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Review Article

Lactotripeptides and antihypertensive effects: a critical review

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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[1]. 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[2–4]. In a large meta-analysis by the Prospective Studies Collaboration[5], 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[6]. Also SBP lower by 2 mmHg is associated with lower IHD and CVD death rates by 4–5 %[7]. The results suggested that for the large majority of individuals, whether hypertensive or normotensive, a lower BP should eventually confer a lower risk of CVD.

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[8–10]. 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[11]. 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[12]. The best characterized peptides found in fermented milk are peptides with the amino acid sequence isoleucine–proline–proline (IPP; $IC_{50} = 5\,\mu mol/l$), and valine–proline–proline (VPP; $IC_{50} = 9\,\mu mol/l$). About twenty human studies

Abbreviations: ACE, angiotensin-converting enzyme; BP, blood pressure; DBP, diastolic blood pressure; IPP, isoleucine–proline–proline; NOAEL, no observable adverse effect level; SBP, systolic blood pressure; VPP, valine–proline–proline.

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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 *Aspergillus oryzae* protease, added to a carrier (vegetable and fruit juice[18,19] and tablets[20]). 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 treatment 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[28,29]. 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 in women and men[30]. A large number of the individuals with high BP are either overweight and/or elderly. However, sex, age and BMI were not analysed separately for associations with BP.

What is the lowest and highest effective dosage tested in human trials?

The lowest dosage of lactotripeptides that was proven effective in man was 3·07 mg/d in subjects with high-normal BP or mild hypertension. Daily intake of sour milk with this amount of peptides resulted in a maximal significant lowering of SBP by 4·4 mmHg and DBP by 2·8 mmHg after 10 weeks of intake as compared with placebo drink[18].

The highest effective dosage of lactotripeptides was evaluated in a safety study, and consisted of 52·5 mg/d[31]. 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[25]. 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 dose produced the biggest BP decrease. A regional difference may account for this apparent discrepancy, as will be discussed later.

Mizuno *et al.* [20] were the first, and so far the only research group, to study dose-dependent effects of lactotripeptides on the extent of BP lowering in subjects with high-normal BP and mild hypertension. In hypertensives, intake of 1·8, 2·5 and 3·6 mg lactotripeptides during 6 weeks resulted in greater decreases of SBP in the test group compared with placebo; by 6·5, 7·9 and 12·2 mmHg, respectively. No differences in DBP in the test group compared with the placebo group were observed during the treatment period. In subjects with high-normal BP, intake of lactotripeptides did not affect BP.

In conclusion, effective dosages of lactotripeptides range from 3·07 to 52·5 mg/d. A dose–effect relationship was found in the one study that specifically addressed this relation, but from a comparison of studies using different doses, no clear dose–effect relation can be established.

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

In all clinical trials, the current BP classification recommended by the WHO[32] 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.* [19] 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.* [18] 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.* [25]. 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.

In none of the trials with normotensives were statistically significant BP changes found[21,26]. 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[33].

Thus, efficacy of lactrotripeptides 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[34]. 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 screening to baseline[17,33,35]. In a trial by Seppo *et al.* [16], 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.

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Table 1. Overview of human trials on antihypertensive effects of lactotripeptides

