Invited review

Milk and milk-derived peptides combat against hypertension and vascular dysfunction: a review

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Summary   Epidemiological studies have revealed that consumption of milk and fermented dairy products is inversely associated with elevated blood pressure and with many of the risk factors of the metabolic syndrome. Previously, calcium was thought to be behind this phenomenon, but during the last 20 years, convincing evidence emerging from experimental, epidemiological and intervention studies has highlighted the important role of the small peptides formed during fermentation processes. This review provides an overview of the potential blood pressure lowering components present in dairy products with a special focus on casein-derived tripeptides.

Introduction

Hypertension is a major risk factor for the development of stroke, heart failure, ischaemic heart and renal diseases and is often associated with the metabolic syndrome. Drug treatment can be effective, but compliance is poor for two important reasons: hypertension, even if moderate, is often symptomless and the drugs used to treat hypertension can cause unpleasant adverse effects. Therefore, nutritional advice and treatment, for example reductions in salt intake and alcohol consumption, in addition to physical exercise, are beneficial. Epidemiological or prospective follow-up studies (Engberink *et al.*, 2009; for reviews see McGrane *et al.*, 2011; Raiston *et al.*, 2012; Chrysant & Chrysant, 2013; Buendia *et al.*, 2018; Lee *et al.*, 2018) as well as short-term intervention studies (van Meijl & Mensink, 2011) have demonstrated that the consumption of dairy products, especially low-fat products, is inversely associated with the blood pressure level. Interestingly, although the systolic blood pressure values of both groups of Caucasians increased during the 9-year follow-up in the Atherosclerosis Risk in Communities Study, it was found that the blood pressure levels of those consuming three or more daily servings of low-fat milk increased by 2.7 mmHg less than of those consuming less than one weekly serving. However, in African Americans, the intake of dairy products was not associated with any changes in blood

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pressure (Alonso *et al.*, 2009). Furthermore, a meta-analysis (Soedamah-Muthu *et al.*, 2012) of prospective cohort studies indicated that low-fat dairy and milk could contribute to the prevention of hypertension. Calcium has traditionally been regarded as being the beneficial component present in dairy products. In fact, its role in essential hypertension has been claimed to be even more important than that of sodium (McGarron, 1985). The role of electrolytes, for example, the high levels of calcium, potassium and magnesium as well as the low amounts of sodium present in milk products, has also been discussed (Massey, 2001). During the last 25 years, some short peptides formed in bacterial or enzymatic fermentation have attracted more and more interest due to their angiotensin converting enzyme type 1 (ACE1) inhibiting properties.

   This review will mainly focus on these bioactive tripeptides but it also examines briefly the possible role of other compounds present in milk which, at least theoretically, may beneficially influence blood pressure. For the literature search we used PudMed, Ovid Medline and Scopus databases.

Milk-derived bioactive peptides

Due to its high protein content, milk is a good source of potentially bioactive peptides. At present, bioactive peptides are known to be formed from both the two main proteins in milk: caseins (alpha-, beta- and kappa-casein) and whey proteins, for example, alpha-lactalbumin and beta-lactoglobulin. This review will

focus only on antihypertensive peptides although dairy products have been shown to contain peptides which potentially could influence many other physiological processes in addition to the cardiovascular system (see Park & Nam, 2015; Korhonen & Pihlanto-Leppälä, 2016; Mohanty *et al.*, 2016).

ACE-inhibiting peptides

The antihypertensive effects of milk-derived peptides are believed to be mainly attributable to the inhibition of angiotensin converting enzyme type 1 (ACE1). In fact, many ACE inhibitory peptides have been identified in dairy products from different source of milk, such as cow (Mullally *et al.*, 1997; Sieber *et al.*, 2010; Rodriguez-Figueroa *et al.*, 2012), camel (Moslehishad *et al.*, 2013; Yahya *et al.*, 2017), goat (Chen *et al.*, 2018) and mare (Chen *et al.*, 2010). So far of three tripeptides, isoleucine-proline-proline (Ile-Pro-Pro), valine-proline-proline (Val-Pro-Pro) and leucine-proline-proline (Leu-Pro-Pro), have been the most extensively evaluated (Nakamura *et al.*, 1995; Masuda *et al.*, 1996). ACE1 is the key enzyme in the renin-angiotensin system (RAS), and its inhibition reduces the level of one of the body's most potent vasoconstrictors, angiotensin II, thus ACE1 inhibition is able to reduce the blood pressure. The first orally active ACE-inhibiting medicine, captopril, was approved by the FDA in 1981.

