

Is protein the forgotten ingredient: effects of higher compared to lower protein diets on cardiometabolic risk factors. A systematic review and meta-analysis of randomised controlled trials

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ATHEROSCLEROSIS

Is Protein the Forgotten Ingredient: Effects of Higher Compared to Lower Protein Diets on Cardiometabolic Risk Factors – a Systematic Review and Meta-Analysis of Randomised Controlled Trials

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Abstract

Background and aims: Higher protein (HP) diets may lead to lower cardiometabolic risk compared to lower protein (LP) diets. This systematic review and meta-analysis aims to investigate the effects of HP vs. LP diets on cardiometabolic risk factors in adults, using most up-to-date evidence from randomised controlled trials (RCTs).

Methods: Systematic searches were conducted in electronic databases, up to November 2020. Random effects meta-analyses were conducted to pool the standardised mean differences (SMD) and 95% confidence intervals (CI). The main outcomes were weight loss, body mass index (BMI), waist circumference, fat mass, systolic and diastolic BP, total cholesterol, HDL- and LDL-cholesterol, triacylglycerol, fasting glucose and insulin, and glycated haemoglobin.

Results: Fifty-seven articles reporting on 54 RCTs were included, involving 4,344 participants (65% female, mean age: 46 (SD 10) years, mean BMI: 33 (SD 3) kg/m²), with a mean study duration of 18 weeks (range: 4 to 156 weeks). Compared to LP diets (range protein (E%): 10-23%), HP diets (range protein (E%): 20-45%) led to more weight loss (SMD -0.13, 95% CI: -0.23, -0.03), greater reductions in fat mass (SMD -0.14, 95% CI: -0.24, -0.04), systolic BP (SMD -0.12, 95% CI: -0.21, -0.02), total cholesterol (SMD -0.11, 95% CI: -0.19, -0.02), triacylglycerol (SMD -0.22, 95% CI: -0.30, -0.14) and insulin (SMD -0.12, 95% CI: -0.22, -0.03). No significant differences were observed for the other outcomes.

Conclusions: Higher protein diets showed small, but favourable effects on weight loss, fat mass loss, systolic blood pressure, some lipid outcomes and insulin, compared to lower protein diets.

Introduction

Dietary proteins are important sources of energy and essential amino acids, necessary for various bodily processes, including tissue growth and maintenance [1]. The effects of dietary protein on human health are determined by several factors, including quantity, quality (animal protein/plant protein) and source; animal (red and white meat, fish, eggs and dairy) or plant-based (nuts, legumes, grains). In terms of quantity, current European and US dietary recommendations for protein intake generally advise ≥ 0.8 g/kg body weight (BW)/day for adults [2, 3] and growing evidence suggests an even higher intake for elderly (1.0-1.2 g/kg BW/day) [4-6]. When expressed in percentage of the total energy intake (energy-percent (E%)), the Nordic Nutrition Recommendations established a desirable daily protein intake of 10-20 E% for adults [7]. Other dietary guidelines provide similar recommendations, including those from the UK [8], the Netherlands [9] and German-speaking countries (Germany, Austria and Switzerland) [10].

The impact of increasing dietary protein intake on cardiometabolic disease risk is still not clearly defined and remains controversial. High protein diets have been promoted for decades for weight loss purposes, prevention of obesity and its metabolic consequences, yet have been documented to increase the risk of cardiovascular disease (CVD) mortality [11, 12] and type 2 diabetes (T2D) [13]. High protein diets have been reported to promote atherogenesis in animal models [14]. Mechanistically, protein ingestion acutely increases blood amino acid concentrations, circulating monocytes, and tissue macrophages, including those residing in the atherosclerotic plaque. This, in turn, leads to acute elevation of macrophage mechanistic target of rapamycin (mTOR) signalling, causing plaque progression [14]. High protein intake is also reported to increase insulin-like growth factor-1 (IGF-1) and to activate the mTOR-S6 kinase signalling pathway, while protein deficiency is sensed by unloaded transfer ribonucleic acid (tRNA) activating the protective general amino acid control nonderepressible-2 (GCN2) kinase

pathway which induces an activating transcription factor4 (ATF4) mediated protective integrated stress response [15]. High protein intake, therefore, leads to proliferation and insulin resistance in short lived animal and cell culture studies. Whether this applies to long living species is uncertain, but there are epidemiological data suggesting that elevated protein intake may be deleterious in younger people, but advantageous in older people [16, 17].

Prior meta-analyses of randomised controlled trials (RCTs) among adults suggest that higher protein (HP) diets may lead to improvements in weight loss and lower cardiometabolic risk, compared to lower protein (LP) diets [18, 19]. Wycherley et al. (2012) conducted a meta-analysis of 24 RCTs (1,063 adults, mean study duration: 12 weeks) and found that high protein diets (31 E%) led to more reductions in body weight (weighted mean difference (WMD) -0.79 kg), fat mass (WMD -0.87 kg), triacylglycerol concentrations (WMD -0.23 mmol/L) and a significant increase in fat-free mass (WMD 0.43 kg), compared to standard protein diets (18 E%) [18]. However, this review only included energy restricted intervention studies and a challenge with interpretation of these results is that energy restriction *per se* has a major impact on appetite, energy intake, and body weight and thereby on markers of obesity, which limits the ability to understand the independent effect of dietary protein. In addition, this review was limited by heterogeneity of the study population which included both free-living and patient groups. Santesso et al. (2012) conducted a meta-analysis of 74 RCTs among free-living adults with at least 5% difference in contribution from protein between the diets, without considering energy restriction [19]. Compared to the LP group (18 E%), the HP group (27 E%) demonstrated greater reductions in body weight (standardised mean difference (SMD) -0.36), BMI (SMD -0.37), waist circumference (SMD -0.43), blood pressure (systolic: SMD -0.21 and diastolic: SMD -0.18) and triacylglycerol concentration (SMD -0.51) [19], but the effects were considered small. However, this meta-analysis had relatively large heterogeneity for the outcomes (range I^2 : 42-85%) and included studies published prior to 2012 and studies with

very-low carbohydrate dietary interventions. The reported results from these types of diets may lead to overestimation of the intervention effect, as very-low carbohydrate diets and the associated higher intake of other macronutrients, such as saturated fatty acids (SFA) and lower fibre intakes, may have an effect on cardiometabolic risk factors, independently of protein intake (e.g. significant decreases in body weight and triacylglycerol concentration, significant increases in total cholesterol, high-density lipoprotein-cholesterol (HDL-c) and low-density lipoprotein-cholesterol (LDL-c)) [20]. Since the 2012 review [19], fourteen studies have been published on the effect of HP compared to LP diets on various cardiometabolic risk factors (e.g. body weight, blood pressure, lipid outcomes) [21-34]. Therefore, a renewed analysis with up-to-date evidence is warranted.

The present systematic review and meta-analysis aims to evaluate the effects of HP vs. LP diets on a wide range of cardiometabolic risk factors in adults from the general population, using the totality of the current evidence from RCTs.

Materials and methods

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [35]. A protocol for this systematic review and meta-analysis was not previously published.

Search strategy

Literature searches were conducted in PubMed, Web of Science and Scopus, in addition to checking of reference lists of retrieved articles and previously published meta-analyses [18, 19, 36, 37]. The searches involved a range of keywords for dietary protein, body weight and anthropometrics, body composition, blood pressure, blood lipids, markers of glucose metabolism and trial design. A detailed search strategy can be found in **Supplementary table**

1. All English language studies that met the eligibility criteria were selected, up to November 2020.

Eligibility criteria

RCTs among adults ≥ 18 years with no presence of chronic medical conditions (including T2D, CVD, kidney diseases), as described by papers, were included. Trials also met the following criteria to be eligible for inclusion: 1) intervention consisted of provision of foods or dietary advice for a higher protein (HP) diet; 2) the comparator consisted of provision of foods or dietary advice for a lower protein (LP) diet; 3) duration of study was at least 4 weeks; 4) one of the following outcomes was assessed: body weight (weight loss), anthropometrics (body mass index (BMI), waist circumference), body composition (fat mass), blood pressure (systolic blood pressure (SBP), diastolic blood pressure (DBP)), fasting blood concentrations of lipids (plasma or serum total cholesterol, HDL-c, LDL-c, triacylglycerol concentrations), markers of glucose metabolism (fasting plasma/serum glucose and insulin, glycated haemoglobin (HbA_{1c})).

