Review Paper / Derleme Makale

Blood Pressure Lowering Effect of Fermented Milk Products

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ABSTRACT

Hypertension (HTN) is a major risk factor for the development of cardiovascular diseases. Therefore, there is a need to lower blood pressure (BP) to reduce the risk of these degenerative diseases. Fermented milks contain several potential factors that can lower BP including calcium and microbial metabolites particularly the angiotension-converting enzyme (ACE) inhibitory peptides and γ-aminobutyric acid. Animal studies clearly demonstrated the BP lowering effect of fermented milk while results from clinical trials were controversial due to a large number of variables that should be considered in clinical trials. An overview on the antihypertensive effect of fermented milk products is presented and discussed in this review.

Keywords: Fermented milks, Hypertension, Calcium, ACE-inhibitory peptides, γ-Aminobutyric acid

Fermente Süt Ürünlerinin Kan Basıncını Düşürücü Etkisi

ÖZ

Hipertansiyon (HTN), kardiyovasküler hastalıkların oluşumu için önemli bir risk faktörüdür. Bu nedenle, söz konusu dejeneratif hastalıkların riskini azaltmak için kan basıncını düşürme ihtiyacı vardır. Fermente edilmiş sütler, kalsiyum ve mikrobiyal metabolitler, özellikle de anjiyotensin-dönüştüren enzim (ACE) inhibitörü peptitler ve γ-aminobütirik asit dahil olmak üzere, kan basıncını azaltabilen birkaç potansiyel faktör içerir. Hayvan çalışmaları, fermente edilmiş sütün kan basıncını azaltma etkisini açıkça gösterirken, klinik çalışmalarda elde edilen sonuçlar, klinik çalışmalarda dikkate alınması gereken çok sayıda değişkenden dolayı tartışmalıdır. Bu makalede fermente süt ürünlerinin antihipertansif etkisine genel bir bakış sunulmakta ve tartışılmaktadır.

Anahtar Kelimeler: Fermente sütler, Hipertansiyon, Kalsiyum, ACE-inhibitörü peptitler, γ-aminobütirik asit

INTRODUCTION

Hypertension (HTN) is a persistent elevation of blood pressure which is not caused by underlying cardiac, endocrine, or renal disease [1]. It is a major risk factor for the development of cardiovascular diseases including stroke, coronary heart disease, heart failure, and end-stage renal disease. For this reason, there is a need to lower blood pressure (BP) in order to reduce the risk of cardiovascular diseases. Hypertension is divided into two stages; stage 1 includes patients with systolic blood pressure (SBP) 140–159 mmHg or diastolic blood pressure (DBP) 90–99 mmHg, and stage 2 includes patients with SBP≥160 mmHg or DBP≥100 mmHg. HTN is widely prevalent in Egypt [2] and worldwide [3].The use of antihypertensive agents aims to bring and to stabilize blood pressure to as close to as possible the normal BP range. Even with small decrease in SBP as 6 mmHg a significant decrease in the potential risk of stroke (14%) was reported [4]. The role of food and food constituents in ameliorating HTN has been the subject of extensive research during the past decades. Evidences have been clearly presented about the relation between some foods and food constituents and

hypertension. For example, the Dietary Approaches to Stop Hypertension (DASH) provided evidence that nutrients, food groups, and whole eating patterns can influence blood pressure [4]. This trial adapted a diet high in fruits and vegetables; or a combination diet with high fruits, vegetables and low-fat dairy products to reduce the incidence of HTN. The greatest benefits for clinically significant decreases in SBP and DBP were obtained with combination diet group, which include 2 to 3 servings of low-fat dairy products per day.

Recent studies gave evidence that consumption of dairy products is involved in the regulation of blood pressure [5]. The potential benefits of dairy food have generally been ascribed to the major nutrients provided by dairy including calcium, potassium, magnesium and vitamin D. The Framingham Heart Study [6] with approximately 15 years of follow-up showed an inverse association of dairy intake with the annual change of the average BP and long-term development of incident HTN. A reduction of 6% (95% CI 1.10) in risk incidence of HTN was reported to be associated with each additional serving of yogurt. Therefore, consumption of dairy products may have the potential benefit control of BP and prevention or delay the onset of HTN.

A special and growing interest in fermented milk has been witnessed during the last decade as food that exhibit several potential health effects including lowering

BP and reducing the incidence of HTN. This has been attributed mainly to the release of inhibitory peptides against angiotension converting enzymes (ACE) from milk proteins by microbial fermentation [7].

The present review aims to give an overview and update on the relation between the consumption of fermented milks and BP and incidence of HTN.