Bioactive peptides can be formed in three different ways from milk proteins: (i) during the fermentation process by certain lactic acid bacteria or yeasts, (ii) during food processing by enzymatic hydrolysis and (iii) by gastrointestinal enzymes. Food processes can be controlled to ensure that they produce and even enrich certain peptides but the third route is a spontaneous physiological process that cannot be controlled.

Lee *et al.* (2007) tested the effects of consumption of a milk drink supplemented with whey peptides in mildly hypertensive subjects. The 12-week trial did not find evidence of any blood pressure reduction as compared to controls, although whey peptides had earlier been shown to have ACE inhibitory activity *in vitro* (Mullally *et al.*, 1997; FitzGerald & Meisel, 1999). Chobert *et al.* (2005) tested the ACE inhibitory activity of the whey protein, β-lactoglobulin from ovine milk, after its digestion with trypsin. They revealed that the hydrophilic, smaller peptides were more potent ACE inhibitors than beta-lactoglobulin itself, although the fragments they tested contained several different peptides.

In the study by Ashar & Chand (2004) fermented Dahi milk enriched with peptide Ser-Lys-Val-Tyr-Pro was fed for 8 weeks to hypertensive subjects from three different age groups: under 45, 45–55 and over 55 years old. The authors conclude that blood pressure was decreased in the middle-aged group (45–55 years old) when compared to placebo group. Also, serum cholesterol levels decreased in all the subjects consuming fermented milk product.

ACE-inhibiting peptides have been isolated from various cheeses (Sieber *et al.*, 2010). For instance, Saito *et al.* (2000) tested the ACE inhibitory activity of different cheeses and evaluated their antihypertensive effects using animal models. The peptide content of the most potent cheese, 8-month-old Gouda, was investigated and pure peptides were isolated and examined. Two peptides – Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln and Tyr-Pro-Phe-Pro-Gly-Pro-Ile-Pro-Asn from α- and β-casein displayed ACE inhibitory and slight antihypertensive activity. Gómez-Ruiz *et al.* (2006) identified peptide fragments different Spanish cheeses. They examined the ACE inhibitory activity of cheeses and chemically synthesised proline in the C-terminal consisted peptides from cheese variates. All tested peptides showed ACE inhibitory action, however, peptide Asp-Lys-Ile-His-Pro was the most potent.

The impact of probiotic bacteria on the content of the bioactive peptides has been investigated during the cheese ripening process. Ong *et al.* (2007) tested the ACE inhibitory activity of Cheddar cheese when certain probiotic lactic acid bacteria were added during the ripening process. They measured the ACE inhibitory activity of the chromatographically separated fractions of the cheese. The peptide content of the most potent fractions was assayed and found to be linked with an amino acid sequence present in different caseins. Ryhänen *et al.* (2001) investigated the ACE inhibitory activity of cheese supplemented with probiotic bacteria and showed that the ripening time affected the ACE inhibitory activity. The most potent peptide sequence was obtained from α_{s1}-casein.

Ile-Pro-Pro, Val-Pro-Pro and Leu-Pro-Pro

When milk is fermented with *Lactobacillus helveticus*, or with common yeast *Saccharomyces cerevisiae*, three tripeptides, Ile-Pro-Pro, Val-Pro-Pro and Leu-Pro-Pro, are released from beta-casein. It has been demonstrated with different experimental hypertension models that the administration of tripeptide-containing products can lower elevated blood pressure (Table 1). The main mechanism of action is believed to be the inhibition of ACE. However, it seems that consumption of these tripeptides affects also other blood pressure lowering mechanisms such as NO bioavailability (Hirota *et al.*, 2011) and the angiotensin (1–7)-Mas-receptor-axis (Siltari *et al.*, 2016). ACE inhibition also prevents the breakdown of bradykinin which may act synergistically with the reduced formation of angiotensin II to lower the blood pressure by increasing arterial vasodilatation (Siltari *et al.*, 2017).

