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SYSTEMATIC REVIEWS AND META-ANALYSES

The effects of whey protein on blood pressure: A systematic review and dose-response meta-analysis of randomized controlled trials

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KEYWORDS
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Abstract *Aims:* This systematic review and dose-response meta-analysis was conducted to summarize data from available clinical trials on the effects of whey protein (WP) supplementation on blood pressure (BP) in adults.
Data synthesis: A comprehensive literature search was conducted in the electronic databases PubMed, Web of Science, ProQuest, Embase, and SCOPUS from inception to October 2022. Weighted mean differences (WMD) and 95% confidence intervals (CI) were calculated to assess pooled effect sizes. Heterogeneity between studies was assessed using the Cochran's Q test and I^2. Subgroup analysis was performed to assess potential sources of heterogeneity. The dose −response relationship was assessed using fractional polynomial modeling. Of the 2,840 records, 18 studies with 1,177 subjects were included. Pooled analysis showed that whey protein supplementation resulted in a significant reduction in systolic blood pressure (WMD: −1.54 mmHg; 95% CI: −2.85 to −0.23, p = 0.021), with significant heterogeneity between studies (I^2 = 64.2%, p < 0.001), but not for diastolic blood pressure (DBP) (WMD: −0.27 mmHg; 95% CI: −1.14, 0.59, p = 0.534) with high heterogeneity between studies (I^2 = 64.8%, p < 0.001). However, WP supplementation significantly reduced DBP at a dose of >30 g/day, in RCTs that used WP isolate powder for their intervention, in sample sizes ≤100, in studies with an intervention duration of ≤10 weeks, and in those studies that were conducted in patients with hypertension and had participants with a BMI of 25–30 kg/m^2.
Conclusion: This meta-analysis demonstrated that WP intake significantly reduced SBP levels. Further large-scale studies are needed to specify the exact mechanism, and optimal dosage of WP supplementation to obtain a beneficial effect on BP.

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1. Introduction

High blood pressure (hypertension or HTN) is a condition in which blood pressure (BP) rises inappropriately and severely damages numerous body organs [1,2]. It can lead to a variety of health complications, including stroke, myocardial infarction, retinopathy, cardiovascular disease (CVD), and renal failure [3–6]. In 2010, one-third of the world's adult population, or 1.3 billion people, suffered from HTN [7]. This number is expected to reach 1.56 billion by 2025 [8]. Annually, approximately 9.4 million people worldwide die from HTN-related problems, 51% from stroke, and 45% from heart disease [9]. Given the increased risk of chronic disease associated with HTN, it is the most common cause of morbidity and represents a significant economic burden to society [10–12]. For the treatment and management of HTN, the community offers a number of options. Medications and lifestyle changes, particularly nutritional management and exercise, are typical treatments [13]. In addition, long-term use of antihypertensive medications often reduces their effectiveness. Given these limitations, there is considerable interest in the discovery of new antihypertensive drugs with minimal side effects that can be used as adjunctive techniques in the treatment and control of HTN [14]. Natural products have recently played a unique role in many diseases because of their nutritional and therapeutic properties [15,16].

One of these natural compounds is whey protein (WP), which is popular and has few side effects. Epidemiological studies demonstrate an inverse association between dairy product consumption and the risk of CVD or mortality [17,18]. Dairy products contain bioactive substances that have important properties such as reducing inflammation and oxidative stress and regulating BP [19]. Milk-derived proteins and bioactive peptides may be responsible for the observed effects; however, the chemicals underlying these effects have not been fully elucidated [20–22]. WP has attracted considerable interest as a biologically active protein that can combat cardiometabolic diseases such as HTN, diabetes mellitus, dyslipidemia, obesity, and oxidative stress [20–22].

According to the data, WP inhibits angiotensin-converting enzyme (ACE) and alters BP and vascular reactivity [23]. Indeed, ACE inhibitors act on the renin–angiotensin system, whose main function is the regulation of BP [24]. Despite the ACE inhibitory and apparent antihypertensive properties of WP, the vascular effects of WP are still not well understood [25,26]. It has been found that consumption of 30 g of WP over a 12-week period significantly lowered BP in prehypertensive and mildly hypertensive adults who were also overweight and obese [27]. Pal and Ellis [28] reported that BP decreased after both WP and casein consumption for 12 weeks, but arterial stiffness improved only after WP consumption. These results suggest that WP may have a stronger cardiovascular effect than casein. Unlike casein, WP is transported intact into the small intestine because of its rapid emptying, allowing it to participate in a range of bioactivities both in the circulation and in the intestine after its excretion. As a result, the postprandial occurrence of amino acids in plasma is much higher than that for casein. WP also consists of a heterogeneous group of proteins such as lactoperoxidase, β-lactoglobulin, lactalbumin, and immunoglobulin and has a high content of branched-chain amino acids, which is probably responsible for its efficient metabolism after ingestion [29,30]. However, further studies are needed to clarify whether WP may be useful in preventing arterial stiffness and related CVD or not.

However, further studies are needed to clarify whether WP or the branched-chain amino acids in WP are responsible for the beneficial effect on arterial stiffness. In a 2017 meta-analysis for overweight and obese individuals, the results of this analysis showed that WP supplementation appears to improve body weight, total fat mass, and some CVD risk factors in overweight and obese patients [31]. In the meantime, several other randomized controlled trials (RCTs) have been published, some of which are large in size and high in quality and have also provided conflicting results. In addition, after the last meta-analysis published in 2017, at least seven more studies were published. Thus, the current study was designed as a comprehensive systematic review and dose–response meta-analysis of published RCTs to assess the effects of WP supplementation on systolic blood pressure (SBP) and diastolic blood pressure (DBP) in adults.

2. Method

This systematic review and meta-analysis were conducted according to the criteria of the recommended reporting elements for systematic reviews and meta-analyzes (PRISMA) (Sup. Table 1). The review protocol was registered in the PROSPERO database of Systematic Reviews (registration number: CRD42022343332).

2.1. Search strategy

The study was designed based on the following criteria: Population, Intervention, Comparison, and Outcome. The population was a human model; the intervention was WP treatment; the comparison was no treatment or placebo; the outcomes were vascular responses to WP; and the study methodology was a clinical trial. International databases, such as Web of Sciences, PubMed, Embase, SCOPUS, and Cochrane Library, as well as Google Scholar, were

searched without time limit until October 2022 using the medical terms non-MESH and MESH. Major search terms included “whey proteins” OR “whey” AND “hypertension” OR “blood pressure” OR “HTN” OR “hypertensive” OR “BP” OR “systolic blood pressure” OR “diastolic blood pressure” OR “SBP” OR “DBP.” In addition, we reviewed the reference lists of eligible articles to avoid omitting relevant studies.

