ORIGINAL ARTICLE

Effects of milk proteins on blood pressure: a meta-analysis of randomized control trials

Khemayanto Hidayat[1], Hong-Zhen Du[2], Jing Yang[1], Guo-Chong Chen[1], Zheng Zhang[1], Zeng-Ning Li[2] and Li-Qiang Qin[1]

Certain foods or their components are widely used in the prevention and/or management of cardiovascular disease. Milk proteins have been suggested to have hypotensive properties. A number of clinical trials have been carried out to evaluate the effect of milk proteins from whole foods and supplements on blood pressure (BP). However, the effect of milk proteins on BP is not well understood. Therefore, we conducted a meta-analysis of randomized control trials to provide insight into and robust evidence concerning the overall impact of milk proteins on BP. The PubMed and Cochrane databases were searched for literature concerning the effects of milk proteins on BP up to May 2016. A random effects model was used to calculate the pooled estimates and 95% confidence intervals of effect sizes. The final analysis included seven randomized control trials involving 412 participants. Overall, milk protein interventions significantly lowered systolic BP by − 3.33 mm Hg (95% confidence interval − 5.62, − 1.03) and diastolic BP by − 1.08 mm Hg (95% confidence interval − 3.38, − 0.22). There was no statistical evidence of publication bias across the studies. In conclusion, this meta-analysis provides further evidence that milk proteins slightly but significantly lower both systolic and diastolic BP.

INTRODUCTION

Cardiovascular disease (CVD) is a group of disorders that affects the heart and blood vessels and is the leading cause of mortality worldwide. In 2008, CVD accounted for ~ 17.5 million deaths worldwide; CVD mortality has been projected to rise to 23.6 million by 2030.[1] CVD risk factors consist of non-modifiable (that is, age, gender and family history) and modifiable (that is, hypertension, hyperlipidemia, physical inactivity, overweight and obesity) risk factors.[2] The latter category is mainly related to an unhealthy lifestyle and diet.[3] Certain foods or their components are widely used in the prevention and/or management of disease, particularly in CVD.[4] As mentioned, hypertension is a modifiable risk factor of CVD. Therefore, any component of foods that possesses hypotensive effects on blood pressure (BP) may act as a potential therapeutic in the prevention or management of CVD. Milk proteins have been suggested to have hypotensive properties.[5] A number of clinical trials have been carried out to evaluate the effect of milk proteins from whole foods and supplements on BP.[6–13] However, the sample size of these trials was small, the quality and duration of trials varied widely, and, most importantly, the effect of milk proteins on BP was not clarified. Therefore, we conducted a meta-analysis of randomized controlled trials (RCTs) to

provide insight into and robust evidence concerning the overall impact of milk proteins on BP.

MATERIALS AND METHODS
Search strategy
This meta-analysis was planned, conducted and reported according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines.[14] We searched the PubMed and Cochrane databases for literature concerning the effects of milk proteins from whole foods and supplements on BP published through May 2016. We used the following search algorithm: ('milk' OR 'whey' OR 'casein') AND ('blood pressure' OR 'hypertension' OR 'systolic blood pressure' OR 'diastolic blood pressure'). The search strategy had no language, publication date or publication-type restrictions. The reference lists of previous reviews were examined to complement the search. We also searched the Google Scholars database to confirm that no studies were missed. Additionally, we contacted the authors of the primary studies for further information.

Eligibility criteria
To be included in this meta-analysis, the studies had to meet the following inclusion criteria: (a) RCTs that lasted at least 2 weeks; (b) one or more intervention groups received intact milk protein supplementation (that is, whey protein not whey-derived peptides, casein not casein-derived peptides) or

[1]Department of Nutrition and Food Hygiene, School of Public Health, Soochow University, Suzhou, China and [2]Department of Nutrition, The First Hospital of Hebei Medical University, Shijiazhuang, China
Correspondence: Professor Z-N Li, Department of Nutrition, The First Hospital of Hebei Medical University, Shijiazhuang 050031, China.
E-mail: lizengning@126.com
or Professor L-Q Qin, Department of Nutrition and Food Hygiene, School of Public Health, Soochow University, Suzhou 215123, China.
E-mail: qinliqiang@suda.edu.cn
Received 4 July 2016; revised 4 August 2016; accepted 22 August 2016; published online 13 October 2016

non-fermented food containing milk proteins (that is, milk) and were compared with a non-milk protein placebo; (c) trials reported effects on systolic BP and/or diastolic BP and (d) mean age of participants ⩾ 18 years. We excluded trials that used fermented milk because we considered that the hypotensive effect of fermented milk is largely attributed to the presence of microorganisms found in it.[15] Trials that did not report CVD risk factors, vascular function or BP as a primary outcome were also excluded.

