

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

Article

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1 **ATHEROSCLEROSIS**

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

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45 **Abstract**

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

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

56 **Results:** Fifty-seven articles reporting on 54 RCTs were included, involving 4,344 participants
57 (65% female, mean age: 46 (SD 10) years, mean BMI: 33 (SD 3) kg/m²), with a mean study
58 duration of 18 weeks (range: 4 to 156 weeks). Compared to LP diets (range protein (E%):10-
59 23%), HP diets (range protein (E%): 20-45%) led to more weight loss (SMD -0.13, 95% CI: -
60 0.23, -0.03), greater reductions in fat mass (SMD -0.14, 95% CI: -0.24, -0.04), systolic BP
61 (SMD -0.12, 95% CI: -0.21, -0.02), total cholesterol (SMD -0.11, 95% CI: -0.19, -0.02),
62 triacylglycerol (SMD -0.22, 95% CI: -0.30, -0.14) and insulin (SMD -0.12, 95% CI: -0.22, -
63 0.03). No significant differences were observed for the other outcomes.

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

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68

69 **Introduction**

70 Dietary proteins are important sources of energy and essential amino acids, necessary for
71 various bodily processes, including tissue growth and maintenance [1]. The effects of dietary
72 protein on human health are determined by several factors, including quantity, quality (animal
73 protein/plant protein) and source; animal (red and white meat, fish, eggs and dairy) or plant-
74 based (nuts, legumes, grains). In terms of quantity, current European and US dietary
75 recommendations for protein intake generally advise ≥ 0.8 g/kg body weight (BW)/day for
76 adults [2, 3] and growing evidence suggests an even higher intake for elderly (1.0-1.2 g/kg
77 BW/day) [4-6]. When expressed in percentage of the total energy intake (energy-percent
78 (E%)), the Nordic Nutrition Recommendations established a desirable daily protein intake of
79 10-20 E% for adults [7]. Other dietary guidelines provide similar recommendations, including
80 those from the UK [8], the Netherlands [9] and German-speaking countries (Germany, Austria
81 and Switzerland) [10].

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

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

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

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

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

132 **Materials and methods**

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

136 **Search strategy**

137 Literature searches were conducted in PubMed, Web of Science and Scopus, in addition to
138 checking of reference lists of retrieved articles and previously published meta-analyses [18, 19,
139 36, 37]. The searches involved a range of keywords for dietary protein, body weight and
140 anthropometrics, body composition, blood pressure, blood lipids, markers of glucose
141 metabolism and trial design. A detailed search strategy can be found in **Supplementary table**

142 1. All English language studies that met the eligibility criteria were selected, up to November
143 2020.

144 **Eligibility criteria**

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

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

165

166 **Study selection and data extraction**

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

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

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

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

192 **Risk of bias within studies**

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

198 **Data analysis**

199 The mean difference in the outcomes between the intervention and control group were
200 calculated as standardised mean difference (SMD) and weighted mean difference (WMD) with
201 95% CI. The SMD and WMD were calculated based on mean and SD of the change from
202 baseline values. If not reported, then these were calculated using the reported data. The
203 $\text{mean}_{\text{change}}$ was calculated by subtracting the baseline values from the postintervention values.
204 The $\text{SD}_{\text{change}}$ was calculated using the following formula; $\text{SD}_{\text{change}} = \sqrt{(\text{SD}_{\text{baseline}}^2 +$
205 $\text{SD}_{\text{postintervention}}^2) - (2 \times r \times \text{SD}_{\text{baseline}} \times \text{SD}_{\text{postintervention}})}$ [45], where r represented the correlation
206 coefficient between the baseline and postintervention values and was assumed to be 0.5 and
207 led to more conservative estimates (wider 95% CI).

208 Random-effects models were carried out to pool the data of RCTs, which examined the effects
209 of HP compared to LP diets on changes in weight loss, weight regain, BMI, waist
210 circumference, fat mass, lean mass, SBP, DBP, total cholesterol, HDL-c, LDL-c,
211 triacylglycerol, glucose, insulin, HOMA-IR and HbA_{1c}, accounting for within and between
212 study variance [46]. Between-study heterogeneity was evaluated using the I² statistic (%) [47],
213 where I² of $\leq 30\%$, between 30% and 50%, between 50% and 75% and $\geq 75\%$ were considered,

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

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

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

230 **Results**

231 **3.1 Study selection process**

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

237

238 3.2 Study characteristics

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

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

249 The achieved relative intake of dietary protein, carbohydrate, and total fat (E%) were, on
250 average, 28% (range: 20 to 45%), 41% (range: 25 to 55%) and 31% (range: 20 to 43%) in the
251 HP group and 18% (range: 10 to 23%), 54% (range: 36 to 66%) and 28% (range: 20 to 45%)
252 in the LP group (**Supplementary table 3**). The mean (SD) total daily energy intakes were
253 1,764 (455) kcal and 1,768 (462) kcal in the HP and LP groups, respectively (**Supplementary**
254 **table 3**).