2.2. Study selection and eligibility criteria

Two researchers (AK and MV) reviewed the papers for inclusion after the initial identification of publications. The titles and abstracts of all identified studies were first reviewed using the selection criteria, followed by searching and assessing the full-text versions of potentially relevant publications for eligibility requirements. Finally, all RCTs (either in parallel or crossover design) that investigated the effects of WP supplementation on SBP and DBP in adults were selected for analysis. Disagreements in study selection were resolved through dialog with a third investigator (MAF). Studies that met the following criteria were included in this meta-analysis: (a) study designs were published RCTs; (b) WP isolate, hydrolysate, or concentrate was the intervention; (c) participants were 18 years of age or older; (d) the intervention duration was at least 2 weeks; (e) the study had a comparable control or placebo group; and (f) the study evaluated SBP and DBP outcomes. Studies were removed if they were [1] RCTs with a treatment duration of less than 2 weeks [2]; studies in which WP was used in combination with other dietary supplements; and [3] conference abstracts, case reports, experimental studies, observational studies, animal studies, and *in vitro* studies.

2.3. Data extraction

The following data were extracted from each study: first author name, publication year, study location, sample size, type and dose of WP supplementation, and duration of intervention. The analysis included longer interventions when measurements were made at multiple time points. When we had questions about the articles or data, we contacted the corresponding authors by email. Two authors (AK and MV) independently extracted the data to minimize potential errors. Disagreements were resolved through consensus discussions.

2.4. Risk of bias in the studies

The risk of bias in each study was assessed using a procedure developed by the Cochrane Collaboration that includes selection bias, detection bias, reporting bias, performance bias, discontinuation bias, and other biases [32]. In each study, the quality of evidence for each outcome was rated as low risk, high risk, or unknown risk of bias. It was determined whether the overall risk of bias for each study was high, low, or unclear. Disagreements regarding the possibility of bias were resolved through dialog.

2.5. Grade assessment

Two reviewers (M.V. and V.M.) independently rated the certainty of the evidence for the outcomes using the GRADE system, a method for grading the quality of evidence and the strength of recommendations, which is classified as high, moderate, low, or very low [33]. GRADE ratings were lowered based on the following factors: (1) risk of bias was downgraded if any of the factors used to assess risk of bias were rated as high and the majority of included studies were rated as high; (2) imprecision was downgraded if the 95% confidence interval (CI) for the effect estimate intersected with zero; (3) inconsistency was downgraded if there was substantial heterogeneity ($I^2 > 50\%$ and $p < 0.1$) that could not be explained by sensitivity or subgroup analysis; (4) indirectness was downgraded if there were any influential factors that limited the interpretation of the results; and (5) publication bias was downgraded if there was a substantial change in the evidence of publication bias based on Begg’s test or Egger’s test ($p < 0.05$).

2.6. Data synthesis and analysis

Version 16 of Stata was used for the meta-analysis. P values of 0.05 were considered statistically significant. Effect size was calculated as (measure at the end of the follow-up in the intervention group—measure at the beginning of the intervention in the intervention group)—(measure at the end of follow-up in the control group—measure at the beginning of the follow-up in the control group). Standard deviation (SD) was calculated using the following formula: [SD = square root (SD pretreatment) 2 + (SD posttreatment) 2 − (2 R SD pretreatment SD posttreatment)] [34]. SD was calculated by the following formula when only the standard error of the mean was reported: $D = SE \times \sqrt{n}$, where n is the number of participants. Effect sizes were characterized by the WMD and the 95% CI. The random-effects model was used to estimate the overall effect from the effect sizes. Heterogeneity between studies was evaluated by Cochrane’s Q test and I^2 index. To find possible causes of heterogeneity between studies, we performed a preplanned subgroup analysis based on age, gender, dose, and duration. A sensitivity analysis was performed by removing individual studies from the meta-analysis to determine whether the overall effects depended on specific studies. Visual funnel plots were also used to examine publication bias [35]. As a result of publication bias, the “trim-and-fill” method was used to fill in potentially missing studies if publication bias was identified. Nonlinear dose−response regression and meta-regression were performed to examine the association between overall effect size and sample size, WP dose (g/day), and duration of intervention (week).

3. Results

3.1. Selected studies and systematic review

As shown in Fig. 1, the systematic search originally identified 2840 studies, of which 1732 studies were duplicated and another 1027 studies were unrelated and excluded from the initial screening of title and abstract. Of these, 63 studies were excluded for the following reasons: (1) studies that investigated the effect of WP in combination with other drugs or supplements (n = 25); (2) studies without a placebo group (n = 9); (3) studies in children (n = 6); (4) studies with a different design (n = 22); (5) the same studies with different articles (n = 3). Finally, 18 studies were included in the present systematic review and meta-analysis [25,27,28,36–50]. A total of 18 studies published between 2010 and 2020 were included in the current meta-analysis. The detailed characteristics of the included RCTs are shown in Table 1. The mean age of the 1177 participants included in the current study was 51.8 years. The intervention duration ranged from 4 to 48 weeks. Eleven of the studies supplemented WP with powders, five with beverages, and two with whey yogurt and capsules. Six trials were conducted in the United States [36,39,42,43,46,49], two in Denmark [45,50], two in Brazil [47,48], two in Australia [28,38], one in the United Kingdom [25], one in Germany [37], one in China [27], one in Iran [41], and one in Japan [44]. Of the included RCTs, some subjects had a different baseline health status, including overweight and obesity, prehypertension and HTN, metabolic syndrome, hemodialytic, sarcopenic obesity, abdominal obesity, and obesity and HTN, whereas three did not report participant health status.

3.2. Risk of bias assessment and grade assessment

Most of the RCTs included in this meta-analysis did not explain allocation concealment, performance bias, and detection bias in detail, which affected study quality. Nine of the 18 RCTs were classified as being of good quality, six as being of moderate quality, and three as being of poor quality. The results of the risk of bias of the RCTs according to the Cochrane criteria are shown in Table 2. The GRADE profile for certainty of evidence is included in Table 3. The DBP was rated as moderate quality because of serious limitations in imprecision. The evidence for the SBP was of high quality.