Data extraction and quality assessment

Using a standardized data-collection form, the following study characteristics were extracted from each study: (1) first author's last name, publication year and country of origin; (2) participant characteristics, including mean age, sex, treatment with antihypertensive drugs and preexisting disease or medical condition; (3) trial characteristics (trial design, blinding, trial duration, source of milk proteins, intervention dose and type of control); (4) baseline mean systolic BP and diastolic BP and (5) BP measurement details (position (that is, seated or supine), location (arm), device and number of measurements). The Jadad score, a scale that ranges from 0 to 5 according to the descriptions of randomization, blinding and reporting of participant withdrawals, was used to measure the quality of each trial.[16] Two investigators independently performed the literature search, data extraction and quality assessment. Any discrepancies regarding inclusion were resolved by consensus.

Statistical analysis

Milk proteins from whole foods or supplements were considered the intervention arm in this meta-analysis. In cases in which the multi-arm interventions included milk proteins with other agents (that is, vitamin D, calcium and lycopene) and plain milk proteins (that is, plain milk and whey protein isolate), we used plain milk proteins as the intervention arm.[8,9] The net changes of each outcome in the intervention and control groups were reported as differences between mean values at baseline and post intervention. If necessary, standard errors, confidence intervals (CIs) and P values were converted to s.d. for the analysis. s.d. for changes from baseline in each group were obtained. Studies with no reported s.d. values had their values imputed using a standard formula.[17] If only s.d. for the baseline and final values were provided, we computed s.d. for net changes using the method proposed by Follmann et al.[18] in which a correlation coefficient of 0.5 was assumed. We calculated s.d. values in studies by Pal et al.[7] and Figueroa et al.[11] using reported standard error values.

The degree of heterogeneity across trials was assessed using Q and I^2 statistics. For the Q statistics, $P < 0.1$ was considered statistically significant; and for the I^2 statistics, the following conventional cutoff points were used: <25% (low heterogeneity), 25–50% (moderate heterogeneity) and >75% (severe heterogeneity). Both Begg's rank correlation test and Egger's linear regression test were performed to investigate potential publication bias.[19] If evidence of publication bias was observed, the trim and fill method was applied to correct the bias.[20] A random effects model was used to calculate the pooled estimates and 95% CIs of effect sizes. To explore the possible influences of study and participant characteristics on combined effect sizes, subgroup and meta-regression analyses were performed according to the mean age of the participants, sample size, trial duration, use of antihypertensive medication, dose of protein and type of protein intake. In addition, we conducted sensitivity analyses to investigate the influence of a single trial on the overall effect estimated by omitting one trial in each turn. All analyses were performed using STATA version 11.0 (StataCorp, College Station, TX, USA). A P value <0.05 was considered to be statistically significant, unless otherwise specified.

RESULTS

Study characteristics

We included seven RCTs[6–12] that fully met our eligibility criteria for this meta-analysis. A flow chart of the study selection process, including reasons for exclusion, is presented in Figure 1. The characteristics of the included trials are presented in Table 1. The included studies were published between 2009 and 2016. The sample size of individual trials varied from 20 to 130 participants, reaching a total of 412 participants. Trial duration ranged from 4 weeks to 2
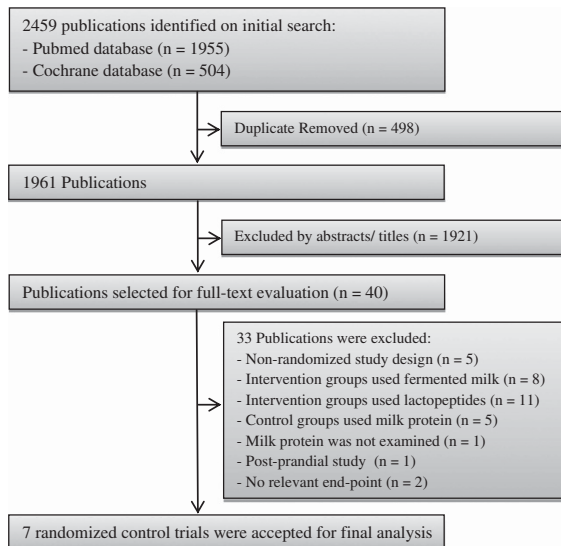

Figure 1 Flow chart or the selection of articles for inclusion in the present meta-analysis.

