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Meta-analyses

The effects of whey protein supplementation on indices of cardiometabolic health: A systematic review and meta-analysis of randomized controlled trials

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SUMMARY

Introduction: The increasing prevalence of cardiometabolic diseases highlights the urgent need for practical interventions to mitigate their associated public health burden. Whey protein supplementation has emerged as a potential intervention for improving markers of cardiometabolic health. The aim of this systematic review and meta-analysis was to examine the effect of whey protein ingestion on cardiometabolic profile in adults.

Methods: A systematic search was conducted in PubMed, Web of Science, Scopus, and Cochrane Library from inception until June 2024. Eligible RCTs compared the effect of whey protein supplementation compared to placebo or a carbohydrate-based control on markers of cardiometabolic health. Using the random effects inverse-variance model, we estimated the mean difference (MD) in blood pressure, high- and low-density lipoproteins (HDL-cholesterol, LDL-cholesterol), total cholesterol, triglycerides, and homeostatic model assessment for insulin resistance (HOMA-IR) index.

Results: This meta-analysis included 21 RCTs. Whey protein supplementation had no effect on HDL-cholesterol concentration but did elicit a reduction in LDL-cholesterol in individuals aged <50 years ($P < 0.01$) and when combined with exercise (MD: −5.38, 95 % confidence interval (95 % CI): −8.87 to −1.88, $I^2 = 0$ %, $P < 0.01$). Total cholesterol was reduced with interventions that combined whey protein supplementation and exercise (MD: −8.58, −14.32 to −2.83, $I^2 = 55$ %, $P < 0.01$), irrespective of age, protein dose, and body mass index ≥25 kg/m^2 (MD: −6.71, 95 % CI: −11.60 to −1.83, $I^2 = 74$ %, $P < 0.01$). Whey protein supplementation of ≥12 weeks was associated with reduced triglyceride levels (MD: −6.61, 95 % CI: −11.06 to −2.17, $I^2 = 70$ %, $P < 0.01$). There was no clinically relevant effect of whey protein supplementation on blood pressure and HOMA-IR, however, changes pertinent to HDL-cholesterol, total cholesterol, and triglyceride reduction were primarily displayed in healthy adults.

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Conclusions: Whey protein supplementation may be an effective intervention for reducing LDL and total cholesterol levels, particularly in healthy, overweight/obese adults aged <50 years, with the greatest benefits observed when combined with exercise. Healthy adults also showed a benefit regarding triglyceride levels.

1. Introduction

Cardiometabolic risk factors such as obesity, hypertension, insulin resistance, and dyslipidemia are precursors of type 2 diabetes and cardiovascular disease. These diseases lead to a reduced quality of life and increased mortality, and at a significant cost to health care services [1]. The increased prevalence of these conditions has attracted global interest from researchers and clinical practitioners to investigate the efficacy of non-pharmacological interventions aimed at mitigating this significant public health and financial burden. Indeed, the prevalence of hypertension in adults aged 30–79 years has doubled over the last three decades [2], while it is estimated that >6000 individuals per 100,000 present with type 2 diabetes worldwide [3].

A key component of non-pharmacological treatments to improve cardiometabolic profile is dietary manipulation. High dietary fibre intake and caloric restriction have been proposed as dietary strategies to reduce cardiometabolic disease risk, as mediated by improvements in diet quality and reductions in excess body fat respectively [4–6]. Moreover, a decrease in consumption of saturated fatty acids and higher intake of polyunsaturated fatty acids (i.e., omega-3) may represent alternative nutritional modifications to improve cardiometabolic health in some populations [7,8].

Evidence is accumulating regarding the clinical utility of dietary protein interventions in reducing cardiometabolic/cardiovascular disease risk. The primary nutritional role of dietary protein is the provision of amino acids for the synthesis of new functional proteins, including skeletal muscle [9]. Dietary protein intake also impacts other psycho-physiological indices of metabolic health, including body composition, satiety, cognition, immune and cardiometabolic health [10–12]. Specifically, whey protein as a by-product of cheese manufacturing and 'high-quality' protein source has been purported to improve markers of metabolic and cardiovascular health via multiple mechanisms linked with the increased delivery of bio-active peptides (defined as the fragments of amino acid sequences in a protein that provide biological functions beyond their nutritional value) [13]. Accordingly, studies have reported whey protein ingestion to elicit improvements in postprandial glycemic and insulin control [14] which may be attributed to enhanced β-cell function leading to elevated levels of plasma glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) in middle-aged adults with type 2 diabetes [15,16]. Consistent with these findings, several meta-analyses have demonstrated improvements in postprandial glycemic control and markers of cardiometabolic health (i.e., blood pressure, HbA1c, lipoproteins) following whey protein supplementation of varying doses (4–55 g) compared to control in individuals with obesity and/or hypertension [17–23].

To date, no study has systematically examined the short- and longer-term impact of whey protein supplementation compared with a placebo carbohydrate-based control on multiple markers of cardiometabolic health. Therefore, the aim of this systematic review and meta-analysis is to evaluate the effectiveness of whey protein supplementation on blood pressure, lipoprotein profile, and glycemic control in adults.

2. Methods

This systematic review and meta-analysis was performed according to PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines [24]. The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) database (CRD42023439602).

2.1. Search strategy

Two reviewers (KP and KKT) searched PubMed, Scopus, Web of Science, and the Cochrane library independently from the beginning of each respective search tool until June 2024 using the following terms: "whey protein" AND "lipoproteins" OR "blood pressure" OR "insulin resistance" (Table S1). Discrepancies in the literature search process were resolved by a third investigator (KSK). The following inclusion criteria were applied: (1) studies were randomized controlled trials (RCTs) with participants aged 18 years old and above irrespective of health status; (2) the intervention group received whey protein supplementation for at least 4 weeks; and (3) the control group received a carbohydrate-based control or placebo (i.e., maltodextrin). In cases where the whey protein group received an additional intervention, such as adjunctive micronutrients or exercise, the placebo control was required to employ an identical intervention. Studies were excluded if (1) they were not RCTs; (2) the RCT was an open-label trial; and (3) a full-text manuscript for the RCT was not available. The search was limited by identifying human studies only. Reference lists of reviews focusing on protein nutrition and cardiometabolic health were also scanned, and additional studies were identified that were relevant to this topic.

2.2. Data extraction and risk of bias

Two investigators (KP and KKT) extracted data independently. Extracted data included first author name, affiliated country, date of publication, study design, age, sex, health status, sample size, outcomes of interest, dose and duration of whey protein and control, and dietary intake assessment (energy and protein intake). Disagreements between authors were resolved by a third investigator (KSK). Where numerical data were not reported, graphical values were calculated using WebPlotDigitizer 4.6 software [25]. The quality of included studies was assessed using the Cochrane risk-of-bias 2 tool for randomized trials (RoB 2) and evaluated by two independent reviewers (KKT and KSK). Risk of bias using the RoB 2 tool included assessment of the following criteria of bias in RCTs: (1) randomization process, (2) deviations from intended interventions, (3) missing outcome data, (4) measurement of the outcome, and (5) selection of the reported result. Based on the RoB 2 tool scoring system, study quality was defined as low risk of bias, some concerns, and high risk of bias.