255 3.3 Changes in body weight, anthropometrics, and body composition

256 A total of 48 trials with 3,346 participants provided data on weight loss, 3 on weight regain
257 (774 participants), 27 on BMI (2,012 participants) and 26 on waist circumference (2,669
258 participants). Meta-analysis revealed statistically significant effects on weight loss with HP
259 compared to LP diets, with a pooled SMD of -0.13 (95% CI: -0.23, -0.03). There was moderate
260 to low heterogeneity across the trials ($I^2 = 38\%$, $p=0.004$) (**Figure 2**). This is equivalent to an
261 increase in weight loss of 0.64 kg (95% CI: -1.12, -0.17, $I^2 = 53\%$, $p=0.000$) with HP compared

262 to LP diets. Significant intervention effects were observed for weight loss when the participants
263 were under 50 years of age, with a pooled SMD of -0.17 (95% CI: -0.31, -0.03)
264 (**Supplementary table 5**). The meta-analysis also revealed less weight regain in the HP
265 compared to LP groups (pooled SMD -0.18, 95% CI: -0.32, -0.04, $I^2 = 0%$, $p=0.6$)
266 (**Supplementary table 4**). However, removal of Larsen et al. (2010) from the analysis, a trial
267 contributing most weight to the pooled estimate, attenuated the results for weight regain
268 (pooled SMD -0.08, 95% CI: -0.39, 0.24, $I^2=0%$, $p=0.5$). The pooled analyses across trials
269 showed a tendency towards an effect in favour of the HP diet for a lower BMI (pooled SMD -
270 0.11, 95% CI: -0.23, 0.01, $I^2 = 31%$, $p=0.1$) and waist circumference (pooled SMD -0.11, 95%
271 CI: -0.23, 0.01, $I^2 = 44%$, $p=0.006$), but these were not statistically significant (**Supplementary**
272 **table 4**).

273 Meta-analysis of 35 trials with 2,580 participants showed a significant reduction in fat mass
274 with HP compared to LP diets (pooled SMD -0.14, 95% CI: -0.24, -0.04). There was low
275 heterogeneity across the trials ($I^2 = 28%$, $p=0.1$) (**Figure 3**). The pooled WMD for reduction
276 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.
277 Significant intervention effects were found for fat mass when participants were 50 years of age
278 or older (pooled SMD -0.15, 95% CI: -0.26, -0.03) (**Supplementary table 5**). Meta-analysis
279 of 30 trials involving 2,418 participants showed no significant differences between the diets
280 for lean mass (pooled SMD 0.06, 95% CI: -0.06, 0.17, $I^2 = 39%$, $p=0.013$) (**Supplementary**
281 **table 4**).

282 **3.4 Changes in blood pressure**

283 A total of 26 trials with 1,813 participants provided data for the meta-analysis for SBP and
284 DBP. A reduction in SBP and DBP was found with HP compared to LP diets (pooled SMD
285 -0.12, 95% CI: -0.21, -0.02, $I^2 = 0.0%$, $p=0.9$) (**Figure 4**) and (pooled SMD -0.09, 95% CI:

286 -0.19, 0.01, $I^2 = 9\%$, $p=0.3$) (**Supplementary table 4**), respectively, although this did not reach
287 statistical significance for DBP. The pooled WMD for reduction in SBP was 1.16 mm Hg (95%
288 CI: -2.13, -0.20, $I^2=0\%$, $p=0.8$) with HP compared to LP diets. The intervention effects were
289 borderline significant for SBP when participants were under 50 years of age (pooled SMD -
290 0.12, 95% CI: -0.23, -0.00) (**Supplementary table 5**) and the study duration was under 12
291 weeks (pooled SMD -0.15, 95% CI: -0.30, -0.00) (**Supplementary table 6**).

292 **3.5 Changes in blood lipid concentrations**

293 A total of 41 trials (2,303 participants) reported data on total cholesterol, 42 trials (2,452
294 participants) on HDL-c, 42 trials (2,516 participants) on LDL-c and 43 trials (2,530
295 participants) on triacylglycerol. Meta-analysis demonstrated a reduction in total cholesterol
296 with HP compared to LP diets (pooled SMD -0.11, 95% CI: -0.19, -0.02) (**Figure 5a**). Results
297 were consistent across all 41 trials with low heterogeneity ($I^2 = 1\%$, $p=0.5$). No significant
298 differences between the diets were observed for HDL-c (pooled SMD 0.10, 95% CI: 0.01, 0.20,
299 $I^2 = 19\%$, $p=0.1$) or LDL-c (pooled SMD 0.01, 95% CI: -0.08, 0.10, $I^2 = 20\%$, $p=0.1$). A
300 significant reduction in triacylglycerol was found with HP compared to LP diets (pooled SMD
301 -0.22, 95% CI: -0.30, -0.14) and pooled trial data for this outcome was homogeneous ($I^2 = 0\%$,
302 $p=0.9$) (**Figure 5b**). Translated to an effect in clinical units, a greater reduction in total
303 cholesterol of 0.08 mmol/L (95% CI: -0.13, -0.03, $I^2=0\%$, $p=0.5$) and a greater reduction in
304 triacylglycerol of 0.12 mmol/L (95% CI: -0.16, -0.08, $I^2=0\%$, $p=0.8$) was observed with HP
305 compared to LP diets. Significant intervention effects were observed for total cholesterol when
306 participants were 50 years of age or older (pooled SMD -0.16, 95% CI: -0.29, -0.02)
307 (**Supplementary table 5**) and the study duration was under 12 weeks (pooled SMD -0.25, 95%
308 CI: -0.40, -0.09) (**Supplementary table 6**). For triacylglycerol, the intervention effects were
309 significant when participants were female (pooled SMD -0.25, 95% CI: -0.40, -0.11)
310 (**Supplementary table 7**), the study duration was under 12 weeks (pooled SMD -0.32, 95%