A predefined difference in contribution of at least 3 E% from protein between the HP and LP diets was chosen, with HP diets being at least 3 E% higher than LP diets, a minimum contrast in protein intake between the diets as suggested by the Health Council of the Netherlands [9]. Data on the mean dietary protein intakes at the end of the intervention were considered as it represented the treatment intakes over the entire study period. Protein needed to be consumed from foods. For this reason, trials in which protein supplements or meal replacements were used were excluded. Furthermore, studies that compared very-low carbohydrate diets (<25 E% from carbohydrate) with high carbohydrate diets were excluded as well as studies with co-interventions e.g. structured exercise programmes or high intensity resistance training.

Study selection and data extraction

Sourced articles were imported into ENDNOTE X9 and Covidence Online Software [38]. The titles and abstracts were screened by three independent reviewers (IF, CDW and YDV) to check for eligibility criteria, with discrepancies resolved by consensus. If multiple publications were identified on the same trial, only data of the original publications were included.

Data were extracted by YDV and independently double-checked by IF and CDW, with inconsistencies resolved by consensus. Data were independently extracted using Covidence and a predesigned form that included author name, publication year, study design, country undertaken, studied outcomes, sample size, participant characteristics, intervention characteristics, dietary characteristics, study duration and context, reporting of urine urea or nitrogen data (yes/no), reporting of power calculation (yes/no) and funding source. Means, standard deviations (SD), standard errors (SE) or 95% confidence intervals (CI) of the change from baseline values, baseline values and postintervention values were extracted. Graphed data were extracted using WebPlotDigitizer version 4.4 [39].

For trials involving multiple arms, only data from the most relevant intervention and comparison group were extracted. For crossover trials, only data of the period before crossover were considered to avoid any carry-over effects due lack of reporting on washout period between the dietary phases within the crossover trials [40, 41]. If the intervention of a trial involved a weight loss period followed by a weight maintenance period, then data from the end of the weight maintenance period were extracted. Only data at the end of the intervention were extracted in which food intake were precisely measured and reported [42, 43]. If results from both per-protocol-analysis and intention-to-treat analysis (ITT) were reported, then data from the ITT were extracted. SI conversion factors were used: To convert cholesterol to mmol/L, mg/dl were multiplied by 0.0259; to convert triacylglycerols to mmol/L, mg/dl were multiplied

by 0.0113; to convert glucose to mmol/L, mg/dl were multiplied by 0.0555; to convert fasting insulin to pmol/L, μ IU/mL were multiplied by 6.

Risk of bias within studies

To evaluate the methodological quality of the individual studies, the Cochrane Collaboration's revised tool was used to assess the risk of bias in the randomised trials (RoB 2.0) [44]. The RoB 2.0 consists of 5 domains for the assessment of individual randomised trials: randomisation process, deviations from intended interventions, missing outcome data, measurement of the outcome and selection of the reported result.

Data analysis

The mean difference in the outcomes between the intervention and control group were calculated as standardised mean difference (SMD) and weighted mean difference (WMD) with 95% CI. The SMD and WMD were calculated based on mean and SD of the change from baseline values. If not reported, then these were calculated using the reported data. The $\text{mean}_{\text{change}}$ was calculated by subtracting the baseline values from the postintervention values. The $\text{SD}_{\text{change}}$ was calculated using the following formula; $\text{SD}_{\text{change}} = \sqrt{(\text{SD}_{\text{baseline}}^2 + \text{SD}_{\text{postintervention}}^2) - (2 \times r \times \text{SD}_{\text{baseline}} \times \text{SD}_{\text{postintervention}})}$ [45], where r represented the correlation coefficient between the baseline and postintervention values and was assumed to be 0.5 and led to more conservative estimates (wider 95% CI).

Random-effects models were carried out to pool the data of RCTs, which examined the effects of HP compared to LP diets on changes in weight loss, weight regain, BMI, waist circumference, fat mass, lean mass, SBP, DBP, total cholesterol, HDL-c, LDL-c, triacylglycerol, glucose, insulin, HOMA-IR and HbA_{1c}, accounting for within and between study variance [46]. Between-study heterogeneity was evaluated using the I^2 statistic (%) [47], where I^2 of $\leq 30\%$, between 30% and 50%, between 50% and 75% and $\geq 75\%$ were considered,

representing low, moderate, substantial and considerable heterogeneity, respectively [45]. The overall effect estimates of the trials in the meta-analysis models were presented in the forest plots, stratified by SD_{change} reported or obtained from standard errors or confidence intervals and SD_{change} imputed using a correlation coefficient.

Sensitivity analysis was performed to assess the influence of excluding trials that were judged to be at high risk of bias. Heterogeneity between the studies was analysed using subgroup analyses on sex, age (<50 years vs. ≥ 50 years) and study duration (<12 weeks vs. 12 weeks-24 weeks vs. ≥ 24 weeks). Multivariate meta-regression analyses were conducted to investigate the influence of weight loss on the effect of HP compared to LP diets on outcomes for which significant results were found, including fat mass, SBP, total cholesterol, triacylglycerol, and insulin.

Risk of publication bias for each outcome were assessed visually and quantitatively using funnel plots and Egger's weighted regression test [48], respectively. Trim and fill method was used [49], if evidence for publication bias was found. Analyses were performed in Stata Statistical Software version 15.0 and two-sided p at 0.05 were considered to be statistically significant in the analyses.

Results

3.1 Study selection process

A total of 7,104 records were initially identified, of which 6,951 were excluded after title and abstract screening (**Figure 1**). Thirty-one duplicates were removed, leaving 122 full text articles. Of these, 57 articles reporting on 54 RCTs were included in the meta-analyses, excluding 65 articles. More detailed information on the study selection process can be found in **Figure 1**.

3.2 Study characteristics

The characteristics of the 54 RCTs are described in **Supplementary table 2**. The studies evaluated a total of 4,344 participants and the percentage of women was 65% (**Supplementary table 3**). The mean (SD) age and BMI were 46 (10) y (range: 23 to 70 y) and 33 (3) kg/m² (range: 24 to 39 kg/m²), respectively (**Supplementary table 3**).

Of the 54 RCTs included, 51 were parallel trials and 3 had a cross-over design (**Supplementary table 3**). The mean (SD) study duration was 18 (23) weeks (range: 4 to 156 weeks). Trials were conducted in North America (n=26), Australia and Oceania (n=14), Europe (n=10), Asia (n=3) or Europe, Australia, and New Zealand (n=1). The mean (SD) dropout rate or loss to follow-up was 17 (17) % (range: 0-70%). Thirty-five out of 54 trials did not receive funding from an industrial source (**Supplementary table 3**).

The achieved relative intake of dietary protein, carbohydrate, and total fat (E%) were, on average, 28% (range: 20 to 45%), 41% (range: 25 to 55%) and 31% (range: 20 to 43%) in the HP group and 18% (range: 10 to 23%), 54% (range: 36 to 66%) and 28% (range: 20 to 45%) in the LP group (**Supplementary table 3**). The mean (SD) total daily energy intakes were 1,764 (455) kcal and 1,768 (462) kcal in the HP and LP groups, respectively (**Supplementary table 3**).