Table 1 Summary of tripeptide feeding in experimental models of hypertension

Study	Experimental model	Duration (weeks)	Peptide dailydose (mg kg^{-1})	SBP compared to controls (mmHg)	Endothelium-dependent vascular relaxation compared to controls	Notes
Nakamura *et al.* (1995)	SHR	8 h	0.9	−18	Not measured	Single dose
Nakamura *et al.* (1996)	SHR	16	dns	−19	Not measured	Diet contained sour milk powder containing tripeptides
Masuda *et al.* (1996)	SHR	6 h	0.4	−26	Not measured	Single dose
Sipola *et al.* (2001)	SHR	12	2.5–3.5	−17	Not measured	
Sipola *et al.* (2002)	SHR	14	1 ± 0.5	−21	Not measured	Daily dose/rat
Jauhiainen *et al.* (2005)	SHR	9	1.5 ± 0.1	−17	No differences	Daily dose/rat
Jauhiainen *et al.* (2010a, 2010b)	dTGR	3	4.1 ± 1.1	−19	Not measured	
Jäkälä *et al.* (2009c)	SHR	8	2.8–5.2	−14	No differences	
Jäkälä *et al.* (2009a)	G-K + salt	8	4.6–6.6	−12	Improved	
Jäkälä *et al.* (2010)	SHR	8	6.3–8.7	−14	No differences	
Kim *et al.* (2010)	SHR	8	dns	dns	Not measured	Orally administered powder containing tripeptides (0.6%) 10 mg kg^{-1} daily
Ehlers *et al.* (2011)	SHR	6	4.1 ± 0.1	−16	Improved	Experimental drink which contained plant sterols (daily dose 1.9 g kg^{-1})
Ehlers *et al.* (2012)	SHR	8	4.2 ± 0.1	−18	No differences	Experimental drink which contained plant sterols (daily dose 2 g kg^{-1})
Siltari *et al.* (2017)	SHR	6	4.7–5.1	−20	No differences	Daily dose/rat

dns, data not shown; dTGR, double transgenic rats harbouring human renin and human angiotensinogen genes; G-K + salt, salt loaded Goto-Kaki-zaki rats; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats.

Not only blood pressure is lowered by tripeptide feeding but there are also improvements in the endothelium-dependent vascular function (Jäkälä et al., 2009a; Ehlers et al., 2011; Nonaka et al., 2014) (Table 1). Furthermore, the endothelium-dependent vascular relaxation persisted when mesenteric artery rings from hypertensive animals were bathed in a buffer containing Ile-Pro-Pro at +4C even after 24 h (Jäkälä et al., 2009b). Hirota et al. (2011) showed that Ile-Pro-Pro and Val-Pro-Pro could relax pre-contracted isolated aorta rings of normotensive animals and this dilatation could be abolished by removal of the endothelium, by blocking nitric oxide formation or by treatment with bradykinin type 2 receptors. Thus, these tripeptides seem to induce the formation of nitric oxide and other vasodilating compounds. Recently, it was reported that there were increases in the plasma bradykinin concentration of normotensive volunteers after an acute intake of a high dose of the tripeptides (Nussberger et al., 2018).

In clinical trials, tripeptide-containing dairy products have lowered blood pressure of mildly/borderline hypertensive humans (see Nongonierma & FitzGerald, 2015; Park & Nam, 2015; Korhonen & Pihlanto-Leppälä, 2016; Mohanty et al., 2016; Marcone et al., 2017). Table 2 illustrates the main findings from all of the meta-analyses from milk peptides conducted so far. Turpeinen et al. (2013) analysed 19 clinical trials with a total of over 1500 patients and concluded that the overall antihypertensive effect of tripeptides was −4.0 mmHg in the systolic blood pressure (SBP) and −1.9 mmHg in the diastolic blood pressure (DBP). If the analysis was restricted only to studies conducted in Asian populations (Chanson-Rolle et al., 2015), then the overall blood pressure lowering effect of tripeptide consumption was even higher: −5.6 mmHg in SBP and −2.6 mmHg in DBP. One meta-analysis restricted to only the European studies (Cicero et al., 2013) claimed that the blood pressure lowering effect was slightly lower (−1.3 mmHg in SBP and −0.6 mmHg in DBP) than the values obtained in analyses involving all the clinical studies.