3.3. WP supplementation and SBP

Eighteen eligible studies with 26 study arms, comprising 898 cases and 1,080 controls, examined the effects of WP supplementation on SBP. Combining their results according to the random-effects model showed that SBP was significantly reduced after the intervention (WMD: −1.54 mmHg; 95% CI: −2.85 to −0.23, p = 0.021), with significant heterogeneity between studies (I^2 = 64.2%, p < 0.001). (Fig. 2A). Supplementation with WP isolate powder at a dose of >30 g/day in subjects with HTN, a

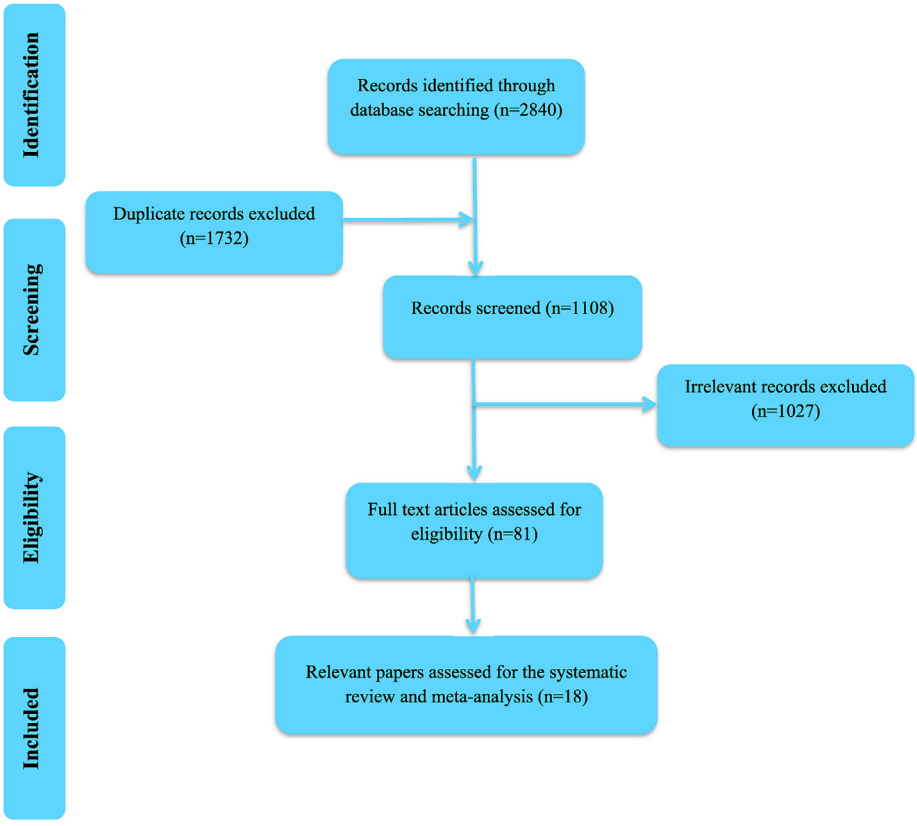

Figure 1 Flow diagram of the study.

Table 1 General characteristics of the included studies.

Author (ref)	Year/ Location	Subjects	Participants		Gender (Male/ Female)	Mean age		Mean BMI		Design	Supplement	Comparator	Dose, g/day	Duration (week)	Main results
			Case	Control		Case	Control	Case	Control						
Pal et al. [29]	2010/ Australia	Overweight and obesity	25	25	Both	48.5 ± 10	48.4 ± 7.5	32.0 ± 4	30.6 ± 4	RSBC	Powder	Glucose powder	54	6	Significant reduction in SBP
Fluegel et al. [35]	2010/US	Prehypertension and hypertension	36	35	Both	20.4 ± 1.7	20.7 ± 1.9	25.1 ± 2.6	24.2 ± 2.4	RC	Beverage	Unmodified whey beverage	28	6	Only participants with previously high blood pressure had their SBP and DBP reduced by whey protein drinks
Gouni-Berthold et al. [36]	2011/ Germany	Metabolic syndrome	83	88	Both	52.9 ± 10.3	53.9 ± 9.5	30.8 ± 4.2	31.3 ± 4.0	RDBPC	Whey yoghurt	Low-fat yoghurt	15.3	12	No significant change was seen
Hodgson et al. [37]	2011/ Australia	Unclear	101	95	F	74.3 ± 2.7	74.3 ± 2.6	26.3 ± 3.8	27.2 ± 3.9	RDBPC	Beverage	Low-protein beverage	30	48	No significant change in SBP and DBP
Weinheimer et al. [38]	2012/US	Overweight and obesity	71	70	Both	47 ± 8.1	49 ± 7.0	30.4 ± 2.6	29.9 ± 2.7	RDBPC	Powder	Nonprotein powder	20	36	SBP and DBP were unchanged
Petyaev et al. [39]	2012/ Russia	Prehypertension	10	10	Both	57.8 ± 3.5	51.1 ± 5.2	25.9 ± 2.8	26.8 ± 5.7	RC	Capsule	Capsule	0.07	4	No significant change in SBP and DBP
Sheikholeslami Vatani et al. [40]	2012/Iran	Overweight	9	10	M	23 ± 2	21 ± 1	26.5 ± 1.2	27.2 ± 1.6	RSBC	Powder	Carbohydrate powder	38.57	6	No changes were observed in SBP and DBP
Figueroa et al. [41]	2013/US	Obesity and hypertension	11	11	F	28 ± 3.31	31.2 ± 6.63	34.3 ± 4.64	33.5 ± 3.98	RDBPC	Powder	Carbohydrate powder	30	4	Significant reduction in SBP
Arciero et al. [42]	2016/US	Overweight and obesity	12	9	Both	48 ± 13.86	52 ± 3	32 ± 6.93	33 ± 3.46	RC	Powder	Food protein	20–25	16	Significant reduction in SBP
Fekete et al. [26]	2016/UK	Elevated blood pressure	19	19	Both	52.9 ± 9.15	52.9 ± 9.15	27.1 ± 3.49	27.1 ± 3.49	RDB	Powder	Carbohydrate powder	49.6	8	Significant reduction in SBP and DBP
Kataoka et al. [43]	2016/ Japan	Hypertension	10	11	M	69 ± 3.16	69 ± 3.32	22 ± 3.16	23 ± 3.32	RC	Beverage	Glucose beverage	4.28	8	Significant reduction in DBP
Kjølbæk et al. [44]	2017/ Denmark	Overweight and obesity	39	38	Both	42.2 ± 9.32	38.7 ± 10.8	33.0 ± 3.43	33.3 ± 2.94	RDBC	Powder	Carbohydrate powder	45	24	No differences were observed in SBP and DBP
Nabuco et al. [46]	2018/ Brazil	Older women	22	23	F	67.5 ± 5.2	66.5 ± 7.1	26.3 ± 5.2	23.8 ± 3.7	RDBPC	Beverage	Carbohydrate beverage	27.1	12	No changes were observed in SBP and DBP
Jeong et al. [45]	2019/US	Hemodialytic	38	34	Both	56.6 ± 13.0	54.4 ± 12.3	30.6 ± 7.1	31.5 ± 7.6	RC	Powder	Non-nutritive beverage	12.85	24	No significant change in SBP and DBP
Nabuco et al. [47]	2019/ Brazil	Sarcopenic obesity	13	13	F	68.0 ± 4.2	70.1 ± 3.9	26.4 ± 3.0	27.4 ± 3.0	RDBPC	Beverage	Maltodextrin beverage	15	16	No significant change in SBP and DBP

(*continued on next page*)

Table 1 (*continued*)