POTENTIAL FACTORS RESPONSIBLE FOR BLOOD PRESSURE LOWERING EFFECT OF FERMENTED MILKS

The potential BP lowering effect of fermented milks arises from the combined effects of BP lowering milk constituents (calcium, potassium and magnesium) and bacterial metabolites (Fig 1). Fermented milks contain all the milk calcium but mainly in the soluble form of calcium lactate with the same bioavailability of calcium in milk [8]. Also, fermented milks contain all the milk potassium and magnesium contents known with their BP lowering effect. Milk proteins are rich sources of bioactive peptides and during fermentation and storage several BP lowering peptides are released which play an important role in BP lowering effect of fermented milks. Other bacterial metabolites such as gamma-aminobutyric acid have potential lowering effect on BP.
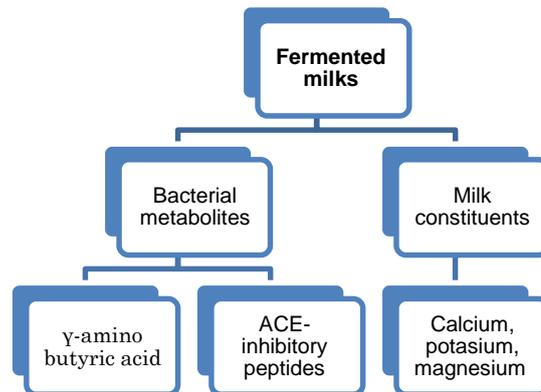

Figure 1. Potential blood pressure lowering factors in fermented milks

Calcium

A significant (P<0.05) linear decrease in SBP and DBP was found with increasing the intake of dairy calcium in both men (7543 subject) and women (8053 subjects) aged 25–69 y [9]. High BP was significantly (P<0.001) related to a decrease (3-10%) in age-adjusted intake of calcium from dairy sources between the highest and the lowest BP groups. They also found that vitamin D intake had no significant effect of on BP. They concluded that the small effect of calcium on BP could have a significant primary prevention effect on cardiovascular diseases.

Green et al. [10] evaluated the effect of high-calcium skim milk and potassium-enriched high calcium skim milk on BP of healthy people compared with non-enriched skim milk. They recorded small hypotensive

effect of potassium-enriched high calcium skim milk in adults aged >40 y.

In a prospective cohort study that covered 28886 US women aged≥45 years [11] investigated the associations of the intake of dairy products, calcium, and vitamin D with the incidence of hypertension. The risk of hypertension decreased in the higher quintiles of dietary calcium (0.87) and dietary vitamin D (0.95), but calcium or vitamin D supplements had no effect. Adjustment for dietary calcium significantly attenuated the inverse association of low-fat dairy intake with risk of hypertension, whereas adjustment for dietary vitamin D did had no effect on the association.

Inverse associations between dairy product consumption and SBP and DBP have been observed in cross-sectional studies [5] whereas calcium, potassium

and magnesium have been the most notably dairy nutrients to have a BP lowering effect. However, inconsistent results were obtained from randomized clinical trials examining the effect of calcium and the combination of calcium, potassium and magnesium on BP. Modest reductions (-1.27 to -4.6 mmHg for SBP, and -0.24 to -3.8 mm Hg for DBP) were generally found. The role of calcium in lowering BP has been explained on the basis that low calcium intake increases intracellular calcium concentrations which in turn increases 1,25-dihydroxyvitamin D(3) and parathyroid hormone (PTH). This cause calcium influx into vascular smooth muscle cells leading to greater vascular resistance.

Hilpert et al. [12] fed adults with stage 1 hypertension a dairy-rich, high fruits and vegetables diet (DFV), a high fruits and vegetables (FV) diet and an average Western diet (control) for 5 weeks each. They concluded that consumption of dairy products beneficially affect the erythrocyte (Ca) (i) resulting in improved BP which they defined as (Ca) (i) response.

Metabolites of Microbial Fermentation

Lactic acid bacteria (LAB) are commonly used in the manufacture of yogurt and other fermented milk products. The traditional and probiotic cultures used include thermophilic and mesophilic strains of *Streptococcus*, *Lactococcus* and *Lactobacillus* species. LAB are fastidious organisms that require various nutrients to survive and grow. Among these various nutrients, amino acids are essential for their growth. LAB metabolizes the large proteins found in milk to obtain free essential amino acids with the help of their proteolytic system [13].

To date, the proteolytic systems of few LAB strains such as *Lactococcus (Lc) lactis, Lactobacillus (Lb.) helveticus* and *Lb. delbrueckii* ssp. *bulgaricus* have been studied and characterized. *Lc. lactis* ssp. *lactis* is a LAB that has a well-studied and characterized proteolytic system [13] including cell wall proteinases and intracellular endo and amino acid peptidases. The cell wall proteinases of the Lactococcus strains are serine proteases that are grouped into at least two types; the PI-type that hydrolyses β-CN and has little activity for α_{S1}-CN, and the PIII-type that hydrolyses both caseins [14]. *Lb. helveticus* proteinases are also serine proteinases that can be grouped into PI- and PIII-types. Proteolysis of milk proteins by LAB involves three steps. In the first step, the proteolytic LAB break down the large protein molecules into 4-18 amino acid residues termed oligo-peptides with the help of their cell wall associated extracellular proteinase. In the second step, the oligo-, di-, and tripeptides are transported across the cell wall and internalized by the LAB cells through oligopeptide (Opp), dipeptide (Dpp) and tripeptide (DtpT) transport systems, respectively. In the third step the internalized peptides are further broken down and release free amino acids with the help of intracellular peptidases in what is an important step in the metabolism of LAB. The presence of peptidases like PepX are important for the degradation of casein-derived high proline oligopeptides

because of their capability to hydrolysis of proline-containing sequences. The release of the ACE-inhibitory peptides was positively correlated with the extracellular proteinases indicating the proteolysis of casein by these enzymes to be the most important step for the production of the bioactive peptides [15].