3.3 Changes in body weight, anthropometrics, and body composition

A total of 48 trials with 3,346 participants provided data on weight loss, 3 on weight regain (774 participants), 27 on BMI (2,012 participants) and 26 on waist circumference (2,669 participants). Meta-analysis revealed statistically significant effects on weight loss with HP compared to LP diets, with a pooled SMD of -0.13 (95% CI: -0.23, -0.03). There was moderate to low heterogeneity across the trials ($I^2 = 38\%$, $p=0.004$) (**Figure 2**). This is equivalent to an increase in weight loss of 0.64 kg (95% CI: -1.12, -0.17, $I^2 = 53\%$, $p=0.000$) with HP compared

to LP diets. Significant intervention effects were observed for weight loss when the participants were under 50 years of age, with a pooled SMD of -0.17 (95% CI: -0.31, -0.03) (**Supplementary table 5**). The meta-analysis also revealed less weight regain in the HP compared to LP groups (pooled SMD -0.18, 95% CI: -0.32, -0.04, $I^2 = 0\%$, $p=0.6$) (**Supplementary table 4**). However, removal of Larsen et al. (2010) from the analysis, a trial contributing most weight to the pooled estimate, attenuated the results for weight regain (pooled SMD -0.08, 95% CI: -0.39, 0.24, $I^2=0\%$, $p=0.5$). The pooled analyses across trials showed a tendency towards an effect in favour of the HP diet for a lower BMI (pooled SMD -0.11, 95% CI: -0.23, 0.01, $I^2 = 31\%$, $p=0.1$) and waist circumference (pooled SMD -0.11, 95% CI: -0.23, 0.01, $I^2 = 44\%$, $p=0.006$), but these were not statistically significant (**Supplementary table 4**).

Meta-analysis of 35 trials with 2,580 participants showed a significant reduction in fat mass with HP compared to LP diets (pooled SMD -0.14, 95% CI: -0.24, -0.04). There was low heterogeneity across the trials ($I^2 = 28\%$, $p=0.1$) (**Figure 3**). The pooled WMD for reduction in fat mass was 0.55 kg (95% CI: -0.92, -0.17, $I^2 = 28\%$, $p=0.1$) in favour of the HP diet. Significant intervention effects were found for fat mass when participants were 50 years of age or older (pooled SMD -0.15, 95% CI: -0.26, -0.03) (**Supplementary table 5**). Meta-analysis of 30 trials involving 2,418 participants showed no significant differences between the diets for lean mass (pooled SMD 0.06, 95% CI: -0.06, 0.17, $I^2 = 39\%$, $p=0.013$) (**Supplementary table 4**).

3.4 Changes in blood pressure

A total of 26 trials with 1,813 participants provided data for the meta-analysis for SBP and DBP. A reduction in SBP and DBP was found with HP compared to LP diets (pooled SMD -0.12, 95% CI: -0.21, -0.02, $I^2 = 0.0\%$, $p=0.9$) (**Figure 4**) and (pooled SMD -0.09, 95% CI:

-0.19, 0.01, $I^2 = 9\%$, $p=0.3$) (**Supplementary table 4**), respectively, although this did not reach statistical significance for DBP. The pooled WMD for reduction in SBP was 1.16 mm Hg (95% CI: -2.13, -0.20, $I^2=0\%$, $p=0.8$) with HP compared to LP diets. The intervention effects were borderline significant for SBP when participants were under 50 years of age (pooled SMD -0.12, 95% CI: -0.23, -0.00) (**Supplementary table 5**) and the study duration was under 12 weeks (pooled SMD -0.15, 95% CI: -0.30, -0.00) (**Supplementary table 6**).

3.5 Changes in blood lipid concentrations

A total of 41 trials (2,303 participants) reported data on total cholesterol, 42 trials (2,452 participants) on HDL-c, 42 trials (2,516 participants) on LDL-c and 43 trials (2,530 participants) on triacylglycerol. Meta-analysis demonstrated a reduction in total cholesterol with HP compared to LP diets (pooled SMD -0.11, 95% CI: -0.19, -0.02) (**Figure 5a**). Results were consistent across all 41 trials with low heterogeneity ($I^2 = 1\%$, $p=0.5$). No significant differences between the diets were observed for HDL-c (pooled SMD 0.10, 95% CI: 0.01, 0.20, $I^2 = 19\%$, $p=0.1$) or LDL-c (pooled SMD 0.01, 95% CI: -0.08, 0.10, $I^2 = 20\%$, $p=0.1$). A significant reduction in triacylglycerol was found with HP compared to LP diets (pooled SMD -0.22, 95% CI: -0.30, -0.14) and pooled trial data for this outcome was homogeneous ($I^2 = 0\%$, $p=0.9$) (**Figure 5b**). Translated to an effect in clinical units, a greater reduction in total cholesterol of 0.08 mmol/L (95% CI: -0.13, -0.03, $I^2=0\%$, $p=0.5$) and a greater reduction in triacylglycerol of 0.12 mmol/L (95% CI: -0.16, -0.08, $I^2=0\%$, $p=0.8$) was observed with HP compared to LP diets. Significant intervention effects were observed for total cholesterol when participants were 50 years of age or older (pooled SMD -0.16, 95% CI: -0.29, -0.02) (**Supplementary table 5**) and the study duration was under 12 weeks (pooled SMD -0.25, 95% CI: -0.40, -0.09) (**Supplementary table 6**). For triacylglycerol, the intervention effects were significant when participants were female (pooled SMD -0.25, 95% CI: -0.40, -0.11) (**Supplementary table 7**), the study duration was under 12 weeks (pooled SMD -0.32, 95%

CI: -0.46, -0.18) and between 12 and 24 weeks (pooled SMD -0.20, 95% CI: -0.31, -0.08) but not when it was 24 weeks or longer (**Supplementary table 6**).

3.6 Changes in markers of glucose metabolism

Thirty-four trials (2,592 participants) were included in the meta-analysis on glucose, 28 trials (2,270 participants) with data on insulin, 19 trials (1,674 participants) on HOMA-IR and 3 trials (152 participants) on HbA_{1c}. Pooled analysis showed a statistically significant lowering effect of HP diets on insulin, compared to LP diets (pooled SMD -0.12, 95% CI: -0.22, -0.03), with low heterogeneity across the trials ($I^2 = 13\%$, $p=0.3$) (**Figure 6**). Significant intervention effects of the were observed for insulin when participants were female (pooled SMD -0.37, 95% CI: -0.58, -0.17) (**Supplementary table 7**), and in the subgroup of 50 years of age or older (pooled SMD -0.15, 95% CI: -0.27, -0.02) (**Supplementary table 5**). No significant differences between the diets were found for glucose (pooled SMD -0.01, 95% CI: -0.11, 0.12, $I^2 = 43\%$, $p=0.003$), HOMA-IR (pooled SMD -0.05, 95% CI: -0.22, 0.11, $I^2 = 56\%$, $p=0.001$) or HbA_{1c} (pooled SMD -0.02, 95% CI: -0.49, 0.45, $I^2 = 52\%$, $p=0.1$) (**Supplementary table 4**).

3.7 Sensitivity analyses

Removal of Keogh et al. (2007) [50], a study judged to be high risk of bias, from the analyses did not change the overall effect estimates of the outcome measures.

3.8 Meta-regression analyses

Multivariate meta-regression analysis showed no major influence of body weight change on the observed association between dietary protein intake and the outcome measures (**Supplemental table 5**).

3.9 Risk of bias within and between studies

The overall risk of bias of the included trials ranged from ‘low’ to ‘some concerns’ and ‘high’. Most trials showed concerns about the selection of the reported result due to not publishing details on the pre-specified analysis plans and the randomisation process (e.g., sequence concealment) (**Supplementary Figure 1**). Only 1 of 54 trials demonstrated high risk of bias [50] (**Supplementary Figure 1**). Assessment of the risk of bias of the included trials can be found in **Supplementary table 6**. No evidence for publication bias was found in the meta-analyses for the outcomes, except for triacylglycerol (Egger’s test $p=0.0$) (**Supplementary figures 2-7**). However, trim and fill analyses revealed no major change in the observed overall effect estimate (pooled SMD -0.14, 95% CI: -0.21, -0.07).