years. The mean age of participants ranged from 23.4 to 61.1 years. Although a crossover design was not an exclusion criterion, all included studies had similar designs. All trials reported complete systolic and diastolic BP data. The quality among seven trials was diverse, with four studies[8,10–12] classified as high quality (Jadad score ⩾ 3) and three studies[6,7,9] classified as low quality (Supplementary Table 1). The characteristics of the participants enrolled in these trials varied across the studies. Notable differences in population characteristics included overweight adults in one study,[7] hypercholesterolemic adults in one study,[8] pre-hypertensive adults in one study,[9] obese sedentary women in one study[11] and overweight adults with a metabolic syndrome in one study.[12] Regarding the sex distributions of the participants, two studies were conducted exclusively in men,[6,10] one evaluated only women[11] and the remaining four included both sexes.[7–9,12] Most included participants were pre-hypertensive as indicated by mean BP levels at baseline. Two[6,7] of the included studies were carried out in Australia, one[11] in the United States, one[12] in South Korea, one[8] in Greece, one[9] in Russia and one[10] in Iran. The protein source of the supplementation was different in each study, consisting of whey protein isolate, sodium caseinate, low-fat milk and reduced-fat fortified milk through diet. The dose of milk proteins varied from 70 mg per day to 82.5 g per day. Regarding the control groups, two studies[7,11] used carbohydrates as placebo and two[8,10] used no supplementation. In the other two studies,[6,12] the control group continued their usual diet.

Effects of milk proteins on BP

Compared with placebo, milk protein intervention was associated with an average net change ranging from − 1.0 to − 7.0 mm Hg for systolic BP and from − 4.0 to 0.3 mm Hg for diastolic BP. Most trials showed a trend toward BP reduction; however, no single study reached statistical significance. The pooled effect size was − 3.33 mm Hg (95% CI − 5.62, − 1.03) for systolic BP (Figure 2), with no evidence of heterogeneity ($I^2 = 0\%$, $P = 0.754$). Furthermore, the pooled effect size was − 1.08 mm Hg (95% CI − 3.38, − 0.22) for diastolic BP (Figure 3), with no evidence of heterogeneity ($I^2 = 0\%$, $P = 0.959$). Neither Begg's rank correlation nor Egger's linear test suggested the presence of publication bias regarding the effects of milk proteins on systolic

Table 1 Characteristics of the included studies

First author, year	Population	Mean age in years (no. of participants)	Sex	Country	BP medication	Design	Length	Intervention	Control	Extra protein	Baseline BP (mm Hg)	BP measurement; position, location, device, no. of measurements
Daly RM and Nowson CA[6]	Healthy older men	I: 61.3 (73); C: 61.2 (67)	M	Australia	Yes	R, P, O	2 years	Reduced-fat fortified milk	Usual diet	13.2 g per day	123.7/69.5 vs. 120.4/71	Seated, arm, automated blood pressure monitor (Vital Care 506DXN; Criticare System, Waukesha, WI, USA), 4
Pal S and Ellis V[7]	Overweight adults	I1: 48.5 (25); I2: 48 (20); C: 48.4 (25)	MW	Australia	No	R, P, S	12 weeks	Whey protein isolate and sodium caseinate	Glucose	54.2 g per day	119.3/64.1 (I1) vs. 114/66 (C); 118.1-/66.8 (I2) vs. 114/66 (C)	Supine, arm, calibrated sphygmomanometer (Dinamap, Compact T, Critikon, Germany), 3
Petrogianni et al.[8]	Hypercholesterolaemic adults	I: 47.2 (36); C: 49.5 (25)	MW	Greece	No	R, P, D	12 weeks	Low-fat milk	No supplementation	17.5 g per day	130.5/83.2 vs. 126/79.4	Seated, automated sphygmomanometer (Omron M6 Blood Pressure Monitor, Tokyo, Japan), 1
Petyaev et al.[9]	Prehypertensive patient	I: 57.8 (10); C: 51.1 (10)	MW	Russia	No	R, P, O	4 weeks	Whey protein isolate	Placebo	70 mg per day	120–139/80–89	Seated, 3
Vatani DS and Golzar FAK[10]	Healthy men	I: 23 (10); C: 21 (10)	M	Iran	No	R, P, S	6 weeks	Whey protein isolate	No supplementation	82.5 g per day	121/83 vs. 120/84	NA
Figueroa et al.[11]	Obese sedentary women	I1: 28 (11); I2: 31 (11); C: 28 (11)	W	United States	No	R, P, D	4 weeks	Whey or casein	Carbohydrate	30 g per day	131/77 (I1) vs. 128/75 (C); 130/78 (I2) vs. 128/75 (C)	Supine, arm, automatic device (VP-2000; Omron Healthcare, Vernon Hills, IL, USA), 2
Lee et al.[12]	Overweight adults with the metabolic syndrome	I: 50.4 (28); C: 49.5 (30)	MW	Korea	Yes	R, P, O	6 weeks	Low-fat milk	Habitual diet	12 g per day	124.8/80.4 vs. 122.9/80.5	Arm, mercury sphygmomanometer

