Milk Peptides and Blood Pressure\textsuperscript{1,2}

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Abstract

Epidemiological studies suggest that milk consumption and dietary intake of dairy proteins are inversely related to the risk for hypertension. Also, some intervention studies have shown a blood pressure-lowering effect of milk products and dairy proteins. Milk peptides are formed from milk proteins by enzymatic breakdown by digestive enzymes or by the proteinases formed by lactobacilli during the fermentation of milk. Several milk peptides have been shown to have antihypertensive effects in animal and in clinical studies. The most studied mechanism underlying the antihypertensive effects of milk peptides is inhibition of angiotensin-converting enzyme. Milk peptides may also have other additional mechanisms to lower blood pressure such as opioid-like activities and mineral-binding and antithrombotic properties. The future challenge is to identify the antihypertensive components in milk and their mechanisms of action and thus to find more possibilities for using these constituents and products as a dietary treatment of hypertension. J. Nutr. 137: 825S–829S, 2007.

Milk and blood pressure

\textbf{Epidemiological studies.} Epidemiological studies suggest that consumption of milk and milk product is inversely related to the risk for hypertension. The association between milk consumption and blood pressure was reported in the analysis of first National Health and Nutrition Examination Survey (NHANES I) (1). In this cross-sectional study with over 10,000 persons, low consumption of milk products was associated with a high incidence of hypertension. The prevalence of hypertension has also been reported to be twice as high in middle-aged men in Puerto Rico who drank no milk as in middle-aged men in Puerto Rico who drank at least 1 L milk/d (2). Also, the association between calcium and dairy products and metabolic syndrome was seen in a study with 10,006 women participating: the Women’s Health Study (3). The consumption of milk has been shown to be lower in hypertensive than normotensive persons in American and Italian population studies as well (4,5). Milk product consumption has also been shown to relate to the risk of stroke. During a 22-y follow-up of 3,000 men, the consumption of milk was related to a lower rate of thromboembolic stroke (6). In this study and in a prospective Nurses’ Health Study with 85,000 American women (7), the inverse association between calcium intake and stroke was stronger for dairy calcium than for nondairy calcium. It is therefore possible that some components of milk other than calcium, e.g., other electrolytes, proteins, or peptides, had been important in relation to the incidence of stroke. However, a beneficial relation between consumption of milk and incidences of hypertension and stroke has not been seen in all studies (8,9).

\textbf{Intervention studies.} Intervention studies have investigated the effects of milk and milk products on blood pressure. One of the most remarkable studies is the Dietary Approaches to Stop Hypertension (DASH) trial with almost 500 normotensive or mildly hypertensive subjects, which showed that a diet rich in fruits, vegetables, and low-fat dairy products (the so-called combination diet) was found to reduce blood pressure significantly. Mean systolic blood pressure and mean diastolic blood pressure decreased by 5.5 mm Hg and 3.0 mm Hg, respectively, in the total study cohort. Among the hypertensive subjects, the reductions were even greater. Mean systolic blood pressure decreased by 11.4 mm Hg, and mean diastolic blood pressure by 5.5 mm Hg. Blood pressure was lowered more on the combination diet than on the diet rich only in fruits and vegetables (10,11). In a follow-up study (DASH II), in which sodium intake was restricted to a maximum of 1.5 g/d, mean systolic blood pressure fell further in the normotensive subjects, by 7.1 mm Hg (12).

Although it has also been shown in some other intervention studies that consumption of milk products reduces blood pressure (13–16), the relation has not been demonstrated in all studies (17).
**Protein and blood pressure**

Cow’s milk contains many constituents including electrolytes, proteins, and peptides, which could affect blood pressure beneficially. Milk contains ~3.5% protein, which consists of caseins (80%) and whey proteins (20%). Caseins have been classified as α-, β-, and κ-caseins. Whey contains β-lactoglobulin, α-lactalbumin, and several minor proteins with different biological activities such as enzymes, mineral-binding properties, and immunoglobulins (18).

Some epidemiological studies suggest that protein intake is inversely related to the risk for hypertension (19–21). Two cross-sectional studies, the Honolulu Heart Program study and the Intersalt study, have shown inverse relations between protein intake and blood pressure (20,21). Association between protein intake and the risk of stroke was also seen in the Nurses’ Health Study (7). However, in some epidemiological studies, no inverse relation between high protein intake and blood pressure has been seen (22).