Discussion

The present systematic review and meta-analyses of 54 RCTs have shown favourable but small effects of HP vs. LP diets on weight loss, fat mass, SBP, total cholesterol, triacylglycerol, and fasting insulin among adults over a mean follow-up of 4-5 months. Findings of these meta-analyses suggest that intake of higher dietary protein (28 E%) (range: 20-45%) compared to lower dietary protein (18 E%) (range: 10-23%) could lead to more weight loss and reductions in fat mass, SBP, total cholesterol, triacylglycerol, and fasting insulin. No significant differences between the diet were found for BMI, waist circumference, lean mass, HDL-c, and LDL-c, DBP, glucose, HbA_{1c} and insulin resistance estimated by HOMA-IR.

Comparison with other reviews

Previous systematic reviews and meta-analyses that compared the effect of higher vs. lower protein diets, irrespective of the source of protein, on various health outcomes generally support our results [18, 19, 51]. Our findings suggest that higher protein diets can lead to improvements

in weight loss [18, 19] and reduction in fat mass [18], compared to lower protein diets. There was no clear effect on BMI and waist circumference, which is in contrast with the meta-analysis by Santesso et al. (2012) [19], who found small to moderate effects. Apart from more studies and participants included in our meta-analysis, we also excluded trials that were included in Santesso's review. This involved very-low carbohydrate dietary interventions, very low-fat and low-fat or high-fat dietary comparisons and studies with co-interventions, which could explain part of the discrepancies. Surprisingly, there was no effect on lean body mass, which is in contrast with the meta-regression by Krieger et al. (2006) [52], who included single arms from observational studies and RCTs, and previous meta-analyses, which only considered energy-restricted dietary interventions [18, 53]. In terms of blood pressure, significant effects were observed of HP diets lowering SBP, but not on DBP, which is partly in line with previous studies [19, 51], who found beneficial effects on both SBP and DBP. In line with previous studies, we found greater reduction in triacylglycerol with HP vs. LP diets, with no significant differences in HDL-c and LDL-c [18, 19]. However, we observed a borderline significant lower total cholesterol after HP compared to LP diets. In terms of diabetes related outcomes, our study suggests no clear effects of HP diets on glucose and HbA_{1c}, which is in line with previous studies [18, 19, 36]. However, we did observe small improvements in fasting insulin with HP compared to LP diets, in line with previous publications [36, 37].

Possible explanations

Several lines of evidence have suggested potential mechanisms underlying the effect of dietary protein intake on changes in intermediary CVD risk factors. An increase in dietary protein intake may prevent weight regain and obesity [54]. It is suggested that higher protein intake during energy restriction or energy balance may have beneficial effect on body weight loss and subsequent weight maintenance [55]. The negative energy balance is a result of decreased energy intake and increased energy expenditure, which can be explained by the satiating effects

of protein and preservation of fat-free mass (FFM), respectively [55]. Furthermore, dietary protein, irrespective of the type, may also have a blood-pressure lowering effect [56]. There is evidence which demonstrates that bioactive peptides can inhibit the activity of angiotensin converting enzyme (ACE), a key component of the renin-angiotensin system (RAS), that mediates systemic hypertension. The ACE-inhibitory activity and peptides have been observed from protein isolates e.g. whey protein isolates [57, 58] and from other food sources e.g. dairy, fish, meat, egg products, soybeans, rice and nuts [59]. The link between dietary protein intake and blood lipid concentrations is more limited. A crossover RCT among healthy men and women revealed that a high protein, high fat hypercaloric diet significantly changed body composition, lowered intrahepatic lipids and circulating triacylglycerol concentrations, compared to a standard protein diet [60]. In addition, previous studies reported effective lowering of cholesterol concentrations with diets that included lean beef as a major protein source [61-63]. Previous double-blinded randomised, 3 way-crossover intervention study investigating the impact of intact milk protein supplementation have found that whey protein and calcium-caseinate intakes decreased total cholesterol, but only whey protein reduced triacylglycerol, compared to the controls [57]. A systematic review that compared the effects of animal vs. plant protein sources on features of metabolic syndrome indicated that soy protein (with isoflavones), but not soy protein alone or other plant proteins, led to greater lowering in total cholesterol and LDL-c, compared to animal-sourced protein [64]. This is partly supported by findings of a meta-analysis of 112 RCTs that showed that substitution of animal protein by plant protein led to reductions in LDL-c, non-HDL-c and ApoB [65]. Dietary proteins may have lipid lowering effects, which may be dependent on the food source, although the exact underlying mechanisms still need to be determined.

Strengths and limitations

An important strength of this systematic review and meta-analysis is the standard systematic methodology used in the identification, selection, reporting, synthesis, and interpretation of the studies. An elaborated predefined search syntax was used, and data were independently extracted using predefined forms and verified by multiple reviewers. Our review is the most comprehensive on this topic using the most up-to-date literature. Another strength is the inclusion of many trials with low between-study heterogeneity and little to no evidence for publication bias. Trial data for our outcomes were relatively more homogenous compared to data used by Santesso et al. (2012) [19], with I^2 varying between 0% and 56%. This may be due to very-low carbohydrate studies that were excluded from our review but were included in Santesso's analyses. Another strength is that this meta-analysis is based on RCTs across various populations with varying health statuses (e.g., healthy people, people with overweight, obesity, hypertension, hyperinsulinemia, hyperlipidaemia, metabolic syndrome (MetS), polycystic ovary syndrome and prediabetes), representing real-life situations. Additionally, we used a careful approach to calculate the change-from-baseline SD for the study outcomes, resulting in lower effect sizes, which were presented separately in the forest plots. The separate presentation of imputed and reported SD was performed previously [19] and showed similar effect sizes.

This systematic review and meta-analysis also has several limitations, which include the relatively limited data available to evaluate the effect of dietary protein intake on weight regain, HOMA-IR and HbA_{1c}. Another limitation is that it is difficult to determine whether the effects of higher protein diets are due to protein or the reductions in other macronutrients, including carbohydrate or fats, although we made every attempt to control for this by excluding trials with very-low carbohydrate dietary interventions and trials with very low-fat and low-fat or high-fat dietary comparisons. Another limitation of this study is the inclusion of intermediary

outcomes, not hard clinical outcomes, although these outcomes play an important role in the development of diseases. To date, limited studies with trial design have been conducted on the effects of higher protein intake on hard clinical outcomes in high-risk populations [66, 67]. Previous evidence from the PREDIMED Study in people at high risk of CVD demonstrated that Mediterranean diets, supplemented with either extra-virgin olive oil or nuts, similarly reduced CVD risk by approximately 30% [66] and T2D risk by 50% [67] compared with the control diet, after follow-up for at least 4 years. Recently, results on T2D incidence in the PREVIEW Study have been reported after follow-up for 3 years [68]. The authors found no difference in the 3-year incidence in T2D between an ad-libitum high protein, low-GI diet and an ad libitum moderate protein, moderate-GI diet, in participants with prediabetes [68], which could be explained by the large and fast initial weight loss (which was still partially present after 3 years) [68]. More RCTs are needed that investigate the long-term effects of higher vs. lower protein diets on incidence of type 2 diabetes and CVD-associated events in high-risk populations. In addition, the results of this meta-analysis may not be generalized to other populations such as people with chronic diseases. A recently published 3-month randomised controlled study among 76 overweight and obese patients with heart failure and diabetes mellitus (72.4% male, mean age: 57.7 years) have shown that high protein diets (30 E%) led to significantly greater reductions in HbA_{1c} levels, total cholesterol and triacylglycerol concentrations, SBP and DBP, compared to standard protein diets (15 E%) [69]. These findings suggest that a HP diet may be more effective in lowering cardiometabolic risk in these populations. Another limitation is that the SDs were not always reported in the publications. If these were reported, then the effect sizes would most likely be more precise. Furthermore, we were not able to investigate the effects of protein from different food sources on our outcomes due to lack of reporting of intake of the main source of protein in most of the articles. A re-analysis of the DIOGENES Study suggests potential differential effects of protein from

different sources on weight maintenance and cardiometabolic risk factors [70], but more research is needed in this area.