Abbreviations: BP, blood pressure; C, control; D, double blind; I, intervention subjects; I½, intervention subjects groups 1 and 2; M, men; NA, not available; O, no blinding; P, parallel; R, randomized; S, single-blind; W, women.
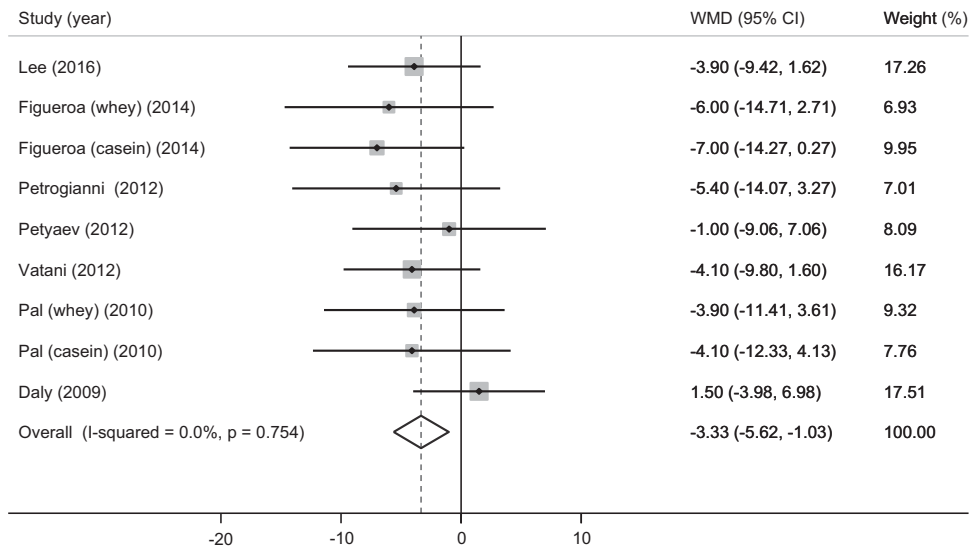
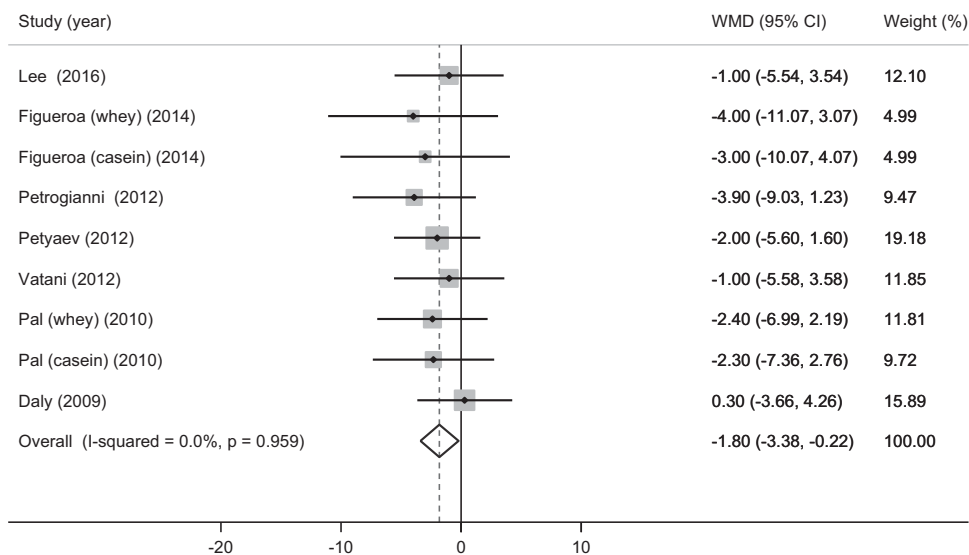| Study (year)		WMD (95% CI)	Weight (%)
Lee (2016)		-3.90 (-9.42, 1.62)	17.26
Figueroa (whey) (2014)		-6.00 (-14.71, 2.71)	6.93
Figueroa (casein) (2014)		-7.00 (-14.27, 0.27)	9.95
Petrogianni (2012)		-5.40 (-14.07, 3.27)	7.01
Petyaev (2012)		-1.00 (-9.06, 7.06)	8.09
Vatani (2012)		-4.10 (-9.80, 1.60)	16.17
Pal (whey) (2010)		-3.90 (-11.41, 3.61)	9.32
Pal (casein) (2010)		-4.10 (-12.33, 4.13)	7.76
Daly (2009)		1.50 (-3.98, 6.98)	17.51
Overall (I-squared = 0.0%, p = 0.754)		-3.33 (-5.62, -1.03)	100.00

-20   -10   0   10

Figure 2 Forest plot of the results of a random effects meta-analysis shown as pooled mean differences with 95% CIs on systolic blood pressure (weighted mean difference: -3.33 mm Hg, 95% CI -5.62, -1.03, $I^2 = 0\%$, P-heterogeneity = 0.754). A full color version of this figure is available at the *Hypertension Research* journal online.

