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

Tiina Jauhiainen, Heikki Vapaatalo, Tuija Poussa, Sinikka Kyrönpalo, Martin Rasmussen, and Riitta Korpela

**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 © 2005 American Journal of Hypertension, Ltd.

**Key Words:** Biologically active tripeptides, *Lactobacillus helveticus*, fermented milk, ambulatory blood pressure measurement, hypertension.
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 \textit{L. helveticus} group received a dose of 150 mL twice daily, of the \textit{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 \textit{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 \textit{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 spectrophotometrically.

### Table 1. Demographic characteristics of the study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>\textit{Lactobacillus helveticus} ((n = 53))</th>
<th>Control ((n = 55))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>51 (12)</td>
<td>55 (11)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.5 (17.8)</td>
<td>80.7 (15.7)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>169 (10)</td>
<td>169 (9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.6 (5.5)</td>
<td>28.3 (4.1)</td>
</tr>
<tr>
<td>Number of women, %</td>
<td>34, %</td>
<td>38 (69)</td>
</tr>
</tbody>
</table>

Mean (SD).

### Table 2. Nutritional composition of the \textit{Lactobacillus helveticus} product and the control product

<table>
<thead>
<tr>
<th></th>
<th>\textit{Lactobacillus helveticus} Product</th>
<th>Control Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ/100 g)</td>
<td>320</td>
<td>160</td>
</tr>
<tr>
<td>Protein (g/100 g)</td>
<td>3.5</td>
<td>3.0</td>
</tr>
<tr>
<td>Fat (g/100 g)</td>
<td>0.07</td>
<td>0.4</td>
</tr>
<tr>
<td>Carbohydrate (g/100 g)</td>
<td>15</td>
<td>5.7</td>
</tr>
<tr>
<td>Calcium (mg/100 g)</td>
<td>230</td>
<td>100</td>
</tr>
<tr>
<td>Potassium (mg/100 g)</td>
<td>510</td>
<td>150</td>
</tr>
<tr>
<td>Magnesium (mg/100 g)</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Sodium (mg/100 g)</td>
<td>41</td>
<td>36</td>
</tr>
<tr>
<td>Ile-Pro-Pro (mg/100 g)</td>
<td>7.5</td>
<td>—</td>
</tr>
<tr>
<td>Val-Pro-Pro (mg/100 g)</td>
<td>10</td>
<td>—</td>
</tr>
</tbody>
</table>
using the artificial substrate method and C-reactive protein was determined using a turbidimetric immunoassay (Wako Chemicals, Neuss, Germany) with a detection limit of 0.1 mg/L. At every visit the subjects were weighed, and body mass index (BMI) was calculated at the beginning of the study.

**Test and Control Products**

The test product was *Lactobacillus helveticus* LBK-16H fermented milk with a high concentration of peptides (Ile-Pro-Pro 7.5 mg/100 g and Val-Pro-Pro 10 mg/100 g) (Valio Ltd., Helsinki, Finland). The peptide content of the fermented milk products was determined by the method of Masuda et al.,6 collecting the peptide fraction by gel filtration chromatography (Superdex Peptide HR 10/30, Amersham Pharmacia Biotech, Bucks, UK) and analyzing it by reversed phase HPLC at 214 nm (Novapak C18, Waters Alliance HPLC, Milford, MA).

The control product was fermented by a normal mesophilic *Lactococcus* sp. mixed culture. The test products were poured into 1-L blank cartons and marked with the study codes.

**Ethics**

The Ethics Committee of the Department of Medicine, Tampere University Central Hospital, approved the study protocol. All the subjects received both written and oral information regarding the trial and gave their written consent.

**Inclusion and Compliance**

Thirty-one of 139 subjects were excluded during the run-in period because they did not fulfill the inclusion criteria. Five subjects from the *L. helveticus* group and six subjects from the control group withdrew from the study during the run-in period. Fourteen subjects did not want to participate in the 24-h ABPM. Ninety-four subjects (34 men and 60 women) were finally included in the statistical analysis.

**Sample Size**

The intended sample size was based on the primary hypothesis. A sample of about 100 patients (50 in each group) was required to detect differences in change between the intervention and control groups of 5 mm Hg systolic BP ($P = .05$, power = 90%).

