Review Article

Lactotripeptides and antihypertensive effects: a critical review

Esther Boelsma and Joris Kloek

1 TNO Quality of Life, Business Unit Biosciences, PO Box 360, 3700 AJ, Zeist, The Netherlands
2 DSM Food Specialties, Department of Nutrition and Health, PO Box 1, 2600 MA, Delft, The Netherlands

(Received 14 March 2008 – Revised 3 September 2008 – Accepted 15 September 2008 – First published online 5 December 2008)

Hypertension or high blood pressure is a significant health problem worldwide. Typically, lifestyle changes, including adopting a healthy diet, are recommended for people with an elevated blood pressure. Lactotripeptides are bioactive milk peptides with potential antihypertensive properties in man. These peptides, as part of a food product or as nutraceutical, may contribute to the prevention and treatment of hypertension.

This paper reviews the current evidence of the blood pressure control properties of lactotripeptides in man. Blood pressure-lowering effects of lactotripeptides are typically measured after 4–6 weeks of treatment. However, in some cases, a blood pressure response has been observed after 1–2 weeks. Maximum blood pressure reductions approximate 13 mmHg (systolic blood pressure) and 8 mmHg (diastolic blood pressure) after active treatment compared with placebo, and are likely reached after 8–12 weeks of treatment. Effective dosages of lactotripeptides range from 3.07 to 52.5 mg/d. Evidence indicates that lactotripeptides are only effective at elevated blood pressure; no further lowering of normal blood pressure has been observed. Concomitant intake of antihypertensive medication does not seem to influence the potency of lactotripeptides to lower blood pressure. Similarly, ethnicity has not been found to influence the extent of lactotripeptide-induced blood pressure lowering. Based on the currently available data, lactotripeptides appear to be safe and effective. Thus, they can be part of a healthy diet and lifestyle to prevent or reduce high blood pressure.

Blood pressure: Antihypertensive effects: Lactotripeptides: Milk peptides

CVD and its related complications affect a significant proportion of the world’s population. The risk of developing CVD is directly related to blood pressure (BP) level. Prolonged reductions of diastolic blood pressure (DBP) of 5, 7.5 and 10 mmHg were respectively associated with at least 34, 46 and 56 % less stroke and at least 21, 29 and 37 % less CHD. In a large meta-analysis by the Prospective Studies Collaboration, it was estimated that a 10 mmHg lower usual systolic blood pressure (SBP) or 5 mmHg lower usual DBP would, in the long term, be associated with about 40 % lower risk of stroke death and about 30 % lower risk of death from IHD or other vascular causes. Extending these observations to small reductions in DBP of about 2 mmHg would result in a 14 % reduction in the risk of stroke, and 6 % reduction in the risk of CHD. Also SBP lower by 2 mmHg is associated with lower IHD and CVD death rates by 4–5 %.

Adoption of a healthy lifestyle is important for the prevention of high BP and is an indispensable part of the management of hypertension. The application of specific foods or food components in the prevention and/or treatment of disease are of particular relevance in the management of CVD. High BP, or hypertension, is a controllable risk factor in the development of a range of cardiovascular conditions. Therefore, any food component that has the ability to reduce BP is a potential candidate component in the prevention/treatment of CVD.

Milk proteins contain angiotensin-converting enzyme (ACE) inhibitory peptides encrypted within their primary structures. These peptides can be released by enzymatic hydrolysis either during gastrointestinal digestion or during food processing. The sequences of the individual milk proteins displaying ACE inhibitory activity in vitro are reviewed elsewhere. The best characterized peptides found in fermented milk are peptides with the amino acid sequence isoleucine–proline–proline (IPP; IC₅₀ = 5 μmol/l), and valine–proline–proline (VPP; IC₅₀ = 9 μmol/l). About twenty human studies...
have been published linking the consumption of products containing lactotripeptides (here defined as IPP and VPP) with significant reductions in both SBP and DBP.

Consumption of products enriched with lactotripeptides has risen slowly since their introduction into the Japanese market in 1997. BP-lowering products containing lactotripeptides are currently on the market in the USA, Spain, UK, Finland, Switzerland, Italy, South Korea, Japan, Iceland and Portugal. Standard use of such products provides on average 5 mg/d lactotripeptides.