Author (ref)	Year/ Location	Subjects	Participants		Gender (Male/ Female)	Mean age		Mean BMI		Design	Supplement	Comparator	Dose, g/day	Duration (week)	Main results
			Case	Control		Case	Control	Case	Control						
Yang et al. [28]	2019/ China	Hypertension	27	27	Both	42.3 ± 11.60	43.8 ± 11.76	24.1 ± 3.10	24.33 ± 2.39	RC	Powder	Maltodextrin powder	15	12	Reduction in SBP and DBP with a borderline statistical significance
Lefferts et al. [48]	2020/ US	Unclear	53	46	Both	69 ± 7	67 ± 6	27.9 ± 5.6	27.0 ± 3.9	RDBPC	Powder	Maltodextrin powder	50	12	Significant reduction in DBP
Fuglsang-Nielsen et al. [49]	2020/ Denmark	Abdominal obesity	17	17	Both	65	64	29.4 ± 3.7	29.1 ± 3.6	RDBPC	Powder	Maltodextrin beverage	30.1	12	No significant change in SBP and DBP

mean age of <50 years, and an intervention duration of ≤10 weeks resulted in a significant reduction in HTN in studies that had the control intervention type carbohydrate placebo and in participants with a body mass index of 25–30 kg/m^2 (Table 4). Overall estimates were not affected by study exclusion, according to sensitivity analysis. Egger's and Begg's tests ($p = 0.447$ and $p = 0.567$, respectively) revealed no small study effect. An asymmetric scatter of studies was also evident from visual inspection of the funnel plot (Fig. 2B). Therefore, a trim-and-fill analysis was performed using 26 effect sizes (no imputed study) (WMD: −1.54 mmHg; 95% CI: −2.85 to −0.23, $p = 0.021$; $p < 0.05$).

3.4. WP supplementation and DBP levels

The effect of WP supplementation on DBP was investigated in 18 studies (with 26 study arms with 898 cases and 1,080 controls). The pooled effect size indicated that DBP did not change after WP supplementation (WMD: −0.27 mmHg; 95% CI: −1.14, 0.59, $p = 0.534$), with large heterogeneity between studies ($I^2 = 64.8\%$, $p < 0.001$) (Fig. 3A). WP supplementation significantly decreased DBP at a dose of >30 g/day, in RCTs that used WP isolate powder for their intervention, in sample sizes ≤100, in studies with an intervention duration of ≤10 weeks, and in those studies that were conducted in patients with HTN and had participants with a BMI of 25–30 kg/m^2 (Table 4). There was no significant difference in the overall estimate after each study was excluded using sensitivity analysis. No effects of small studies were detected using Egger's and Begg's tests ($p = 0.573$ and $p = 0.724$, respectively). Visual inspection of the funnel plot revealed asymmetric scatter and publication bias (Fig. 3B). Therefore, a trim-and-fill analysis was performed, but no imputed studies were added (WMD: −0.27 mmHg; 95% CI: −1.14, 0.59, $p > 0.05$).

3.5. Meta-regression

A meta-regression analysis was performed to investigate a possible association between SBP and DBP reduction, WP dosage (g/day), sample size, and duration of intervention (weeks). There was a linear relationship between dose and absolute changes in DBP ($p = 0.017$) (Suppl. Fig. 1) and between duration and absolute changes in SBP ($p = 0.025$) (Suppl. Fig. 2), but not for other cases ($p > 0.05$) (Suppl. Fig. 3–6). Meta-regression analysis showed that WP supplementation significantly altered DBP in a linear fashion depending on the dose used, with higher doses enhancing the trend found to decrease DBP. In addition, meta-regression analysis showed that there was a significant linear relationship between study duration and SBP. That is, with increasing study duration, SBP increases.

3.6. Nonlinear relationship between dose and duration of intervention and changes in BP

In the nonlinear dose-response analysis, a trend toward a significant effect of study duration on SBP was observed

Table 2 Results of risk of bias assessment for randomized clinical trials included in the current meta-analysis.

Study	Random sequence generation	Allocation concealment	Reporting bias	Other sources of bias	Performance bias	Detection bias	Attrition bias	Overall quality
Pal et al., 2010	L	U	L	L	L	H	L	Fair
Fluegel, Shultz et al., 2010	L	L	L	L	L	L	L	Good
Dovgalevsky et al., 2012	L	L	L	L	H	U	U	Poor
Sheikholeslami Vatani 2012	L	U	L	L	H	U	U	Poor
Gouni-Berthold et al., 2012	L	L	L	L	L	L	L	Good
Hodgson et al., 2012	L	L	L	L	L	L	L	Good
Weinheimer et al., 2012	L	H	L	L	L	L	L	Fair
Figueroa et al., 2014	L	H	L	L	L	L	H	Fair
Arciero et al., 2016	L	H	L	L	L	H	L	Fair
Fekete et al., 2016	L	L	L	L	L	L	L	Good
Kataoka et al., 2016	L	H	L	L	H	H	H	Poor
Kjølbæk et al., 2017	L	L	L	L	L	L	L	Good
Nabuco et al., 2019	L	L	L	L	L	L	L	Good
Jeong et al., 2019	L	L	L	L	H	H	L	Fair
Nabuco et al., 2019	L	L	L	L	L	L	L	Good
Yang et al., 2019	L	L	L	L	L	L	L	Good
Lefferts et al., 2020	L	H	L	L	L	L	L	Fair
Fuglsang-Nielsen et al., 2020	L	L	L	L	L	L	L	Good

[1]Each study was assessed for risk of bias using the Cochrane Risk of Bias Assessment Tool. Domains of assessment were included random sequence generation, allocation concealment, reporting bias, performance bias, detection bias, attrition bias, and other sources of bias. Each domain was scored as "high risk" if it contained methodological flaws that may have affected the results, "low risk" if the flaw was deemed inconsequential, and "unclear risk" if information was insufficient to determine. If a study got "low risk" for all domains, it considered as a high-quality study with totally low risk of bias.

Table 3 GRADE approach summary of findings and quality of evidence assessment.

Outcome measure	Summary of findings		Quality of evidence assessment (GRADE)					
	No of patients (trials)	WMD* (95% CI)	Risk of bias[a]	Inconsistency[b]	Indirectness[c]	Imprecision[d]	Publication bias[e]	Quality of evidence[f]
Blood pressure								
SBP (mmHg)	1978 [18]	−1.54 (−2.85, −0.23)	Not serious	Not serious	Not serious	Not serious	Not serious	High
DBP (mmHg)	1978 [18]	−0.27 (−1.14, 0.59)	Not serious	Not serious	Not serious	serious	Not serious	Moderate

SBP = Systolic blood pressure; DBP = Diastolic blood pressure.
[a] Risk of bias based on the Cochrane Risk of Bias Tool. This tool assesses selection bias, performance bias, detection bias, attrition bias, and reporting bias. Only one study had clear selection bias, performance bias, and detection bias. Four of 14 studies had attrition bias.
[b] Downgraded if there was a substantial unexplained heterogeneity ($I^2 > 50\%$, $P < 0.10$) that was unexplained by meta-regression or subgroup analyses.
[c] Downgraded if there were factors present relating to the participants, interventions, or outcomes that limited the generalizability of the results.
[d] Optimal information size was not met, or the 95% CI include the null value lower and upper bounds of the 95% CI were <0.95 and >1.05, respectively.
[e] Downgraded if there was an evidence of publication bias using funnel plot.
[f] Since all included studies were meta-analyses of randomized clinical trials, the certainty of the evidence was graded as high for all outcomes by default and then downgraded based on prespecified criteria. Quality was graded as high, moderate, low, and very low.