**Statistical Methods**

The within-subject changes in BP from baseline to the intervention were calculated. The changes in BP were analyzed by using a *t* test for independent samples and the results are given as treatment differences with 95% confidence intervals (CI). Analysis of covariance (ANCOVA) was also used to compare the groups as BP variables from ABPM during the intervention. In these analyses baseline

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**Table 3. Blood pressure changes from baseline (at end of the 4-week run-in period) to the end of the 10-week treatment period**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline</th>
<th>Change by Weeks 10</th>
<th>Baseline</th>
<th>Change by Weeks 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-h ambulatory blood pressure:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>47</td>
<td>133(10)</td>
<td>156(7)</td>
<td>156(12)</td>
</tr>
<tr>
<td>Systolic (mm Hg)</td>
<td>130 (7)</td>
<td>83 (10)</td>
<td>88 (8)</td>
<td>88 (10)</td>
</tr>
<tr>
<td>Diastolic (mm Hg)</td>
<td>94 (6)</td>
<td>63 (6)</td>
<td>65 (6)</td>
<td>65 (6)</td>
</tr>
<tr>
<td>Office blood pressure:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of subjects</td>
<td>53</td>
<td>55 (9)</td>
<td>55 (9)</td>
<td>55 (9)</td>
</tr>
<tr>
<td>Systolic (mm Hg)</td>
<td>143 (7)</td>
<td>138 (9)</td>
<td>138 (9)</td>
<td>138 (9)</td>
</tr>
<tr>
<td>Diastolic (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>94 (6)</td>
<td>93 (6)</td>
<td>93 (6)</td>
<td>93 (6)</td>
</tr>
</tbody>
</table>

* Analysis of covariance - Baseline value as covariable.
values were used as covariates. The corresponding baseline was included as a continuous covariate. The results are given as mean ± SD. The data were analyzed with SPSS (version 11.5 SPSS Inc., Chicago, USA).

Results

BP

The baseline systolic and diastolic BP values in repeated office BP measurements were, 148.4 ± 8.1 /93.5 ± 6.2 mm Hg and 150.5 ± 8.3 /93.2 ± 6.1 mm Hg, in the L. helveticus group and the control group, respectively. However in the 24-h ABPM the average baseline systolic and diastolic BP values were clearly lower (P < .001) being 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 (P = .27 systolic BP, P = .076 diastolic BP) (Table 3).

In the office BP measurements (n = 108), the treatment effect on systolic BP was −2.0 (95% CI: −6.0 to 2.1), P = .34. The treatment effect on diastolic BP was +1.0 (95% CI: −1.3 to 3.2), P = .40 (Table 3).

In the 24-h ABPM (n = 94), both systolic and diastolic BP decreased more in the L. helveticus group compared to the control group during the 10-week intervention period (Fig. 1). The mean decrease in systolic BP was −4.7 mm Hg in the L. helveticus group and −0.6 mm Hg in the control group. The mean decrease in diastolic BP was −2.7 mm Hg in the L. helveticus group and −0.9 mm Hg in the control group (Table 3). The treatment effect on systolic BP was −4.1 (95% CI: −6.6 to −1.8, P < .001). The treatment effect on diastolic BP was −1.8 (95% CI: −3.7 to 0.0, P = .048) (Table 3).

For the subjects that met 24-h ambulatory criteria for hypertension (n = 39) (systolic BP >135 mm Hg and diastolic BP ≥85 mm Hg), ABPM the treatment effect on systolic BP was −3.8 (95% CI: −7.1 to −0.4), P = .028 and on diastolic BP −1.7 (95% CI: −4.8 to 1.3), P = .26.
The treatment effect for normotensive subjects in ABPM (white coat hypertension) \( n = 55 \) on systolic BP was \(-3.5\) (95% CI: \(-6.5\) to \(-0.6\)), \( P = .02 \), and on diastolic BP, \(-1.5\) (95% CI: \(-3.7\) to \(-0.8\)), \( P = .19 \).

**Other Variables**

Body weight did not change during the 10-week period (0.49 kg in the \( L. \) helveticus group \( v 0.02 \) kg in the control group).

After 10 weeks of treatment, there was no significant difference in the safety laboratory analyses or serum lipid values and ACE activity (Table 4). At baseline, C-reactive protein levels were 2.6 ± 3.6 mg/L in the \( L. \) helveticus group and 2.0 ± 2.3 mg/L in the control group. The mean change during the intervention period was \(-0.54\) mg/L (95% CI: \(-1.8\) to \(-0.7\), \( P = .37 \)) in the \( L. \) helveticus group \( n = 23 \) and \(+0.53\) mg/L (95% CI: \(-0.4\) to \(+1.5\), \( P = .25 \)) in the control group \( n = 21 \).