Context and implications for future research

The present systematic review and meta-analysis of 54 RCTs in adults demonstrated that HP diets compared to LP diets had small but favourable effects on weight loss, fat mass loss, systolic blood pressure and some lipid outcomes, which are relevant markers for CVD risk. Decreases in fasting insulin was also observed with HP compared to LP diets, but the effect was small. The amount of dietary protein in HP and LP diets in this meta-analysis is according to the Acceptable Macronutrient Distribution Range (AMDR) for protein, which is 10%-35% of the total energy intake [2], except for one RCT [71]. Our results suggest that a modest increase in the proportion of dietary protein within the diets may have, small but beneficial effects on intermediary risk factors of CVD. Future high quality RCTs are needed that focus on the effects of HP diets on weight regain and diabetes related outcomes (e.g. insulin resistance and HbA_{1c}). Future studies should also investigate the effectiveness of HP compared to LP diets in people with chronic diseases. More research is also needed on the potential differential effects of protein from specific food sources on cardiometabolic risk factors.

Our study showed that a higher protein diet had no detrimental effects and some beneficial effects, although these were clinically small. Future work is needed on the long-term effects of a higher protein diet on cardiometabolic risk factors and hard clinical outcomes.

Conflict of interest

YDV received funding from the Rank Prize Funds, the Dutch Dairy Association and the Danish Dairy Research Foundation. The funding sources were not involved in the study design, the collection, analyses, and interpretation of data and in the writing of the report. All the other authors declare no conflict of interest.

Author contributions

YDV, AR, AFHP and SSSM conceived and designed the review. YDV, IF and CDW performed the literature search and screened the data. YDV extracted the data. IF and CDW verified the extracted data. YDV analysed and interpreted the data. SSSM supervised YDV with data analyses and interpretation. YDV wrote the draft manuscript. AR, AFHP, JAL, DIG and SSSM critically revised the manuscript for important intellectual content. All authors gave final approval of the version to be submitted and any revised version.

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713 **Keywords**

714 Cardiometabolic; Meta-analysis; Protein diet; Randomised controlled trial; Systematic review

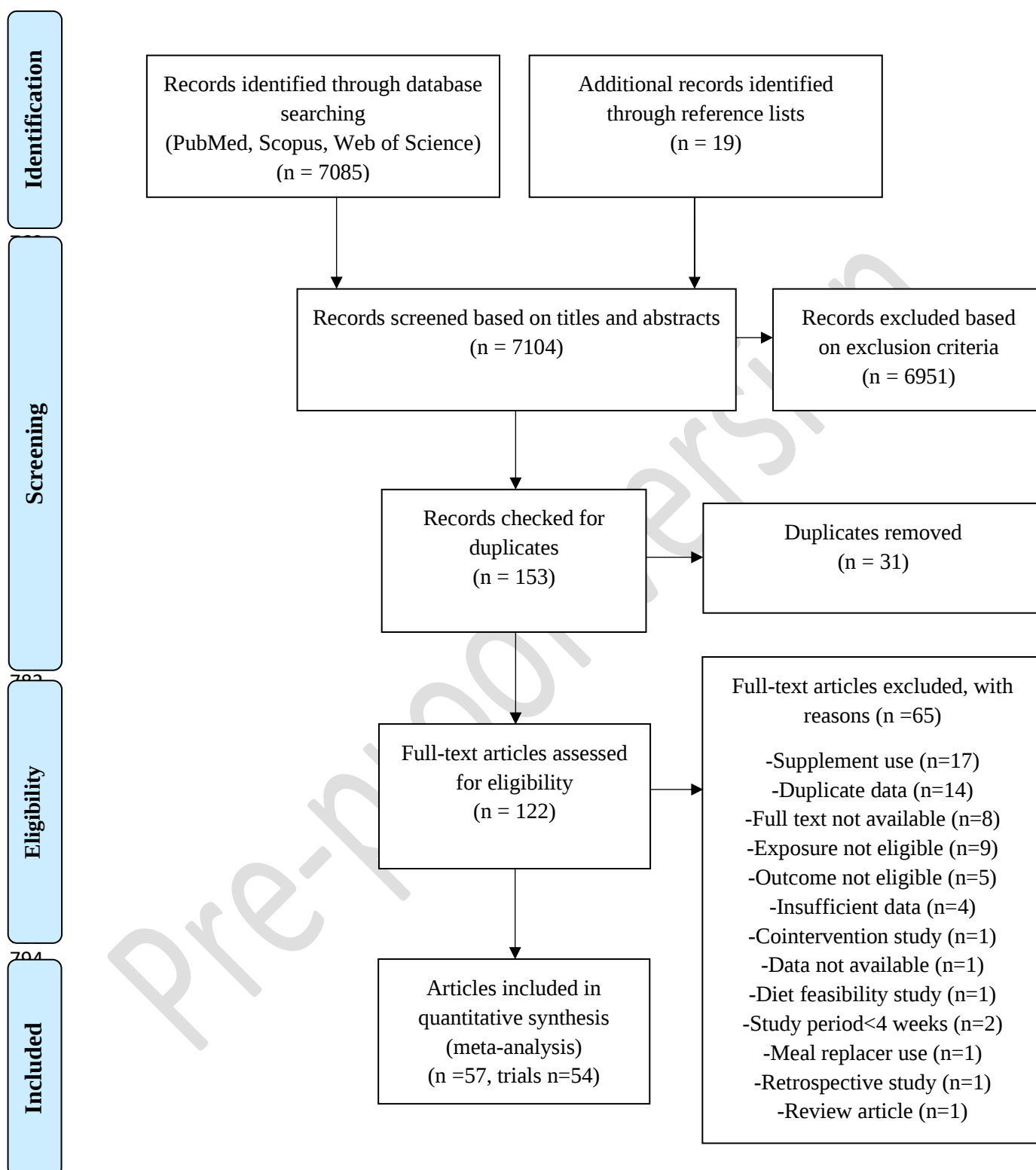
715 **Abbreviations**

716	ACE	Angiotensin converting enzyme
717	AMDR	Acceptable macronutrient distribution range
718	ATF4	Activating transcription factor 4
719	BMI	Body mass index
720	BP	Blood pressure
721	BW	Body weight
722	CI	Confidence interval
723	CVD	Cardiovascular disease
724	DBP	Diastolic blood pressure
725	E%	Energy percent
726	FGF21	Fibroblast growth factor 21
727	FFM	Fat free mass
728	GCN2	General amino acid control nonderepressible-2
729	HbA _{1c}	Glycated haemoglobin
730	HDL-c	High density lipoprotein cholesterol
731	HOMA-IR	Homeostatic model assessment of insulin resistance
732	HP	Higher protein

733	IGF-1	Insulin-like growth factor 1
734	ITT	Intention-to-treat
735	LDL-c	Low density lipoprotein cholesterol
736	LP	Lower protein
737	MetS	Metabolic syndrome
738	mTOR	Mechanistic target of rapamycin
739	RAS	Renin-angiotensin system
740	RCT	Randomised controlled trial
741	SBP	Systolic blood pressure
742	SD	Standard deviation
743	SE	Standard error
744	SFA	Saturated fatty acids
745	SMD	Standardised mean difference
746	tRNA	Transfer ribonucleic acid
747	T2D	Type 2 diabetes
748	UK	United Kingdom
749	US	United States
750	WMD	Weighted mean difference

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805 **Figure 1** Flowchart of article selection process