The meta-analysis conducted by Qin et al. (2013) reported similar results: −1.7 mmHg SBP and −0.8 mmHg DBP. However, their sub-analyses revealed interesting findings that is that the blood pressure lowering impact of tripeptides varied not only between the Asian and European populations but also between the study designs (single or double blinded), baseline blood pressure level of subjects (pre-hypertensive or hypertensive) and even the source of the peptide (fermentation or enzymatic hydrolysis) was

Table 2 Summary of meta-analysis of clinical studies on bioactive peptide containing products

Meta-analysis	No. of trials	No. of subjects	All subjects (change in mmHg (95% CI))		Pre-hypertensive (change in mmHg (95% CI))		Hypertensive (change in mmHg (95% CI))		Notes
			SBP	DBP	SBP	DBP	SBP	DBP	
Xu *et al.* (2008)	12	623	−4.8 (−6 to −3.7)	−2.2 (−3.1 to −1.3)	−3.2 (−4.4 to −1.9)	−1.5 (−3 to 0)	−5.6 (−6.4 to −4.8)	−2.5 (−3.6 to −1.4)	
Pripp (2008)	17	826	−5.5 (−7.12 to −3.14)	−2.42 (−3.82 to −1.03)	dns	dns	dns	dns	Analysis included also other food peptide interventions as well as those that were milk-derived
Cicero *et al.* (2011)	18	1691	−3.73 (−6.7 to −1.76)	−1.97 (−3.85 to −0.64)	dns	dns	dns	dns	
Turpeinen *et al.* (2013)	22	1532	−4.0 (−5.9 to −2.1)	−1.9 (−3.1 to −0.8)	dns	dns	dns	dns	
Qin *et al.* (2013)	28	1919	−1.66 (−2.48 to −0.84)	−0.76 (−1.31 to −0.2)	−0.59 (−1.32 to 0.14)	−0.04 (−1.11 to 1.04)	−2.36 (−3.58 to −1.14)	−1.56 (−2.78 to −0.33)	
Cicero *et al.* (2013)	15	1306	−1.28 (−2.09 to −0.48)	−0.59 (−1.18 to −0.01)	dns	dns	dns	dns	Only European populations
Fekete *et al.* (2015)	30	2200	−2.95 (−4.17 to −1.73)	−1.51 (−2.21 to −0.8)	−2.68 (−3.61 to −1.74)	−1.38 (−2.13 to −0.63)	−3.0 (−4.71 to −1.29)	−1.9 (−3.38 to −0.42)	
Chanson-Rolle *et al.* (2015)	18	1194	−5.63 (−6.87 to −4.39)	dns	−3.43 (−4.65 to −2.21)	dns	−8.35 (−10.25 to −6.44)	dns	Only Asian populations

DBP, diastolic blood pressure; dns, data not shown; SBP, systolic blood pressure.

crucial. A fermentation process may produce also different bioactive peptides or compounds other than those of interest. This means that products manufactured by fermentation may have a greater impact due to the fact that they may also contain some other, still to be characterised, blood pressure lowering compounds. Nevertheless, tripeptides originating from different production methods (enzymatic hydrolysis or fermentation with *Lactobacillus helveticus*) were roughly equally successful in lowering blood pressure as compared to water consumption in hypertensive rats (SHR) (Jäkälä *et al.*, 2010).

It is not clear, why tripeptide consumption seems to be more efficient in Asian populations but one can speculate that lifestyle related factors, such as diet and genetic factors may play a role. For instance, cheeses contain tripeptides which are formed during the cheese ripening process (Sieber *et al.*, 2010). Cheeses have a more important part in the daily diet in Europe than in Asia. Thus, a European population is most likely consuming dairy products and thus bioactive tripeptides already before clinical trials and will not benefit so much from supplementation with some specific peptide-enriched dairy product.