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

313 **3.6 Changes in markers of glucose metabolism**

314 Thirty-four trials (2,592 participants) were included in the meta-analysis on glucose, 28 trials
315 (2,270 participants) with data on insulin, 19 trials (1,674 participants) on HOMA-IR and 3
316 trials (152 participants) on HbA_{1c}. Pooled analysis showed a statistically significant lowering
317 effect of HP diets on insulin, compared to LP diets (pooled SMD -0.12, 95% CI: -0.22, -0.03),
318 with low heterogeneity across the trials ($I^2 = 13\%$, $p=0.3$) (**Figure 6**). Significant intervention
319 effects of the were observed for insulin when participants were female (pooled SMD -0.37,
320 95% CI: -0.58, -0.17) (**Supplementary table 7**), and in the subgroup of 50 years of age or
321 older (pooled SMD -0.15, 95% CI: -0.27, -0.02) (**Supplementary table 5**). No significant
322 differences between the diets were found for glucose (pooled SMD -0.01, 95% CI: -0.11, 0.12,
323 $I^2 = 43\%$, $p=0.003$), HOMA-IR (pooled SMD -0.05, 95% CI: -0.22, 0.11, $I^2 = 56\%$, $p=0.001$)
324 or HbA_{1c} (pooled SMD -0.02, 95% CI: -0.49, 0.45, $I^2 = 52\%$, $p=0.1$) (**Supplementary table**
325 **4**).

326 **3.7 Sensitivity analyses**

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

329 **3.8 Meta-regression analyses**

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

333

334 **3.9 Risk of bias within and between studies**

335 The overall risk of bias of the included trials ranged from ‘low’ to ‘some concerns’ and ‘high’.
336 Most trials showed concerns about the selection of the reported result due to not publishing
337 details on the pre-specified analysis plans and the randomisation process (e.g., sequence
338 concealment) (**Supplementary Figure 1**). Only 1 of 54 trials demonstrated high risk of bias
339 [50] (**Supplementary Figure 1**). Assessment of the risk of bias of the included trials can be
340 found in **Supplementary table 6**. No evidence for publication bias was found in the meta-
341 analyses for the outcomes, except for triacylglycerol (Egger’s test $p=0.0$) (**Supplementary**
342 **figures 2-7**). However, trim and fill analyses revealed no major change in the observed overall
343 effect estimate (pooled SMD -0.14, 95% CI: -0.21, -0.07).

344 **Discussion**

345
346 The present systematic review and meta-analyses of 54 RCTs have shown favourable but small
347 effects of HP vs. LP diets on weight loss, fat mass, SBP, total cholesterol, triacylglycerol, and
348 fasting insulin among adults over a mean follow-up of 4-5 months. Findings of these meta-
349 analyses suggest that intake of higher dietary protein (28 E%) (range: 20-45%) compared to
350 lower dietary protein (18 E%) (range: 10-23%) could lead to more weight loss and reductions
351 in fat mass, SBP, total cholesterol, triacylglycerol, and fasting insulin. No significant
352 differences between the diet were found for BMI, waist circumference, lean mass, HDL-c, and
353 LDL-c, DBP, glucose, HbA_{1c} and insulin resistance estimated by HOMA-IR.

354 *Comparison with other reviews*

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

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

376 *Possible explanations*

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

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

406

407

408 *Strengths and limitations*

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

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

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

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

460 *Context and implications for future research*

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

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

477

478 **Conflict of interest**

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

483 **Author contributions**

484 YDV, AR, AFHP and SSSM conceived and designed the review. YDV, IF and CDW
485 performed the literature search and screened the data. YDV extracted the data. IF and CDW
486 verified the extracted data. YDV analysed and interpreted the data. SSSM supervised YDV
487 with data analyses and interpretation. YDV wrote the draft manuscript. AR, AFHP, JAL, DIG
488 and SSSM critically revised the manuscript for important intellectual content. All authors gave
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713 **Keywords**