(coefficiency = −2.04, P-nonlinearity = 0.025) (Fig. 4A). Based on the dose-response evaluation, there was a significant nonlinear relationship between the duration of WP supplementation and SBP (P-nonlinearity = 0.025). The trend toward an increase in SBP persisted until 25 weeks of the intervention, after which this effect reversed. However, there was no nonlinear relationship between effect size and treatment dose for SBP (Fig. 4B). In addition, WP supplementation did not significantly alter DBP levels as a function of dose and duration (P-nonlinearity >0.05) (Fig. 5A and B).

4. Discussion

To the best of our knowledge, the present systematic review and meta-analysis is the first study to investigate the effects of WP supplementation on BP. From the overall results, although supplementation with WP resulted in a statistically significant reduction in BP in the intervention groups compared with the control groups, this reduction was not clinically significant. A clinically significant reduction in BP is considered to be a reduction in SBP of

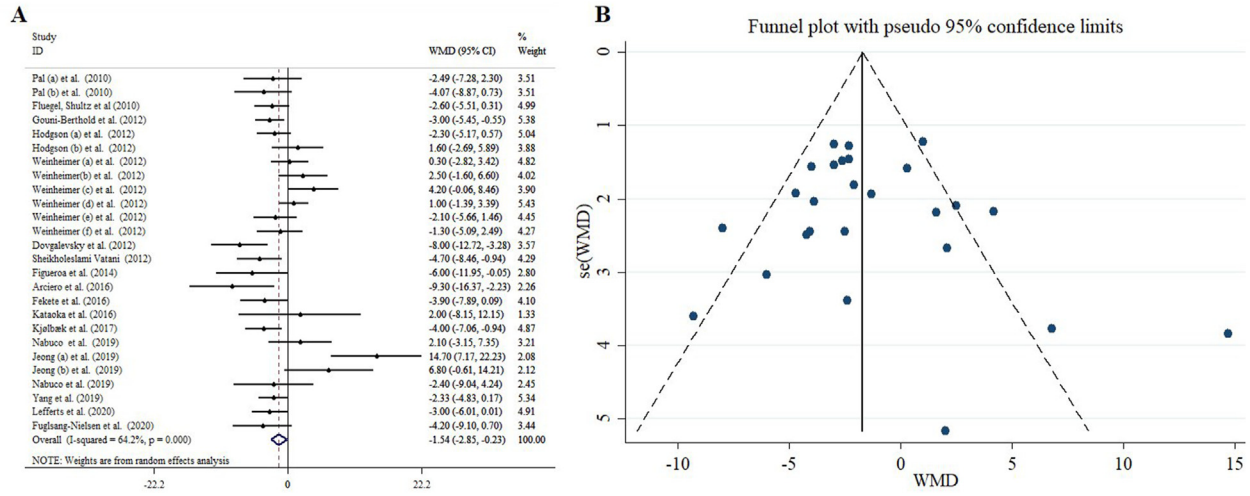

Figure 2 Forest and funnel plot with weighted mean difference and 95% confidence intervals (CIs) for the effect of whey protein supplementation on systolic blood pressure (SBP).

≥10 mmHg or a reduction in DBP of ≥5 mmHg during a follow-up period of 3 months [51].

It has been shown that lowering SBP and DBP by ≥ 2 mmHg can lead to a significant reduction in the incidence of CVD in healthy people and in people with HTN [52]. Moreover, in this study, no significant changes in DBP were observed after WP supplementation compared with a control group. The current study showed that administration of WP at a dosage of >30 g/day resulted in an effect on SBP and DBP. Also, administration of WP for up to 25 weeks leads to an effect on SBP and DBP. Oberoi et al. indicated that BP of healthy elderly men decreased after consumption of beverages containing 30 g and 70 g of WP, with a significant decrease occurring between 120 and 180 min after WP consumption [54].

The present study summarized 18 RCTs that examined the effect of WP supplementation in relation to numerous outcomes in a wide range of diseases, namely, polycystic ovarian syndrome (PCOS), sarcopenic obesity, hemodialysis, metabolic syndrome, overweight and obesity, prehypertension, and HTN. To obtain a conclusive result, subgroup analyses were performed based on the average age of participants, duration of intervention, dosage of supplementation, type of intervention, study population, type of control groups, sample size, average BMI of subjects, and study quality. The results of the subgroup analysis suggested that the average age of participants, study quality, and WP supplementation dosage were not promising factors influencing the association between WP and SBP. Subgroup analyses revealed that an intervention duration of <20 weeks had a stronger effect on HTN than studies with a duration of >20 weeks. In addition, studies involving both sexes showed more significant effects of WP on HTN than studies involving either women only or men only. Another important factor that could influence the overall results is the form of supplementation. In this regard, the subgroup analysis showed that WP in the form of powder had no significant effect on HTN. In other words, other forms of supplementation showed promising effects on the relationship between WP and SBP. Considering the average BMI of the participants, WP supplementation was more effective in reducing HTN in healthy and overweight individuals. In addition, WP supplementation showed positive effects on DBP in overweight individuals.

It should be noted that different health conditions could also significantly alter the effectiveness of WP supplementation on the outcomes studied. For example, WP administration had more promising effects on HTN in individuals with HTN, metabolic syndrome, overweight and obesity, and in individuals undergoing hemodialysis. On the other hand, WP had a more significant lowering effect on BP in subjects with HTN and hemodialysis. Interestingly, studies with small sample sizes (≤100) showed a stronger effect on SBP. These conflicting results could be due to several factors, including different doses of supplementation, different populations, and types of intervention. In this systematic review and meta-analysis, the Cochrane risk of bias assessment tool was applied to assess the quality of the included studies. The risk of bias results show that 78% of the studies were low risk, whereas 11% of the studies were high risk and unclear. Therefore, it can be concluded that the results of the present study are reliable. In addition, the results of Egger's test and funnel plots showed no evidence of publication bias for SBP and DBP.

4.1. Mechanisms of action of WP on BP

Functional foods are ingredients that have health benefits beyond their nutritional value. Because of their potential to alleviate or avert certain noncommunicable diseases, an increasing number of studies on functional foods are currently being conducted [53]. WPs are recognized as an excellent source of nutrients and are characterized by the content of certain bioactive components [53]. Previous evidence suggests that WP supplementation has beneficial effects on numerous components of the metabolic syndrome [54]. For instance, a recent systematic review and meta-analysis demonstrated the beneficial effects of WP

Table 4 Subgroup analyses for the effects of whey protein supplementation on blood pressure.