The release of these internal enzymes from dead cells may play an important role in the qualitative and quantitative composition of the protein derived products in fermented milk. The autolysis rate of the dead LAB cells and release of the internal enzyme system varies widely which determine the importance of these enzymes in protein degradation products of fermented milk.

Angiotention-converting Enzyme Inhibitory Peptides

LAB produces small bioactive peptides of different biological activities as intermediate or secondary metabolites during proteolysis of milk protein. Peptides exhibiting inhibitory activity on angiotension-converting enzyme (ACE) have received special attention as LAB metabolites that have direct effect on hypertension. The most important metabolic pathway in the control of blood pressure is the renin-angiotensin system [16]. It plays a fundamental role in blood pressure by converting angiotensin I into angiotensin II, a potent vasoconstrictor; in the mean time it hydrolyzes the vasodilator peptides bradykinin and kallidin. Therefore, the antihypertensive effect of fermented is affected by the used strain and the conditions of fermentations and post-fermentation changes.

LAB Strain

Qualitative and quantitative differences in the released ACE-inhibitory peptides in fermented milk by the used LAB strains were reported.

Yamamoto *et al.* [17] found that most of the milk fermented by *Lb. helveticus* or *Lb. delbrueckii* subsp. *bulgaricus* to show higher ACE- inhibitory activity than those fermented by *Lb. casei, Lb. acidophilus, Lb. delbrueckii* subsp. *lactis, Str. thermophilus, Lc. lactis* subsp. *cremoris,* or *Lc. lactis* subsp. *lactis.*

Wild stains of LAB isolated from raw milk were tested for their ability to produce ACE-inhibitory activity in fermented milk [18]. Four *Enterococcus faecalis* of the isolated strains exhibited high ACE inhibitory activity than other isolated LAB strains.

Donkor et al. [19] assessed the growth characteristics and release of ACE-inhibitory activity of LAB and probiotic strains of *Lb. acidophilus* (two strains), *Bifidobacterium* (*B. lactis* and *B. longum*), *Str. theromophilus* and *Lb. delbreukii* ssp *bulgaricus* grown in fermented milk. The extent of proteolysis varied among strains and appeared to be time dependant. All the cultures released peptides with in vitro ACE-inhibitory activity during growth with *B. longum* Bl 536

and *Lb. acidophilus* L10 having IC_{50} values of 0.196 and 0.151 mg/mL, respectively.

Nielsen et al. [20] screened 13 LAB strains for their ACE-inhibitory activity in fermented milk. They found that the tested *Str. thermophilus* and *Lb. acidophilus* strains did not give rise to significant ACE inhibitory activity. The four *Lc. lactis* strains behaved similarly in fermentation, proteolysis and ACE-inhibition. The products made with the seven *Lb. helveticus* strains varied. They found positive relation between the proteolytic activity of the tested strains and the developed ACE-inhibitory activity.

Pihlanto et al. [21] tested 25 LAB strains including *Lb. acidophilus*, *Lb. casei*, *Lb. helveticus*, *Lb. jensenii*, *Lb. reuteri*, *Lb. rhamnosus*, *Lc. lactis* ssp. lactis, *Lb. raffinolactis* and *Leuconostoc mesenteroides* ssp. *cremoris* of their ACE-inhibitory activity in fermented milk. The variable ACE inhibitory potencies of these strains were correlated to the degree of protein hydrolysis.

Compared with other lactic acid bacteria, *Lb.casei* YIT 9029 and *B. bifidum* MF 20/5 were able to induce strong ACE inhibitory activity [22]. *Lb. casei* (Lc210), *B. animalis* ssp12 (Bb12), *Lb. delbrueckii* ssp. *bulgaricus* (Lb11842) and *Lb. acidophilus* (La2410) were grown in 12% of reconstituted skim milk (RSM) or 4% of whey protein concentrates (WPC-35) with (0.14%) or without addition of the microbial derived enzyme Flavourzyme [23]. All tested strains showed higher proteolytic activity and produced more antihypersensitive peptides in RSM medium than in WPC medium. Combination with Flavourzyme, also increased LAB growth and proteolytic and ACE-I activities. Of the four strains used the performance Bb12 and La2410 was better than Lc210 and Lb11842.

Rasika et al. [24] reported that the ACE inhibitory activity of the milk samples fermented with single or mixed cultures of *Lb. lactis* ssp. *lactis* NBRC 12007 and *S. cerevisiae* K7 were significantly (P<0.05) different.