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

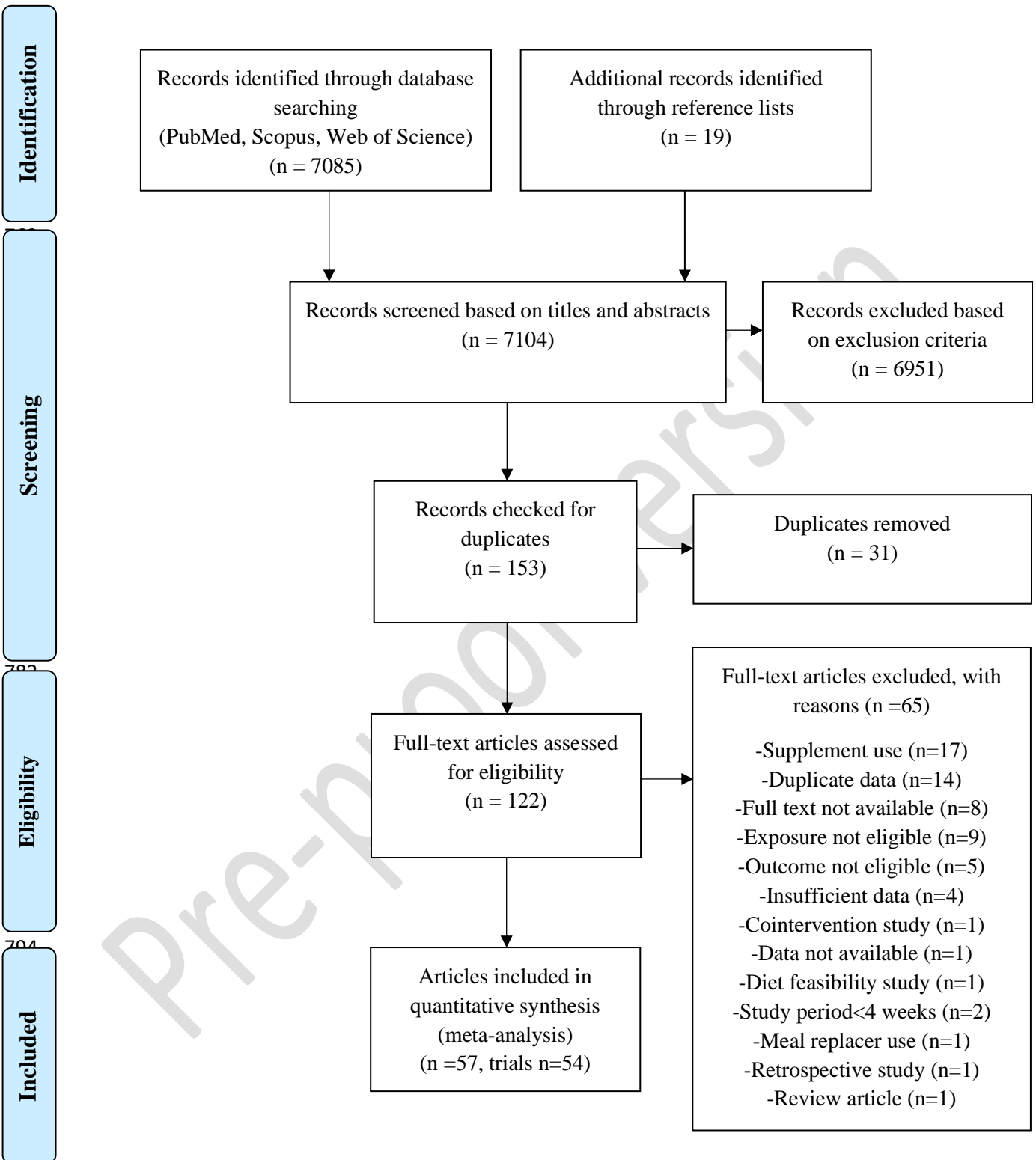
715 **Abbreviations**

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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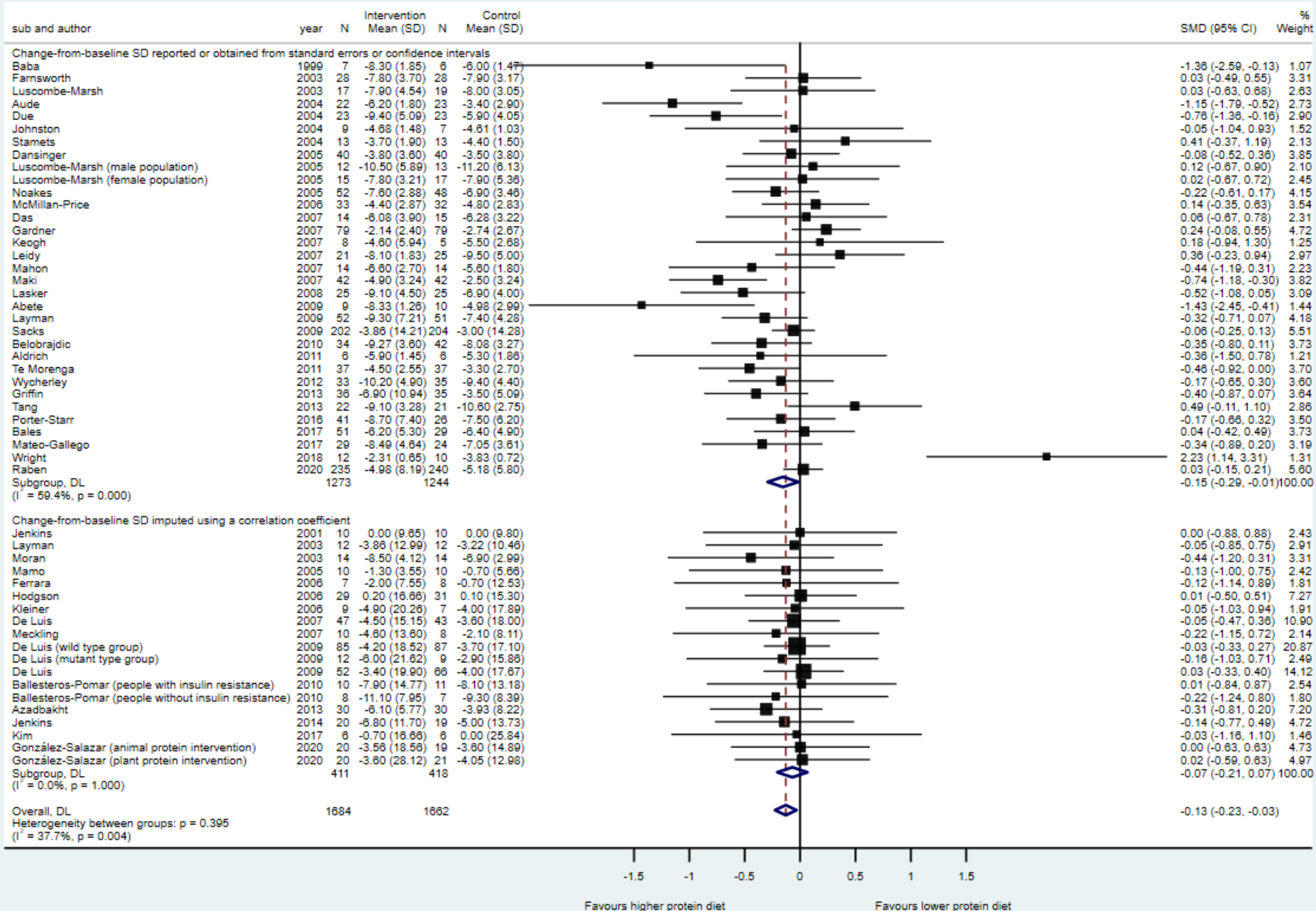