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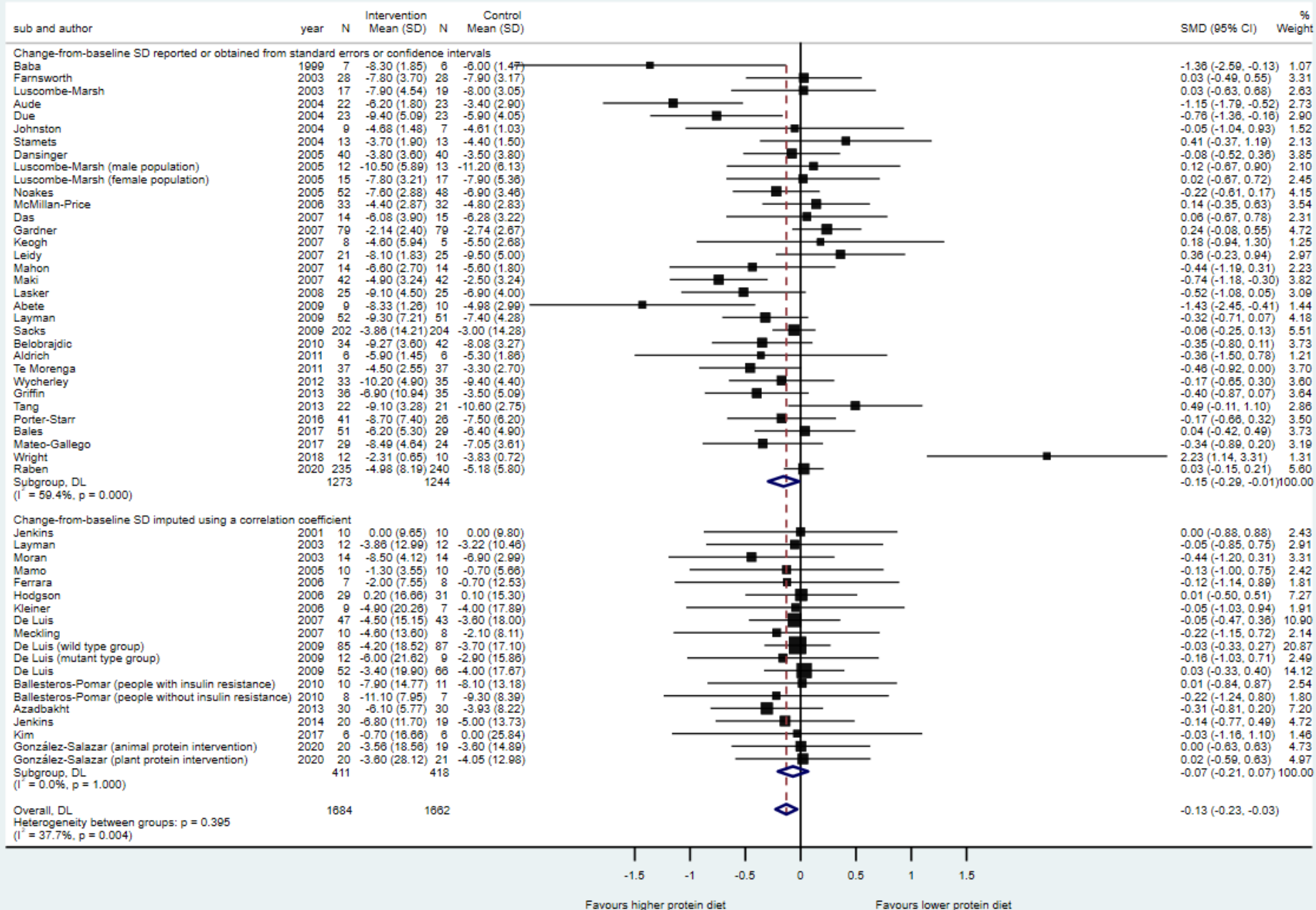


Figure 2. Standardised mean difference (SMD) and 95% confidence interval (CI) in weight loss between the intervention and control groups on the effect of a higher protein diet.

Pre-proof version

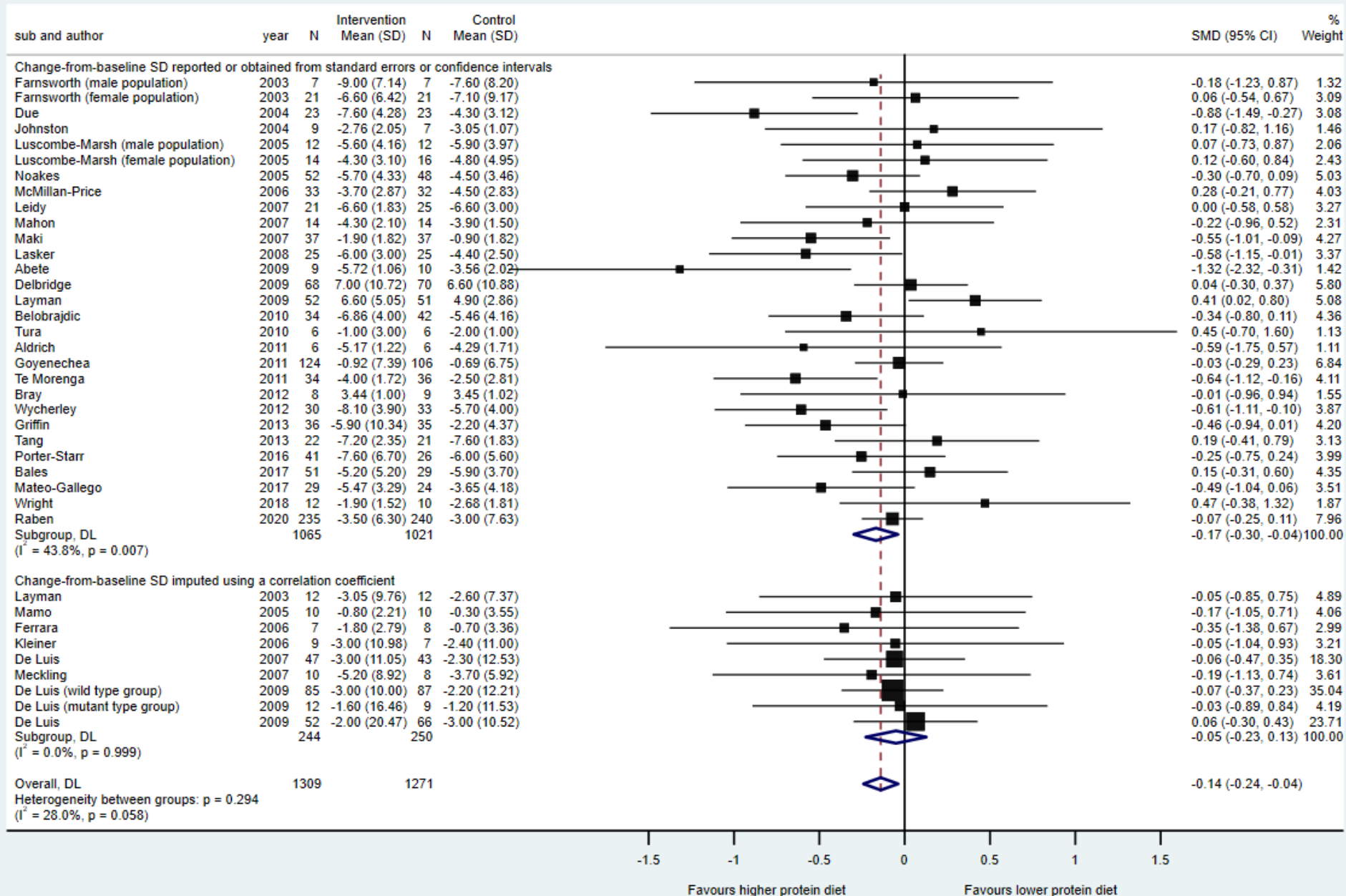


Figure 3. Standardised mean difference (SMD) and 95% confidence interval (CI) in fat mass between the intervention and control groups on the effect of a higher protein diet.