Out of a total of 59 *Lb. helveticus* strains isolated from traditional fermented dairy products, three strains expressed the highest ACE-inhibitory activity [25]. One of these three strains (*Lb. helveticus* IMAU80872) showed better tolerance to gastrointestinal proteases and thermostability of its ACE-inhibitory activity in fermented milks. Six peptides have been identified three of which i.e. ALPM, VAGTWY, IPI were previously identified as ACE-inhibitory peptides.

Effect of Fermentation and Post-Fermentation Conditions

The composition of the medium, and fermentation storage conditions have significant effects on the release of ACE-activity in fermented milk.

Effect of the type of milk. Sultan et al. [26] prepared yogurts from goat, sheep, cow and buffalo's milk. The water soluble peptide fractions of these yogurts were extracted and their antihypertensive and antioxidant activities were quantified at different intervals during storage. Yogurt made from goat milk exhibited highest antihypertensive and antioxidant activities as compared to yogurts prepared from other milks.

Effect of milk fortification. Leclerc *et al.* [27] found that fortification of milk with casein increased the ACE-inhibitory activity developed in fermented milk prepared by the use of *Lb. helveticus* but fortification of milk with whey proteins did not give the same effect.

Effect of pH. The effect of pH at the end of fermentation on the developed ACE-inhibitory activity was controversial. Nielsen et al. [20] concluded that fermentation with *Lb. helveticus* should be terminated at pH 4.6 in order to release the highest ACE-inhibitory activity in fermented milk. Extending fermentation to pH 3.5 reduced the developed ACE-inhibitory activity. In the mean time, they found that fermentation with lactococcus should be continued until pH 4.3 to achieve the optimum ACE-inhibitory activity in fermented milk. Pihlanto et al. [21] found that modification of the fermentation conditions or pH control had no effect of the developed ACE-inhibitory activity by the 25 tested LAB strains. Cold storage was found to increase dramatically the ACE-inhibitory activity of some products [20].

Effect of fermentation temperature. Otte et al. [28] reported that temperature significantly affect the growth, lysis and release of ACE-inhibitory activity of fermented milk prepared using *Lc. lactis* or *Lb. helveticus.* The profile of the released peptides was almost unchanged by fermentation temperature or cell lysis. They suggested that the cell wall proteinase to be the primary catalyst in the release of ACE-inhibitory peptides. The ionic calcium released during milk fermentation could contribute to the ACE-inhibitory activity of fermented milks [22]. Li et al. [29] found that the fermentation temperature, inoculum level and rotation speed were the most significant factors affecting the production of ACE inhibition in milk fermented with *Kluyveromyces marxianus.* They found that the optimum conditions for maximum ACE-inhibition activity production (81.23%) were incubation at 32ºC, initial pH of 6.5, inoculation level of 6% and rotation speed of 189 rpm.

Released ACE-Inhibitory Peptides

β-Casein is considered the main source for the released ACE-inhibitory peptides by the action of microbial fermentation. However, other casein fractions and whey proteins have been reported to generate ACE-inhibitory peptides during microbial fermentation. The ACE-inhibitory peptides consist of 2-20 amino acid residues that differ widely in their potency. The activity of ACE-inhibitory peptides (IC_{50}) is usually expressed as "the concentration (mg/mL or µM/mL) that inhibit 50% of the enzyme.

1. Lactotripeptides

The most extensively studied ACE-inhibitory peptides released in fermented milk have been the tripeptides IPP and VPP which have the highest inhibitory activity compared to other ACE-inhibitory peptides. These peptides are produced in milk fermented by *Lb. helveticus* [30, 31]. The IPP corresponds to the β-CN (f74–76) and has IC_{50} of 5 µM/mL and VPP corresponds to the β-CN (f 84–86) and has IC_{50} of 9 µM/mL.

2. Other ACE-inhibitory peptides

Several ACE-inhibitory peptides have been identified from milks fermented with different microbial strains (Table 1). These peptides were mainly derived from β-CN and exhibited variable ACE inhibitory potencies.

Table 1. ACE-inhibitory peptides other than lactotripeptides identified in fermented milks