Figure 2. Standardised mean difference (SMD) and 95% confidence interval (CI) in weight loss between the intervention and control groups on the effect of a higher protein diet.

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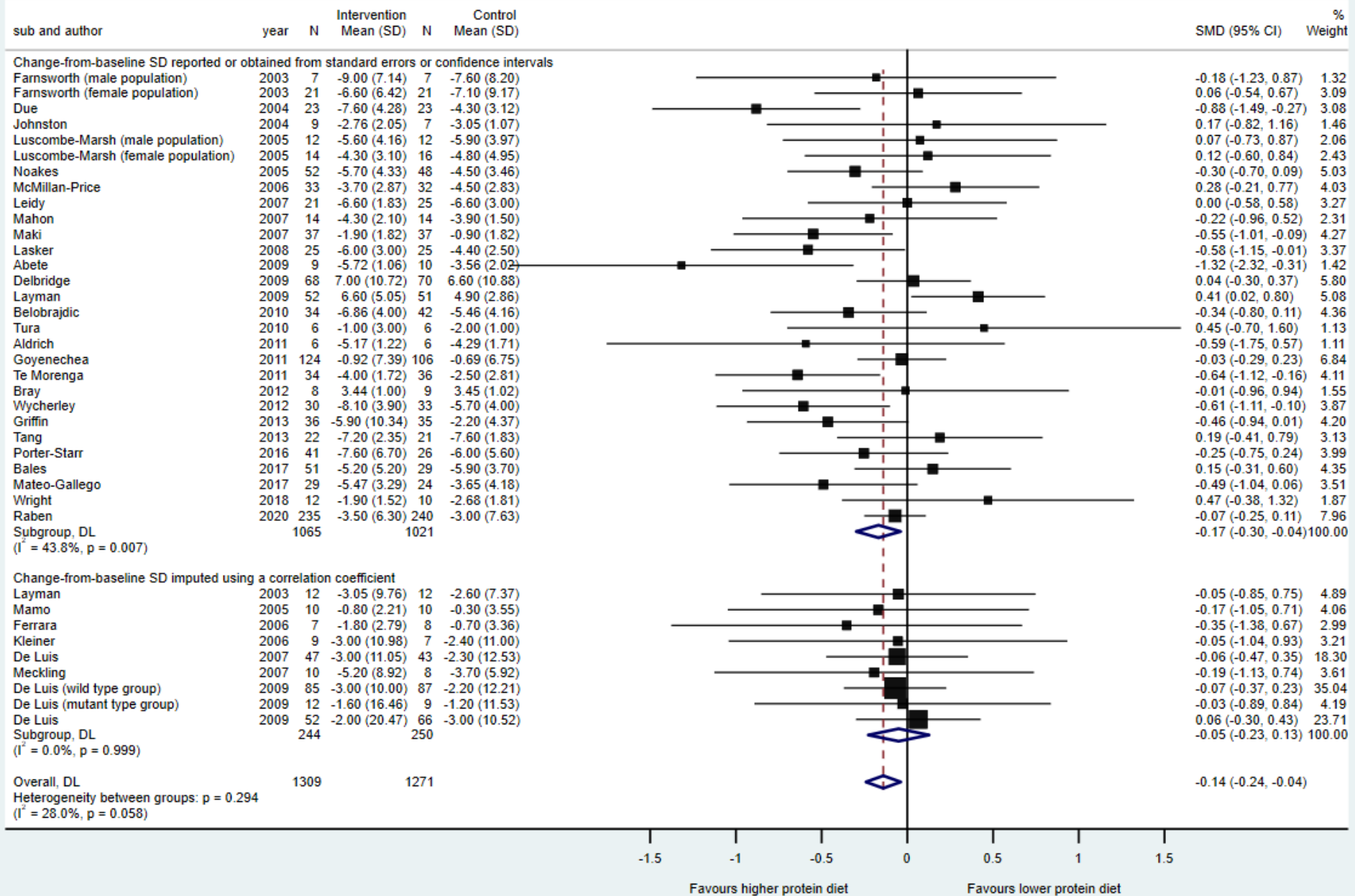


Figure 3. Standardised mean difference (SMD) and 95% confidence interval (CI) in fat mass between the intervention and control groups on the effect of a higher protein diet.