This review outlines the current evidence of the BP control properties of lactotripeptides in man. The relation between these milk-derived peptides and BP was discussed previously (13–15). However, not all of the reviews have expressed BP-lowering effects relative to placebo treatment. Furthermore, a number of interesting questions have remained unanswered. This review article aims at clarifying issues such as the duration of intake required to obtain a BP effect, the maximum BP effect, effective dosages of lactotripeptides, the relation between height of baseline BP and attainable effect, and the influence of antihypertensive medication and race. Aspects of bioavailability, safety and proposed mechanisms of action will be addressed as well.

In all clinical trials performed in (mildly) hypertensive subjects, by the time of the last visit, SBP had fallen significantly from baseline in the groups that ingested the product containing lactotripeptides, but also in the placebo groups often a trend was seen towards a decreasing BP (16,17). The consequence is that a significant decrease of BP compared with baseline does not mean that there is a significant difference between the test product and placebo. Generally, the nutritional composition of the placebo and test products was similar, with the difference that the active ingredient was not present in the placebo products. In most clinical trials, the test products consisted of sour milk prepared by fermenting skim milk using Lactobacillus helveticus and/or Saccharomyces cerevisiae. As a placebo mostly artificially acidified milk was used. In three other trials, test products consisted of casein hydrolysate, generated using L. helveticus and/or Aspergillus oryzae. For the corresponding placebo products the same carrier was used without the casein hydrolysate. Therefore, a comparison between test product and placebo would be more appropriate for evaluation of a true treatment effect. Similarly, Itakura et al. (21) observed only a trend towards greater lowering of SBP in the test product group compared with the placebo group throughout the treatment period, whereas absolute changes of SBP were already significantly different from baseline after 2 weeks of treatment with the test product. In other trials, similar effects were found throughout the treatment period (22–24). Even in the absence of any treatment, BP may decrease, as was observed after a 4-week run-in period in a study by Seppo et al. (16). These findings stress the power of a so-called placebo effect. Alternatively, they may be indicative of a regression-to-the-mean effect following selection of study subjects with an elevated BP. To control for these phenomena, in the present review, BP-lowering effects are expressed against placebo rather than starting BP. In the present paper, BP changes will only be reviewed if they were compared to placebo; not if only a comparison with baseline BP was made in the original articles. What is the time–effect relation of blood pressure lowering by lactotripeptides?

Trials in hypertensive subjects, in which BP measurements were taken at relatively early time-points, demonstrated significantly lower SBP values (8–10 mmHg) and DBP values (6–7 mmHg) after 1–2 weeks of active treatment compared with placebo treatment (22–25). Aihara et al. (25) reported a trend for a BP-lowering effect even after 1 d of treatment with the peptides compared with placebo, but it took another week for the effects to reach significance.

Most clinical trials have assessed BP-lowering effects at multiple points over time. Generally, maximum duration of treatment was 8 weeks. From these data, it becomes apparent that the largest part of the total BP reduction takes place in the first 1–2 weeks of treatment. Thereafter, a further gradual lowering is seen, but to a lesser extent than in the first period (23–25). For example, Aihara et al. (25) have observed such patterns. In that study, lactotripeptides induced a gradual lowering of SBP compared to control treatment by 7.8, 10.5, 10.6 and 11.2 mmHg after 1, 2, 3 and 4 weeks of active treatment, respectively. Kajimoto et al. (23) demonstrated a comparable profile: SBP decreased by 7.6 mmHg after a 1-week intake of lactotripeptides compared with placebo and gradually thereafter to 13 mmHg after 8 weeks. In this respect even more clear were BP results reported by Hirata et al. (24) showing a BP lowering by 10 mmHg already after 2 weeks and by 12.1 mmHg after 6 more weeks of treatment compared with placebo. In general, changes in DBP were smaller and curves of BP effects in time were more flat compared with changes in SBP.

A few interventions evaluated BP-lowering effects after treatment periods that lasted longer than the generally applied 8 weeks. Sano et al. (18) demonstrated a decrease of SBP by 3.3 mmHg after 8 weeks of intake of 3.1 mg/d lactotripeptides compared with placebo and a slightly further decrease by 4.4 and 4.1 mmHg after 10 and 12 weeks, respectively, in subjects with high-normal BP and subjects with hypertension. DBP was 2.8 mmHg lower compared with placebo after 8 weeks of treatment, and remained nearly constant at 10 and 12 weeks. The study with the longest treatment period in hypertensive subjects that was published lasted 5 months (17). An overall trend effect over 5 months was observed yielding a significant mean difference of −6.7 mmHg SBP and a trend of −3.6 mmHg DBP between the test product and placebo group. BP effects did not become larger with a prolonged treatment time.