			Characteristics of the subjects				Treatment				BP changes			
											SBP		DBP	
Author and reference	Design	Duration (weeks)	Number and sex	Age (years)	BMI (kg/m^2)	Baseline BP* (SBP/DBP)	IPP (mg/d)	VPP (mg/d)	Source tripeptides	Formula	mmHg	Sig.	mmHg	Sig.
Seppo *et al.* [16]	R, p-c, d-bld, parallel	8	10 (3 M, 7 F) Test group	49	27·3	148/94	2·25	3–3·75	*L. helv.* LBK-16 H	1 × 150 ml milk drink	10·8	Y	6·9	Y
			7 (2 M, 5 F) Placebo group	46	27·0	148/93	0	0						
Seppo *et al.* [17]†	R, p-c, d-bld, parallel	21	22 (10 M, 12 F) Test group	51	85·6 kg	155/97	2·25	3·0	*L. helv.* LBK-16 H	1 × 150 ml milk drink	5·9	N	3·7	N
			17 (9 M, 8 F) Placebo group	48	77·6 kg	152/96	0	0			Mean 6·7	Y	Mean 3·6	N
Sano *et al.* [18]‡	R, p-c, d-bld, parallel	12	72 (28 M, 44 F) Test group	51	24	138/85	1·60	1·47	*A. oryzae* casein hydrolysate	1 × 200 ml vegetable and fruits juice	4·1	Y	2·5	Y
			72 (29 M, 43 F) Placebo group	50	24	138/85	0	0						
			20 Test group	56		147/88	1·60	1·47			~6	Y	~3	N
			20 Placebo group				0	0						
			52 Test group	49		135/84	1·60	1·47			~3	Y	2	Y
			52 Placebo group				0	0						
Sano *et al.* [19]	R, p-c, d-bld, parallel	4	22 (11 M, 11 F) Test group	47	23·7	133/81	4·80	4·41	*A. oryzae* hydrolysate	3 × 200 ml vegetable and fruit juice	5·6	N	3·2	N
			21 (10 M, 11 F) Placebo group	47	23·5	134/14	0	0						
			8 Test group			148/87	4·80	4·41			7·7	Y	6·4	Y
			8 Placebo group			148/90	0	0						
			8 Test group			135/82	4·80	4·41			3·6	N	1·8	N
			8 Placebo group			133/82	0	0						
			6 Test group			108/70	4·80	4·41			2	N	1·4	N
			5 Placebo group			111/71	0	0						
Mizuno *et al.* [20]§	R, p-c, s-bld, parallel	6	21 (11 M, 10 F) Test group	40	23·1	150/89	0·86	0·89	*A. oryzae* hydrolysate	1 × 2 tablets	6·5	N	2·6	N
			21 (9 M, 12 F) Test group	42	23·7	149/89	1·20	1·27			7·9	Y	2·4	N
			21 (10 M, 11 F) Test group	46	22·5	149/89	1·76	1·86			12·2	Y	3·4	N
			20 (10 M, 10 F) Placebo group	46	23·0	150/89	0	0						
	R, p-c, s-bld, parallel	6	12 (3 M, 9 F) Test group	47	25·0	135/82	0·86	0·89	*A. oryzae* hydrolysate	1 × 2 tablets	0·3	N	−0·7	N
			12 (3 M, 9 F) Test group	50·2	23·5	134·5/81·7	1·20	1·27			0·5	N	−0·8	N
			12 (3 M, 9 F) Test group	42·9	25·9	134·2/81·6	1·76	1·86			2	N	−0·6	N
			12 (3 M, 9 F) Placebo group	42·6	24·0	133·2/81·6	0	0						

Table 1. *Continued*

Author and reference	Design	Duration (weeks)	Characteristics of the subjects: Number and sex	Age (years)	BMI (kg/m^2)	Baseline BP* (SBP/DBP)	Treatment: IPP (mg/d)	VPP (mg/d)	Source tripeptides	Formula	BP changes: SBP mmHg	SBP Sig.	DBP mmHg	DBP Sig.
Itakura *et al.* [21]‖	R, p-c, d-bld, parallel	8	9 M Test group	54	64 kg	157/102	1·1	1·5	*L. helv.* + *S. cer.*	1 × 100 g milk drink	7·6	N	2	N
			9 M Placebo group	55	67 kg	156/102	0	0						
			13 (10 M, 3 F) Test group	35	64 kg	118/77	1·1	1·5	*L. helv.* + *S. cer.*	1 × 100 g milk drink	~4	N	~3	N
			13 (10 M, 3 F) Placebo group	37	66 kg	119/78	0	0						
Kajimoto *et al.* [22]	R, p-c, d-bld, parallel	8	42 (29 M, 13 F) Test group	46	23·8	150/91	1·64	2·52	*L. helv.* CM4	1 × 2 tablets	10·1	Y	3·6	N
			39 (30 M, 9 F) Placebo group	46	22·5	150/92	0	0						
Kajimoto *et al.* [23]	R, p-c, d-bld, parallel	8	31 (16 M, 15 F) Test group	51	25·4	148/94	1·58	2·24	*L. helv.* + *S. cer.*	2 × 150 g milk drink	13	Y	8·4	Y
			33 (17 M, 16 F) Placebo group	49	24·7	148/95	0	0						
Hirata *et al.* [24]	R, p-c, d-bld, parallel	8	16 (8 M, 8 F) Test group	52	25·2	157/94	1·60	2·66	*L. helv.* + *S. cer.* + *L. helv.* CM4	1 × 120 g milk drink	12·1	Y	5·8	N
			16 (7 M, 9 F) Placebo group	50	24·0	158/95	0	0						
Aihara *et al.* [25]¶	R, p-c, d-bld, parallel	4	20 (13 M, 7 F) Test group	50	24·0	137/85	4·7	8·3	*L. helv.* CM4	1 × 6 tablets	3·2	N	5	Y
			20 (13 M, 7 F) Placebo group	53	24·3	137/85	0	0						
			20 (16 M, 4 F) Test group	52	24·9	149/93	4·7	8·3	*L. helv.* CM4	1 × 6 tablets	11·2	Y	6·5	N
			20 (16 M, 4 F) Placebo group	52	25·1	147/92	0	0						
Kajimoto *et al.* [26]	R, p-c, d-bld, parallel	8	15 (6 M, 9 F) Test group	52	23·0	158/93	1·52	2·53	*L. helv.* + *S. cer.* + *L. helv.* CM4	1 × 160 g milk drink	13·2	Y	7·8	Y
			15 (6 M, 9 F) Placebo group	51	24·7	157/93	0	0						
Nakamura *et al.* [27]	R, p-c, d-bld, parallel	12	53 (17 M, 36 F) Test group	39	22·0	134/79	1·48	2·26	*L. helv.* + *S. cer.* + *L. helv.* CM4	2 × 150 g milk drink	4	Y	3·5	Y
			53 (17 M, 36 F) Placebo group	38	22·0	135/78	0	0						
Jauhiainen *et al.* [31]**	R, p-c, d-bld, parallel	10	53 (35 M, 18 F) Test group	51	28·6	133/83	30	22·5	*L. helv.* LBK-16H	2 × 150 ml milk drink	4·1	Y (amb.)	1·8	Y
			55 (34 M, 21 F) Placebo group	55	28·3	130/80	0	0			2	N (off.)	1	N
Yasuda *et al.* [33]	R, p-c, d-bld, parallel	1	10 (5 M, 5 F) Test group	31	21·4	117/71	11·5	17·7	*L. helv.* CM4	1 × 14 tablets	2·6	N	2	N
			10 (5 M, 5 F) Placebo group	31	20·4	114/66	0	0						