2.3. Outcomes of interest

The following outcomes of cardiometabolic health were included in analyses: systolic blood pressure (SBP), diastolic blood pressure (DBP), high-density lipoprotein-cholesterol (HDL-cholesterol), low-density lipoprotein-cholesterol (LDL-cholesterol), total cholesterol, and the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) index.

2.4. Statistical analysis

A random-effects meta-analysis was employed for all main outcome measurements. All meta-analyses were performed using RevMan (Review Manager, v5.4; The Cochrane Collaboration), and data are presented as the mean difference (MD) or standardized mean difference (SMD), the respective 95 % confidence intervals (CIs), and effect size. Quantitative data were treated as continuous measurements, and changes in outcomes from baseline to follow-up were compared between groups to determine mean differences. The random-effects model and the inverse-variance approach were used to determine statistical significance. Any differences in baseline and follow-up outcome data for which standard deviations were unavailable were estimated using a correlation coefficient calculated from a known change from the baseline standard deviation obtained from a similar study. In cases where no data were available, the SD of changes from baseline was imputed using a correlation coefficient (Corr) of 0.7 and a sensitivity analysis for each outcome was made using a Corr value of 0.5. To calculate the change in SD from baseline for both groups, the following formula was used:

$$SD_{E,change} = \sqrt{SD^2_{E,baseline} + SD^2_{E,final} - \left(2 \times Corr \times SD_{E,baseline} \times SD_{E,final}\right)}$$

Where $SD_{E,change}$ represents the SD of the mean changes from baseline, $SD_{E,baseline}$ represents the SD of the pre-intervention assessment, $SD_{E,final}$ corresponds to the post-intervention SD, and Corr represents the correlations between the baseline and final measurements.

Statistical heterogeneity across studies was assessed using the overlap of their 95% CIs expressed as a measurement of Cochran's Q (χ^2 test) and I^2. Data were classified as moderately heterogeneous when I^2 values ranged from 50 to 74.9 % and as highly heterogeneous when values were 75 % and above. Furthermore, sensitivity analysis was performed to evaluate the robustness of reported statistical results by discounting the effects of studies with increased risk of bias, starch carbohydrate as a control, and studies that did not assess and control for dietary intake. Subgroup analyses were conducted based on whey protein with exercise vs. whey protein alone, age (<50 years vs. ≥ 50 years), treatment duration (<12 weeks vs. ≥ 12 weeks), whey protein dose (≤35 g/day vs. high dose > 35 g/day), BMI (<25 kg/m^2 vs. ≥25 kg/m^2), and placebo vs. non-placebo controls. The meta-analysis of data was synthesized using Cochrane's Review Manager (RevMan 5.4.1) software and a p value < 0.05 was utilized to determine statistically significant differences. Multiple sensitivity analyses were performed for each model by systematically excluding studies to determine if any findings were influenced by removed studies. Meta-regressions were conducted using a random-effects model based on age, energy, and protein intake, using STATA/MP 13.0. Publication bias was assessed by inspecting funnel plots and performing the Egger's test [26]. In case of publication bias, the trim-and-fill method was used, adjusting for the potential effect of unpublished (trimmed) studies [27].

3. Results

3.1. Search results

The initial literature search provided 2279 publications. Following exclusion of 648 duplicates, 1631 publications were screened from which 1579 were marked as ineligible. Overall, from the 40 RCTs that were screened, six studies had no treatment as a comparator, five studies had a placebo or control group that did not meet the inclusion criteria, four studies used multiple nutrients as an intervention, one study was open-label, one study used data from an identical cohort included in our analysis, one study had no outcomes of interest, and one was an acute study. Finally, 21 RCTs were included in the systematic review and meta-analysis [28–48] (Fig. 1). A detailed description of the characteristics of the included studies is presented in Table 1.

3.2. Blood pressure

Our meta-analysis revealed no statistically significant effects of whey protein (n = 326) on SBP (n = 371) (k = 12; MD: −1.52, 95% CI: −3.51 − 0.47, I^2 = 76 %, P = 0.14) (Fig. 2) or DBP (n = 371) (k = 12; MD: −1.05, 95% CI: −2.58 − 0.49, I^2 = 81 %, P = 0.18) (Fig. 3) vs. placebo or carbohydrate-based control. However, subgroup analysis revealed a significant reduction of SBP in individuals <50 years of age (P = 0.03). Details for subgroup and sensitivity analyses are presented in Table S2.

3.3. Lipoprotein profile

No statistically significant effects of whey protein were observed (n = 300) on HDL-cholesterol (n = 327) (k = 13; MD: −0.82, 95% CI: −2.17 − 0.53, I^2 = 43 %, P = 0.23) (Fig. 4) or LDL-cholesterol (n = 318) (k = 12; MD: −2.07, 95% CI: −4.10 to −0.03, I^2 = 6 %, P = 0.05) (Fig. 5) profiles vs. placebo or carbohydrate-based control. Subgroup analyses revealed a significant reduction in LDL-cholesterol concentration in individuals aged <50 years (P = 0.008) with whey protein combined with exercise (P = 0.003), while HDL-cholesterol concentration was reduced following a treatment duration of 12 weeks or more (P = 0.02). No impact of other subgroup parameters was observed on lipoprotein levels (Table S2).

3.4. Total cholesterol and triglyceride levels

This meta-analysis revealed an effect of whey protein (n = 300) on total cholesterol concentration vs. placebo or carbohydrate-based control (n = 327) (k = 13; MD: −6.35, 95% CI: −10.96 to −1.74, I^2 = 71 %, P = 0.007) (Fig. 6). Multiple subgroup analyses displayed statistically significant reductions in total cholesterol concentration with regards to age (<50 years, P = 0.02; ≥50 years,