**Adverse Events**

Adverse events were registered from all the subjects \( n = 108 \) who participated in the intervention period. Thirteen of 53 subjects in the \( L. \) helveticus group and 12 of 55 in the control group reported adverse effects \( P = .820 \). In the \( L. \) helveticus group the adverse events were pain in the stomach, flatulence, abdominal bloating, flu, pain in the knee, inflammation of the urinary tract, gynecological inflammation, and dislike of the test product. In the control group, the adverse events were pain in the stomach, flatulence, abdominal bloating, diarrhea, flu, broken ankle, and inflammation of the urinary tract.

**Discussion**

In this randomized, placebo-controlled study \( L. \) helveticus fermented milk containing bioactive tripeptides, in daily use, lowered systolic and diastolic BP of hypertensive subjects more effectively than the control product without the peptides.

In this trial we also compared usability of two BP measurements, of ABPM and office BP. An important finding was that the office baseline systolic BP values were, on average, \( >15 \) mm Hg and diastolic BP values were \( >10 \) mm Hg higher than the ambulatory values. In the present study the main BP marker was the ABPM values. Analyses of the subjects that met 24-h ambulatory criteria for hypertension showed statistically significant reduction in systolic BP. Systolic BP decreased by \(-7.6\) mm Hg and diastolic BP by \(-4.1\) mm Hg in the \( L. \) helveticus group. This kind of BP reduction is considered epidemiologically significant from a public health point of view. The results of controlled trials of antihypertensive drugs have shown that the risk of stroke is reduced by 40% when the mean reduction in diastolic BP is 6 mm Hg.

The absorption of bioactive tripeptides in the \( L. \) helveticus product from the gastrointestinal tract has been shown.\(^6^,\)^11 Ile-Pro-Pro and Val-Pro-Pro have been found in the aorta of SHR after oral administration of fermented milk containing these tripeptides.\(^6^\)

It has been suggested that the antihypertensive mechanism of Ile-Pro-Pro and Val-Pro-Pro tripeptides is related ACE inhibition.\(^2^,\)^6,\(^7^\) In our study, the \( L. \) helveticus product contained somewhat more calcium, potassium, and magnesium than the control product. Therefore, we cannot exclude the possibility that a part of the BP-lowering effect is related to the minerals; however, it does not explain the whole difference between the groups. In recent meta-analysis of clinical trials calcium supplementation (1000 to 2000 mg/d) decreased systolic BP \(-1.44\) mm Hg and diastolic BP \(-0.84\) mm Hg.\(^5^\) In the meta-analysis of 33 controlled clinical studies the potassium supplementation (about 2.9 g/d) reduced systolic BP \(-3.11\) mm Hg and diastolic BP \(-1.97\) mm Hg.\(^5^\) Ile-Pro-Pro and Val-Pro-Pro tripeptides,\(^4^\) like the normal milk components, caseinophosphopeptides,\(^5^\) increase the absorption of calcium.

There were no differences in ACE activity between the groups as the tripeptides are much weaker ACE inhibitors than commonly used antihypertensive drugs and the antihypertensive effect could be partly mediated through other biological mechanisms. It is also possible that in plasma ACE activity the small differences could not be seen due to large individual variation.

Inflammation may be an important mechanism or parallel phenomenon for the development of hypertension and already slightly elevated C-reactive protein levels \((>3.0\) mg/L\) are a risk factor for coronary heart disease.\(^1^6^\) In the present study, C-reactive protein levels were in the normal range. In the \( L. \) helveticus group it decreased slightly, and in the control group, it increased. Although these results are statistically not significant, the finding on C-reactive protein is interesting and needs further evaluation.

The subjects reported similar adverse events in both groups and there were no changes in the laboratory values, which is an important observation from the safety point of view. According to this study the \( L. \) helveticus product and even a high amount of tripeptides can be considered as a safe alternative for the dietary treatment of hypertension.

**Acknowledgments**

We wish to thank Leena Seppo, MSC for organizing work in the beginning of the study. Elina Lausvaara for preparing the study products, and Mimi Ponsonby, MA, for correcting the language.

**References**