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Lactobacillus helveticus Fermented Milk Lowers Blood Pressure in Hypertensive Subjects in 24-h Ambulatory Blood Pressure Measurement

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Background: The present study was carried out to evaluate the blood pressure (BP)-lowering effect and the safety aspects of *Lactobacillus helveticus* LBK-16H fermented milk with high tripeptide doses on hypertensive subjects using 24-h ambulatory measurements (ABPM).

Methods: In a randomized, double blinded placebo-controlled parallel group study, 94 hypertensive patients not receiving any drug treatment were given 150 mL twice daily of either *L. helveticus* LBK-16H fermented milk with a high concentration of tripeptides (Ile-Pro-Pro 7.5 mg/100 g and Val-Pro-Pro 10 mg/100 g) or a control product, for 10 weeks after a 4-week run-in period. Twenty-four-hour ABPM were taken at the beginning and at the end of the intervention period. The average baseline systolic and diastolic BP values were 132.6 ± 9.9/83.0 ± 8.0 mm Hg in the *L. helveticus* group and 130.3 ± 9.6 /80.2 ± 7.0 mm Hg in the control group.

Results: There was a mean difference of −4.1 ± 0.9 mm Hg in systolic ($P = .001$) and a −1.8 ± 0.7 mm Hg in diastolic BP ($P = .048$) between the *L. helveticus* group and the control group. There was no difference in the sum of the adverse events ($P = .820$).

Conclusions: *Lactobacillus helveticus* LBK-16H fermented milk containing bioactive peptides, in daily use, does have a BP-lowering effect in hypertensive subjects and is thus a potential for the dietary treatment of hypertension. Am J Hypertens 2005;18:1600–1605

Key Words: Biologically active tripeptides, *Lactobacillus helveticus*, fermented milk, ambulatory blood pressure measurement, hypertension.

The milk protein-derived biologically active peptides isoleucyl-prolyl-proline (Ile-Pro-Pro) and valyl-prolyl-proline (Val-Pro-Pro) prevent the development of hypertension in spontaneously hypertensive rats (SHR)[1–3] and lower blood pressure (BP) in mildly hypertensive subjects.[4,5] It has been suggested that the mechanism of the antihypertensive effect of these tripeptides may at least, in part, be the inhibition of the angiotensin-converting enzyme (ACE).[2]

The Ile-Pro-Pro and Val-Pro-Pro tripeptides have been shown to lower ACE activity in the aorta in SHR after a single oral administration and after a long-term intake[6,7] and increase plasma renin activity (PRA) in SHR.[2]

In previous studies, a daily intake of 2.25 mg of Ile-Pro-Pro and 2.55 to 3.75 mg of Val-Pro-Pro has reduced systolic BP by 7 to 10 mm Hg and diastolic BP by 4 to 7 mm Hg more than the control product in humans.[4,5] These tripeptide doses reduced BP without adverse events, but the effects of higher doses on BP have not been evaluated. Twenty-four-hour ambulatory BP measurement (ABPM) has not been used in any of the previous studies, although it is regarded as being the most reliable method of BP measurement, without white coat hypertension effect.[8]

The aim of the present study was to evaluate the BP-lowering effect and possible adverse events of *Lactobacillus helveticus* LBK-16H fermented milk with a high tripeptide concentration on hypertensive subjects by using 24-h ABPM.

Methods

Subjects

Ninety-four hypertensive subjects participated in this double blind randomized placebo-controlled study. Subjects

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The test milks were provided by Valio Ltd.

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Table 1. Demographic characteristics of the study subjects

Characteristics	Group	
	***Lactobacillus helveticus* (*n* = 53)**	**Control (*n* = 55)**
Age (y)	51 (12)	55 (11)
Weight (kg)	82.5 (17.8)	80.7 (15.7)
Height (cm)	169 (10)	169 (9)
BMI (kg/m^2)	28.6 (5.5)	28.3 (4.1)
Number of women, %	34, %	38 (69)

Mean (SD).

with systolic BP in office BP measurement of between 140 and 180 mm Hg and diastolic BP of between 90 and 110 mm Hg were included. Exclusion criteria were BP-lowering medication, unstable coronary artery disease, diabetes mellitus, malignant diseases, alcohol abuse, milk allergy, and pregnancy. Demographic characteristics did not differ between the groups ($P > .05$) and the demographic data of the subjects are presented in Table 1.

Design

The subjects were randomly allocated to two treatments after a 4-week run-in period. The *L. helveticus* group received a dose of 150 mL twice daily, of the *L. helveticus* product containing bioactive tripeptides throughout the 10-week intervention period. The control group received the same amount of the control product similar to the test drink without the two tripeptides, and less calcium, potassium, magnesium, and sodium than the *L. helveticus* product (Table 2). After the intervention period there was a 4-week follow-up period. During the run-in and follow-up periods the subjects received 150 mL twice daily of fermented milk product different from the *L. helveticus* product or the control product.

Subjects were asked to fill in a form about their daily use of the test products. The subjects were also asked, at every visit, whether they had marked any adverse events.

BP Measurement

At the beginning and end of the intervention period BP was measured with an automatic 24-h BP recorder (SpaceLab ABP 90207, Redmont, CA) four times an hour during the daytime and twice an hour during the night. The measurement was accepted if at least 80% of the readings were successful, otherwise the measurement was repeated. Office BP was measured nine times during the study period. The same physician used a fully automatic BP recorder (Omron M4, Omron Matsusaka Co., Ltd., Kyoto, Japan) for the BP measurements from the left arm after a 7-min rest in a sitting position in the morning. If the difference between these first two measurements was more than 5 mm Hg in systolic BP, further measurements were done. The subjects were asked to avoid exercise that day and not to eat, drink coffee, or smoke for 1 h before the BP measurement. The mean of the last two measurements from the run-in period defined the baseline level and the mean of the last two BP level during the intervention period was used as the response variable in the statistical analyses.

Blood Sampling

Blood samples were taken at the beginning and at the end of the intervention phase after an overnight (12 h) fast. The following variables were analyzed: serum lipid pattern (total, LDL-cholesterol, HDL-cholesterol, and triglycerides), ACE activity, C-reactive protein, and safety laboratory analyses (blood cell count, serum creatinine, urate, and gamma glutamyl transferase). Total cholesterol, HDL cholesterol, and triglycerides were measured enzymatically, LDL was calculated by the Friedewald equation.[9] The ACE activity was determined spectophotometrically

Table 2. Nutritional composition of the *Lactobacillus helveticus* product and the control product

	***Lactobacillus helveticus* Product**	**Control Product**
Energy (kJ/100 g)	320	160
Protein (g/100 g)	3.5	3.0
Fat (g/100 g)	0.07	0.4
Carbohydrate (g/100 g)	15	5.7
Calcium (mg/100 g)	230	100
Potassium (mg/100 g)	510	150
Magnesium (mg/100 g)	31	11
Sodium (mg/100 g)	41	36
Ile-Pro-Pro (mg/100 g)	7.5	—
Val-Pro-Pro (mg/100 g)	10	—