Study (year)		WMD (95% CI)	Weight (%)
Lee (2016)		-1.00 (-5.54, 3.54)	12.10
Figueroa (whey) (2014)		-4.00 (-11.07, 3.07)	4.99
Figueroa (casein) (2014)		-3.00 (-10.07, 4.07)	4.99
Petrogianni (2012)		-3.90 (-9.03, 1.23)	9.47
Petyaev (2012)		-2.00 (-5.60, 1.60)	19.18
Vatani (2012)		-1.00 (-5.58, 3.58)	11.85
Pal (whey) (2010)		-2.40 (-6.99, 2.19)	11.81
Pal (casein) (2010)		-2.30 (-7.36, 2.76)	9.72
Daly (2009)		0.30 (-3.66, 4.26)	15.89
Overall (I-squared = 0.0%, p = 0.959)		-1.80 (-3.38, -0.22)	100.00

-20   -10   0   10

Figure 3 Forest plot of the results of a random effects meta-analysis shown as pooled mean differences with 95% CIs on diastolic blood pressure (weighted mean difference: -1.08 mm Hg, 95% CI -3.38, -0.22, $I^2 = 0\%$, P-heterogeneity = 0.959). A full color version of this figure is available at the *Hypertension Research* journal online.

(Begg, $P = 0.211$; Egger, $P = 0.216$) or diastolic (Begg, $P = 0.6$; Egger, $P = 0.398$) BP.

Subgroup analyses and sensitivity analyses

The results of subgroup analyses stratified by mean age of participants, sample size, trial duration, use of antihypertensive medication, dose of protein and type of protein intake are presented in Table 2. Modest BP reductions were observed in trials that included <70 participants, in trials with younger participants, in trials with a shorter duration, in trials with a higher dose of protein and in trials that used milk protein supplements. We performed sensitivity analyses to test the robustness of the findings. Sensitivity analyses examining the impact of a single trial on the overall results by omitting one trial each in turn yielded a range from -2.92 mm Hg (95% CI -5.34, -0.50) to -4.35 mm Hg

(95% CI -6.88, -1.83) for systolic BP, and from -1.58 mm Hg (95% CI -3.24, 0.07) to -2.19 mm Hg (95% CI -3.92, -0.48) for diastolic BP.

DISCUSSION

To our knowledge, the present meta-analysis is the first quantitative review of randomized trials evaluating the effects of milk proteins from whole foods and supplements on BP. Findings from the present study showed that milk proteins, compared with placebo, produced a significant reduction of 3.33 mm Hg in systolic BP and 1.08 mm Hg in diastolic BP. The magnitudes of the BP reductions reported in this study were relatively modest. On a population level, even a small reduction in BP could have important public health benefits and cardiovascular

Table 2 Subgroup analyses according to participant or trial characteristics

		Change in SBP					Change in DBP			
Subgroup	No.	Effect	95% CI	P-value	P-value*	No.	Effect	95% CI	P-value	P-value*
Mean age										
≥ 50	3	− 1.15	− 4.65, 2.36	0.396	0.151	3	− 0.97	− 3.27, 1.33	0.701	0.361
< 50	4	− 4.96	− 7.99, − 1.93	0.989		4	− 2.54	− 4.72, − 0.37	0.970	
No. of participants										
≥ 70	2	− 1.21	− 5.11, 2.69	0.386	0.230	2	− 1.23	− 3.80, 1.35	0.608	0.599
< 70	5	− 4.45	− 7.28, − 1.61	0.925		5	− 2.14	− 4.12, − 0.15	0.939	
Duration										
≥ 12 weeks	3	− 1.92	− 5.47, 1.64	0.449	0.343	3	− 1.83	− 3.99, 0.33	0.609	0.970
< 12 weeks	4	− 4.33	− 7.33, − 1.33	0.854		4	− 1.77	− 4.07, 0.54	0.947	
Antihypertensive medication										
Yes	2	− 1.18	− 6.48, 4.10	0.174	0.222	2	− 0.26	− 3.24, 2.72	0.672	0.272
No	5	− 4.47	− 7.31, − 1.63	0.754		5	− 2.39	− 4.26, − 0.54	0.987	
Protein dose										
> 15 g per day	4	− 4.96	− 7.99, − 1.93	0.989	0.151	4	− 2.54	− 4.72, − 0.37	0.959	0.361
< 15 g per day	3	− 1.15	− 4.65, 2.36	0.396		3	− 0.97	− 3.27, 1.33	0.701	
Type of protein intake										
Supplements	4	− 4.36	− 7.36, − 1.35	0.931	0.332	4	− 2.33	− 3.31, − 1.34	0.989	0.570
Whole food	3	− 2.01	− 6.15, 2.12	0.271		3	− 0.73	− 2.89, 1.42	0.444	

Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.
P value for heterogeneity; *P** value for heterogeneity between groups according to meta-regression.

consequences.[21] A 3.3 mm Hg decrease in systolic BP and a 1.4 mm Hg decrease in diastolic BP has been shown to be associated with a 22% reduction in risk of cardiovascular mortality, heart attack or stroke.[22]

A meta-analysis by Rebholz *et al.*[23] suggests that dietary protein intake from animals and vegetables leads to lower BP. There are several reasons why we need to specifically examine the effect of milk proteins and BP. Milk and dairy products are widely consumed around the world on a daily basis. Moreover, it is generally accepted that milk protein is the most commonly used protein supplement. Furthermore, intact milk proteins and milk-protein-derived peptide supplementations have been shown to reduce BP.[5] Thus, further evidence of the impact of milk proteins on BP is needed.

There is ample evidence supporting the benefits of milk and dairy products on BP. This positive effect has been largely attributed to the functional components found in milk and dairy products, including protein, bioactive peptides, calcium, potassium and magnesium.[7,24,25] The composition of milk protein is ~ 80% casein and 20% whey.[26] The potential mechanism responsible for the hypotensive effects of milk proteins is believed to involve the inhibition of angiotensin-converting enzyme activity,[5,27,28] the enzyme that catalyzes the conversion of angiotensin I to angiotensin II, which leads to arterial vasodilation. Whey contains potent angiotensin-converting enzyme inhibitory peptides, known as lactokinins that inhibit angiotensin-converting enzyme, whereas casokinins are casein-derived inhibitors of angiotensin-converting enzyme.[29,30] Animal studies have revealed that lactokinins and casokinins reduce BP in spontaneously hypertensive rats, with Systolic BP reduction ranging from 2 to 34 mm Hg.[31,32] Nevertheless, the mechanism underlying the hypotensive effect of milk proteins on BP warrants further research.

Subgroup analyses showed that participants who consumed higher doses of milk proteins had greater reductions in BP than participants who consumed lower doses. It is possible that a higher overall protein intake did lead to this beneficial effect.[33–37] Moreover, higher dietary protein intake (particularly from tryptophan-rich foods such as milk) may also contribute to increased amino acid tryptophan levels, which have been shown to reduce BP in animal studies.[38,39] Unfortunately, we could not provide a dose recommendation for the prevention of hypertension in this meta-analysis. Future observational studies conducted on both normotensive and hypertensive populations are required to determine the appropriated dose of milk proteins.

Interestingly, when we further stratified the data by type of protein intake, there was no noteworthy change in the BP of participants who consumed milk protein from whole foods. In contrast, those who consumed milk proteins from supplements experienced significant reductions in BP. These findings were reasonable because both casein and whey protein supplements are in concentrated form and therefore contain more protein and peptides than regular milk. As mentioned, higher dietary protein intake may help reduce BP. Thus, we hypothesized that the higher the intake of protein and bioactive peptides, the stronger the hypotensive effect on BP will be.

Furthermore, participants who were not taking antihypertensive medication experienced significant reductions in BP, whereas no change was observed in participant taking antihypertensive medication. There are several reasons to explain this difference. First, it has been suggested that the effect of non-pharmacological measures may became greater as BP is elevated in the absence of antihypertensive drugs.[40] Second, hypertensive participants who were taking antihypertensive medication may have also changed their dietary behavior or

the dose of antihypertensive medication to further lower BP, which may contribute to this difference. Therefore, it is essential for participants taking antihypertensive medication to maintain stable doses of daily medication to avoid the confounding effects of alterations in medication regime on BP.

Unsurprisingly, significant reductions in BP were observed in trials with younger participants, whereas no noteworthy change was observed in trials with older participants. It has been suggested that the rise in BP is an inevitable consequence of aging.[41] When we looked more closely at the daily dose of milk proteins in these groups, we found that the dose of milk proteins in the older participants was lower than in the younger participants. Based on these findings, we hypothesized that the beneficial effects of milk proteins in older participants might only occur with higher doses.

