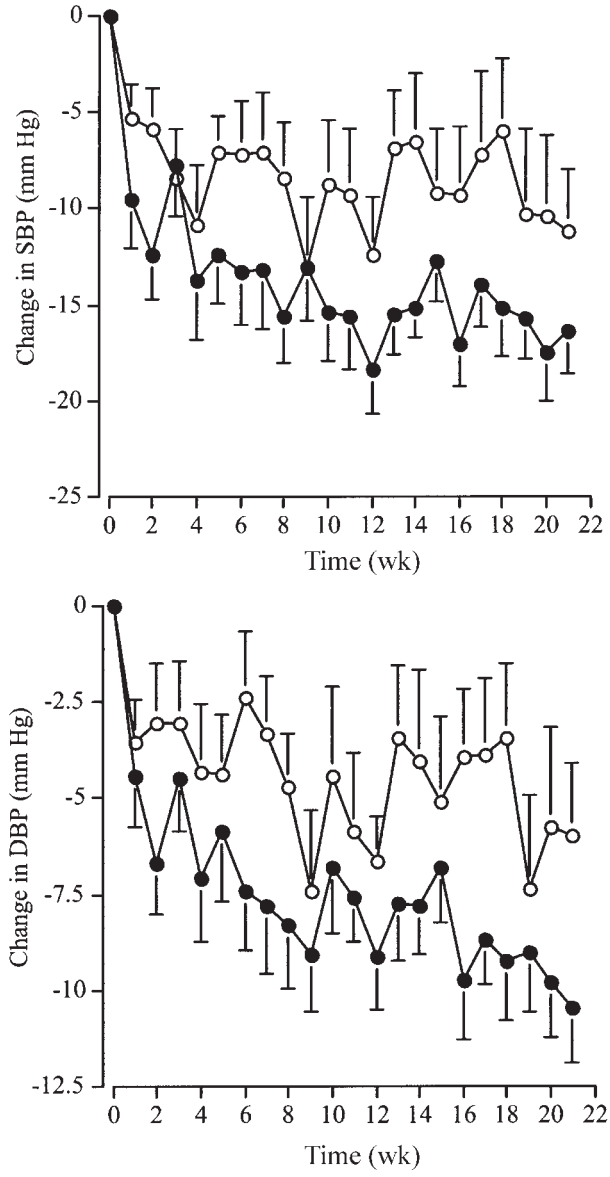

FIGURE 1. Mean (±SEM) change in systolic blood pressure (SBP) and diastolic blood pressure (DBP) from baseline during the 21 wk of treatment in the test product (●; n = 19) and control (○; n = 17) groups.

and *3*) to test the interaction between period and treatment. The possible effects of baseline demographic characteristics (sex, weight, age, and use of antihypertensive medication) of the subjects were also analyzed. Changes in blood pressure from baseline were compared between the groups by adjusted means based on an analysis of covariance with baseline characteristics as the covariates. SPSS (version 10.1; SPSS Inc, Chicago) was used for the statistical analyses.

RESULTS

The changes in weekly systolic and diastolic blood pressure values are presented in **Figure 1**. The baseline values for systolic blood pressure ($\bar{x}$ ± SEM) in the test and control groups were 152 ± 2.7 and 149 ± 2.7 mm Hg, respectively; those for diastolic blood pressure were 96 ± 1.1 and 95 ± 1.4 mm Hg, respectively.

Both systolic and diastolic blood pressure decreased more in the test product group than in the control group. There was a mean difference of 6.7 ± 3.0 mm Hg in systolic blood pressure and a mean difference of 3.6 ± 1.9 mm Hg in diastolic blood pressure between the groups (**Table 3**). According to the analysis of variance for repeated measurements, the treatment effect (test product compared with control product) was significant for systolic blood pressure (P = 0.030) and tended to be significant for diastolic blood pressure (P = 0.059). The interaction effects and the period effects were nonsignificant.

In **Table 4**, the weekly changes in blood pressure are combined with the monthly results. When all of the observation points were included in the analysis, the mean decrease in both systolic (difference between the groups from –3.4 to –6.3 mm Hg) and diastolic (difference between the groups from – 2.0 to –3.7 mm Hg) blood pressure was greater, but not significantly so, in the test product group than in the control group.

The AUC takes into account both the size and temporal persistence of the reduction in blood pressure. The AUC (mm Hg · mo) for the decrease in systolic blood pressure during weeks 1–21 was ($\bar{x}$ ± SEM) −308 ± 34 for the test product group compared with −192 ± 55 for the control product group. The corresponding treatment difference was −116 (95% CI: −243, 11; P = 0.071). Similarly, the AUC for the decrease in diastolic blood pressure was greater in the test product group than in the control group: −169 ± 22 compared with −108 ± 31. The corresponding treatment difference was −61 (95% CI: −137, 14; P = 0.107). If ambulatory measurements were not included in the baseline values, the corresponding treatment differences were −4.4 (95% CI: −9.6, 0.7; P = 0.091) for systolic blood pressure and −2.7 (95% CI: −6.3, 0.9; P = 0.13) for diastolic blood pressure.