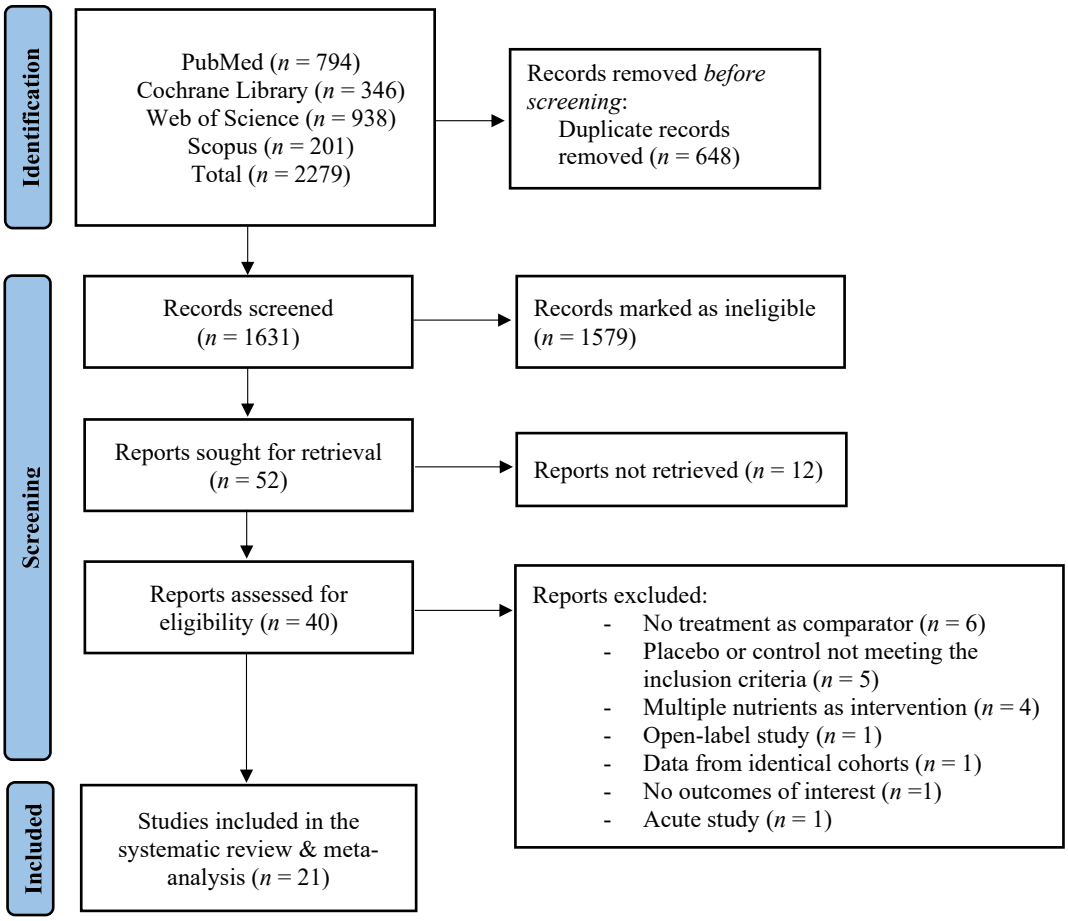

Fig. 1. Flowchart of the employed literature search.

<0.001), BMI >25 kg/m^2 (P = 0.007), treatment dose (>35 g/d, P < 0.001; ≤35 g/d, P < 0.001), non-placebo control (P = 0.04), and whey protein combined with exercise (P = 0.003). Sensitivity analyses also observed a significant reduction in total cholesterol, (i) after the removal of starch carbohydrates as a control (P = 0.007), (ii) when studies did not control for dietary intake (P = 0.04), (iii) in studies with increased risk of bias (P = 0.04), and (iv) in studies that were based on a Corr value of 0.5 (P = 0.002). In addition, our main analysis revealed no effects of whey protein (n = 300) on triglyceride concentrations vs. placebo or carbohydrate-based control (n = 327) (k = 13; MD: −3.20, 95% CI: −8.03 − 1.62, I^2 = 78 %, P = 0.19) (Fig. 7). Subgroup analysis showed a significant effect of ≥12 weeks whey protein supplementation in reducing triglyceride concentrations (P = 0.004). Significant effects of whey protein supplementation on triglyceride concentrations were observed in comparison to a non-placebo control (P = 0.003) and based on the exclusion of studies with increased risk of bias (P = 0.006). Details of the overall results are presented in Table S2.

3.5. HOMA-IR

Our meta-analysis revealed no effect of whey protein (n = 171) on HOMA-IR vs. placebo or carbohydrate-based control (n = 232) (k = 8; MD: 0.24, 95% CI: −0.27 − 0.76, I^2 = 82 %, P = 0.35) (Fig. 8). Similarly, no changes in HOMA-IR were observed with whey protein supplementation following subgroup and sensitivity analyses (Table S2).

3.6. Risk of bias assessment

Two studies [29,42] were classified as having some concerns based on RoB 2 tool, as shown in Table S3. Specifically, 13 studies exhibited some concerns due to insufficient information on treatment allocation [28,30,32,33,35–38,41,42,46–48], one study had increased risk of bias due to deviations from the intended intervention [32], and three studies [29,31,42] had an increased risk due to missing outcome data. Finally, one study also had some concerns due to missing outcome data and the selection of reported results [29].

3.7. Meta-regressions and publication bias

Meta-regressions based on sex, energy, and protein intake did not reveal any confounding impact on any outcome of interest (Table S4). In addition, publication bias was not identified among outcomes of interest (Table S5).

3.8. Impact of comorbidities

To explore further the impact of whey protein supplementation on cardiometabolic markers, we employed analyses based on health status. Not statistically significant changes were observed from whey protein vs. placebo in relation to blood pressure in both adults with overweight and/or obesity (SBP; k = 7; MD: −2.19, 95% CI: −4.86 − 0.48, I^2 = 80 %, P = 0.11 − DBP; k = 7; MD: −1.98, 95%

Table 1
Characteristics of the included studies.

Author Year Country	Study design	Population	N of participants (F/M)		Intervention	Dose and duration	Comparator (Type)	Age	
			Intervention group	Control group				Intervention group	Control group
Kim 2023 Korea	RCT Double blind	Sedentary males	18 (0/18)	16 (0/16	WP	60 g/4 weeks	Placebo (CHO)	23.9 ± 2.5	24.8 ± 3
Soares 2023 Brazil	RCT Triple blind	Males with T2D	14 (0/14)	14 (0/14)	WP + RT	20 g/12 weeks	Placebo (maltodextrin)	68.1 ± 4.5	68.9 ± 4.1
Dos Santos 2022 Brazil	RCT Single blind	Older adults with HF	17 (4/13)	16 (3/13)	WP	30 g/12 weeks	Placebo (maltodextrin)	64 (61–67)	61 (58–78)
Reljic 2022 Germany	RCT Double blind	Healthy adults	24 (13/11)	23 (13/10)	WP + LOW-HIIT	40 g/8 weeks	Placebo (maltodextrin) + LOW-HIIT	30 ± 7.8	32.5 ± 8.0
Mertz 2021 Denmark	RCT Single blind	Healthy adults	50 (22/28)	36 (18/18)	WP	40 g/52 weeks	Placebo (maltodextrin + sucrose)	70.3 ± 4.3	69.6 ± 3.9
Fuglsang-Nielsen 2021 Denmark	RCT Double blind	Adults with obesity	All: 15	All: 16	WP + low fiber	60 g/12 weeks	Maltodextrin + low fiber	64 (58–68)	
Gaffney 2021 New Zealand	RCT Double blind	Adults with overweight/ obesity & T2D	12 (0/12)	12 (0/12)	WP + MMIT	40 g/10 weeks	Placebo (maltodextrin & sucrose) + MMIT	53.6 ± 5.6	57.8 ± 5.2
Lefferts 2020 USA	RCT Double blind	Older adults	53 (26/27)	46 (19/27)	WP	50 g/12 weeks	Placebo (maltodextrin)	69 ± 7	67 ± 6
Nabuco 2019 Brazil	RCT Double blind	Older women	13 (13/0)	13 (13/0)	WP + RT	35 g/12 weeks	Placebo (maltodextrin) + RT	68 ± 4.2	70.1 ± 3.9
Fernandes 2018 Brazil	RCT Double blind	Older women	16 (16/0)	16 (16/0)	WP + RT	35 g/12 weeks	Placebo (maltodextrin)	67.3 ± 4.1	67.8 ± 4
Kjølbæk 2017 Denmark	RCT Double blind	Adults with overweight/ obesity	55 (45/10)	58 (43/15)	WP	45 g/12 weeks	Placebo (maltodextrin)	41.2 ± 10.2	38.3 ± 11.5
Stojkovic 2017 USA	RCT Double blind	Older women	38 (38/0)	46 (46/0)	WP	20 g/78 weeks	Placebo (maltodextrin)	68.9 ± 0.9	69.3 ± 0.9
Kataoka 2016 Japan	RCT Single blind	Older men with hypertension	10 (10/0)	11 (11/0)	WP + cycling exercise	10 g/3× per week/8 weeks	Placebo (glucose)	69 ± 1	69 ± 1
Hulmi 2015 Finland	RCT Double blind	Healthy men	22 (0/22)	21 (0/21)	WP + RT	30 g/12 weeks	Placebo (maltodextrin)	31.4 ± 1.4	36.4 ± 4.2
Ormsbee 2015 South Africa	RCT Double blind	Women with obesity	13 (13/0)	10 (10/0)	WP + RT + HIIT	30 g/4 weeks	Placebo (maltodextrin) + RT + HIIT	29.3 ± 1.2	27.7 ± 2.3
Figueroa 2013 USA	RCT Double blind	Women with obesity & high blood pressure	11 (11/0)	11 (11/0)	WP + AT + RT	30 g/4 weeks	Placebo (Maltodextrin) + AT + RT	28 ± 1	31 ± 2
Hodgson 2012 Australia	RCT Double blind	Older women	109 (109/0)	110 (110/0)	WP	30 g/104 weeks	Placebo (maltodextrin)	74.3 ± 2.7	74.3 ± 2.6
Pal 2012 Australia	RCT Single blind	Adults with overweight/ obesity	25 (21/4)	25 (22/3)	WP	54 g/12 weeks	Placebo (glucose)	48.5 ± 2.0	48.4 ± 1.5
Vatani 2012 Iran	RCT Single blind	Male students with overweight	9 (0/9)	10 (0/10)	WP + RT	90 g/6 weeks	Placebo (orange juice powder)	23 ± 2	21 ± 1
Weinheimer 2012 USA	RCT Double blind	Adults with overweight/ obesity	84 (50/34)	30 (18/12)	WP + CET	60 g/39 weeks	Placebo (maltodextrin)	50 ± 7.1	49 ± 7.0
DeNysschen 2009 USA	RCT Double blind	Men with overweight & hypercholesterolemia	10 (0/10)	9 (0/9)	WP + RT	26.6 g/12 weeks	Placebo (complex CHO)	38 (21–50)	