There are several limitations to this meta-analysis. First, the sample sizes of the individual trials were relatively small, which limited the capacity of randomization to minimize the potential influences of confounding factors. Second, the validity of our meta-analysis depended upon the quality of the individual studies. Although all studies were randomized, parallel trials, the allocation concealment, quality of randomization, details of withdrawals and details of BP measurement were not always reported. Third, only seven studies were eligible for this meta-analysis, all of which were conducted in participants with specific medical conditions, such as hypercholesterolemia, overweight and obese with metabolic syndrome, which may limit the generalization of the findings. Fourth, the included studies were predominantly conducted in Western populations, which are known to have higher milk and dairy product intake compared with other populations. Therefore, these findings may not be completely generalizable to other populations, particularly those consuming very low levels of milk proteins. Finally, the format in which the data were reported in each study varied widely (for example, two of the included studies reported standard errors instead of s.d.), which made data extraction difficult and may have influenced the extracted result. Therefore, results from our meta-analysis should be interpreted with caution. Despite these limitations, to date, few RCTs have investigated the effect of milk proteins on BP. Thus, this current meta-analysis provides a comprehensive overview of the previous published literature addressing the effect of milk proteins on BP. This analysis also highlights the need for further interventions to investigate the effect of milk proteins on BP and hypertension, which may help scientists, policy makers and the industry determine the value of milk protein as an effective strategy for hypertension prevention or adjuvant antihypertensive therapy.

In contrast to the antihypertensive medications that often cause negative side effects on health,[42,43] with proper dosage, food-derived proteins with hypotensive properties are relatively safer for consumption by individuals with a variety of other disease conditions. Although it is too early to recommend milk proteins as a supplement or alternative to pharmaceutical medications for hypertension, this meta-analysis provides further evidence that milk proteins slightly but significantly lower both systolic and diastolic BP. Future large-scale, long-term, well-designed RCTs with long durations and large sample sizes are needed to scientifically validate the claimed effects and to better delineate the hypotensive activities of milk proteins, particularly in populations with high BP.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 World Health Organization (2015). http://www.who.int/cardiovascular_diseases/about_cvd/en/. Accessed May 2016.
2 World Heart Federation (2012). http://www.world-heart-federation.org/press/fact-sheets/cardiovascular-disease-risk-factors/. Accessed May 2016.
3 Forman D, Bulwer BE. Cardiovascular disease: optimal approaches to risk factor modification of diet and lifestyle. Curr Treat Options Cardiovasc Med 2006; 8: 47–57.
4 Moore LL. Functional foods and cardiovascular disease risk: building the evidence base. Curr Opin Endocrinol Diabetes Obes 2011; 18: 332–335.
5 Fekete AA, Givens DI, Lovegrove JA. The impact of milk proteins and peptides on blood pressure and vascular function: a review of evidence from human intervention studies. Nutr Res Rev 2013; 26: 177–190.
6 Daly RM, Nowson CA. Long-term effect of calcium-vitamin D(3) fortified milk on blood pressure and serum lipid concentrations in healthy older men. Eur J Clin Nutr 2009; 63: 993–1000.
7 Pal S, Ellis V. The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals. Obesity 2010; 18: 1354–1359.
8 Petrogianni M, Grammatikaki E, Kalogeropoulos N, Peristeraki A, Moschonis G, Pitsavos C, Antonopoulou S, Manios Y. Additional benefit in CVD risk indices derived from the consumption of fortified milk when combined with a lifestyle intervention. Public Health Nutr 2014; 17: 440–449.
9 Petyaev IM, Dvogalevsky PY, Klochkov VA, Chalyk NE, Kyle N. Whey protein lycosome formulation improves vascular functions and plasma lipids with reduction of markers of inflammation and oxidative stress in prehypertension. ScientificWorldJournal 2012; 2012: 269476.
10 Vatani DS, Golzar FAK Changes in antioxidant status and cardiovascular risk factors of overweight young men after six weeks supplementation of whey protein isolate and resistance training. Appetite 2012; 59: 673–678.
11 Figueroa A, Wong A, Kinsey A, Kalfon R, Eddy W, Ormsbee MJ. Effects of milk proteins and combined exercise training on aortic hemodynamics and arterial stiffness in young obese women with high blood pressure. Am J Hypertens 2014; 27: 338–344.
12 Lee YJ, Seo JA, Yoon T, Seo I, Lee JH, Im D, Lee JH, Bahn KN, Ham HS, Jeong SA, Kang TS, Ahn JH, Kim DH, Nam GE, Kim NH. Effects of low-fat milk consumption on metabolic and atherogenic biomarkers in Korean adults with the metabolic syndrome: a randomised controlled trial. J Hum Nutr Diet 2016; 29: 477–486.
13 Pal S, Ellis V. Acute effects of whey protein isolate on blood pressure, vascular function and inflammatory markers in overweight postmenopausal women. Br J Nutr 2011; 105: 1512–1519.
14 Moher D, Liberati A, Tetzlaff J, Altman DGPRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009; 6: e1000097.
15 Dong JY, Szeto IM, Makinen K, Gao Q, Wang J, Qin LQ, Zhao Y. Effect of probiotic fermented milk on blood pressure: a meta-analysis of randomised controlled trials. Br J Nutr 2013; 110: 1188–1194.
16 Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Control Clin Trials 1996; 17: 1–12.
17 Higgins J, Green S. Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0. The Cochrane Collaboration. http://www.cochrane-handbook.org. Accessed 20 June 2014.
18 Follmann D, Elliott P, Suh I, Cutler J. Variance imputation for overviews of clinical trials with continuous response. J Clin Epidemiol 1992; 45: 769–773.
19 Egger M, Davey Smith G, Schneider M, Minder C Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315: 629–634.
20 Duval S Tweedie RTrim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 2000; 56: 455–463.
21 Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. Hypertension 2014; 64: 897–903.
22 Sleight P, Yusuf S, Pogue J, Tsuyuki R, Diaz R, Probstfield J Heart Outcomes Prevention Evaluation (HOPE) study. Blood-pressure reduction and cardiovascular risk in HOPE study. Lancet 2001; 358: 2130–2131.
23 Rebholz CM, Friedman EE, Powers LJ, Arroyave WD, He J, Kelly TN. Dietary protein intake and blood pressure: a meta-analysis of randomized controlled trials. Am J Epidemiol 2012; 176: S27–S43.
24 McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. Science 1984; 224: 1392–1398.
25 Kris-Etherton PM, Grieger JA, Hilpert KF, West SG. Milk products, dietary patterns and blood pressure management. J Am Coll Nutr 2009; 28: 103S–119S.
26 Hoffman JR, Falvo MJ. Protein – which is best? J Sports Sci Med 2004; 3: 118–130.
27 Pal S, Radavelli-Bagatini S. The effects of whey protein on cardiometabolic risk factors. Obes Rev 2013; 14: 324–343.