After termination of treatment, BP gradually returned to baseline values within 2–4 weeks (16,21,23,26,27). In the study by Hirata et al. (24) even at 2 weeks after completion of intake, a significantly lower SBP (by 8.4 mmHg) was still observed in the subjects that had received the test product compared with those that received placebo. This difference, however, was smaller than at 8 weeks of intake (SBP was 12.1 mmHg lower than placebo) and disappeared at 4 weeks after completion of intake. Subjects with high-normal BP or mild hypertension, treated for 12 weeks with 3/07 mg lactotripeptides, demonstrated still a significantly lower SBP at 2 weeks (by 2.4 mmHg) and 4 weeks (by 2.5 mmHg) after completion of intake as compared with the placebo group (18). Several antihypertensive drugs are known to cause a rapid and abnormal elevation of BP,
so-called rebound, after treatment stops, but from the data mentioned earlier, no such effects become apparent after intake of lactotripeptides.

In conclusion, first significant effects of lactotripeptides on BP in hypertensive subjects are observed after 1–2 weeks of treatment with dosages as low as 3-8 mg/d. Maximum BP-lowering effects of lactotripeptides approximate 13 mmHg SBP and 8 mmHg DBP active treatment v. placebo, and are likely reached after 8–12 weeks of treatment. Lactotripeptides exert a gradual effect on BP lowering after start of intake and return of BP after end of treatment as well.

Table 1 presents a summary of all human trials on antihypertensive effects of lactotripeptides. In the table, sex, age and BMI are included since there is a strong association of age and BMI and elevated BP. Concerning sex, the incidence of hypertension is markedly higher in men than in age-matched, premenopausal women. After menopause, this relationship no longer exists, and the incidence is comparable matched, premenopausal women. After menopause, this relationship no longer exists, and the incidence is comparable.

In none of the trials with normotensives were statistically significant BP changes found. Even at the highest dosage of lactotripeptides used in normotensives, which included a total of 29-2 mg/d during a period of 7 d, no BP-lowering effects by lactotripeptides were observed.

Thus, efficacy of lactotripeptides could only be demonstrated in subjects with (slightly) elevated BP. This is in line with findings in BP-lowering studies using pharmaceutical interventions. In such settings, BP decreases are also larger at higher starting BP values, although a small residual BP-lowering effect is still observed even at normotensive starting values. The fact that this latter finding is not true for lactotripeptides may be due to their lower potency compared to BP-lowering medication. A further explanation for the more pronounced effects of lactotripeptides on BP in subjects with elevated BP may be that if subjects have been screened for a higher-than-normal BP, regression to the mean may occur after screening, giving a BP decrease that is independent of the intervention (but that would not be corrected for if no placebo comparison is made). This artifact is likely more pronounced with higher starting values. Indeed, in a number of trials a decrease of BP was observed from baseline. In a trial by Seppo et al., a marked BP decrease of mildly hypertensive subjects was observed during the 4-week run-in period, which was explained by training the subjects to BP measurements.

Why are lactotripeptides only effective in subjects with higher blood pressure?

In all clinical trials, the current BP classification recommended by the WHO was used. With respect to effectiveness of the lactotripeptides in different BP categories, clinical trials have been carried out in subjects with (mild) hypertension, either or not using antihypertensive medication, in subjects with high-normal BP and subjects with normal BP.

It appears that lactotripeptides are effective in reducing BP provided that starting BP is at least high-normal. Sano et al. who performed a trial including three BP categories illustrated this: mild hypertensive, high-normal BP and normal BP. Daily consumption of 600 ml juice containing in total 9-21 mg lactotripeptides during 4 weeks induced a significant decrease of both SBP (by 7-7 mmHg) and DBP (by 6-4 mmHg) in the mild hypertensive group as compared to the placebo drink.