	NO	WMD (95% CI)[1]	P-within[2]	I^2 (%)[3]	P-heterogeneity[4]
Whey protein supplementation on SBP					
Overall	26	−1.54 (−2.85, −0.23)	0.021	64.2	<0.001
Age (years)					
<50	12	−1.88 (−2.87, −0.88)	<0.001	52.5	0.017
≥50	14	−1.49 (−2.58, −0.39)	0.008	72.0	<0.001
Gender					
Women	5	−1.29 (−3.24, 0.66)	0.193	36.9	0.175
Men	1	2.00 (−8.15, 12.15)	0.699	-	-
Both	20	−1.79 (−2.59, −0.99)	<0.001	69.7	<0.001
Intervention duration (week)					
≤10	7	−3.98 (−5.60, −2.35)	<0.001	0.0	0.430
10−20	11	−1.95 (−2.99, −0.91)	<0.001	34.4	0.123
>20	8	0.32 (−1.04, 1.69)	0.642	78.4	<0.001
Dosage of whey protein (g/day)					
≤30	13	−1.19 (−2.19, −0.18)	<0.001	69.7	<0.001
>30	13	−2.31 (−3.40, −1.22)	<0.001	57.1	0.006
Intervention type					
Whey protein powder	14	−0.63 (−1.66, 0.40)	0.277	72.7	<0.001
Whey-protein isolate powder	5	−3.61 (−5.35, −1.87)	<0.001	0.0	0.946
High-protein beverage	3	−1.70 (−3.54, 0.15)	0.071	28.9	0.245
Hydrolyzed whey protein drink	4	−3.10 (−5.02, −1.17)	<0.001	62.3	0.047
Study population					
Overweight & Obesity	13	−1.27 (−2.33, −0.21)	0.019	57.0	0.005
Hypertension	6	−3.39 (−4.92, −1.85)	<0.001	23.4	0.258
Overweight with Hypertension	2	−1.09 (−3.48, 1.29)	0.368	54.5	0.138
Hemodialysis	2	10.69 (5.41, 15.97)	<0.001	53.5	0.143
Healthy	2	−1.74 (−4.35, 0.88)	0.193	63.3	0.099
Metabolic syndrome	1	−3.00 (−5.45, −0.55)	0.017	-	-
Control intervention type					
Carbohydrate placebo	9	−3.36 (−4.63, −2.08)	<0.001	18.1	0.262
Nonprotein placebo powder	6	0.61 (−0.75, 1.97)	0.379	27.3	0.229
Glucose powder	3	−2.75 (−5.97, 0.47)	0.094	0.0	0.564
Low-protein beverage	2	−1.09 (−3.48, 1.29)	0.368	54.5	0.138
Non-nutritive beverage	2	10.69 (5.41, 15.97)	<0.001	53.5	0.143
Others	4	−3.53 (−5.17, −1.90)	<0.001	14.3	0.320
Sample size					
≤100	19	−2.33 (−3.20, −1.26)	<0.001	69.3	<0.001
>100	7	−0.96 (−2.11, 0.18)	0.097	29.4	0.204
BMI					
≤ 25	3	−2.29 (−4.16, −0.43)	0.016	0.0	0.694
25−30	11	−2.32 (−3.51, −1.12)	<0.001	49.3	0.032
>30	12	−0.99 (−2.08, 0.10)	0.075	76.2	<0.001
Study quality					
Low	9	−3.47 (−5.10, −1.85)	<0.001	79.1	<0.001
High	17	−1.24 (−2.07, −0.41)	0.003	37.9	0.058
Whey protein supplementation on DBP					
Overall	26	−0.27 (−1.14, 0.59)	0.534	64.8	<0.001
Age (years)					
<50	12	−0.45 (−1.14, 0.23)	0.195	56.6	0.008
≥50	14	−0.25 (−0.94, 0.44)	0.481	71.4	<0.001
Gender					
Women	5	−0.21 (−1.47, 1.06)	0.750	33.4	0.199
Men	1	0.00 (−5.18, 5.18)	0.999	-	-
Both	20	−0.38 (−0.91, 0.15)	0.158	70.7	<0.001
Intervention duration (week)					
≤10	7	−1.12 (−2.20, −0.04)	0.041	42.9	0.105
10−20	11	−0.47 (−1.17, 0.22)	0.184	30.9	0.153
>20	8	0.36 (−0.53, 1.24)	0.430	83.1	<0.001
Dosage of whey protein (g/day)					
≤30	13	0.54 (−0.13, 1.21)	0.116	63.8	<0.001
>30	13	−1.36 (−2.07, −0.65)	<0.001	48.5	0.025
Intervention type					
Whey protein powder	14	0.13 (−0.56, 0.82)	0.715	74.1	<0.001
Whey-protein isolate powder	5	−1.58 (−2.68, −0.47)	<0.001	0.0	0.515
High-protein beverage	3	−0.08 (−1.35, 1.18)	0.897	0.0	0.416

(*continued on next page*)

Table 4 (*continued*)

	NO	WMD (95% CI)[1]	P-within[2]	I^2 (%)[3]	P-heterogeneity[4]
Hydrolyzed whey protein drink	4	−0.63 (−1.84, 0.59)	0.314	66.0	0.032
Study population					
Overweight & Obesity	13	−0.15 (−0.86, 0.55)	0.669	53.6	0.011
Hypertension	6	−1.32 (−2.37, −0.27)	0.013	45.7	0.101
Overweight with hypertension	2	−0.54 (−2.16, 1.09)	0.519	1.0	0.315
Hemodialysis	2	7.29 (4.31, 10.26)	<0.001	0.0	0.742
Healthy	2	−1.03 (−2.46, 0.41)	0.160	79.9	0.026
Metabolic syndrome	1	−0.30 (−2.12, 1.52)	0.746	-	-
Control intervention type					
Carbohydrate placebo	9	−1.79 (−2.64, −0.95)	<0.001	51.8	0.035
Nonprotein placebo powder	6	0.55 (−0.32, 1.41)	0.214	42.0	0.125
Glucose powder	3	−1.00 (−3.09, 1.10)	0.351	0.0	0.464
Low-protein beverage	2	−0.54 (−2.16, 1.09)	0.519	1.0	0.315
Non-nutritive beverage	2	7.29 (4.31, 10.26)	<0.001	0.0	0.742
Others	4	−0.11 (−1.28, 1.06)	0.858	0.0	0.629
Sample size					
≤100	19	−0.69 (−1.32, −0.06)	0.032	70.1	<0.001
>100	7	0.15 (−0.62, 0.91)	0.710	24.6	0.241
BMI					
≤ 25	3	−0.12 (−1.47, 1.24)	0.867	0.0	0.610
25−30	11	−1.19 (−1.95, −0.43)	<0.001	25.7	0.199
>30	12	0.33 (−0.39, −1.05)	0.375	77.3	<0.001
Study quality					
Low	9	−1.38 (−2.45, −0.31)	0.012	82.0	<0.001
High	17	−0.09 (−0.63, 0.46)	0.760	27.7	0.139