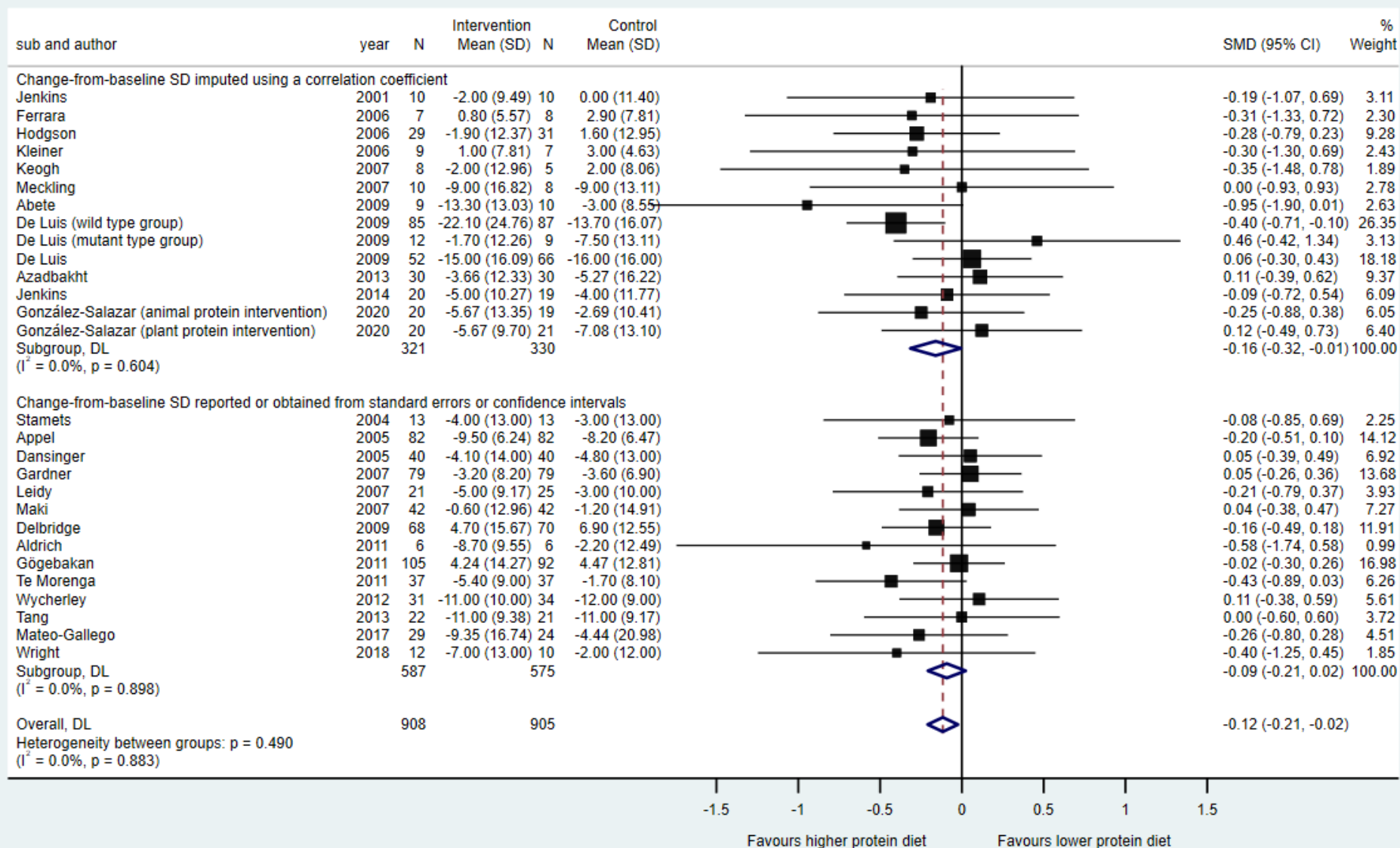


Figure 4. Standardised mean difference (SMD) and 95% confidence interval (CI) in systolic blood pressure between the intervention and control groups on the effect of a higher protein diet.

Pre-proof version

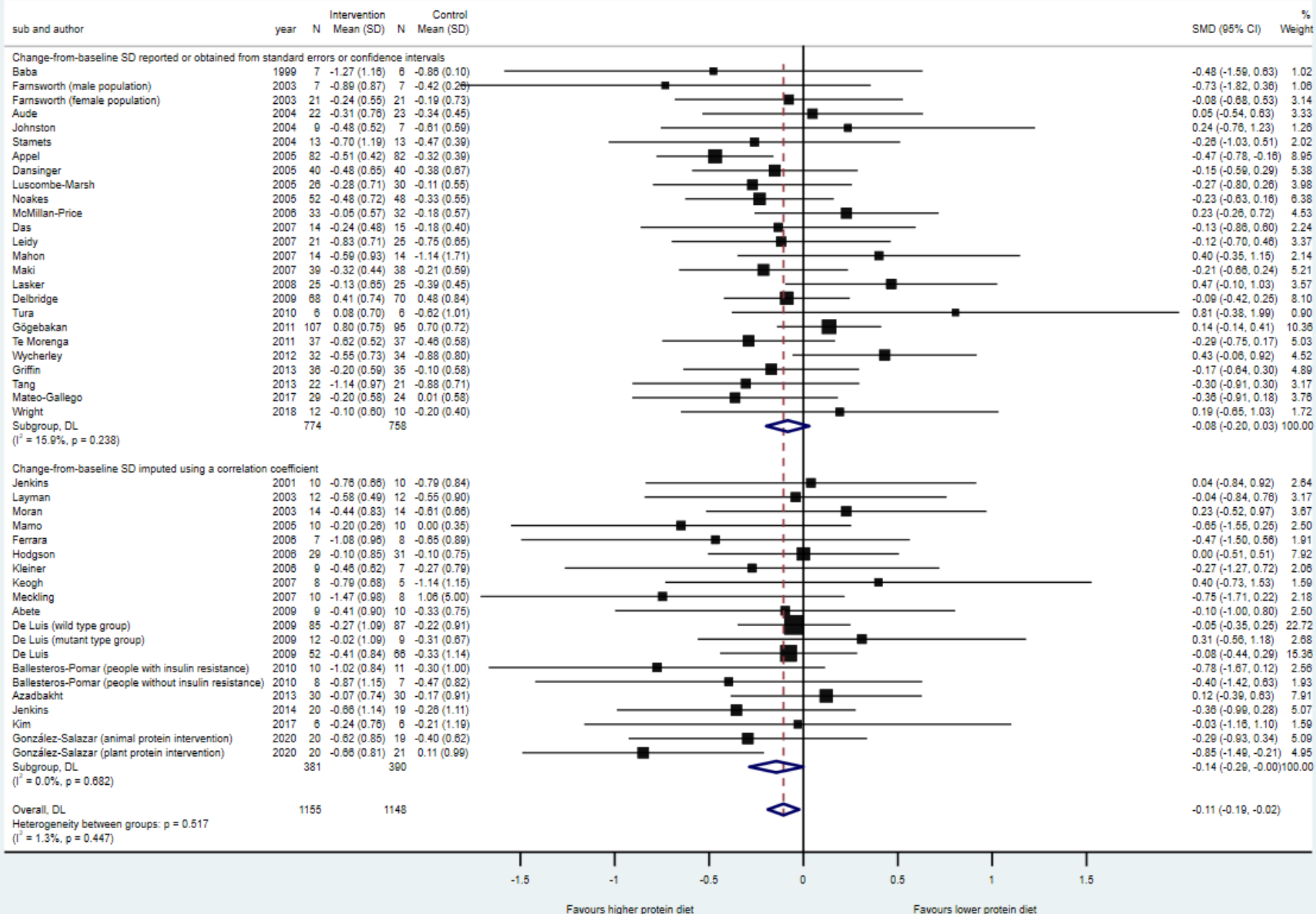


Figure 5a. Standardised mean difference (SMD) and 95% confidence interval (CI) in total cholesterol between intervention and control groups on the effect of a higher protein diet.