Four of the meta-analyses (Xu *et al.*, 2008; Qin *et al.*, 2013; Chanson-Rolle *et al.*, 2015; Fekete *et al.*, 2015) conducted sub-group analyses separately for pre-hypertensive and hypertensive subjects. These analyses revealed that tripeptide feeding lowered blood pressure more efficiently in hypertensive subjects although the reduction was statistically significant also in the pre-hypertensive subjects. When recruiting mildly hypertensive subjects to clinical trials, we noted that out of the nearly 400 mildly hypertensive volunteers (BP as measured by a nurse or physician >140/90 mmHg), only 96 were clearly still hypertensive after the run-in period when assessed by ambulatory 24 h monitoring and ultimately only 89 were enrolled in the yogurt intervention trial based on truly elevated blood pressure (Jauhiainen *et al.*, 2007). This possible pitfall in study population recruitment has not been taken into account in all of the clinical studies.

Based on both the clinical and experimental studies, it does seem to be the case that tripeptide-containing products need to be consumed daily for several weeks before any significant blood pressure reduction can be detected. This was seen also in a subgroup meta-analysis where the analysis was performed based on the duration of the peptide intake (less or more than 8 weeks): the antihypertensive effect of tripeptide ingestion was greater after consumption for 8 weeks or more (Qin *et al.*, 2013). Interestingly, when the 8-week-long treatment with a peptide containing fermented milk was interrupted, the slightly reduced blood pressure returned to the initial level during a 4-week-long follow-up (Seppo *et al.*, 2002).

It seems that tripeptide feeding decreases arterial stiffness in humans when this is assessed with an augmentation index, AIx (Jauhiainen *et al.*, 2010a, 2010b) even without any changes in blood pressure. In the long run, this effect may also decrease blood pressure; however, with or without an effect on blood pressure, the reduced arterial stiffness will decrease the risk of the development of atherosclerosis and the risk of serious cardiovascular events such as stroke and cardiac infarcts. Furthermore, in an *in vitro* model, Aihara *et al.* (2009) showed that pre-incubation of monocytic cells with Val-Pro-Pro decreased the adhesion of monocytes to human endothelial cells. They also showed that Val-Pro-Pro decreased the expression of integrins and reduced the phosphorylation of c- Jun N-terminal kinase (JNK), two important pathways in the adhesion of monocytes to endothelium. The authors concluded that Val-Pro-Pro might possess an anti-atherosclerotic potential in conditions where there is low grade inflammation of the endothelium, like in hypertension and aging.

Bioavailability of food-derived components, that is, their absorption from intestine to the systemic circulation is an important issue when examining the physiological effects of nutrients. Ile-Pro-Pro, Leu-Pro-Pro and Val-Pro-Pro have been detected in the circulation after oral administration of dairy products enriched with these peptides both in experimental and human studies (Jauhiainen *et al.*, 2007; van der Pijl *et al.*, 2008; Kawaguchi *et al.*, 2012; Gleeson *et al.*, 2017). The levels of the detected tripeptides are low; however, but there were some indications that there was an accumulation of peptides in blood pressure regulating organs. Jauhiainen *et al.* (2007) administered radio-labelled Ile-Pro-Pro to spontaneously hypertensive rats (SHR) both intravenously and orally and subsequently radioactivity was detected in aorta, heart and kidneys for as long as 48 h after their administration. Kawaguchi *et al.* (2012) detected labelled Ile-Pro-Pro and Val-Pro-Pro in the endothelium of rats after oral administration of these compounds. They also measured Ile-Pro-Pro and Val-Pro-Pro concentrations in plasma and tissues; liver, kidneys, lung and aorta (concentrations from lowest to highest). Foltz *et al.* (2007) conducted a trial in volunteers; it was found that Ile-Pro-Pro selectively escaped intestinal breakdown and reached the circulation undegraded.

Proline-proline bond containing peptides has been claimed to be resistant to digestion in the gastrointestinal tract. The stability of Ile-Pro-Pro against intestinal degradation was tested using rat intestinal rinses, intestinal proteases and homogenates from intestine and liver (Ohsawa *et al.*, 2008; Gleeson *et al.*, 2015). Ile-Pro-Pro seemed to be rather stable in these *in vitro* setups. Furthermore, Ile-Pro-Pro was not cytotoxic even after chronic exposure to Hep G2 and caco-2 cells (Gleeson *et al.*, 2015).