Data is expressed as mean ± SD or median (interquartile range).
AT, aerobic training; CHO, carbohydrates; HF, heart failure; HIIT, high-intensity interval training; MMIT, mixed mode intense training; RT, resistance training; T2D, type 2 diabetes; WP, whey protein.

CI: −4.23 − 0.27, I^2 = 84 %, P = 0.08) and healthy individuals (SBP; k = 3; MD: −0.94, 95% CI: −2.89 − 1.02, I^2 = 0 %, P = 0.35 − DBP; k = 3; MD: 0.03, 95% CI: −2.34 − 2.40, I^2 = 67 %, P = 0.98) (Figs. S22 and S23). Additionally, HDL-cholesterol was reduced in healthy participants (k = 6; MD: −1.30, 95% CI: −2.30 to −0.30, I^2 = 15 %, P = 0.01), but remained unchanged in those with overweight and/or obesity (k = 5; MD: −0.03, 95% CI: −3.75 − 3.69, I^2 = 69 %, P = 0.99) (Fig. S24). Our analyses did not display a statistically significant change pertaining to LDL-cholesterol to both healthy (k = 6; MD: −1.38, 95% CI: −2.85 − 0.09, I^2 = 0 %, P = 0.07) and adults with overweight and/or obesity (k = 4; MD: −4.97, 95% CI: −11.37 − 1.43, I^2 = 0 %, P = 0.13) (Fig. S25). Reductions related to total cholesterol were shown in healthy participants (k = 6; MD: −8.21, 95%CI: −14.53 to −1.89, I^2 = 79 %, P = 0.01), but not in adults with overweight and/or obesity (k = 5; MD: −4.94, 95% CI: −14.64 − 4.77, I^2 = 72 %, P = 0.32) (Fig. S26). Similar results were depicted in terms of triglycerides (Healthy; k = 6; MD: −8.20, 95% CI: −12.03 to −4.37, I^2 = 57 %, P < 0.01 − With overweight and/or obesity; k = 5; MD: 2.15, 95% CI: −6.35 − 10.65, I^2 = 66 %, P = 0.62) (Fig. S27). Finally, no changes were observed in regards to HOMA-IR

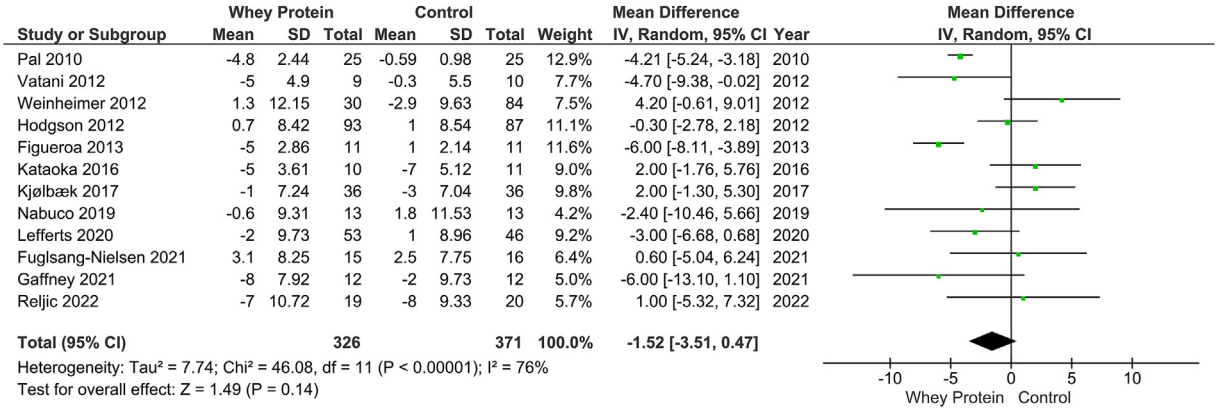

Fig. 2. Effects of whey protein supplementation on systolic blood pressure.

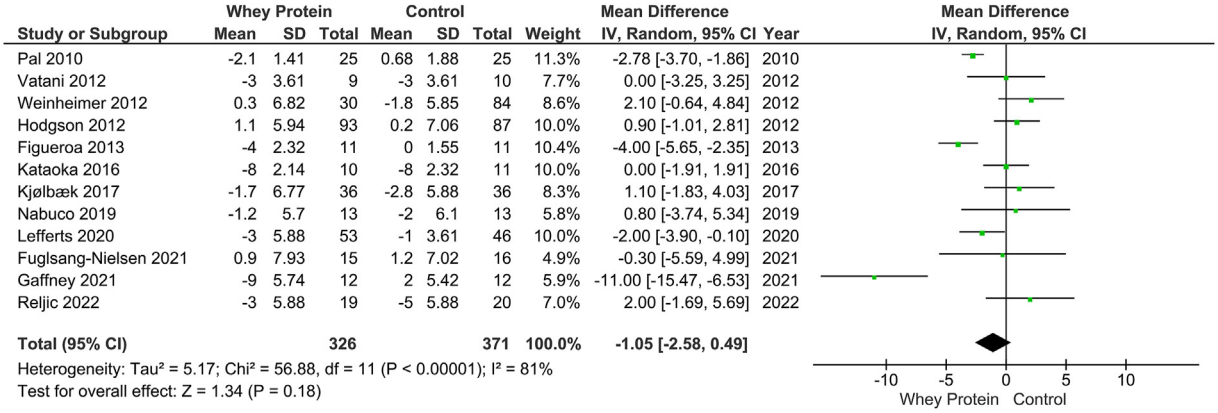

Fig. 3. Effects of whey protein supplementation on diastolic blood pressure.