The possible effects of baseline demographic characteristics (sex, weight, age, and use of antihypertensive medication) on the mean change in blood pressure were analyzed by using several methods, such as stepwise regression analyses and by forcing these variables into the regression models. None of these factors significantly influenced the blood pressure responses.

After 5 mo of treatment, weight loss was not significantly related to the reduction in systolic blood pressure (P = 0.679) or diastolic blood pressure (P = 0.574). The weight loss differences between the groups were nonsignificant. After 23 wk of treatment, the mean (±SD) reduction was 0.8 ± 2.0 kg in the test product group and 0.7 ± 1.8 kg in the control group.

In the control group, the mean changes in systolic and diastolic blood pressure were −8.5 and −3.8 mm Hg, respectively, among untreated subjects and −9.2 and −6.2 mm Hg, respectively, among subjects taking anithypertensive medication. In the test product group, the mean changes were −14.4 and −7.7 mm Hg among untreated subjects and −12.8 and −6.8 mm Hg among treated subjects. The interaction between blood pressure medication and study treatment was nonsignificant (P = 0.717 for systolic blood pressure and P = 0.390 for diastolic blood pressure).

No adverse effects were experienced by the subjects, except that one subject reported abdominal bloating and one reported flatulence; both of these subjects belonged to the control group. None of the withdrawals were considered to be due to the study products.

TABLE 3
Changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) during the intervention period among subjects in the test product (n = 19) and control (n = 17) groups who had blood pressure measurements for each month (per protocol analysis)[1]

	SBP		DBP	
	Test product group	Control group	Test product group	Control group
	mm Hg		*mm Hg*	
Month 2	−15.5 ± 2.1	−7.7 ± 1.9	−7.9 ± 1.4	−4.2 ± 1.3
Month 3	−17.2 ± 1.8	−10.9 ± 2.8	−9.1 ± 1.2	−6.1 ± 1.6
Month 4	−15.7 ± 1.5	−8.8 ± 3.0	−9.0 ± 1.0	−4.9 ± 1.9
Month 5	−15.4 ± 1.9	−9.4 ± 3.2	−9.3 ± 1.1	−5.5 ± 1.8
Test product compared with control				
$\bar{x}$	−6.7 ± 3.0		−3.6 ± 1.9	
95% CI	−12.8, −0.7		−7.4, 0.1	
P for treatment effect	0.030		0.059	

[1] $\bar{x}$ ± SEM. There was no interaction effect or period effect.

DISCUSSION

In a placebo-controlled study of mildly hypertensive subjects, Seppo et al (10) found that *L. helveticus* LBK-16H fermented milk reduced systolic and diastolic blood pressure more in the test group than in the control group (P = 0.05 for systolic and P < 0.05 diastolic blood pressure). *L. helveticus* LBK-16H fermented milk was also shown to significantly attenuate the development of hypertension in spontaneously hypertensive rats in 2 experimental studies (9, 11). We decided to use blood pressure measurements taken by the subjects at home to avoid the "white coat" effect, which is generally known to influence blood pressure measurements taken in a doctor's office. This effect was also seen in the present study: home systolic blood pressure measurements were ≈10 mm Hg lower those taken during the pretrial visits. The high reproducibility and low placebo effect on the home blood pressure measurements in hypertensive subjects were described earlier (12).

The subjects included represented normal consumers of different ages, sexes, and lifestyles. Age, sex, and antihypertensive medication had no significant interaction effects. Subjects were advised not to change their exercise or eating habits during the study, as confirmed by their unchanged body weights.

The intervention time (5 mo) was estimated to be long enough to demonstrate the persistence of the effects shown previously in shorter studies (8, 10). Compliance was good and the product was well tolerated. No adverse effects or symptoms were reported on the questionnaires sent to the volunteers twice during the study. The relatively small number of subjects and the fairly large variation in blood pressure values seen in this and other trials may result in clinically significant mean reductions in blood pressure not reaching statistical significance.

Of the many *Lactobacillus* species, *L. helveticus* strains have a relatively high proteolytic activity. The LBK-16H strain was chosen because of its high proteolytic activity and its capacity to produce bioactive peptides, including Val-Pro-Pro and Ile-Pro-Pro, which have been shown to possess ACE activity (3). It has been suggested that Ile-Pro-Pro and Val-Pro-Pro are stable and are absorbed because of their small size and carboxyl-terminal proline-proline sequence, which is resistant to peptidase (13).