The toxicity of tripeptide feeding has been evaluated in an embryo-foetal development test in rabbits, in a 90-day repeated-dose oral gavage test in rats as well as in a pre- and post-natal development test in rats (Dent *et al.*, 2007) with no serious adverse effects being observed in any of these tests. Other toxicity studies on tripeptide feeding have come to the same conclusion (Beltran-Barrientos *et al.*, 2017). Furthermore, none of the clinical trials have reported any significant adverse effects associated with tripeptide feeding, with the adverse effects of the tripeptide consumption being typical of dairy products such as abdominal discomfort.

Antithrombotic peptides

Studies conducted already in the 1980s and 1990s showed that peptides derived from lactoferrin (Fiat *et al.*, 1989; Qian *et al.*, 1995) and caseins (Jolles *et al.*, 1986; Fiat *et al.*, 1989) inhibited ADP-induced platelet aggregation *in vitro*. Drouet *et al.* (1990) investigated the effects of lactoferrin-derived peptides on platelet aggregation *in vitro* as well as on arteriolar thrombosis *in vivo* in rats and guinea pigs; it was noted that these peptides inhibited both the aggregation of activated platelets and prevented thrombosis. Furthermore, Bal dit Sollier *et al.* (1996) showed that the antithrombotic effect of caseinoglycopeptides was even more marked *in vivo* than *in vitro*; in guinea pigs, the antithrombotic effect was achieved *in vivo* with a lower concentration than that preventing platelet aggregation *in vitro*. In addition, the antithrombotic activity of whey proteins has been investigated; κ-casein macropeptide and its tryptic hydrolysate have been reported to exert an inhibitory activity against platelet aggregation (Manso *et al.*, 2002).

Peptides with opioid-like properties

Milk contains peptides with a natural opioid-like activity; however, these compounds can also be formed *in vitro* during the fermentation process or cheese ripening or they can be formed *in vivo* during gastrointestinal digestion (Nguyen *et al.*, 2015). This review examines only the possible cardiovascular effects of opioid peptides, but it is clear that these compounds have effects on many other physiological functions, such as gastrointestinal motility, food intake, analgesia and immunomodulation (see, Kostyra *et al.*, 2004).

β-Casomorphines are components of β-casein. The most widely studied compound in this family is β-casomorphine-7. The opioid-like peptides formed from α-casein are called α-exophins and those from κ-casein are termed as casoxins. Opioid peptides can be formed also from whey proteins, that is α-lactorphins from α-lactalbumin and β-lactorphins from β-lactoglobulin

(Wada *et al.*, 2017). Han *et al.*, 2013 investigated the effects of β-casomorphine-7 on diabetic cardiomyopathy in a rat model of diabetes. They found that β-casomorphine-7 exerted protective effects on the heart and possessed also antioxidative properties.

Nurminen *et al.* (2000) administered sucutaneously α-lactorphin, a four amino acid peptide derived from α-lactalbumin, to hypertensive and normotensive rats. The blood pressure was reduced similarly in both strains and this effect could be abolished by pre-treatment with the opioid receptor (mu, delta and kappa receptors) antagonist emphasising the role of opioid receptors in the pressor response. Furthermore, Hayashida *et al.*, 2004 investigated the effects of bovine lactoferrin on blood pressure and vascular function in normotensive rats; they reported that an intravenous injection of lactoferrin dose-dependently lowered blood pressure. Once again, the hypotensive effect was blocked by pre-treatment with naloxone. The authors concluded that lactoferrin's hypotensive effect could partly be regulated via opioid receptors, although vascular relaxation was dependent on nitric oxide release from the endothelium.

Other milk components with possible blood pressure lowering activity

In addition to bioactive peptides, dairy products contain many other factors which, at least theoretically, can lower blood pressure and/or improve vascular dysfunction (see Chrysant & Chrysant, 2013).