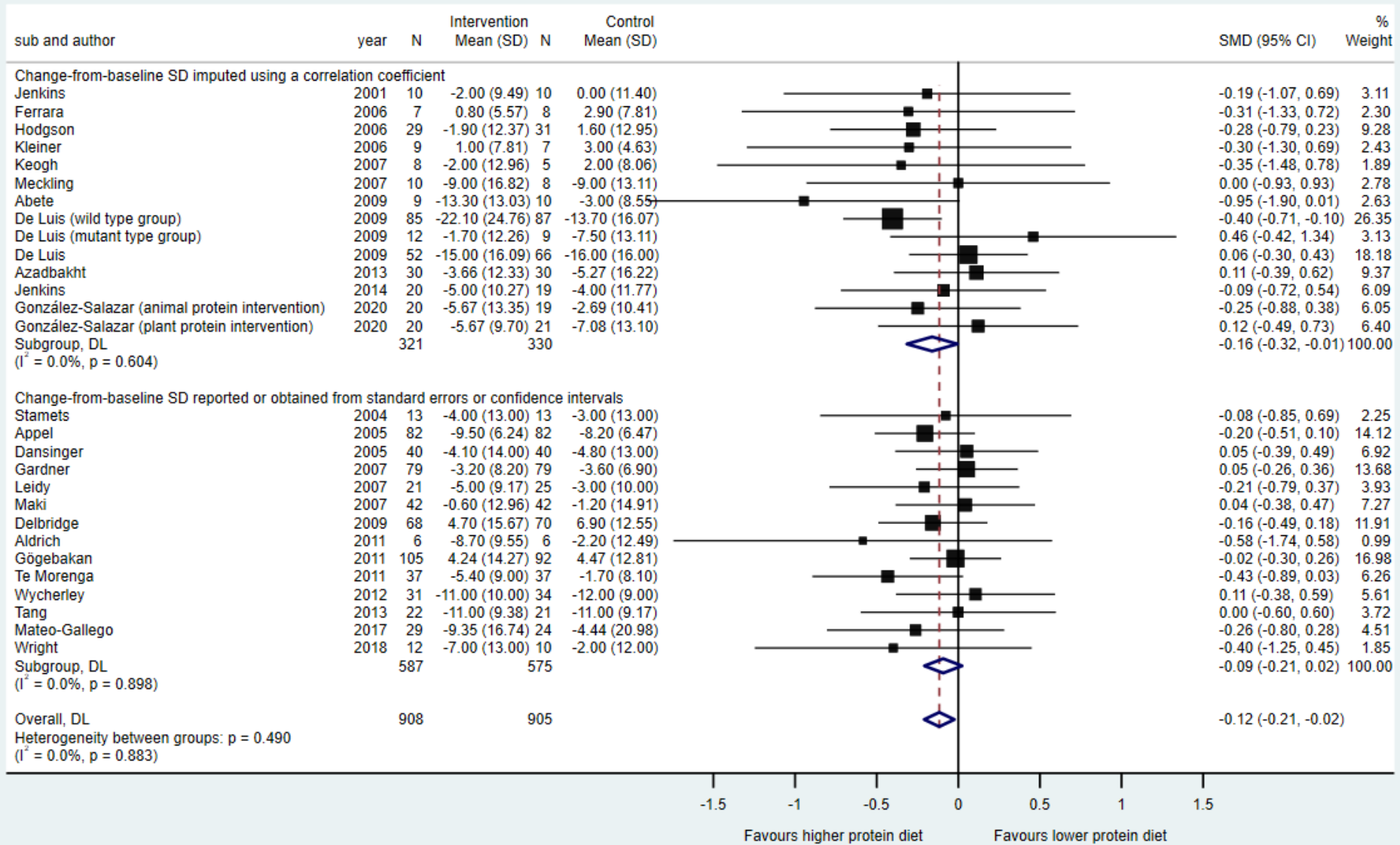


Figure 4. Standardised mean difference (SMD) and 95% confidence interval (CI) in systolic blood pressure between the intervention and control groups on the effect of a higher protein diet.