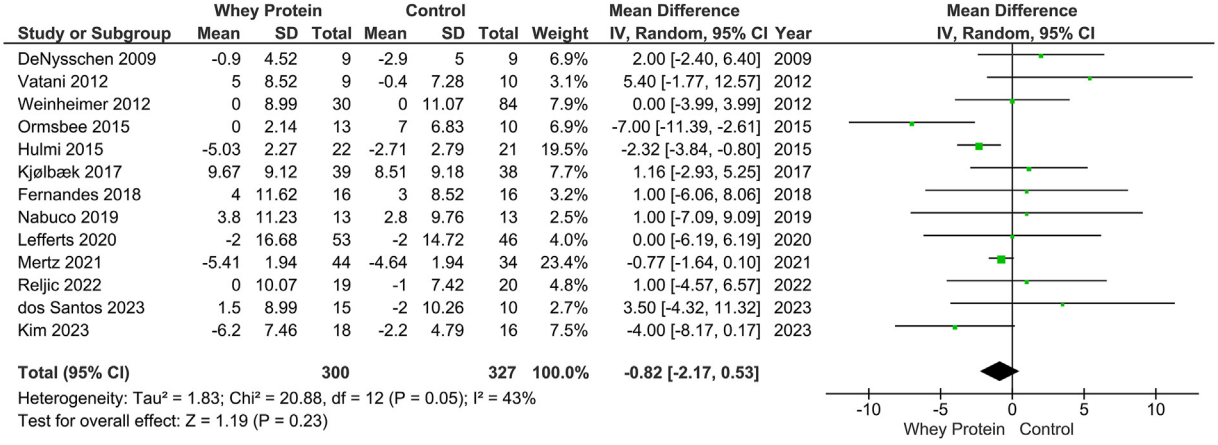

Fig. 4. Effects of whey protein supplementation on HDL-cholesterol levels.

in both adults with overweight and/or obesity ($k = 3$; SMD: 0.33, 95% CI: −0.54 − 1.21, $I^2 = 88$ %, P = 0.46) and type 2 diabetes ($k = 2$; SMD: 0.14, 95% CI: −1.57 − 1.84, $I^2 = 88$ %, P = 0.88) (Fig. S28). Details for all analyses are shown in Table S6.

4. Discussion

To our knowledge, this is the first systematic review and meta-analysis of RCTs that investigated the effect of whey protein supplementation on multiple markers of cardiometabolic health in adults. Our meta-analyses revealed that whey protein supplementation may lower SBP, LDL-cholesterol, and total cholesterol levels compared with placebo, with exercise conferring an additive effect. Moreover, subgroup analyses revealed a greater response to LDL-cholesterol and SBP via whey protein supplementation in adults aged <50 years of age, while reductions in HDL-cholesterol, total cholesterol, and triglyceride were primarily observed in healthy adults as opposed to adults with overweight and/or obesity.

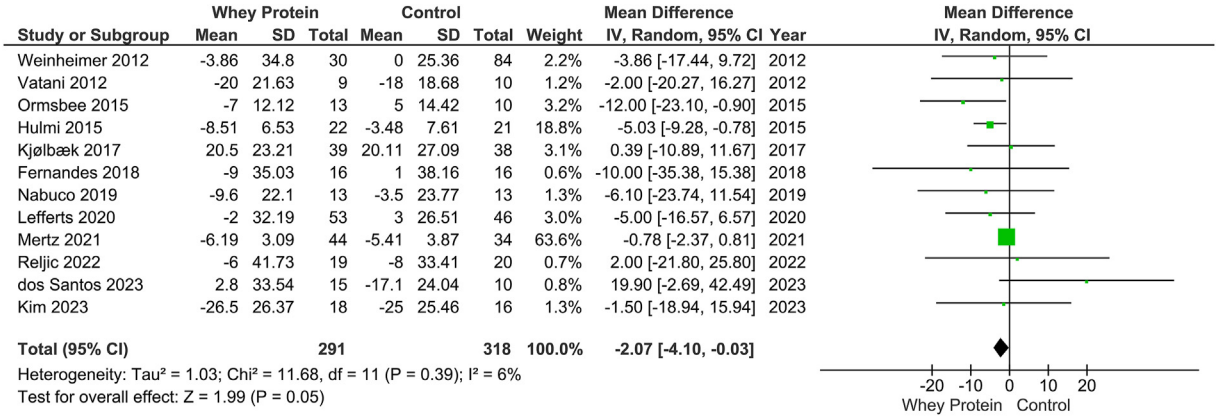

Fig. 5. Effects of whey protein supplementation on LDL-cholesterol levels.

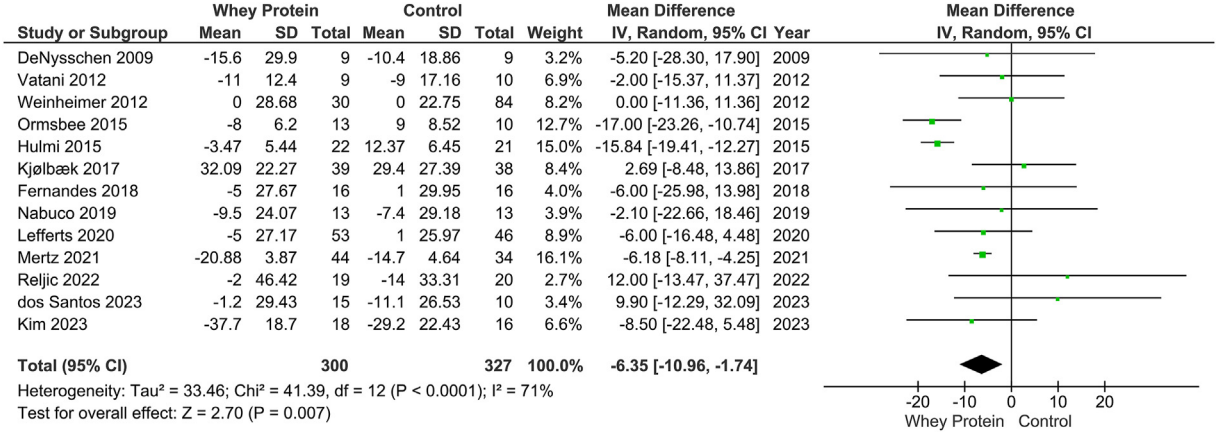

Fig. 6. Effects of whey protein supplementation on total cholesterol levels.