WMD; weighted mean differences; CI, confidence interval; T2DM, type 2 diabetes mellitus; NAFLD, nonalcoholic fatty liver disease.
[1] Obtained from the random-effects model.
[2] Refers to the mean (95% CI).
[3] Inconsistency, percentage of variation across studies due to heterogeneity.
[4] Obtained from the Q-test.

supplementation on glycemic control, BP, and lipid parameters in people with overweight and obesity [55]. Several studies have investigated the mechanism of the antihypertensive effect of WP. Tahavorgar et al. [56] examined the effects of WP compared with soy protein on cardiovascular risk factors over 12 weeks. Their results showed a significant reduction in BP. These results are in agreement with those of the present meta-analysis. On the other hand, the studies by Lee, Skurk [57] showed that WP supplementation had an incremental effect on BP. These conflicting results could be due to different forms of supplementation, duration of intervention, and different study populations. The BP-lowering effect of WP could be due to the composition of its subfractions, which include glycol macropeptides, α-lactalbumin, β-lactoglobulin, and immunoglobulins [58]. These compounds possess several properties. For example,

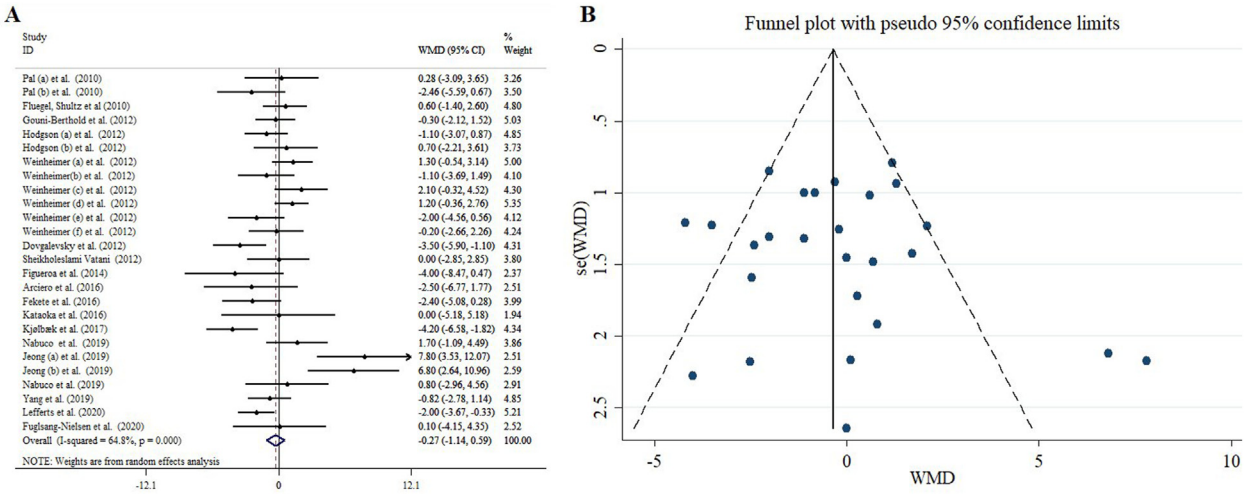

Figure 3 Forest and funnel plot showing weighted mean difference and 95% confidence intervals (CIs) for the effect of whey protein supplementation on diastolic blood pressure (DBP).

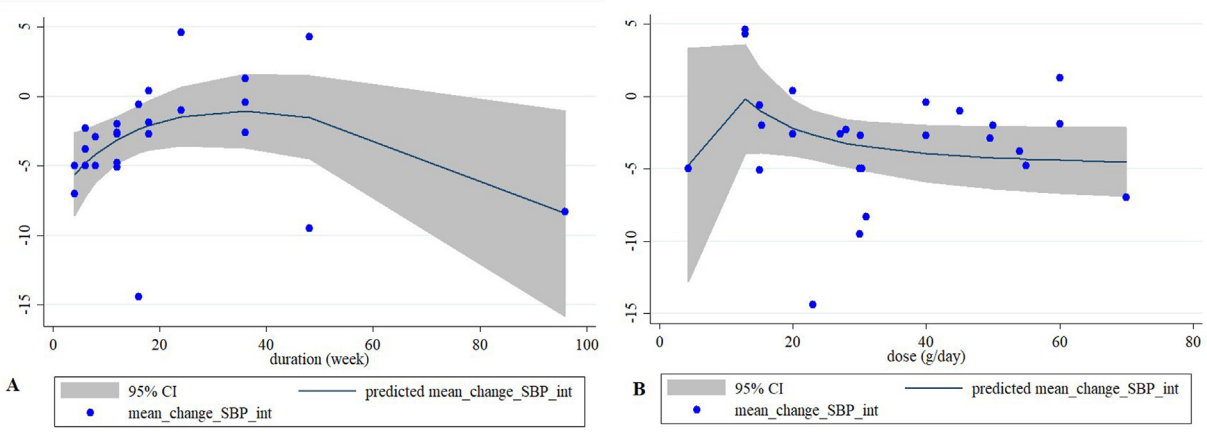

Figure 4 Nonlinear dose-response relationships between whey protein (a) duration of intervention (weeks), (b) dosage (g/d), and unstandardized mean difference in SBP. The 95% CI is shown in the shaded areas.

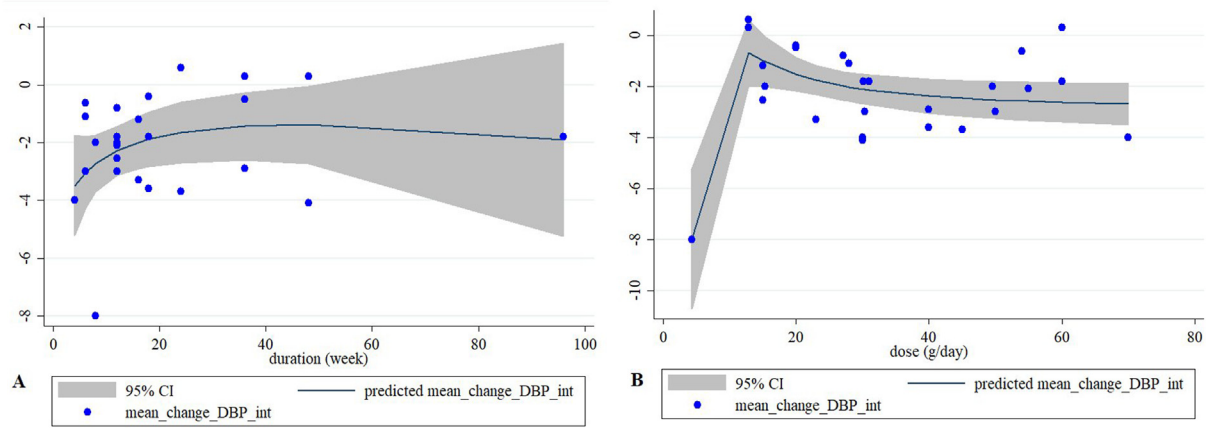

Figure 5 Nonlinear dose-response relationships between whey protein (a) duration of intervention (weeks), (b) dose (g/day), and unstandardized mean difference in DBP. The 95% CI is shown in the shaded areas.