LAB used in fermentation	Structure	Origin	Potency (IC_{50})	Reference
Lb. helveticus CPN4	YP	α_{s1}-CN,β-CN, and κ-CN	720µM/mL	[32]
Lb. bulgaricus	SKVYPFPGPI	β-CN	1.7 mg/mL	[33]
Lb. bulgaricus+ S. thermohilus+ Lc. diacetylactis	SKVYP	β-CN	1.4 mg/mL	
Enterococcus faecalis	LHLPLP LVYPFPGPIPNSLPQNIPP VLGPVRGPFP, VVVPPF, VVVPPF, VRGPFPIIV, LHLPLPL and VLGPVRGPFPIIV	β-CN	5.5 ± 0.4 µM/mL 5.2± 0.3 µM/mL Low and variable	[34]
*Lb. helveticus*130B4	AIPPKKNQD	κ-CN	19.9 µM/L	[35]
Lb. jensenii	LVYPFPGPIHNSLPQN LVYPFPGPIH	β-CN	71 mg/mL 89 mg/mL	[21]
Koumiss flora	YQDPRLGPTGELD, PATQPIVAVHNPVIV, PKDLREN, LLLAHLL, and NHRNRMMDHVH	The 1st peptide from β-CN of mare milk the others unknown	14.53±0.21 µM, 9.82±0.37 µM, 5.19±0.18 µM, and 13.42±0.17 µM/mL respectively	[36]
Two wild *Lc. Lactis Lc. lactis* NRRL B-50571 and 50572	HPHPHLSFMAIPP, SLPQNIPPL	κ-CN β-CN	0.034±0.002 µg/mL and 0.041 ±0.003 µg/mL	[37]
B. bifidum MF 20/5	LVYPFP LPLP	β-CN unknown	132 µM/mL 703 µM/mL	[38]
B. longum KACC91563	YQEPVLGPVRGPFPIIV, GPVRGPFPIIV and QEPVLGPVRGPFPIIV	β-CN		[39]
Kluyveromyces marxianus	VLSRYP IRFF	κ-CN α_{s1}-CN	36.7 µM/mL 116.9 µM/mL	[29]

γ-Aminobutyric acid

γ-Aminobutyric acid (GABA) is a metabolite of microbial fermentation by many LAB strains [40]. It arises from decarboxylation of free L-glutamate by the enzyme glutamic decarboxylase (GAD)

$$L\text{-glutamate} + H^+ \rightarrow GABA + CO_2$$
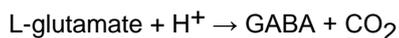
Production of GABA is strictly controlled by pH, temperature and availability of free glutamic acid [40, 41]. The optimum pH and temperature for the production of GABA were reported to be pH 5.0 and 35ºC [40].

GABA has been reported to reduce BP in experimental animals. In spontaneously hypertensive rats, GABA has an antihypertensive effect, possibly through the inhibition of noradrenaline release from sympathetic nerve endings [42].

The BP-lowering effects of GABA and a GABA-enriched non-fat fermented milk product (FMG) were tested by low-dose oral administration to spontaneously hypertensive (SHR/Izm) and normotensive Wistar–Kyoto (WKY/Izm) rats [43]. A single oral dose of GABA or FMG (5 mL/kg; 0.5mg GABA/kg) significantly (P<0.05) decreased the BP of SHR/Izm after 4 to 8 h of administration, but did not increase that of WKY/Izm rats. The hypotensive activity of GABA was dose-dependent from 0.05 to 5.00 mg/kg in SHR/Izm. FMG did not inhibit angiotensin 1-converting enzyme. Furthermore, an FMG peptide-containing fraction from reverse-phase chromatography lacked a hypotensive effect in SHR/Izm rats. These results suggest that low-dose oral GABA has a hypotensive effect in SHR/Izm and that the hypotensive effect of FMG was due to GABA.

A fermented milk containing GABA (FMG) was produced from skim milk that had been fermented by using 2 starters namely: *Lb. casei* strain Shirota and *Lc. lactis* YIT 2027 with added sweeteners. The possible route for the formation of GABA in this fermented milk is that *Lb. casei* strain hydrolyzes milk protein into glutamic acid, and the *Lc. lactis* converts glutamic acid into GABA. Consumption of 100 mL of fermented milk containing 10 mg of GABA daily for 12 week was effective in reducing blood pressure for people with mild hypertension [44].

The components found in GABA-rich fermented milk were compared with those found in control milk fermented without GABA production [45]. The amount of GABA and the amino acid ornithine in GABA-rich fermented milk were much higher (1,216-fold and 27-fold respectively) than that of the control milk. Peptide analysis showed that levels of 6 ACE-inhibitory peptides were also higher in the GABA-rich fermented milk. This indicates that the GABA-producing *Lc. lactis* 01-7 provided fermented milk with other functional components in addition to GABA.

EXPERIMENTAL EVIDENCE OF BP LOWERING EFFECT OF FERMENTED MILK

Animal Studies

Two strains of *Lb. helveticus* were used to produce fermented milk rich in angiotensin converting enzyme (ACE) inhibitors [46]. The two milks were administered by gavage to spontaneously hypertensive rats and the mean arterial BP and heart rate were monitored from 4 to 8h after administration. Unfermented milk and milk fermented with a lactococcal strain that does not produce ACE-inhibitors were used as controls. Highly significant BP lowering effects were observed for milk fermented with the two strains of *Lb. helveticus* than the controls. Also, significant differences in heart rate effects were detected with one of the strains.

The changes in arterial BP of spontaneously hypertensive rats (SHR) by long-term (20 weeks) intake of an *Enterococcus faecalis* CECT 5728-fermented milk were followed [47]. A definite decrease in SBP and DBP was observed in the rats that received the *E. faecalis* CECT 5728-fermented milks. The effect of Ca-enriched fermented milk was slightly more accentuated and more constant than the effect of the un-enriched fermented milk. SBP and DBP increased in the treated SHR when the intake of the fermented milk was stopped.