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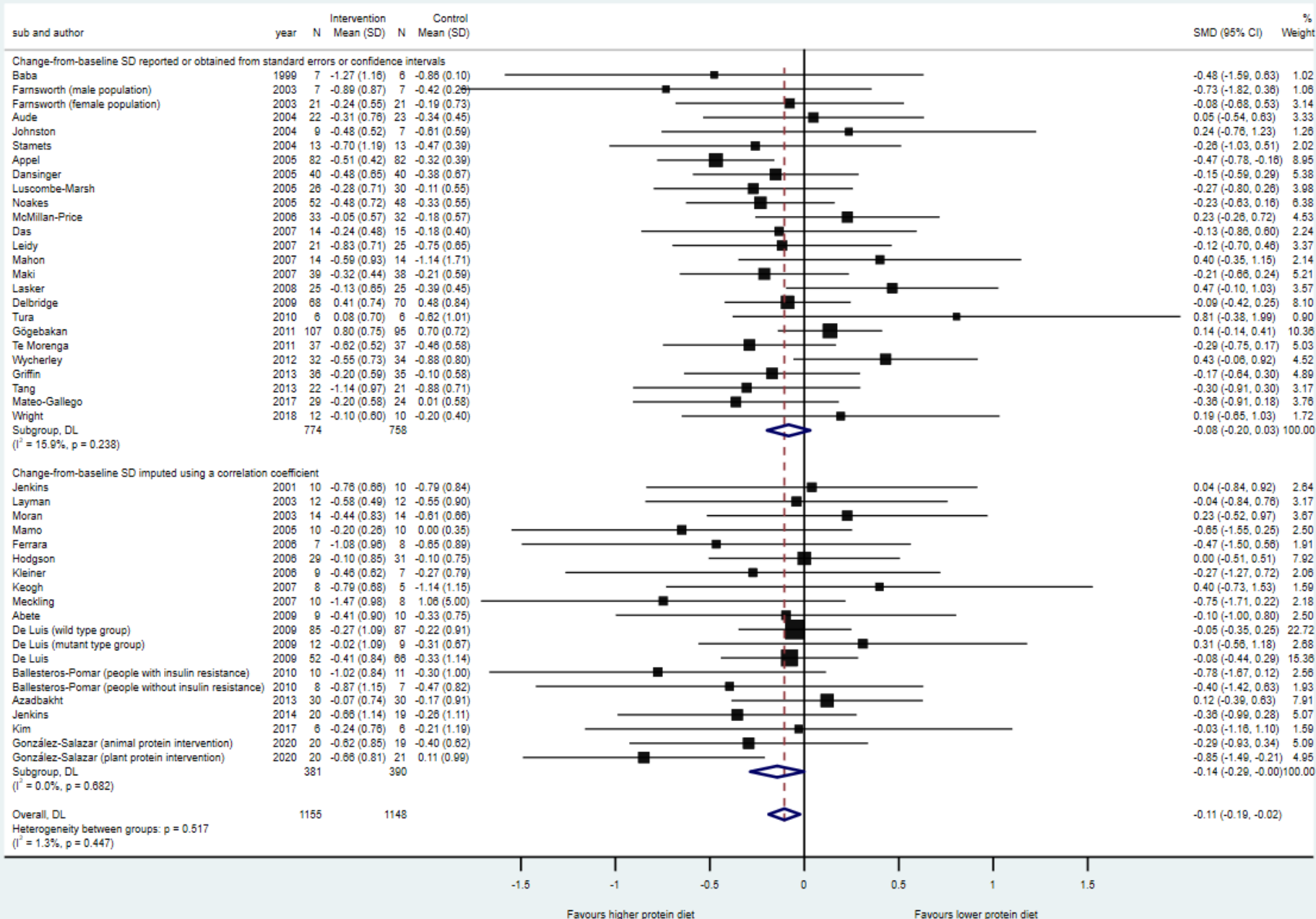


Figure 5a. Standardised mean difference (SMD) and 95% confidence interval (CI) in total cholesterol between intervention and control groups on the effect of a higher protein diet.

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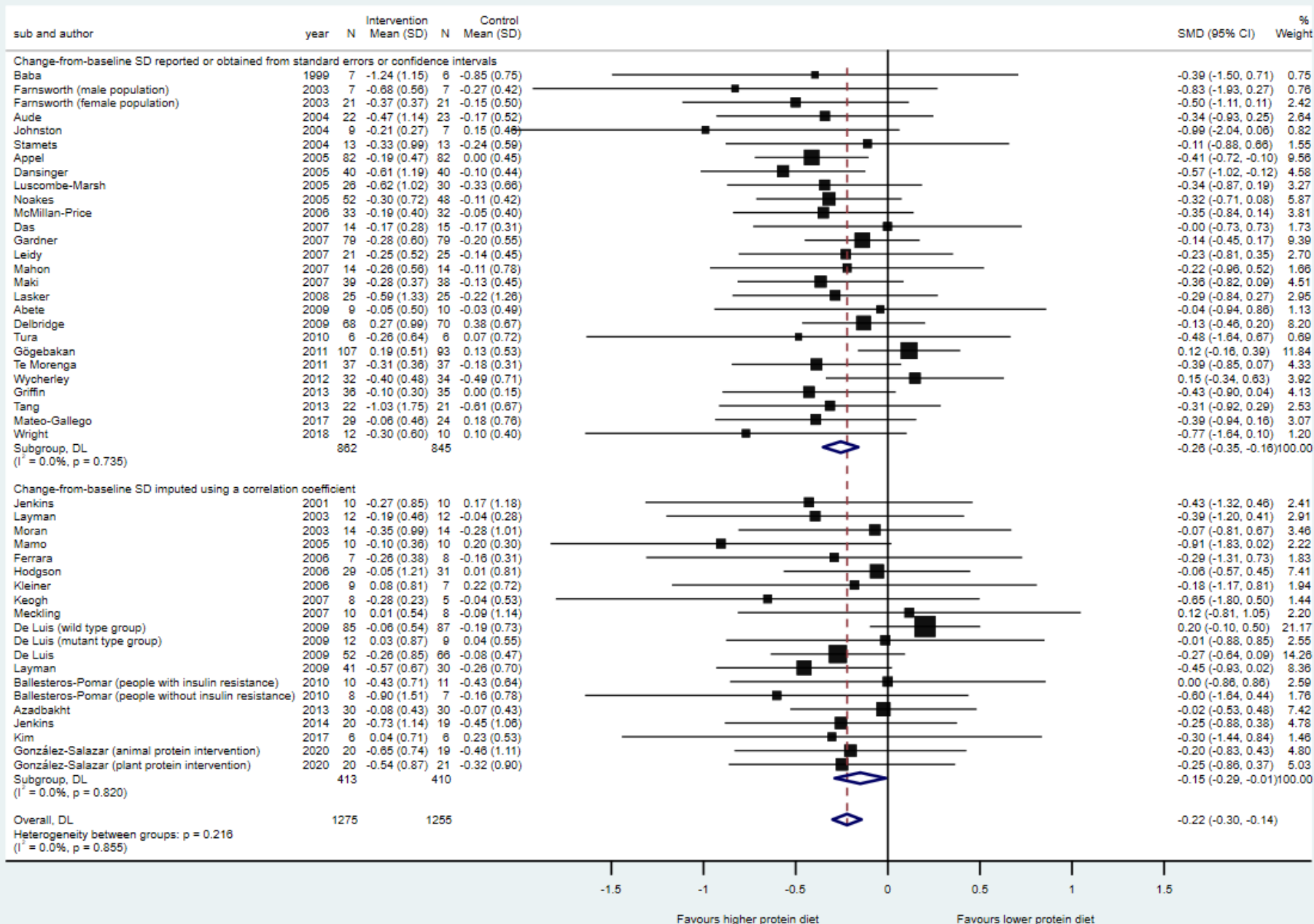