The electrolytes present in dairy products, most notably *calcium*, *potassium* and *magnesium* lower the systolic blood pressure by 1.3–4.6 mmHg and diastolic blood pressure by 0.2–3.8 mmHg in long-term human interventions (see, Kris-Etherton *et al.*, 2009) and higher dietary calcium intake is slighty associated with lower risk of developing hypertension (Jayedi & Zargar, 2018). McGarron was one the pioneers who studied the relationship of calcium and blood pressure; he identified low serum concentrations of ionised calcium in patients with hypertension (see McGarron, 1982). The calcium effect was complex, because a low calcium intake increased the intracellular calcium concentration as well as elevated vitamin D3 and parathyroid hormone concentrations. These changes induced calcium influx into vascular smooth muscle cells and further increased the vascular tone. Acute and chronic effects of calcium are different. Sodium status and possibly even the calcium salt used in the dietary experiments can influence the findings. The role of adrenergic stimulation due to acute hypercalcaemia has been demonstrated both in normotensive and even more clearly in borderline hypertensive subjects (Bianchetti *et al.*, 1983). Since the calcium concentration in milk is modest (approx. 120 mg per 100 mL), it is not anticipated

that the acute blood pressure or vascular effects would be influenced by drinking milk. In epidemiological studies, exact data on the consumption of daily milk and other dairy products are, at best, approximations and most often not correlated with other vasoactive components present in the diet. It seems that it is the balance between sodium and calcium intake which is important. Increased natriuresis has been proposed as a mechanism by which calcium intake may reduce blood pressure (Lasaridis *et al.*, 1989) since this would be accompanied by a reduction in total body water content (Zemel *et al.*, 1988), in contrast to the acute effects of calcium. These observations were supported by experimental findings reported by Mäkynen (1996) showing that the blood pressure of hypertensive rats on a high calcium diet (2.5%) lowered in a 10–12-week-long intervention but a *magnesium* enriched diet alone or in combination with calcium exerted no additive blood pressure lowering effect. In contrast, a dietary potassium and magnesium enriched salt alternative improved further the beneficial cardiovascular effects of an ACE-inhibitor in hypertensive rats (Mervaala *et al.*, 1994). A recent review concludes, based on studies on low (Kostov and Halacheva, 2018) and high magnesium intake, that magnesium supplementation has blood pressure lowering effect (Schutten *et al.*, 2018). However, the contents of magnesium (approx. 11 mg per 100 mL) and potassium (approx. 150 mg per 100 mL) in milk are so low that it is unlikely that their concentrations in plasma and tissue would be influenced by dietary intake in individuals with normal kidney function.

A low level of *vitamin D* in plasma has been postulated to be associated with elevated blood pressure and further cardiovascular events. In an extensive systematic review and meta-analysis (Beveridge *et al.*, 2015), it was concluded that vitamin D supplementation, even at much higher doses than present in milk (1 μg per 100 mL without enrichment), was ineffective as a blood pressure lowering component and should not be considered as hypertension treatment, but it is not known whether it could act synergistically with dairy calcium.

L-arginine is a precursor for the physiological vasodilator nitric oxide (NO) in endothelium. The arginine content in milk is about 100 mg per 100 mL and in cheddar cheese about 250 mg per 100 g. Therefore, milk arginine cannot on its own be a potential blood pressure lowering factor, because in clinical trials when intravenously administered L-arginine was administered as a single dose of either 6 or 30 g, only the higher dose lowered blood pressure and reduced total peripheral resistance in healthy humans (Bode-Böger *et al.*, 1998). Alba *et al.* (2016) hypothesised that acute dairy milk consumption increases endothelial nitric oxide (NO) in testing the cutaneous

vasodilatation in healthy volunteers, but could not show that ingestion of milk would augment NO-dependent vasodilatation in the cutaneous microcirculation.

It can be summarised that with respect to the known vasoactive components of milk, only calcium – in addition to the bioactive peptides – seems to contribute to the beneficial effects of milk on blood pressure and arterial function.

Conclusions

Milk contains a great number of proteins which either during food processing or in the gastrointestinal tract can be degraded into peptides that possess antihypertensive effects. Epidemiological studies have revealed that a high intake of low-fat dairy products and/or fermented dairy products is associated with reduced blood pressure. Although there are several possible mechanisms by which dairy products can lower blood pressure, ACE inhibition by the bioactive peptides has been the most extensively studied mechanism. These peptides and calcium are the most important blood pressure lowering milk components. The antithrombotic and opioid-like effects of the peptides may be involved but more clinical studies are needed to confirm these findings.

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Conflict of interest

All authors declare that there is no conflict of interest.

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