In the high-normal group, SBP and DBP showed a tendency to decrease by 3-6 and 1-8 mmHg, respectively, whereas in the normal BP group no changes were found. Also in another trial, Sano et al. compared BP of subjects with high-normal BP and subjects with mild hypertension after intake of 3-07 mg lactotripeptides during 12 weeks. Relative to placebo, the test product decreased SBP by approximately 6 mmHg in mild hypertensives and approximately 3 mmHg in subjects with high-normal BP. Comparable results were reported by Aihara et al. At the end of a 4-week treatment, in the subjects with mild hypertension, SBP decreased by 11-2 mmHg and DBP tended to decrease by 6-5 mmHg compared with placebo. In subjects with high-normal BP, SBP decreased by 3-2 mmHg and DBP by 5-0 mmHg compared with placebo. These trials demonstrated the positive relation between height of starting BP and effectiveness of the peptides.

The highest effective dosage of lactotripeptides was evaluated in a safety study, and consisted of 52-5 mg/d. After 10 weeks of active treatment, mean SBP in subjects with hypertension decreased by 4-1 mmHg and DBP by 1-8 mmHg. The next highest dose of lactotripeptides that was tested amounted to 13-0 mg/d. After 4 weeks of active treatment, SBP in subjects with mild hypertension decreased by 11-2 mmHg compared to placebo, and DBP tended to decrease by 6-5 mmHg. It is intriguing that the study applying the lower dosage produced the biggest BP decrease. A regional difference may account for this apparent discrepancy, as will be discussed later.
## Table 1. Overview of human trials on antihypertensive effects of lactotripeptides

<table>
<thead>
<tr>
<th>Author and reference</th>
<th>Design</th>
<th>Duration (weeks)</th>
<th>Number and sex</th>
<th>Characteristics of the subjects</th>
<th>Treatment</th>
<th>BP changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SBP</td>
</tr>
<tr>
<td><strong>Baseline BP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>mmHg</td>
</tr>
<tr>
<td><strong>SBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sig.</td>
</tr>
<tr>
<td><strong>DBP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>IPP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>VPP</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Seppo et al. (16)
- **Design**: R, p-c, d-bld, parallel
- **Duration**: 8 weeks
- **Number and sex**: 10 (3 M, 7 F)
- **BMI**: 27.3 kg/m²
- **Baseline BP**: 148/94 mmHg
- **Treatment**: L. helv. LBK-16 H 1 x 150 ml milk drink
- **BP changes**: 10.8 mmHg, Y

### Seppo et al. (17)
- **Design**: R, p-c, d-bld, parallel
- **Duration**: 21 weeks
- **Number and sex**: 22 (10 M, 12 F)
- **BMI**: 85.6 kg
- **Baseline BP**: 155/97 mmHg
- **Treatment**: L. helv. LBK-16 H 1 x 150 ml milk drink
- **BP changes**: Mean 5.9 mmHg, N

### Sano et al. (18)
- **Design**: R, p-c, d-bld, parallel
- **Duration**: 12 weeks
- **Number and sex**: 72 (28 M, 44 F)
- **BMI**: 24 kg
- **Baseline BP**: 138/85 mmHg
- **Treatment**: A. oryzae casein hydrolysate 1 x 200 ml vegetable and fruits juice
- **BP changes**: Mean 4.1 mmHg, Y

### Sano et al. (19)
- **Design**: R, p-c, d-bld, parallel
- **Duration**: 4 weeks
- **Number and sex**: 22 (11 M, 11 F)
- **BMI**: 23.7 kg
- **Baseline BP**: 133/81 mmHg
- **Treatment**: A. oryzae hydrolysate 3 x 200 ml vegetable and fruit juice
- **BP changes**: Mean 5.6 mmHg, N