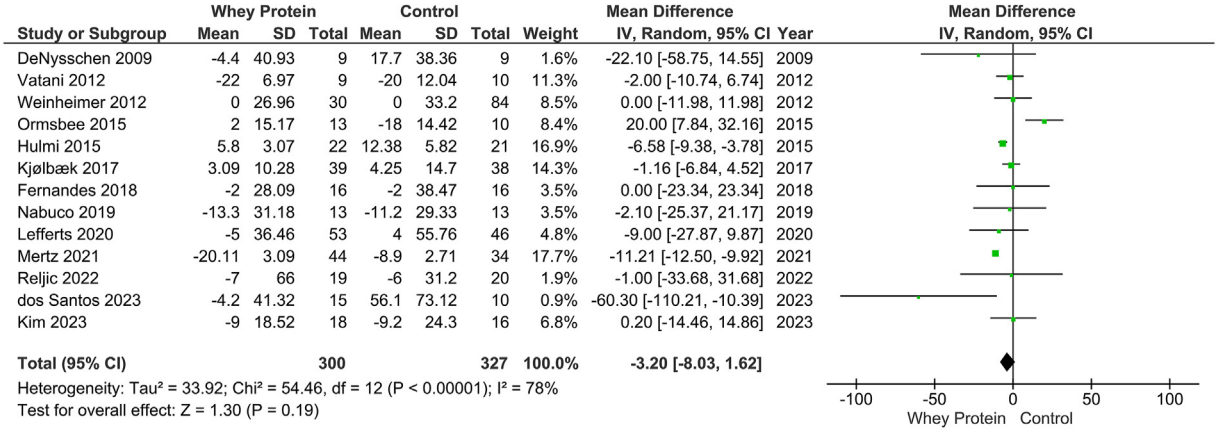

Fig. 7. Effects of whey protein supplementation on triglyceride levels.

Some of our findings underpinned increased heterogeneity, particularly in relation to SPB and DBP, triglycerides, and HOMA-IR (I^2 > 75 %). According to Table S2, significant heterogeneity was observed in the following subgroups: age <50 years (for SBP, triglycerides, and HOMA-IR), BMI ≥25 kg/m^2 (for SBP, DBP, and triglycerides), dose >35 g/day (for HOMA-IR), intervention duration <12 weeks (for HOMA-IR), placebo group (for DBP and HOMA-IR), non-placebo control group (for triglycerides), whey protein alone (for triglycerides), and whey protein combined with exercise (for DBP and HOMA-IR). These factors potentially contribute to the elevated I^2 values observed, beyond what could be explained by meta-regression analyses.

4.1. Whey protein and blood pressure

Based on our systematic review and meta-analysis, we report a reduction in SBP with whey protein supplementation in individuals aged <50 years that may have important implications for

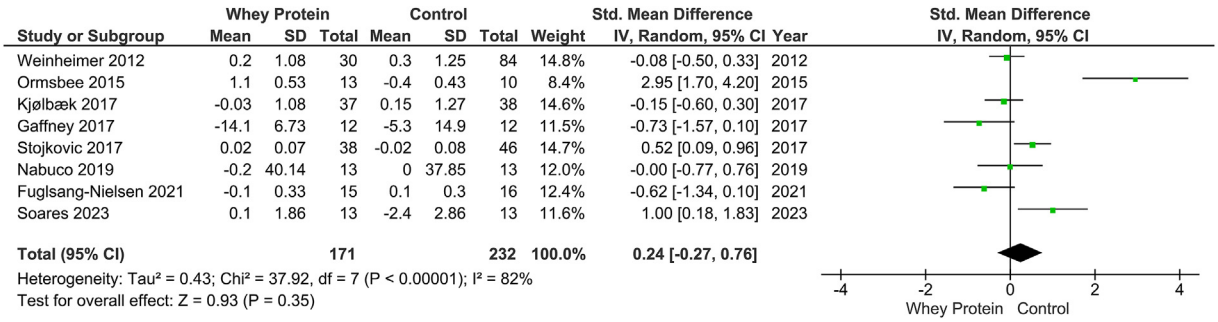

Fig. 8. Effects of whey protein supplementation on HOMA-IR.

cardiometabolic/cardiovascular disease risk. Indeed, for each 5-mmHg reduction in SBP, the risk of developing cardiovascular events has been shown to decrease by ~10 % [49]. Based on this observation, our reported ~3-mmHg reduction in SBP following whey protein supplementation in individuals <50 years of age may extrapolate to an ~6 % reduction in CVD risk. On a mechanistic level, the effect of whey protein supplementation on reduced SBP may be explained by the increased bioavailability of bioactive peptides contained in whey protein. In this regard, whey peptides contain the angiotensin-converting enzyme that exhibits inhibitory properties regarding the renin-angiotensin-aldosterone system [50]. Hence, following whey protein ingestion, the reduced generation of angiotensin II may lead to increased vasodilation and a decrease in SBP, at least in a sub-population of individuals aged <50 years. In contrast, no significant reduction in SBP was observed in adults aged ≥50 years, which may be attributed to confounding factors such as comorbidity status [51] and reduced vascular function associated with ageing [52]. Age-related arterial stiffening may limit vasodilatory responses to whey protein, for which, its effects may not sufficiently reverse this phenomenon [53]. Regarding the non-significant decreases in DBP in younger adults, this could be explained by the lower arteriolar resistance combined with lower arterial stiffening compared to older adults, which has a greater effect on SBP regulation rather than DBP [54].

Ageing is an independent risk factor of endothelial dysfunction that enhances arterial stiffness and impairs endothelial progenitor cell capacity to repair and maintain endothelial layer integrity [55]. These age-related changes in vascular function may diminish blood vessel dilation, thereby limiting the effectiveness of non-pharmacological interventions such as whey protein to improve blood pressure outcomes. However, previous research has demonstrated a favourable effect of whey protein (30 g/day) vs. placebo on microvascular function in older adults with heart failure [56], as well as reduced aortic stiffness in older community-dwelling individuals after 12 weeks of whey supplementation (50 g/day) [40]. In the present study, mean baseline SBP values were 134 mmHg and 123 mmHg in the older and younger subgroup, respectively. Previous research has shown greater reductions in SBP with diet intervention in a cohort that presented with higher baseline values [57], hence our observation of an effect of whey protein supplementation in the younger cohort only may be considered somewhat surprising and indicative that other factors may be prominent regarding the impact of whey protein supplementation in reducing SBP in adults aged 50 years and older.

Lower baseline BMI levels (≥50 years: 24.7 kg/m^2 vs. <50 years: 30.0 kg/m^2) and increased antihypertensive drug use has been shown to override the effectiveness of whey protein supplementation in reducing SBP [58]. This observation is consistent with our findings given that the benefits of whey protein supplementation on SBP were not reported in adults with normal body weight compared to overweight/obesity individuals (<25 kg/m^2: 1.74 [−1.50, 4.97] mmHg, P = 0.29; ≥25 kg/m^2: −2.08 [−4.15, −0.01] mmHg, P < 0.05). Overall, the existing literature generally supports the notion that whey protein could potentially represent an effective non-pharmacological intervention to reduce SBP and therefore reduce cardiometabolic disease risk, making it a potential dietary strategy for managing or preventing hypertension [59–61]. Trials that control for antihypertensive drugs may provide valuable insights into their potential impact in affecting non-pharmacological interventions pertinent to SBP.