The effects of dietary protein on blood pressure have also been investigated in intervention studies (23,24). The results of some of these studies show that a high dietary protein intake was associated with a low incidence of hypertension. However, in some intervention studies no significant effects of level of protein intake on blood pressure were observed [for review, see Meisel (25)].

The antihypertensive mechanism of protein is still unknown. One possibility is the degradation of protein into peptides that have antihypertensive effects. In this article, we focus our discussion on the blood pressure-lowering effect of milk-derived peptides.

**Peptides and blood pressure**

**Peptide production and absorption.** Biologically active peptide fragments are formed when milk proteins are broken down by digestive enzymes or by the proteinases formed by lactobacilli during fermentation of milk (25,26). Lactic acid bacteria are suitable for milk fermentation because they have a proteolytic activity and capacity to produce peptides; e.g., a starter containing a *Lactobacillus helveticus* strain has been reported to produce bioactive peptides (27,28). Milk peptide activities include binding to opioid receptors, inhibition of angiotensin-converting enzyme (ACE), and modification of antithrombotic and immune responses (25,26). Phosphopeptides formed from casein may enhance the absorption of minerals, especially calcium, from the digestive tract into the circulation (25).

Milk peptides that are not degraded in proteolysis can theoretically be absorbed intact. It has been suggested that dipeptides and tripeptides (Ile-Pro-Pro and Val-Pro-Pro) are absorbed in the intestine (29,30). Val-Pro-Pro has been reported to transport across a Caco-2 cell monolayer via paracellular diffusion (30). In addition, the absorption of longer peptides has been studied. The ACE inhibitory peptide lactokinin, Ala-Leu-Pro-Met-His-Ile-Arg, has been found to be transported intact through Caco-2 monolayers (31).

**Animal studies.** Ile-Pro-Pro and Val-Pro-Pro have been shown to reduce blood pressure in spontaneously hypertensive rats (SHR) after a single oral administration (28). They also prevent the development of hypertension in SHR after long-term (12, 13, and 9 wk) oral feeding (32–34). At the end of the 12-wk treatment period, systolic blood pressure was 17 mm Hg lower in the group receiving *L. helveticus* LBK-16H fermented milk containing Ile-Pro-Pro and Val-Pro-Pro than in the control group receiving water (*P* < 0.001) and 12 mm Hg lower in the group receiving the tripeptides in water than in the control group (34). In a 13-wk study, *L. helveticus* LBK-16H fermented milk containing Ile-Pro-Pro and Val-Pro-Pro tripeptides attenuated the development of hypertension more effectively than water or the *L. helveticus* and *S. cerevisiae* fermented milk containing half as much the same peptides than *L. helveticus* LBK-16H fermented milk (33). In a 9-wk study *L. helveticus* fermented milk attenuated the development of hypertension more effectively than pure peptides or minerals and peptides combined (32). It has been shown that α-lactorphin (Tyr-Gly-Leu-Phe) lowers blood pressure dose-dependently in SHR. The blood pressure was measured with continuous radiotelemetric monitoring, and the maximal reductions in systolic and diastolic blood pressure were 23 ± 4 and 17 ± 4 mm Hg, respectively (35). Two *L. helveticus* strains, *L. helveticus* CHCC637 and *L. helveticus* CHCC641, in fermented milk have an ACE inhibition effect. This fermented milk reduced blood pressure more than the control group in SHR (36). Results of some animal studies are summarized in Table 1.