### Mizuno et al. (20)
- **Design**: R, p-c, d-bld, parallel
- **Duration**: 6 weeks
- **Number and sex**: 21 (9 M, 12 F)
- **BMI**: 23.1 kg
- **Baseline BP**: 150/89 mmHg
- **Treatment**: A. oryzae hydrolysate 1 x 2 tablets
- **BP changes**: Mean 6.5 mmHg, N
<table>
<thead>
<tr>
<th>Author and reference</th>
<th>Design</th>
<th>Duration (weeks)</th>
<th>Characteristics of the subjects</th>
<th>Treatment</th>
<th>BP changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Number and sex</td>
<td>Age (years)</td>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Itakura et al. [21]</td>
<td>R, p-c, d-bld, parallel</td>
<td>8</td>
<td>9 M Test group</td>
<td>54</td>
<td>64 kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9 M Placebo group</td>
<td>55</td>
<td>67 kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13 (10 M, 3 F) Test group</td>
<td>35</td>
<td>64 kg</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13 (10 M, 3 F) Placebo group</td>
<td>37</td>
<td>66 kg</td>
</tr>
<tr>
<td>Kajimoto et al. [22]</td>
<td>R, p-c, d-bld, parallel</td>
<td>8</td>
<td>42 (29 M, 13 F) Test group</td>
<td>46</td>
<td>23·8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42 (29 M, 13 F) Placebo group</td>
<td>46</td>
<td>22·5</td>
</tr>
<tr>
<td>Kajimoto et al. [23]</td>
<td>R, p-c, d-bld, parallel</td>
<td>8</td>
<td>31 (16 M, 15 F) Test group</td>
<td>51</td>
<td>25·4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>31 (17 M, 16 F) Placebo group</td>
<td>49</td>
<td>24·7</td>
</tr>
<tr>
<td>Hirata et al. [24]</td>
<td>R, p-c, d-bld, parallel</td>
<td>8</td>
<td>16 (8 M, 8 F) Test group</td>
<td>52</td>
<td>25·2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>16 (7 M, 9 F) Placebo group</td>
<td>50</td>
<td>24·0</td>
</tr>
<tr>
<td>Aihara et al. [25]</td>
<td>R, p-c, d-bld, parallel</td>
<td>4</td>
<td>20 (13 M, 7 F) Test group</td>
<td>50</td>
<td>24·0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 (13 M, 7 F) Placebo group</td>
<td>53</td>
<td>24·3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 (16 M, 4 F) Test group</td>
<td>52</td>
<td>24·9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20 (16 M, 4 F) Placebo group</td>
<td>52</td>
<td>25·1</td>
</tr>
<tr>
<td>Kajimoto et al. [26]</td>
<td>R, p-c, d-bld, parallel</td>
<td>8</td>
<td>15 (6 M, 9 F) Test group</td>
<td>52</td>
<td>23·0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15 (6 M, 9 F) Placebo group</td>
<td>51</td>
<td>24·7</td>
</tr>
<tr>
<td>Nakamura et al. [27]</td>
<td>R, p-c, d-bld, parallel</td>
<td>12</td>
<td>53 (17 M, 36 F) Test group</td>
<td>39</td>
<td>22·0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53 (17 M, 36 F) Placebo group</td>
<td>38</td>
<td>22·0</td>
</tr>
<tr>
<td>Jauhiainen et al. [31]**</td>
<td>R, p-c, d-bld, parallel</td>
<td>10</td>
<td>53 (35 M, 18 F) Test group</td>
<td>51</td>
<td>28·6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53 (34 M, 21 F) Placebo group</td>
<td>55</td>
<td>28·3</td>
</tr>
<tr>
<td>Yasuda et al. [32]</td>
<td>R, p-c, d-bld, parallel</td>
<td>1</td>
<td>10 (5 M, 5 F) Test group</td>
<td>31</td>
<td>21·4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10 (5 M, 5 F) Placebo group</td>
<td>31</td>
<td>20·4</td>
</tr>
<tr>
<td>Author and reference</td>
<td>Design</td>
<td>Duration (weeks)</td>
<td>Number and sex</td>
<td>Age (years)</td>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>------------------</td>
<td>----------------</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Mizushima et al. (^{35})</td>
<td>R, p-c, d-bld, parallel</td>
<td>4</td>
<td>23 M Test group</td>
<td>44</td>
<td>25·1</td>
</tr>
<tr>
<td>Hata et al. (^{36})</td>
<td>R, p-c, d-bld, parallel</td>
<td>8</td>
<td>17 (4 M, 13 F) Test group</td>
<td>77</td>
<td>19·1</td>
</tr>
<tr>
<td>Tuomilehto et al. (^{38})††</td>
<td>R, p-c, d-bld, parallel</td>
<td>10</td>
<td>30 (18 M, 12 F) Test group</td>
<td>51</td>
<td>29·0</td>
</tr>
<tr>
<td>Cross-over</td>
<td>7</td>
<td>17 (10 M, 7 F) Placebo Test</td>
<td>55</td>
<td>28·8</td>
<td>159/98</td>
</tr>
<tr>
<td>Engberink et al. (^{51})‡‡</td>
<td>R, p-c, d-bld, parallel</td>
<td>8</td>
<td>36 (23 M, 12 F) Fermented LTP</td>
<td>59</td>
<td>26·9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32 (22 M, 10 F) Enzymatic LTP</td>
<td>54</td>
<td>26·8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36 (23 M, 13 F) Synthetic LTP</td>
<td>60</td>
<td>27·0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>32 (20 M, 12 F) Placebo</td>
<td>59</td>
<td>26·8</td>
</tr>
</tbody>
</table>
Table 1. Continued