Table 1. *Continued*

Author and reference	Design	Duration (weeks)	Characteristics of the subjects: Number and sex	Age (years)	BMI (kg/m^2)	Baseline BP* (SBP/DBP)	Treatment: IPP (mg/d)	VPP (mg/d)	Source tripeptides	Formula	BP changes: SBP mmHg	SBP Sig.	DBP mmHg	DBP Sig.
Mizushima *et al.* [35]	R, p-c, d-bld, parallel	4	23 M Test group	44	25·1	148/95	1·15	1·98	*L. helv.* + *S. cer.*	1 × 160 g milk drink	1·5	N	1·7	N
			23 M Placebo group	49	24·8	145/92	0	0						
Hata *et al.* [36]	R, p-c, d-bld, parallel	8	17 (4 M, 13 F) Test group	77	19·1	159/89	1·1	1·5	*L. helv.* + *S. cer.*	1 × 95 ml milk drink	9·7	N	4·4	N
			13 (4 M, 9 F) Placebo group	73	21·9	151/87	0	0						
Tuomilehto *et al.* [38]††	R, p-c, d-bld, parallel	10	30 (18 M, 12 F) Test group	51	29·0	153/98	2·4–2·7	2·4–2·7	*L. helv.* LBK-16H	1 × 150 ml milk drink	2·3	N	0·5	N
			29 (18 M, 11 F) Placebo	54	28·1	157/98	0	0						
	Cross-over	7	17 (10 M, 7 F) Placebo Test	55	28·8	159/98					12·3	Y	3·7	N
			22 (14 M, 8 F) Test Placebo	52	28·9	151/97								
Engberink *et al.* [51]‡‡	R, p-c, d-bld, parallel	8	35 (23 M, 12 F) Fermented LTP	59	26·9	140–159 SBP or 90–99 DBP (49 %)	2·1	2·9	NR	1 × 200 ml yoghurt drink	2·8 (off.)	N	2·2 (off.)	N
											−0·9 (home)		NR	
			32 (22 M, 10 F) Enzymatic LTP	54	26·8	140–159 SBP or 90–99 DBP (44 %)	2·7	2·5			−0·5 (off.)	N	0·01 (off.)	N
											−1·8 (home)		NR	
			36 (23 M, 13 F) Synthetic LTP	60	27·0	140–159 SBP or 90–99 DBP (44 %)	2·6	2·5			1·6 (off.)	N	−0·01 (off.)	N
											0·6 (home)		NR	
			32 (20 M, 12 F) Placebo	59	26·8	140–159 SBP or 90–99 DBP (44 %)	0	0						