The short-term oral antihypertensive effect of several peptide isolated from hydrolysate of casein fractions was evaluated [48] in spontaneously hypertensive rats (SHR). These peptides were previously characterized as *in vitro* ACE-inhibitors. SBP and DBP of the rats were measured before administration and also after 2, 4, 6, 8 and 24 h post-administration. The sequences LVYPFTGPIPN, HLPLP, IAK, YAKPVA and WQVLPNAVPAK showed a clear decrease in SBP and DBP in SHR. HPHPHSF caused a significant decrease of the DBP, but did not modify the SBP of SHR in a significant manner. KKYNVPQL did not modify SBP in the SHR, but caused and maintained a slight decrease in DBP in these animals. SBP and DBP returned to baseline values 24h post-administration of all peptides.

The antihypertensive effect of IPP and VPP was evaluated in double transgenic rats (dTGR) harboring human renin and human angiotensinogen genes which develop malignant hypertension due to increased angiotensin II formation [7]. Four week-old dTGR were randomized in three groups the 1st received water (control), the 2nd received fermented milk containing IPP and VPP, and the 3rd received IPP and VPP dissolved in water for three week. The BP of the group that received fermented milk, but not those received the aqueous peptides solution, was reduced by 19mm Hg versus the control group (*P*=0.023). *In vitro* vascular function tests showed that high concentrations of the peptides exhibited ACE inhibitory properties. This study suggests that the antihypertensive peptides were not the only factor responsible for the reduced hypertension.

The influence of yogurt- and probiotic yogurt-based diets on the weight gain, serum lipid profile, and BP were investigated in 14 week old spontaneously hypertensive rats [49]. Animals were subdivided into three groups, the 1st (control) received skim milk diet (Feed-C), the 2nd received skim milk diet supplemented with freeze dried low fat yogurt (Feed-Y), and the 3rd with freeze dried low-fat probiotic yogurt (Feed-PY). At the end of the feeding period (8 weeks) the reduction in SBP of rats fed Feed-Y was 3.7% (–9.5 mm Hg) and 2.7% (–6.4 mm Hg) in those fed Feed-PY while reduction in DBP was 30% (–9.4 mmHg) and 44% (–13.8 mmHg), respectively, in comparison to those fed Feed-C. Also, the levels of total cholesterol and LDL of rats fed the supplemented diets were lower than those fed Feed-C while no changes in the levels of HDL were observed. They concluded that feeding diets supplemented with yogurts exhibited antihypertensive and hypocholesterolemic effects in spontaneously hypertensive rats.

In vivo effects of a fermented milk product containing lactotripeptide IPP and plant sterols on already established hypertension, endothelial dysfunction and aortic gene expression were studied in male SHR [50]. Animals were given either fermented milk rich in IPP and plant sterols, milk or water *ad libitum* for 6 week. Consumption of the enriched milk decreased SBP by 16 mmHg compared with water (P<0.001) and improved endothelial dysfunction and affected signaling pathways related to inflammatory responses in SHR. Milk also had an antihypertensive effect but with an upward trend back towards the baseline SBP values.

The antihypertensive and heart rate (HR)-lowering effect of milk fermented by specific *Lc. lactis* were studied in a murine model [51]. Spontaneously hypertensive male rats (271±14 g) were divided into four randomized groups. Two groups were orally administered with milk fermented by *Lc. lactis* NRRL B-50 571 or *Lc. lactis* NRRL B-50 572 at 35 or 50 mg protein/kg body weight (BW), respectively. The other two groups were fed a saline solution as the negative control and Captoprile

(40 mg/kg BW), a proven ACE inhibitor, as the positive control. The results demonstrated that milk fermented by the two tested *Lc. lactis* presented significant SBP and DBP and HR-lowering effects.

It is evident from these studies that consumption milk fermented with several bacterial strains had significant BP lowering effect in animal models.

Clinical studies

Calpis is a Japanese sour milk fermented with a mixture of *Lb. helveticus* and *Saccharomyces cerevisiae*. The effect of Calpis on BP of 30 elderly hypertensive patients, most of whom were taking antihypertensive medication was studied [52]. Subjects were randomly assigned into two groups, the 1st get a daily intake (95 mL) of the sour milk, and 2nd group ingested the same amount of acidified milk as a placebo for 8 weeks. The SBP and DBP decreased significantly in the sour-milk group after 8 weeks after ingestion whereas no significant changes were observed in BP of the placebo group.

In a clinical trial for 12 weeks period mildly hypertensive patients (16 women and 23 men) aged 28 – 81 y (mean, 54.2 y) received a daily intake of fermented milk containing γ-aminobutyric acid (FMG) or placebo for 1-12 weeks followed by 2 weeks of no intake [44]. The peripheral BP and heart rate of seated patients were measured at week intervals. Also, blood analysis and urinalysis were performed before and after the intake. The BP was significantly decreased within 2 or 4 weeks, and it remained decreased throughout the 12 weeks intake period. For the FMG recipients, the mean decrease after 12 weeks was 17.4±4.3 mmHg in the SBP and 7.2±5.7 mmHg in the DBP. Both of these values differed statistically from baseline levels (P<0.01), and from the placebo group (P<0.05). However, heart rate, body weight, hematological and blood chemistry variables, and urinalysis did not differ between groups.