**Clinical studies.** *L. helveticus* fermented milk containing Ile-Pro-Pro and Val-Pro-Pro tripeptide has also been shown to decrease systolic and diastolic blood pressure in hypertensive subjects (37–41). In a placebo-controlled study on hypertensive subjects, *L. helveticus* and *S. cerevisiae* fermented milk reduced systolic and diastolic blood pressure during the 8-wk intervention more than placebo-fermented milk (37). In an 8-wk placebo-controlled study on 17 hypertensive subjects, systolic and diastolic blood pressures were lowered more in the group receiving *L. helveticus* fermented milk containing Ile-Pro-Pro and Val-Pro-Pro tripeptides than in the control group receiving normal fermented milk fermented with *Lactococcus sp*. mixed culture (40), and in the long-term clinical study (21 wk) as well, systolic and diastolic blood pressure were decreased more in the *L. helveticus* fermented milk group (*n* = 22) than in the control group receiving fermented milk (*n* = 17) (SBP 6.7 ± 3.0, *P* = 0.030, and DBP 3.6 ± 1.9, *P* = 0.059) (39). In a 10-wk study in 94 hypertensive subjects there was a difference of −4.1 ± 0.9 mm

<table>
<thead>
<tr>
<th>Animals</th>
<th>Duration</th>
<th>Product</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR and WKY</td>
<td>200 min</td>
<td>α-Lactorphin</td>
<td>Dose-dependently lowered blood pressure, <em>P</em> &lt; 0.05.</td>
<td>Numinen et al. (35)</td>
</tr>
<tr>
<td>SHR</td>
<td>13 wk</td>
<td>Fermented milk containing tripeptides</td>
<td>Reduced SBP, <em>P</em> &lt; 0.001</td>
<td>Sipola et al. (34)</td>
</tr>
<tr>
<td>SHR</td>
<td>8 h</td>
<td>Fermented milk containing inhibitors of ACE</td>
<td>Reduced blood pressure</td>
<td>Fuglsang et al. (36)</td>
</tr>
<tr>
<td>SHR</td>
<td>14 wk</td>
<td>Fermented milk containing tripeptides</td>
<td>Reduced SBP, <em>P</em> &lt; 0.001.</td>
<td>Sipola et al. (33)</td>
</tr>
<tr>
<td>SHR</td>
<td>9 wk</td>
<td>Fermented milk containing tripeptides</td>
<td>Reduced SBP</td>
<td>Jauhiainen et al. (32)</td>
</tr>
</tbody>
</table>

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6 Abbreviations used: ACE, angiotensin-converting enzyme; RAS, renin-angiotensin system; SHR, spontaneously hypertensive rats.
Hg in systolic ($P = 0.001$) and $-1.8 \pm 0.7$ mm Hg in diastolic blood pressure ($P = 0.048$) between the $L. helveticus$ group and the control group (38). The blood pressure–lowering effect of sour milk containing Ile-Pro-Pro and Val-Pro-Pro was also seen in the placebo-controlled 4-wk study in 46 borderline hypertensive men (42). Tables containing Ile-Pro-Pro and Val-Pro-Pro triptides have been shown to decrease blood pressure in mild or moderately hypertensive subjects (43,44). Milk that has been fermented using $Lb. casei$ and $Lc. lactis$ and that contains $\gamma$-aminobutyric acid (GABA) reduced blood pressure during a 12-wk treatment period. Systolic blood pressure lowered more in the fermented milk group than in the control group, but diastolic blood pressure of the fermented milk group did not differ from that in the control group (45). In another 8-wk-long study, systolic blood pressure was significantly lower in the group receiving yogurt fermented with 2 strains of $Streptococcus thermophilus$ and 2 strains of $Lactobacillus acidophilus$ and in the group receiving yogurt fermented with 1 strain of $Enterococcus faecium$ and 2 strains of $S. thermophilus$ compared with the group receiving yogurt fermented with 2 strains of $S. thermophilus$ and 1 strain of $Lactobacillus rhamnosus$ (46). Results of some clinical studies are summarized in Table 2.