Diastolic blood pressure contributes to mean arterial pressure and therefore represents an important predictive biomarker of cardiometabolic disease risk. While no significant effect of whey protein supplementation on DBP was observed in our analyses, whey protein supplementation has previously been shown to improve endothelial function and arterial stiffness, which contribute to cardiovascular health [62]. Endothelial function is important for maintaining blood vessel integrity and responsiveness, while arterial stiffness influences blood pressure regulation [63]. The positive impact of whey protein on nitric oxide [64] and vasodilation enhancing blood vessel flexibility could explain a potential application in reducing DBP [60]. However, our findings suggest that the potential benefits of whey protein ingestion on cardiovascular health may be limited to a reduction in SBP rather than the modulation of DBP. Interestingly, DBP was relatively constant across RCT sub-populations compared with SBP. While we reported differences in relation to mean baseline SBP between age groups (i.e., ≥50 vs. <50 years), mean baseline DBP was similar (≥50 years: 77.0 mmHg; <50 years: 75.2 mmHg) which could partially explain the lack of effect of whey protein supplementation on DBP. Notwithstanding, we cannot discount a clinically relevant effect of whey protein supplementation on DBP across different BMI classifications following whey protein (≥25 kg/m^2: −1.43 (−3.15, 0.28); <25 kg/m^2: 0.42 (−1.27, 2.12)), further supporting the notion that baseline BMI could be a critical confounder in addressing the impact of whey protein on blood pressure. Nevertheless, it is noteworthy that increases in systolic rather than diastolic blood pressure are more strongly associated with a greater risk of cardiometabolic disease and associated events [65].

4.2. Whey protein and lipoprotein profile

Assessment of lipoprotein profile is suggested to be important for assessing metabolic health status and risk of cardiometabolic disease [66]. Indeed, disorders of lipoprotein metabolism are strongly associated with the development of chronic disease risk and the analysis of lipoproteins and apolipoproteins is important to the assessment of cardiovascular risk [67]. Whey protein supplementation has shown potential benefits for cardiovascular health by regulating cholesterol metabolism [21]. Whey protein may

impact hepatic processes by promoting the production, or reducing the clearance, of HDL-cholesterol, leading to elevated circulating levels that may favor an optimal total cholesterol/HDL ratio [17,68]. Additionally, the amino acid composition of whey, specifically the high essential amino acid content which includes leucine, could contribute to favorable lipid profiles [69]. Whey protein ingestion may also influence lipid metabolism by reducing cholesterol absorption in the intestine through its active components such as beta-lactoglobulin and sphingolipids [70,71]. Whilst we observed no effect of short-term (i.e., <12 weeks) whey protein supplementation on HDL-cholesterol, our analysis did reveal a statistically significant effect when whey protein was consumed for ≥12 weeks. Compared to the shorter treatment duration (<12 weeks; k = 4), this finding may be explained by the higher number of included studies (≥12 weeks; k = 9) which provided our statistical model with sufficient statistical power to detect a significant effect. This notion is important to consider given that the four studies examining the impact of whey protein in <12 weeks led to greater reductions in HDL-cholesterol (−1.13 mg/dL) that tended to reach statistical significance (P = 0.06). Nevertheless, it is noteworthy that the mean reduction in HDL-cholesterol observed after ≥12 weeks (0.87 mg/dL, equating to ~1.7 % reduction) may not confer meaningful clinical relevance. Specifically, HDL-cholesterol could be an independent risk factor for cardiovascular disease, where an increase of 10 mg/L levels may reduce cardiovascular risk by 2–3% [72]. However, it is important to acknowledge the growing concerns around static assessments of HDL-cholesterol, rather than function, as a marker of increased cardiometabolic disease risk [73].

Whilst there is growing consensus that HDL-cholesterol content bears limited relevance to cardiometabolic disease risk [74], blood triglyceride, apolipoprotein B levels, and LDL-cholesterol levels are considered more relevant for CVD risk and chronic disease [75]. Our analyses revealed an ~5 mg/dL reduction in LDL-cholesterol when whey protein ingestion was combined with exercise in adults <50 years of age. According to recent evidence, a mean decrease of ~5 mg/dL following whey protein supplementation would equate to ~1.5 % reduction in all-cause mortality and ~2.5 % risk reduction from coronary heart disease [76]. The synergistic effects of whey protein supplementation and exercise may play a crucial role in mediating these effects [77]. Indeed, studies suggest that combining whey protein ingestion with exercise enhances the expression of LDL receptors, facilitating the clearance of LDL-cholesterol from the circulation [78]. Therefore, our observation of an ~5 % reduction of LDL-cholesterol supports the recommendation for daily ingestion of whey protein in potentially reducing chronic disease risk. Interestingly, the largest reductions in LDL-cholesterol were observed with the combination of whey protein supplementation and exercise, despite no significant changes in HDL-cholesterol. Previous research has reported beneficial effects of both aerobic and resistance exercise on HDL-cholesterol and LDL-cholesterol [79]; however, aerobic exercise tends to be more effective in individuals with low baseline HDL-cholesterol, elevated triglycerides, and abdominal obesity [80], characteristics not representative of the overall population in our included studies. In this context, our analyses in healthy only adults showed a mean decrease in HDL-cholesterol (−1.30 mg/dL), while in those with overweight and/or obesity we observed no changes. Furthermore, the studies that included exercise in their protocols and demonstrated reductions in mean LDL-cholesterol showed variability in treatment doses, intervention durations, and participant ages. In this regard, we found no changes between healthy and participants with overweight and/or obesity. Interestingly, a consistent factor across these studies was the inclusion of resistance exercise, either alone or in combination with aerobic exercise. These findings suggest that exercise combined with whey protein supplementation may offer greater benefits in modulating HDL and LDL-cholesterol levels compared to exercise with placebo, highlighting its potential as a therapeutic intervention. However, further research is needed to explore the synergistic effects of whey protein with exercise and to determine whether resistance exercise provides superior benefits compared to aerobic exercise.

4.3. Whey protein and total cholesterol and triglycerides

The measurement of total cholesterol and triglycerides may represent important markers of cardiometabolic disease risk [75]. In this meta-analysis, whey protein ingestion was consistently shown to improve total cholesterol profiles across all age groups (i.e., above, and below 50 years of age), particularly in overweight individuals, regardless of the daily dosage of whey protein (above or below 35 g/day), in non-placebo-controlled studies, and studies combining whey with exercise. The impact of whey protein on total cholesterol levels may be partially explained by the modulation of hepatic cholesterol synthesis [81,82]. Mean reductions in total cholesterol across groups varied between ~5 and ~12.5 mg/dL following whey protein supplementation across subgroup analyses. A previous meta-analysis reported that a decrease of 0.6 mmol/L (or ~23 mg/dL) in total cholesterol was linked to a reduced ischemic heart disease incidence of 54 % at age 40 years, 39 % at age 50, 27 % at 60, 20 % at 70, and 19 % at 80 [83], which highlights the potential clinical value of our findings. Previous studies have demonstrated the capacity of whey protein ingestion to influence key components of cholesterol homeostasis within the liver [21,62]. Specifically, the ingestion of whey protein may induce its cholesterol lowering effects by orchestrating a downregulation of hepatic cholesterol synthesis, which limits the production of cholesterol precursors [82]. Some of these functions may be prominent in the intestine through small peptides with cholesterol-lowering activity during enzymatic hydrolysis [84]. The combination of whey protein and exercise further activates these pathways, potentially via an increase in lipoprotein lipase activity and improved lipid oxidation [30]. These observations suggest a synergistic effect by which whey protein ingestion and exercise may contribute to the management of total cholesterol levels. Moreover, these findings underscore the potential use of whey protein as a dietary intervention for individuals seeking to modulate their lipid profiles, although it is important to recognize the need for further exploration into the long-term effects and potential variations across diverse populations to establish a broader applicability of these observations.