In a randomized placebo-controlled study, 39 hypertensive patients received 150 mL/day of either *Lb. helveticus* LBK-16H fermented milk or a control product for 21 weeks after a 2 weeks run-in period [53]. During the run-in period, the average baseline SBP and DBP values were 155 and 97 mmHg, respectively and in the test product group were 152 and 96 mmHg respectively in the control group. After the run-in period, BP decreased by 6.7±3.0 mm Hg in SBP (*P*=0.030) and of 3.6±1.9 mmHg (*P*=0.059) in DBP in test product as compared to control groups. They found that demographic factors had no significant effect on the responses.

The BP lowering effect of *Lb. helveticus* LBK-16H fermented milk was evaluated in a randomized, double blinded placebo controlled parallel group study [54]. Hypertensive patients (94 subjects) not receiving any drug treatment were given a dose of 150mL twice daily of either *Lb. helveticus*LBK-16H fermented milk with a high concentration of the lactotripeptides (IPP 7.5

mg/100 g and VPP 10 mg/100g) or a control product, for 10 weeks after a 4 weeks run-in period. Twenty four hr BP was taken at the beginning and at the end of the intervention period. The average baseline systolic and diastolic BP values were 132.6±9.9/83.0±8.0 mmHg in the group receiving *Lb. helveticus* and 130.3±9.6 /80.2±7.0 mmHg in the control group. At the end of the experiment a mean difference of -4.1±0.9 mmHg in SBP (*P*=0.001) and a -1.8±0.7 mmHg in DBP (*P*=0.048) between the *Lb. helveticus* group and the control group. There was no difference in the sum of the adverse events (*P*=0.820).

Boelsma and Kloek [55] reviewed the reported evidences of the BP control properties of lactotripeptides in man. The BP-lowering effects of lactotripeptides were typically evident after 4–6 weeks of treatment but in some cases response was observed after 1–2 week/s. The maximum BP reductions were 13 mmHg and 8 mmHg for SBP and DBP respectively after active treatment compared with placebo, and were reached after 8–12 weeks of treatment. Effective dosages of lactotripeptides ranged from 3.07 to 52.5 mg/day. Lactotripeptides were only effective at elevated BP but no further lowering has been observed of normal BP. Concomitant intake of antihypertensive medication had no effect on the BP lowering potency of lactotripeptides. Also, ethnicity had no influence the lactotripeptide-induced BP lowering effect. Based on the available data they concluded that lactotripeptides appear to be safe and effective in lowering BP and can be part of a healthy diet and lifestyle to prevent or reduce high BP.

A randomized, double-blind placebo-controlled study was conducted of the antihypertensive effect of *Lb. helveticus* fermented milk (FM) in 94 pre-hypertensive and borderline hypertensive subjects [56].The participants were randomized into three treatment groups with a daily intake of 150 mL of FM, 300 mL of FM or placebo (chemically acidified milk). The repeated 24h ambulatory BP measurements were recorded. No statistically significant differences were found in BP between the groups (SBP, P=0.9; DBP, P=0.2). However, the group receiving 300 mL FM had reduced BP across the 8 weeks period in several readings, which could be compatible with a minor antihypertensive effect. Heart rate and lipids remained unchanged between groups. They concluded that milk fermented with *Lb. helveticus* does not pose significant antihypertensive effect.

A double-blinded randomized placebo-controlled study was undertaken [57] including 94 borderline-hypertensive persons to study the effect on human physiology as affected by consumption of *Lb. helveticus* fermented milk. No ACE inhibition of the fermented milk was demonstrated, as none of the components of the renin–angiotensin–aldosteron system was changed. They suggested that the intake of fermented milk decreases sympathetic activity, although not to an extent mediating reductions of BP and heart rate in borderline hypertensive subjects.

Placebo-controlled clinical trials evaluating the anti-hypertensive effect of lactotripeptides were used perform a meta-analysis for the BP lowering effect of lactotripeptides [58]. A total of 18 trials have been identified, the clinical data of which have been clearly reported. Pooled effect of peptides was a reduction of -3.73 mmHg (95% CI: -6.70, -1.76) for SBP and 1.97 mmHg (95% CI:3.85, -0.64) for DBP. The effect was more evident in Asian patients (SBP= -6.93 mmHg (95% CI: -10.95, -2.94); DBP= -3.98 mmHg (95% CI: -5.38, -2.44)) than in Caucasian ones(SBP=-1.17mmHg (95% CI: -2.82, 0.72); DBP=-0.52 mmHg (95% CI: -1.39, 0.13)), and apparently not related to age, baseline BP values, dose of lactotripeptides assumed or length of the treatment. They concluded that VPP and IPP lactotripeptides assumed as functional foods may significantly reduce SBP particularly in Asian subjects, but they suggested this effect should be further investigated in other ethnicities or in association with different dietary pattern.