### Mechanisms of the antihypertensive effects of milk peptides

#### Angiotensin-converting enzyme inhibition.

One mechanism by which milk-derived peptides can reduce blood pressure is inhibition of ACE (27,28,33,47–50). This is the mechanism that has been studied most in relation to the antihypertensive effects of milk peptides. ACE is an enzyme that plays a crucial role in the function of the renin-angiotensin system (RAS). The RAS is an important regulator of blood pressure and fluid and electrolyte balance (51). In the RAS, angiotensin I is converted to angiotensin II by ACE. Angiotensin II is a strong vasoconstrictor involved, e.g., in the regulation of circulation (55). Opioids also affect blood pressure (56). Opioid-like activity has been discovered in many peptide fragments from casein, and the first characterized opioid milk peptide agonist was derived from $\beta$-casein ($\beta$-casomorphin). The peptides with opioid-like activity derived from $\alpha$-casein are called $\alpha$-exorphins, and those derived from $\kappa$-casein are called casoxins. In addition, opioid peptides can be derived from the whey proteins $\alpha$-lactalbumin and $\beta$-lactoglobulin. $\alpha$-Lactorphin, derived from $\alpha$-lactalbumin, has been shown to lower blood pressure in SHR. Because the antihypertensive effect of $\alpha$-lactorphin was completely prevented by an opioid receptor antagonist naloxone, it has been proposed that the antihypertensive effect is mediated via opioid receptors (35).

Some peptides, such as caseinophosphopeptides, have also been shown to increase the solubility of calcium and enhance the absorption of calcium (57,58), and some milk peptides have antithrombotic effects by, e.g., inhibiting the aggregation of ADP-activated platelets (59). This might also have some role in the beneficial cardiovascular effects of milk-derived peptides.

#### Other possible mechanisms.

Several milk peptides have opioid-like activities. Typical opioid peptides originate from 3 precursor proteins: proopiomelanocortin (endorphins), preenkephalin (enkephalins), and prodynorphin (dynorphins). All have the same N-terminal amino acid sequence, Tyr-Gly-Gly-Phe (26). Opioids bind to opioid receptors and have morphine-like effects. The opioid system contains several different endogenous opioid peptides and receptors. Opioids are present in the central nervous system and in peripheral tissues, where they are involved, e.g., in the regulation of circulation (55). Opioids also affect blood pressure (56). Opioid-like activity has been discovered in many peptide fragments from casein, and the first characterized opioid milk peptide agonist was derived from $\beta$-casein ($\beta$-casomorphin). The peptides with opioid-like activity derived from $\alpha$-casein are called $\alpha$-exorphins, and those derived from $\kappa$-casein are called casoxins. In addition, opioid peptides can be derived from the whey proteins $\alpha$-lactalbumin and $\beta$-lactoglobulin. $\alpha$-Lactorphin, derived from $\alpha$-lactalbumin, has been shown to lower blood pressure in SHR. Because the antihypertensive effect of $\alpha$-lactorphin was completely prevented by an opioid receptor antagonist naloxone, it has been proposed that the antihypertensive effect is mediated via opioid receptors (35).

#### Literature Cited


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**Table 2** Examples of clinical trials on the effects of fermented milk on blood pressure

<table>
<thead>
<tr>
<th>Subjects, n</th>
<th>Duration, wk</th>
<th>Product</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>8</td>
<td>Fermented milk containing tripeptides</td>
<td>SBP and DBP ↓ vs. control</td>
<td>Hata et al. (37)</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>Fermented milk containing whey protein</td>
<td>SBP ↓ vs. control</td>
<td>Jolles et al. (59)</td>
</tr>
<tr>
<td>70</td>
<td>8</td>
<td>Fermented yogurt</td>
<td>SBP ↓ vs. control</td>
<td>Agerholm-Larsen et al. (46)</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>Tablets containing tripeptides</td>
<td>SBP and DBP ↓ vs. control</td>
<td>Kajimoto et al. (44)</td>
</tr>
<tr>
<td>80</td>
<td>4</td>
<td>Tablets containing tripeptides</td>
<td>SBP and DBP ↓ vs. control</td>
<td>Aihara et al. (43)</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>Fermented milk containing tripeptides</td>
<td>SBP and DBP ↓ vs. control</td>
<td>Seppo et al. (40)</td>
</tr>
<tr>
<td>39</td>
<td>21</td>
<td>Fermented milk containing tripeptides</td>
<td>SBP and DBP ↓ vs. control</td>
<td>Seppo et al. (39)</td>
</tr>
<tr>
<td>39</td>
<td>12</td>
<td>Fermented milk containing GABA</td>
<td>SBP ↓ vs. control</td>
<td>Inoue et al. (45)</td>
</tr>
<tr>
<td>94</td>
<td>10</td>
<td>Fermented milk containing tripeptides</td>
<td>SBP and DBP ↓ vs. control</td>
<td>Jauhiainen et al. (38)</td>
</tr>
</tbody>
</table>