<table>
<thead>
<tr>
<th>Author and reference</th>
<th>Design</th>
<th>Duration (weeks)</th>
<th>Characteristics of the subjects</th>
<th>Treatment</th>
<th>BP changes</th>
<th>Source triptides</th>
<th>Formula</th>
<th>IP (mg/d)</th>
<th>VPP (mg/d)</th>
<th>SBP</th>
<th>DBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kajimoto et al. (33)</td>
<td>R, p-c, d-bid, parallel</td>
<td>2</td>
<td>21 (10 M, 11 F) Test group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.5</td>
<td>7.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22 (10 M, 12 F) Placebo group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A. oryzae, Aspergillus oryzae; ACE, angiotensin-converting enzyme; BP, blood pressure; d-bid, double blind; DBP, diastolic blood pressure; F, female; IPP, isoleucine–proline–proline; L. helv. Lactobacillus helveticus; LTP, lactotripeptides; M, male; NR, not reported; p-c, placebo controlled; R, randomized; S. cervisiae. SBP, systolic blood pressure; Sig., significant; VPP, valine–proline–proline.

* Degree of hypertension of subjects (SBP/DBP): optimal 130/85 mmHg, high-normal 130–139/85–89 mmHg, mild 140–149/90–99 mmHg, moderate 160–169/100–109 mmHg, severe > 170/110 mmHg.
† Results reported as changes in SBP and DBP after each month of treatment for all subjects (intention-to-treat analysis), and as mean changes over the total intervention period among subjects who had BP measurements for each month (per protocol analysis). BMI was not reported, only body weight (in kg).
‡ Results reported separately for subjects with mild hypertension and subjects with high-normal blood pressure and for both groups combined.
§ Results reported separately for subjects with mild to moderate hypertension and subjects with normal blood pressure, and for all groups combined.
‖ Results reported separately for subjects with mild hypertension and subjects with high-normal blood pressure and with normal blood pressure.
‡‡ Results reported separately for subjects with mild hypertension and subjects with high-normal blood pressure.
\* Results of 24 h ambulatory (amb.) BP measurements and office (off.) BP measurements were reported.
\‡ First part of the study was carried out in parallel design and second part of the study was carried out in crossover design.
\‖ Subjects with BP values ranging from optimal to moderate hypertension were included, but the majority had high-normal BP or mild hypertension and no relevant differences were reported among groups. Results of office (off.) BP and home BP were reported.

Hypertension is a complex multifactor disorder that is thought to result from an interaction between environmental factors and genetic background. Subject characteristics such as age, gender, race/ethnicity can affect BP, including the BP response to specific antihypertensive medication. Although numbers were very small for statistics, the decrease in SBP and DBP in the test group tended to be greater than in the placebo group for all types of medication (mainly calcium antagonists, b-blockers and ACE inhibitors). These findings demonstrate the potency of lactotripeptides to lower BP in addition to medication. Also, the use of antihypertensive medication in the study did not significantly influence the BP responses to lactotripeptides, although no data were given.