A meta-analysis was conducted on the effect of IPP and VPP on SBP in Europeans [59]. The study covered 91 publications of which 14 trials with 15 sets of data (n = 1.306) met the inclusion criteria for the meta-analysis. A random-effects model was used for the analysis. Although not all individual trials showed a statistically significant effect of IPP or VPP in reducing SBP, the combination of all data for the two peptides yielded a statistically significantly greater effect for IPP/VPP than for placebo. The decrease in SBP with IPP/VPP was 1.28 mm Hg (95% CI, –2.09 to–0.48, P=0.0017) and the decrease in DBP was 0.59 mmHg (95% CI, –1.18 to –0.01, P=0.047). The study concluded that the peptides IPP and VPP were effective in moderately reducing SBP in European subjects, as is known for Asian populations. These two peptides could have a role in controlling blood pressure.

Compared to placebo, meta-analysis of fourteen randomized placebo-controlled trials involving 702 participants showed that probiotic fermented milk, produced a significant reduction of 3.10 mmHg (95% CI 24.64, 21.56) in SBP and 1.09 mmHg (95% CI 22.11, 20.06) in DBP [60]. Sensitivity analysis excluded small trials that reported extreme results. Subgroup analyses suggested that consumption of probiotic fermented milk to exhibit a slightly greater effect on SBP in hypertensive participants than in normotensive ones (23.98 v. 22.09 mmHg). Analysis of trials done in Japan showed a greater reduction than those conducted in Europe for both SBP (26.12 v. 22.08 mmHg) and DBP (23.45 v. 20.52 mmHg). The study suggested that probiotic fermented milk has BP-lowering effects in pre-hypertensive and hypertensive subjects.

Fekete et al. [61] performed a comprehensive meta-analysis of data on the blood pressure (BP) lowering effect of casein-derived lactotripeptides (LTP) from all relevant randomized controlled trials (RCT) until May 2014. Thirty RCT met the inclusion criteria, which resulted in 33 sets of data. The pooled treatment effect for SBP was −2.95 mmHg (95% CI: −4.17, −1.73; P<0.001), and for DBP was −1.51 mmHg (95% CI:

−2.21, −0.80; P<0.001). The reduction of BP in Japanese studies was significantly greater, compared with European studies (P=0.002 for SBP and P<0.001 for DBP). The 24 h ambulatory BP (AMBP) response to LTP supplementation was statistically non-significant (P=0.101 for SBP and P=0.166 for DBP). Both publication bias and "small-study effect" were identified. These two factors shifted the treatment effect towards less significant SBP and non-significant DBP reduction after LTP consumption. LTP may be effective in BP reduction, especially in Japanese individuals. However sub-group, meta-regression analyses and statistically significant publication biases suggest inconsistencies.

Wang et al. [6] found that greater intakes of total dairy foods, total low-fat/fat-free dairy foods, low-fat/skimmed milk and yogurt were associated with annual but smaller increments reduction in SBP and a lower risk of projected HTN incidence. However, with the exception of total dairy foods and yogurt, these inverse associations with HTN risk were attenuated as the follow-up time increased. For yogurt, each additional serving was associated with 6% (95% CI 1, 10) reduced risk of incident HTN. Total dairy and total low-fat/fat-free dairy intakes were found to be inversely related to changes in DBP. As part of a nutritious and energy-balanced diet pattern, dairy consumption, may benefit BP control and prevent or delay the onset of HTN.

A meta-analysis for 18 studies (including a total of 1194 subjects) was carried out to evaluate the systolic blood pressure (SBP) lowering effect of the lactotripeptides IPP and VPP [62]. They concluded that consumption of every day potential doses of IPP/VPP can significantly reduce SBP in Japanese subjects with or without overt hypertension.

CONCLUSIONS AND FUTURE TRENDS

The BP lowering effect of fermented milks has been evident from several studies carried out on animals and human subjects but this effect was not confirmed in some other clinical studies. This can be expected from the large number of factors involved in BP lowering effect of fermented milks including the type of fermented milk (e.g. yogurt, kefir), composition of the fermented milk (normal, standardized, fortified), technological treatments.(e.g. heat treatments), microorganisms used in fermentation (e..g traditional starters, probiotics), condition of the fermentation process (e.g. temperature x time), and storage condition (e.g. duration and temperature).

Several meta-analysis studies done gave evidences of low but significant effect of the antihypertensive effect of fermented milks. Therefore, consumption of fermented milks represents potentially important strategy to reduce the risk of hypertension through the lifespan, which could reduce the need for antihypertensive drugs later in life.

However, conducting a properly designed randomized, controlled, double-blind studies that take into consideration all factors involved in BP lowering effect

of fermented milks are important to get a solid evidence for the BP effect of fermented milks. However

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