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Table 1. *Continued*

Author and reference	Design	Characteristics of the subjects				Treatment				BP changes				
		Duration (weeks)	Number and sex	Age (years)	BMI (kg/m^2)	Baseline BP* (SBP/DBP)	IPP (mg/d)	VPP (mg/d)	Source tripeptides	Formula	SBP		DBP	
											mmHg	Sig.	mmHg	Sig.
Kajimoto *et al.*[63]	R, p-c, d-bld, parallel	2	21 (10 M, 11 F) Test group 22 (10 M, 12 F) Placebo group	30 30	21·1 21·9	114/67 114/68	4·5 0	7·9 0	*L. helv.* CM4	1 × 6 tablets	3·3	N	2	N

A. oryzae, *Aspergillus oryzae*; ACE, angiotensin-converting enzyme; BP, blood pressure; d-bld, 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. cerv.*, *Saccharomyces cerevisiae*; SBP, systolic blood pressure; Sig., significant; VPP, valine–proline–proline.

*Degree of hypertension of subjects (SBP/DBP): optimal < 120/80 mmHg; normal < 130/85 mmHg, high-normal 130–139/85–89 mmHg; mild 140–159/90–99 mmHg; moderate 160–179/100–109 mmHg, severe > 179/109 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 hypertension, subjects with high-normal blood pressure, subjects with normal blood pressure, and for all groups combined.

‖ Results reported separately for subjects with mild to moderate hypertension and subjects with normal blood pressure; BMI was not reported, only body weight (in kg).

¶ 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.

Thus, lactotripeptides only seem to be active at elevated BP and not at normal BP values. Evidence indicates that effectiveness is positively associated with BP level, which is in line with existing data for BP-lowering medication[34].

Are lactotripeptides effective in subjects on antihypertensive medication?

The use of lactotripeptides on top of antihypertensive medication was described in two papers[17,36]. As described by Hata *et al.*[36], lactotripeptides can have antihypertensive effects even in addition to the effects of antihypertensive medication. Although numbers were very small for statistics, in stratified analyses by the kind of antihypertensive medication used, 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, β-blockers and ACE inhibitors). These findings demonstrate the potency of lactotripeptides to lower BP in addition to medication. Also Seppo *et al.*[17] reported that the use of antihypertensive medication did not significantly influence the BP responses to lactotripeptides, although no data were given.

Are blood pressure effects of lactotripeptides different in Japanese subjects versus Caucasian subjects?

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 and race/ethnicity can affect BP, including the BP response to specific antihypertensive medication. Indeed, public health studies have observed that the prevalence of hypertension is different in different racial groups. Worldwide, a particularly high prevalence of hypertension was reported in Latin America and the Caribbean, and a number of Asian countries had the lowest prevalence, with the exception of Japan[37].

Most of the BP studies with lactotripeptides have been done in Japanese subjects, and several studies have been done in Finnish subjects[16,17,31,38]. In general, the effects described in Japanese studies on lactotripeptides are larger than those reported in Finnish studies. However, it is unlikely that genetic differences can account for these differential effects. Comparative studies on antihypertensive medication in different races/ethnic groups have demonstrated that pharmacokinetic parameters and haemodynamic effects are essentially the same in Chinese and Japanese subjects compared with Caucasian subjects[39–41].

Thus, despite genetic differences related to hypertension between different ethnic groups, there is no reason to consider Japanese subjects different from Caucasian subjects with respect to BP effects of lactotripeptides. However, a number of other background factors are different between Japanese and Caucasian subjects that may account for variation in BP responses to lactotripeptides, especially dietary factors such as dairy intake[42].

What is the mechanism of action?

Inhibition of ACE is generally believed to be the underlying working mechanism of lactotripeptides[15]. ACE is an enzyme that plays a crucial role in the renin angiotensin

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[35]. 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 and reach 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 ($IC_{50} = 5\,\mu mol/l$) has a higher ACE inhibitory potency than VPP ($IC_{50} = 9\,\mu mol/l$)[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 Jauhiainen *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 Jauhiainen *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),

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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 F_1 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 lactoripeptide-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[19,25,31,33,63].

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 need to be taken for at least 1–2 weeks before BP effects become apparent. Within the first 2 weeks the largest effect is achieved followed by a gradual BP lowering until 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.

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