Our analysis revealed that whey protein supplementation resulted in a significant improvement in triglyceride concentrations. This effect was consistently observed after a 12-week supplementation period and in non-placebo-controlled studies. The reduction in triglyceride levels associated with whey protein consumption can be attributed, at least in part, to a complex relationship of physiological responses that collectively contribute to improved lipid metabolism [85]. The ingestion of whey protein has been shown to increase fat oxidation [86], enhance insulin sensitivity [87], and decrease hepatic triglyceride synthesis in rat models [88]. These mechanisms have similar effects on lowering triglyceride levels, and align with previous studies highlighting the effect of whey protein supplementation in modulating lipid metabolism [89]. Moreover, the favorable effect of whey protein ingestion on fat oxidation suggests a greater metabolic flexibility in terms of a greater propensity to utilize stored triglycerides for energy [86]. Accordingly, greater metabolic flexibility may be facilitated by the specific amino acids contained within whey protein, including branched-chain amino acids that support insulinotropic processes [90], albeit we observed no changes to insulin sensitivity in this meta-analysis (see '*Whey protein and insulin resistance*' below). The

improvement in metabolic flexibility and reduction in hepatic triglyceride synthesis with whey protein may be primarily attributed to the high leucine content within whey protein, especially in overweight/obese or individuals that present with metabolic syndrome [17,91]. It is also noteworthy that the ingestion of higher amounts of protein may reduce the intake of carbohydrates and dietary fats that could also favorably alter metabolic profiles [92]. However, it is critical to consider that the aforementioned findings were driven primarily by healthy adults who benefited to a larger extent as opposed to individuals with overweight and/or obesity (MD of healthy: −8.20 mg/dL vs. of overweight/obesity: 2.15 mg/dL). A reason that may explain such findings could be attributed to the study by Ormsbee et al. (2015), where participants showed a greater reduction of triglycerides in the control vs. the whey protein group. Taking into account energy intake across the study, the control group was consuming approximately 150 kcal/day less vs. baseline, while the intervention group increased it by ~100 kcal/day. These could lead to higher weight gain in conjunction with fat mass, potentially, mediating the results of our analysis which was consisted of only three studies as opposed to other lipoprotein profile markers that included more studies in this subgroup. Nevertheless, the authors reported a much greater reduction of body fat % in the intervention vs. the control group that does not align with our initial hypothesis, highlighting further the limitations occurring in nutrition research in relation to actual vs. reported energy consumption. From that perspective, we speculate that whey protein may confer benefits on triglyceride levels in healthy adults through mechanisms that may not translate in individuals with overweight and obesity. For instance, preliminary research recently has suggested glycomacropeptide, a bioactive compound in whey protein with lipogenesis-regulatory properties, as ineffective in alleviating skeletal muscle and liver tissue ceramide and triglyceride content in mice following a high-fat diet [93].

Whether these results based on this or other compounds in whey protein are applicable in humans with obesity has yet to be determined. Taken together, these findings highlight the diverse pathways through which whey protein may positively impact lipid metabolism, emphasizing its potential as a dietary adjunct for individuals aiming to manage triglyceride levels. However, as a note of caution, the mean reduction (~8 mg/dL or 8.5 % decrease from baseline) in triglyceride levels with whey protein supplementation observed in the present systematic review was relatively modest and may be associated with a negligible decrease in cardiovascular disease risk [94].

4.4. Whey protein and insulin resistance

HOMA-IR is a practical index used to evaluate the interaction between glucose and insulin dynamics and is used to represent a static marker of insulin resistance [95]. Whey protein constitutes a high content of essential amino acids, specifically the branched-chain amino acids, that may modulate insulin secretion [91,96], and exhibits positive effects on pancreatic beta-cell function [97,98]. By enhancing beta-cell function, the ingestion of whey protein may promote insulin secretion and supports glucose homeostasis [98]. These observations may be partly attributed to α-lactalbumin, a whey-protein peptide that may increase glucose uptake and Akt phosphorylation and decrease insulin resistant 3T3-L1 adipocytes [99]. However, our analyses revealed no effects of whey protein on HOMA-IR. This observation is surprising given that whey protein ingestion has been shown to reduce HOMA-IR, indicating a potential role in reducing insulin resistance [100]. Moreover, insulin resistance is a key factor in glucose homeostasis and metabolic disorders and is characterized by diminished cellular responsiveness to insulin [101]. These equivocal findings may be partly explained by the assessment method of insulin resistance. Indeed, HOMA-IR is calculated by an equation that consists of fasting (typically for more than 8 h) blood glucose and insulin concentrations which does not necessarily capture, 1) postprandial responses to whey protein, 2) prolonged glucose concentrations via multiple measurements, as depicted through glycated haemoglobin (HbA1c), and 3) the impact of habitual dietary changes and/or behaviours on fasting glucose and insulin concentrations. Nevertheless, similar observations to our analysis have been displayed in a previous RCT utilising a high-protein-low-carbohydrate diet, in which, although HbA1c was improved, HOMA-IR remained unchanged [102]. This observation may be attributed to persistent, but modest, reductions in postprandial blood glucose levels leading to lower HbA1c, albeit of insufficient magnitude to induce marked changes in insulin resistance as determined by HOMA-IR. Overall, in contrast to other studies [14,98], our findings do not support the notion that whey protein ingestion serves as an effective intervention to modulate postprandial glycaemic responses.

4.5. Considerations

A primary strength of this systematic review and meta-analysis relates to the robust analyses, including multiple subgroup and sensitivity analyses. Moreover, the application of a meta-analysis with homogeneous clinical parameters ensures a cohesive synthesis of data, enhancing the overall comparability of included studies. However, the heterogeneity in frequency, intensity, and types of exercise conducted in the analyzed RCTs emphasizes the need for caution when interpreting results and the requirement for more standardized exercise protocols in future research. Furthermore, the independent impact of increasing protein intake on cardiometabolic markers due to whey protein supplementation, irrespective of the proposed effects of whey protein, should be considered [103]. These considerations are especially prudent given the absence of a protein control in several RCTs included in our systematic review and the potential confounding effects of diet manipulation on other aspects of the diet. Moreover, it is worthy of note that we did not directly assess the impacts of whey protein compared with other dietary proteins in this review. To this point, evidence suggests that plant-based and animal-based dietary proteins can exert differential effects on modifying cardiometabolic risk factors [104]. Finally, various dosages of supplementation were used among studies, raising awareness about the practicality of appropriately incorporating whey protein within individuals' dietary patterns.

5. Conclusions

This systematic review and meta-analysis of RCTs indicates that whey protein supplementation represents a potentially promising dietary intervention for improving cardiovascular and metabolic health. Whey protein supplementation was shown to lower SBP, LDL-cholesterol, and total cholesterol levels, with exercise conferring an additive effect. Moreover, subgroup analyses revealed a more positive response to whey protein supplementation in adults aged below 50 years. Future studies are warranted to explore the efficacy of whey protein supplementation in combination with exercise to modulate cardiometabolic markers at the population level.

Data availability statement

Data is available upon request.

Author contributions

KP conceived the idea; KP, JM assisted with screening and data extraction; KKT and KSK assisted with the risk of bias assessment; KP and NV assisted with the statistical analyses; KP, PM, and OCW wrote the manuscript; PM, JR, CH, ES, DV, and OCW revised the manuscript.

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Conflict of interest

Authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2024.12.003.

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