Thus, lactotripeptides only seem to be active at elevated BP and not at normal BP values. Evidence indicates that effective blood pressure effects of lactotripeptides are different in Chinese and Japanese subjects compared with Caucasians. As described by Hata et al. (36), lactotripeptides can have antihypertensive effects even in addition to the effects of antihypertensive medication. In studies on the effects of lactotripeptides, the decrease in BP and DBP in the test group tended to be greater than in the placebo group for all types of medication (mainly calcium antagonists, b-blockers and ACE inhibitors). These findings demonstrate the potency of lactotripeptides to lower BP in addition to medication. Also, the use of antihypertensive medication in the study did not significantly influence the BP responses to lactotripeptides, although no data were given.
system, which regulates BP and fluid and electrolyte balance. The ACE inhibitory activity of lactotripeptides has mainly been determined in vitro (43). One of the few studies that support their in vivo action demonstrated the presence of IPP and VPP as well as a decreased ACE activity in the aorta after a single oral administration to spontaneously hypertensive rats (44). Sipola et al. (45) demonstrated ACE inhibition by measuring an increased plasma renin activity in spontaneously hypertensive rats after oral intake of IPP and VPP. Changes in angiotensin I and angiotensin II have been reported, but the result was not significant (39). However, ACE inhibitors not only decrease the production of angiotensin II but also decrease the degradation of the vasodilator bradykinin (13). Thus, BP-lowering activity of lactotripeptides may therefore also result from inhibition of bradykinin degradation and/or subsequent increases of vasodilating PG or endothelium-derived relaxing factor(s).

Moreover, lactotripeptides may exert antihypertensive effects through other mechanisms, such as opioid-like activities (46), inhibition of the release of the vasoactive substances such as the vasoconstrictor endothelin-1, eicosanoids and nitric oxide (47). However, involvement of ACE inhibition in these pathways cannot be excluded.

Lactotripeptides have additionally been shown to exert beneficial effects other than lowering systemic BP, such as improvement of vascular endothelial function in subjects with mild hypertension (48). Since there was no change in systemic BP, the authors suggest that the improvement of the vascular endothelial function attributable to VPP and IPP is independent of haemodynamic changes.

Lactotripeptides may exert BP-lowering effects either via ACE inhibition or via non-ACE-dependent pathways, but only limited in vivo evidence is currently available for the physiological basis of their antihypertensive action.

Are lactotripeptides bioavailable?

To exert physiological effects after oral ingestion, it is of crucial importance that lactotripeptides remain active during gastrointestinal digestion and absorption to the cardiovascular system. Proline- and hydroxyproline-containing peptides are relatively resistant to degradation by digestive enzymes. Furthermore, tripeptides containing the C-terminal proline–proline are reported to be resistant to proline-specific peptidases (12). Peptides consisting of two or three amino acids can be absorbed intact from the intestinal lumen into the blood circulation via different mechanisms for intestinal transport (49). The presence of IPP (but not VPP) was recently demonstrated in measurable amounts in the circulation of volunteers that consumed a drink enriched in IPP and VPP (50).

Are differences in composition of the test products important for the blood pressure effect?

In all clinical trials discussed here, the study products contained both IPP and VPP administered either as a fermented milk drink or as tablets. In vitro studies indicate that IPP (IC50 = 5 µmol/l) has a higher ACE inhibitory potency than VPP (IC50 = 9 µmol/l) (11), and may thus be more effective. Moreover, data from a study that assessed the bioavailability of IPP and VPP suggest that IPP may have a better bioavailability than VPP (50). In most products more VPP is present than IPP giving a ratio of approximately 1:1–1:8 VPP:IPP. Only the products used by Sano et al. (18,19) and Jaunhiainen et al. (31) contained slightly less VPP than IPP, giving ratios of 0:8 and 0:9 VPP:IPP, respectively. Recently, a study compared the effects on BP of different lactotripeptide sources, namely a fermented and an enzymatically hydrolysed dairy drink, a dairy drink in which equal amounts of IPP and VPP but no other tripeptides were added, and a dairy drink without further additions (placebo) (51). Since no results on BP were observed in any of the conditions, it is difficult to draw conclusions on the importance of the source of the tripeptides. It appears, however, that beneficial BP effects do not require both lactotripeptides to be present in the product, because a recently conducted study on a product containing IPP but no VPP showed significant BP-lowering effects in Caucasian stage I hypertensive subjects (E Boelsma and J Kloek, unpublished results).