Pre-proof version

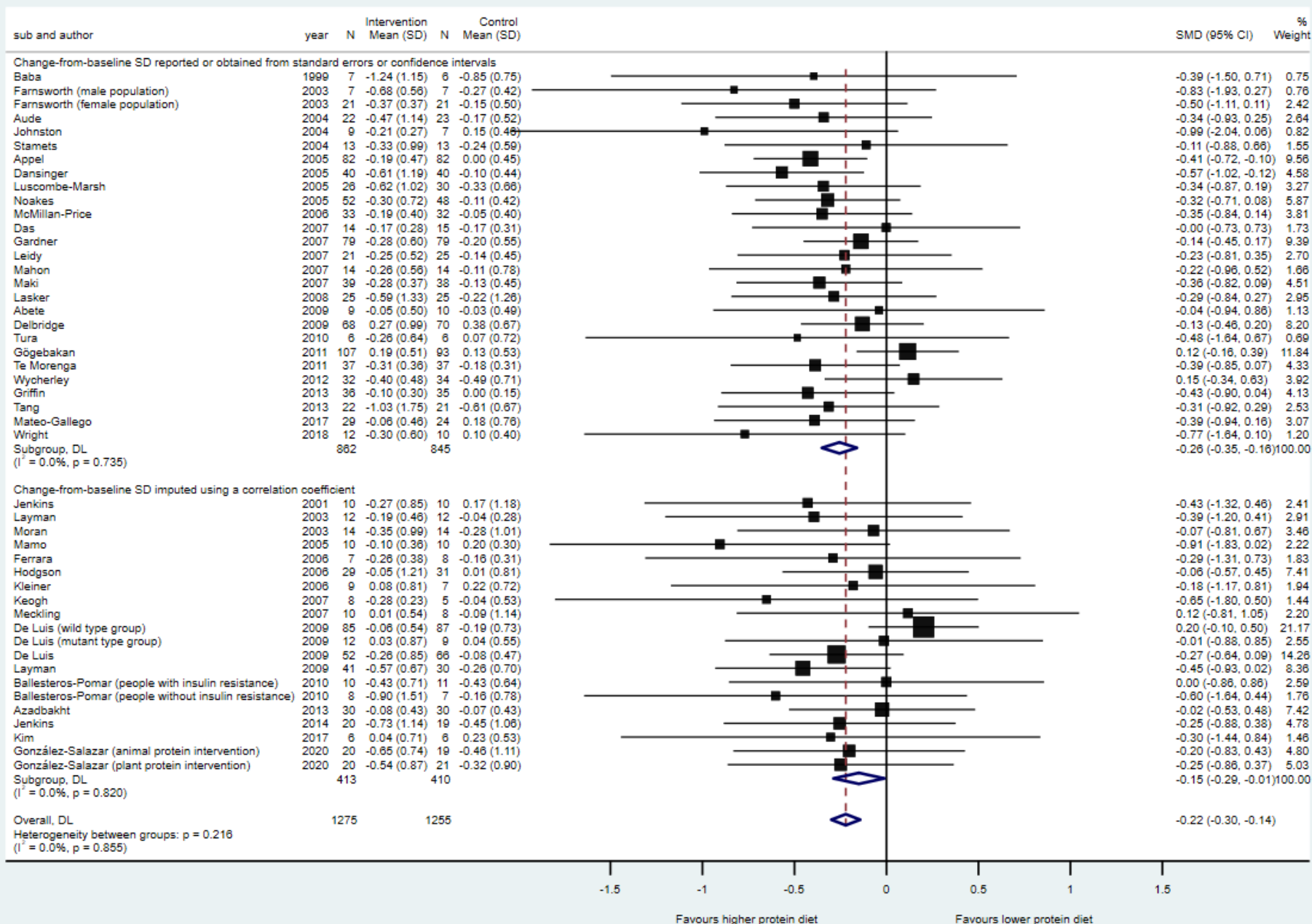


Figure 5b. Standardised mean difference (SMD) and 95% confidence interval (CI) in triacylglycerol between intervention and control groups on the effect of a higher protein diet.

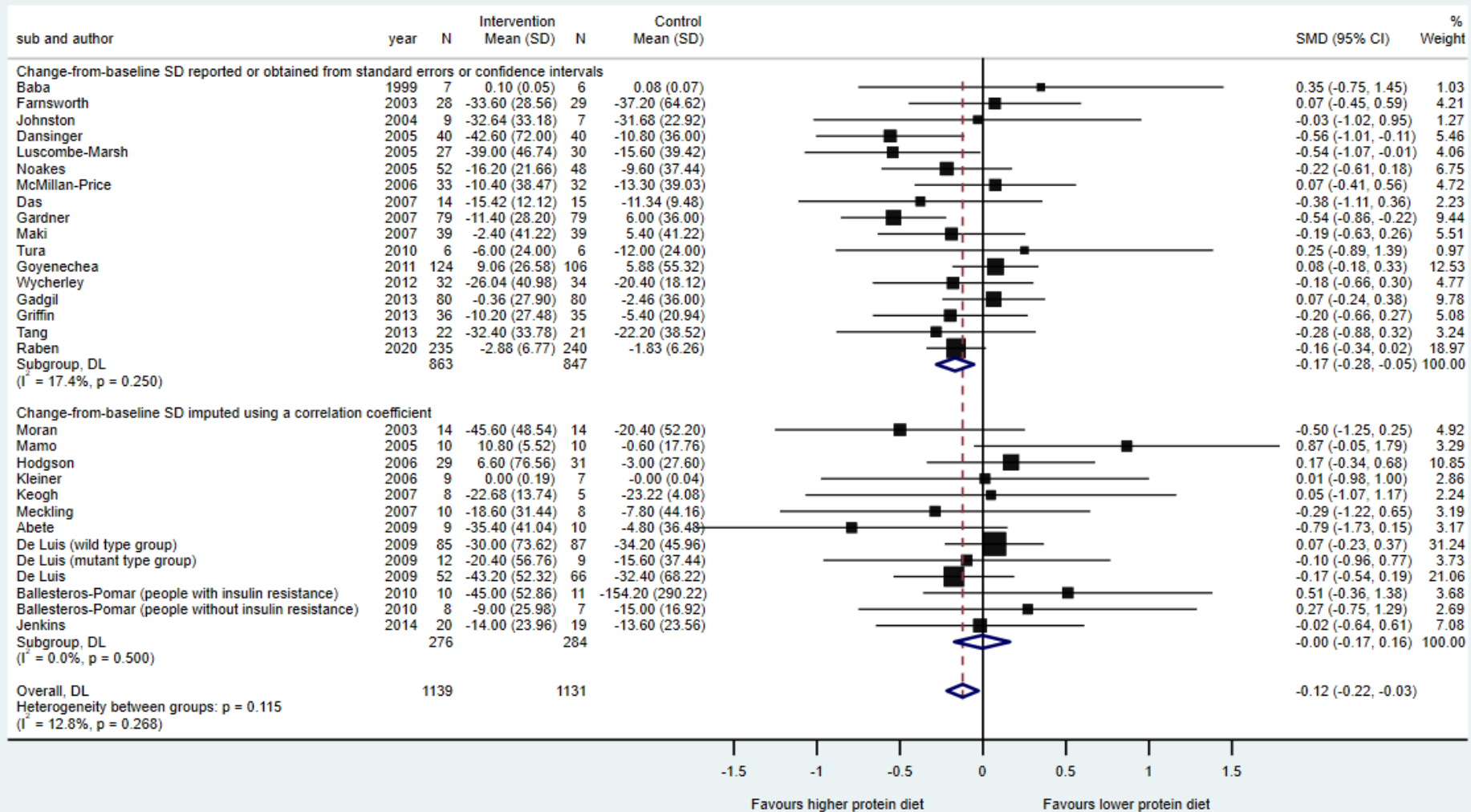


Figure 6. Standardised mean difference (SMD) and 95% confidence interval (CI) in fasting insulin between intervention and control groups on the effect of a higher protein diet.

Pre-proof version