α-lactalbumin and β-lactoglobulin have been shown to have ACE inhibitory effects [58]. ACE is an important component of the renin–angiotensin system (RAS).

This system includes the conversion of the hormone angiotensin I to angiotensin II, which subsequently leads to vasoconstriction [59]. Inhibition of ACE also leads to accumulation of bradykinin, a peptide containing nine amino acid residues that have vasodilatory effects [60]. WP-derived peptides have been shown to inhibit the synthesis of angiotensin II from angiotensin I, which in turn increases the concentration of bradykinin and leads to a greater vasodilatory effect [60,61]. Another important mechanism for the beneficial effects of WP on BP has to do with the production of nitric oxide (NO) [27,58]. Given the role of NO in lowering BP, it is critical to stimulate its production. WP contains cysteine, a key substrate for the formation of the antioxidant glutathione (GSH) [62]. WP-induced GSH leads to a marked reduction in inflammation and oxidative stress, which in turn increases the activity of the NO-generating enzyme eNOS [63]. Thus, WP supplementation reduces BP via multiple mechanisms of action (Fig. 6). The current study has several strengths and limitations. One of the main strengths of this study is the subgroup analyses, which revealed different aspects of the effect of WP supplementation on BP. In addition, potential bias was accounted for using several methods, including Egger's test and the Cochrane Risk of Bias Tool. However, the main limitation of the present study is the heterogeneity of the included studies, especially the different study populations.

4.2. Potential side effects of WP supplementation

WP is one of the most commonly administered supplements, especially in individuals who are physically active and seeking a significant increase in protein intake [64–66]. Therefore, the ideal dose of WP supplementation may vary depending on the individual's level of physical activity, body composition, and goals [67,68]. It has been shown that the ideal dose of WP is between 20 and 25 g/day, resulting in beneficial effects [69]. Therefore, a dose above 40 g/day could cause numerous side effects on various organisms, including kidney, liver, and intestine [69].

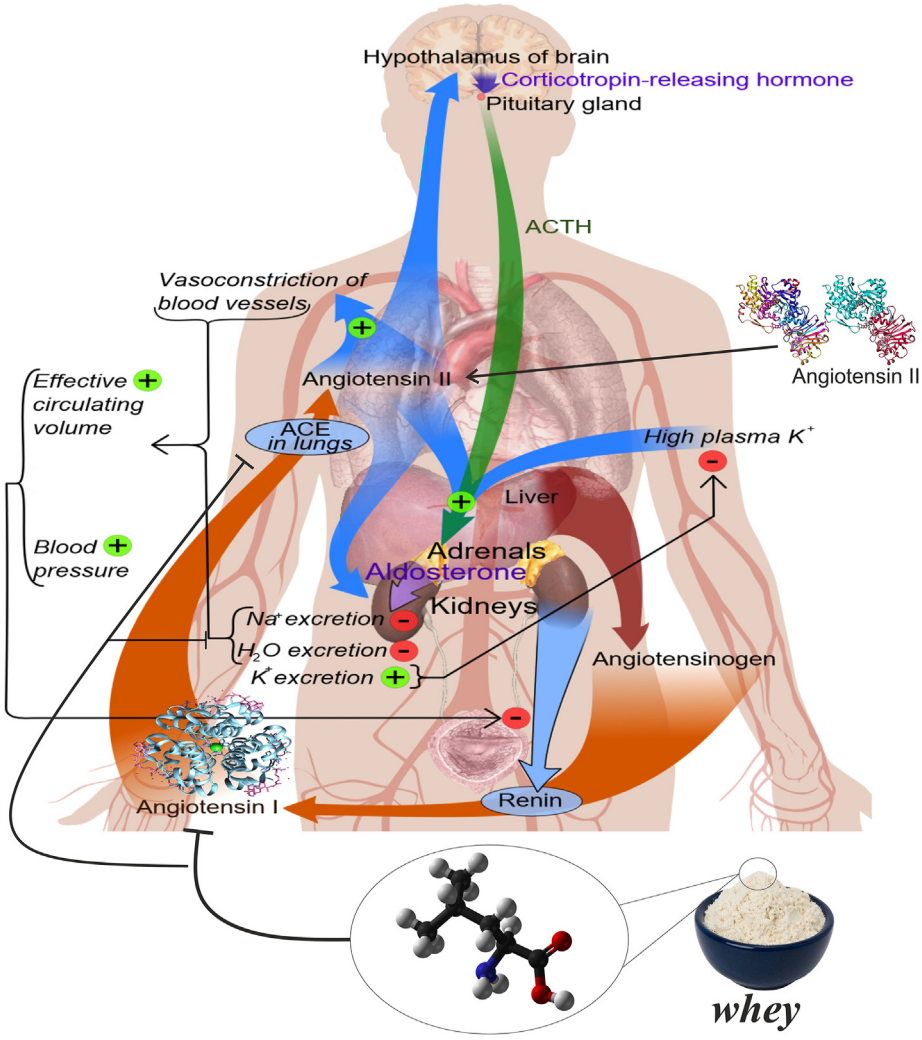

Figure 6 Mechanism of action of whey protein on blood pressure.

5. Conclusion

The current systematic review and meta-analysis found that supplementation with WP could lead to a significant decrease in SBP. However, no significant effect on DBP was found. Therefore, it can be concluded that WP might be a reasonable option for people suffering from hypertension. It should be noted that numerous factors, including the form of supplementation, duration of intervention, and various chronic conditions, may alter the effects of WP. Further large-scale studies with different doses of WP and with longer intervention duration are needed to confirm these findings.

Author contributions

MV and AK designed the study. Literature search and screening of data were performed by AK, MD, and VM. Data extraction and quality assessment were performed independently by MV, AK, SHA, and MAF. HSH, MZ, MV, and AK analyzed and interpreted the data and drafted the manuscript. All authors read and approved the final manuscript.

Statement of ethics

The protocol of the present work was approved by the Ethics Committee of Tabriz University of Medical Sciences (Code: IR.TBZMED.VCR.REC.1402.038, Grant number: 71121).

Declaration of competing interest

Nothing to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2023.05.025.

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