Figure 5b. Standardised mean difference (SMD) and 95% confidence interval (CI) in triacylglycerol between intervention and control groups on the effect of a higher protein diet.

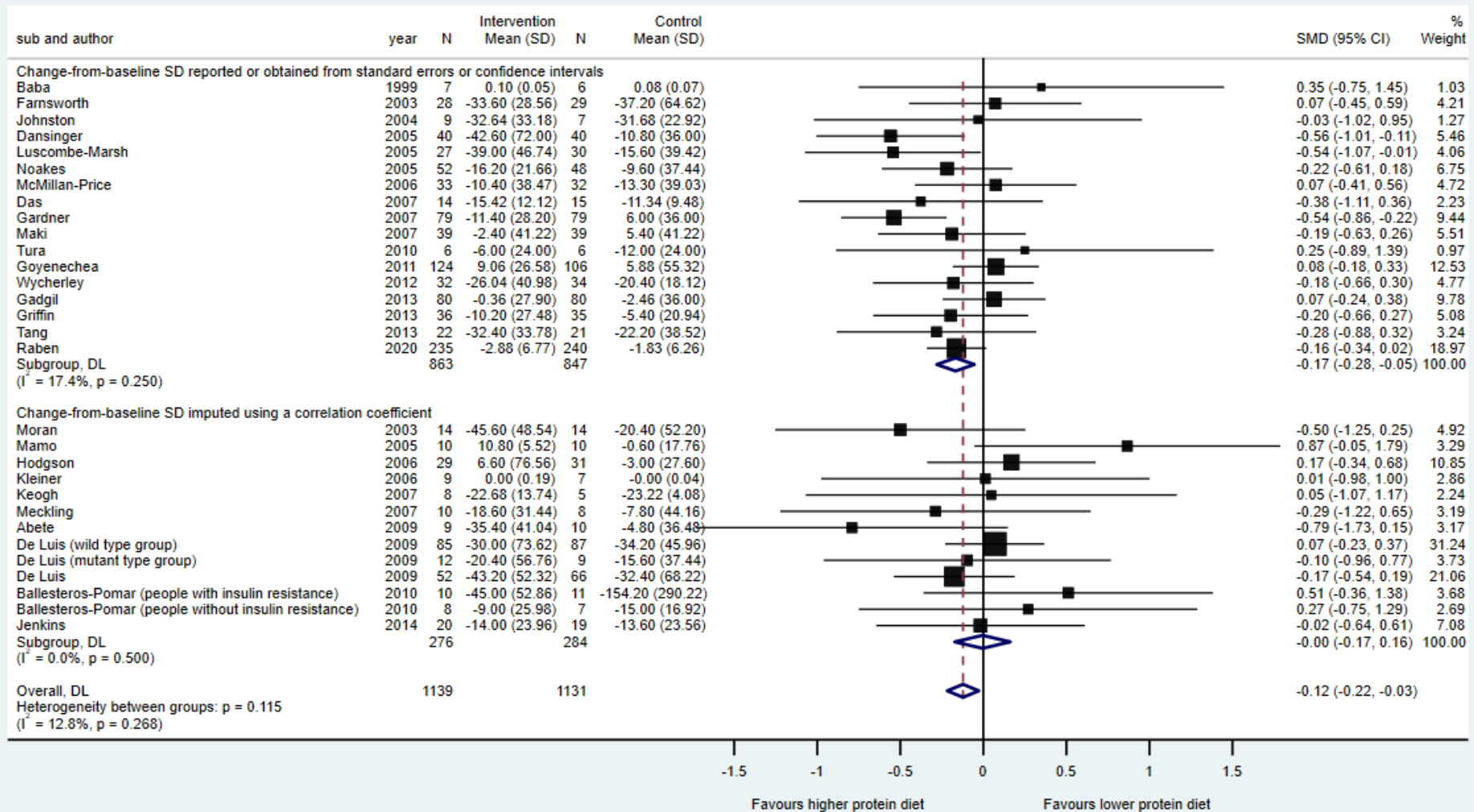


Figure 6. Standardised mean difference (SMD) and 95% confidence interval (CI) in fasting insulin between intervention and control groups on the effect of a higher protein diet.

Pre-proof version