In vitro results showed ACE activity in the test milk, which obviously explains, at least in part, the antihypertensive effects of the product. In ACE inhibition, the 50% inhibitory concentration (IC_{50} value) of Ile-Pro-Pro and Val-Pro-Pro peptides is much

TABLE 4
Changes in systolic blood pressure (SBP) and diastolic blood pressure (DBP) from baseline and the differences between the groups for all subjects (intention-to-treat analysis)

	Test product group	Control group	Test product compared with control	P
SBP (mm Hg)				
Month 1	−11.1 ± 2.2 [22][1]	−7.7 ± 2.0 [17]	−3.4 (−9.6, 2.7)[2]	0.266
Month 2	−13.5 ± 2.1 [22]	−7.7 ± 1.9 [17]	−5.7 (−11.7, 0.2)	0.058
Month 3	−15.6 ± 2.1 [22]	−10.9 ± 2.8 [17]	−4.8 (−11.8, 2.2)	0.174
Month 4	−15.1 ± 1.6 [21]	−8.8 ± 3.0 [17]	−6.3 (−13.2, 0.7)	0.077
Month 5	−15.4 ± 1.9 [19]	−9.4 ± 3.2 [17]	−5.9 (−13.5, 1.6)	0.119
$\bar{x}$ change	−13.8 ± 1.8 [22]	−8.8 ± 2.4 [17]	−5.0 (−10.9, 1.0)	0.098
DBP (mm Hg)				
Month 1	−5.5 ± 1.2 [22]	−3.5 ± 1.3 [17]	−2.0 (−5.5, 1.5)	0.264
Month 2	−6.9 ± 1.5 [22]	−4.2 ± 1.3 [17]	−2.7 (−6.9, 1.4)	0.195
Month 3	−8.2 ± 1.2 [22]	−6.1 ± 1.6 [17]	−2.1 (−6.0, 1.8)	0.285
Month 4	−7.8 ± 1.3 [21]	−4.9 ± 1.9 [17]	−2.9 (−7.4, 1.7)	0.208
Month 5	−9.3 ± 1.1 [19]	−5.5 ± 1.8 [17]	−3.7 (−7.9, 0.5)	0.079
$\bar{x}$ change	−7.3 ± 1.1 [22]	−4.8 ± 1.4 [17]	−2.5 (−6.6, 1.2)	0.169

[1] $\bar{x}$ ± SEM; n in brackets.
[2] $\bar{x}$ (95% CI).

higher than that of ACE-inhibitory drugs but lower than that of most other milk peptides that possess ACE-inhibiting effects (14). It has been shown that after both a single dose of (14) and a longer intervention with (15) a product containing Ile-Pro-Pro and Val-Pro-Pro, the ACE activity of the abdominal aorta is reduced compared with that in control rats. In our own experiments, we found that plasma renin activity increases in rats treated with the *L. helveticus* LBK-16H fermented milk product (11).

Mechanisms other than ACE inhibition by which the *L. helveticus* fermented milk product could lower blood pressure cannot be excluded. One contributing factor of the antihypertensive effect of the test milk might be the higher calcium content of the test product than of the control product. In the test product, the peptide content was produced by a natural lactobacillus fermentation process, without any addition of purified peptides. It remains to be investigated whether larger doses of the peptides could lower blood pressure still further. The natural fermentation process results in the inclusion of live starter bacteria in the test product, and it is possible that this has some additional effect on blood pressure regulation. In a rat model, it was shown that the same fermented milk product as used in the present study attenuated the development of hypertension in spontaneously hypertensive rats, more than did an equal amount of purified peptides (9).

The results show that during the intervention period there was on average a 6.7 ± 3.0-mm Hg difference between the test group and the control group in systolic blood pressure and on average a 3.6 ± 1.9-mm Hg difference in diastolic blood pressure (Table 3). These results confirm the preliminary findings in short-term studies (8, 10; J Tuomilehto et al, unpublished observations, 2002) and in animal models (9, 11) and show that the effect persists in a normal population for ≥21 wk.

In the present study, the average decreases in both systolic and diastolic blood pressure can be considered significant from a public health point of view. The decreases were even greater than those seen in the Heart Outcomes Prevention Evaluation Study, for example, in which a low-dose ACE inhibitor was used (16) or in nonpharmacologic intervention studies (17). In conclusion, the results of the present study showed that *L. helveticus* LBK-16H fermented milk, in normal daily use, has a blood pressure–lowering effect in hypertensive subjects and is thus potentially useful in the dietary treatment of hypertension.

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