28 Nonaka A, Nakamura T, Hirota T, Matsushita A, Asakura M, Ohki K, Kitakaze M. The milk-derived peptides Val-Pro-Pro and Ile-Pro-Pro attenuate arterial dysfunction in L-NAME-treated rats. *Hypertens Res* 2014; **37**: 703–707.

29 FitzGerald RJ, Murray BA, Walsh DJ. Hypotensive peptides from milk proteins. *J Nutr* 2004; **134**: 980–998.

30 FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. *Br J Nutr* 2000; **84**: 33–37.

31 Abubakar A, Saito T, Kitazawa H, Kawai Y, Itoh T. Structural analysis of new antihypertensive peptides derived from cheese whey protein by proteinase K digestion. *J Dairy Sci* 1998; **81**: 3131–3138.

32 Nurminen ML, Sipola M, Kaarto H, Pihlanto-Leppälä A, Piilola K, Korpela R, Tossavainen O, Korhonen H, Vapaatalo H. Alpha-lactorphin lowers blood pressure measured by radiotelemetry in normotensive and spontaneously hypertensive rats. *Life Sci* 2000; **66**: 1535–1543.

33 Buendia JR, Bradlee ML, Singer MR, Moore LL. Diets higher in protein predict lower high blood pressure risk in Framingham Offspring Study adults. *Am J Hypertens* 2015; **28**: 372–379.

34 Altorf-van der Kuil W, Engberink MF, Brink EJ, van Baak MA, Bakker SJ, Navis G, van 't Veer P, Geleijnse JM Dietary protein and blood pressure: a systematic review. *PLoS ONE* 2010; **5**: e12102.

35 He J, Whelton PK. Effect of dietary fiber and protein intake on blood pressure: a review of epidemiologic evidence. *Clin Exp Hypertens* 1999; **21**: 785–796.

36 Liu L, Ikeda K, Sullivan DH, Ling W, Yamori Y. Epidemiological evidence of the association between dietary protein intake and blood pressure: a meta-analysis of published data. *Hypertens Res* 2002; **25**: 689–695.

37 Obarzanek E, Velletri PA, Cutler JA. Dietary protein and blood pressure. *JAMA* 1996; **275**: 1598–1603.

38 Sved AF, Van Itallie CM, Fernstrom JD. Studies on the antihypertensive action of L-tryptophan. *J Pharmacol Exp Ther* 1982; **221**: 329–333.

39 Ardiansyah, Shirakawa H, Inagawa Y, Koseki T, Komai M. Regulation of blood pressure and glucose metabolism induced by L-tryptophan in stroke-prone spontaneously hypertensive rats. *Nutr Metab (Lond)* 2011; **8**: 45.

40 Pietinen P, Aro A. The role of nutrition in the prevention and treatment of hypertension. *Adv Nutr Res* 1990; **8**: 35–78.

41 Pinto E. Blood pressure and ageing. *Postgrad Med J* 2007; **83**: 109–114.

42 Atkinson AB, Robertson JIS Captopril in the treatment of clinical hypertension and cardiac-failure. *Lancet* 1979; **2**: 836–839.

43 Gavras H, Gavras I. Angiotensin converting enzyme inhibitors properties and side effects. *Hypertension* 1988; **11**: 37.

Supplementary Information accompanies the paper on Hypertension Research website (http://www.nature.com/hr)