All products tested in human studies so far contain a number of minerals with known effects on BP, such as calcium, potassium, magnesium and phosphorus. Concerning calcium and magnesium, meta-analyses of randomized, controlled trials yielded an inverse association between intake of these minerals and BP in hypertensive and non-hypertensive subjects (52–55). A negative correlation of potassium with BP has been demonstrated as well (56,57). In a recent study, significant inverse relationships of dietary phosphorus intake with BP were found (58). Although the dosages of the minerals in the sour milk/tablets were much lower than those that were effective in lowering BP in intervention trials, and placebo treatments were controlled for mineral content, it is possible that the minerals present may have induced synergistic effects to reduce BP. Only in the studies by Seppo et al. (16,17) and Jaunhiainen et al. (31) did the placebo products contain 1.5–3.5 times more minerals compared with the sour milk drinks.

Is administration of lactotripeptides safe?

In general, lactotripeptides are considered safe since milk proteins are an essential part of the daily human diet. After ingestion, proteins are hydrolysed in the gastrointestinal tract by proteolytic enzymes derived from the pancreas resulting in the release of dipeptides, tripeptides and free amino acids (59). In addition, the FDA list mentioning specific substances affirmed as ‘generally recognized as safe’ includes casein peptones (60). Recently, a battery of in vitro and in vivo toxicity tests were performed with lactotripeptide-containing products, casein hydrolysate, fermented milk and lactotripeptides (61,62). Results from the in vitro toxicity studies showed that the lactotripeptide-containing products did not exert mutagenic or genotoxic properties. Results from the in vitro toxicity studies, including sub-chronic studies in rats and developmental and reproductive toxicity studies in rats and rabbits, showed that the lactotripeptide-containing products did not induce any treatment-related adverse effects, even in the highest doses tested. Therefore, in the sub-chronic toxicity test in rats exposed to the casein hydrolysate product, the ‘no observable adverse effect level’ (NOAEL) resulted in >1000 mg casein hydrolysate/kg body weight per d (corresponding to 3 mg IPP +3 mg VPP/kg body weight per d),
and for the lactotripeptide product it resulted in >4000 mg lactotripeptides/kg body weight per d (corresponding to 11.2 mg IPP + 10.4 mg VPP/kg body weight per d). Fertility, reproductive performance, embryo toxicity and F1 generation (first offspring) studies in rats were performed and showed a NOAEL of >2000 mg fermented milk/kg body weight per d (corresponding to 3 mg IPP + 3 mg VPP/kg body weight per d). In the embryo-fetal (pre-natal) developmental study in rabbits exposed to the lactotripeptide-containing product, the NOAEL was set for the highest dose tested, 1000 mg lactotripeptide product/kg body weight per d (corresponding to 2.8 mg IPP + 2.6 mg VPP/kg body weight per d). Finally, in a number of human trials, clinical, biochemical and haematological parameters as well as urinalysis, medical examinations and questionnaires were included covering most parameters related to known adverse events of ACE inhibitory drugs. Even doses of lactotripeptides up to 52.5 mg/d did not cause adverse effects and neither did they affect serum clinical chemistry, substantiating the safety of consumption of lactotripeptides.

General conclusion

In the present review, a number of issues on BP-lowering effects of lactotripeptides in man were clarified and current knowledge was updated. Available data demonstrate that lactotripeptides are safe and effective BP-lowering agents with a maximum effect after about 8–12 weeks of treatment. Largest BP-lowering effects approximate 13 mmHg SBP and 8 mmHg DBP active treatment v. placebo. Effective dosages range between 3.07 and 52.5 mg/d, but the existence of a relationship between dose and effect is not yet clear.

Given the facts that lactotripeptides are safe and exert relevant and stable BP-lowering effects within relatively short time periods, they are good candidates to be included in healthy lifestyle changes to prevent or reduce high blood pressure.

Acknowledgements

Y. Ponstein is gratefully acknowledged for her contribution to the safety paragraph of this paper. The authors’ responsibilities were as follows: E. B. reviewed the literature; E. B. and J. K. wrote the manuscript; J. K. approved the final version. The authors did not have a conflict of interest. E. B. is a project manager of clinical trials and consultant at the Business Unit Biosciences, TNO Quality of Life, Zeist, The Netherlands. J. K. is a senior scientist at the Department of Nutrition and Health, DSM Food Specialties, The Netherlands.

References

44. Masuda O, Nakamura Y & Takino T (1996) Antihypertensive peptides are present in aorta after oral administration of sour milk containing these peptides to spontaneously hypertensive rats. J Nutr 126, 3063–3068.
60. FDA (1998) List of substances that added directly to human food. rdb/opa-gras.html
