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**$\beta$ -hydroxy- $\beta$ -methylbutyrate and its impact on skeletal muscle mass and physical function in clinical practice: a systematic review and meta-analysis**

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**Short running head:**  $\beta$ -hydroxy- $\beta$ -methylbutyrate and muscle mass

### **Abbreviations**

ARG, Arginine

BIA, Bioelectrical Impedance Analysis

BUN, Blood Urea Nitrogen

CT, Computed Tomography

GLN, Glutamine

FFM, Fat free mass

HMB,  $\beta$ -hydroxy- $\beta$ -methylbutyrate

ONS, Oral Nutrition Supplement

RCT, Randomized Controlled Trial

SIRS, Systemic Inflammatory Response Syndrome

SMD, Standard Mean Difference

3-MH, 3-methylhistidine

UUN, Urinary Urea Nitrogen

## 1 **Abstract**

## 2 **Background**

3 Loss of skeletal muscle mass and muscle weakness are common in a variety of  
4 clinical conditions with both wasting and weakness associated with an impairment of  
5 physical function.  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) is a nutrition supplement that  
6 has been shown to favourably influence muscle protein turnover and thus has a  
7 potential role in ameliorating skeletal muscle wasting and weakness.

## 8 **Objective**

9 To investigate the efficacy of HMB alone, or supplements containing HMB, on  
10 skeletal muscle mass and physical function in a variety of clinical conditions  
11 characterized by loss in skeletal muscle mass and weakness.

## 12 **Design**

13 A systematic review and meta-analysis of randomized controlled trials reporting  
14 outcomes of muscle mass, strength and physical function was performed. Two  
15 reviewers independently performed screening, data extraction, and risk of bias  
16 assessment. Outcome data were synthesized through meta-analysis using a  
17 random-effects model and data presented as standardized mean differences  
18 (SMDs).

## 19 **Results**

20 Fifteen RCTs were included, involving 2137 patients. Meta-analysis revealed some  
21 evidence to support the effect of HMB alone, or supplements containing HMB, on  
22 increasing skeletal muscle mass (SMD = 0.25; 95% CI -0.00, 0.50; Z = 1.93; P =  
23 0.05;  $I^2=58\%$ ) and strong evidence to support improving muscle strength (SMD =

24 0.31; 95% CI 0.12, 0.50; Z = 3.25; P = 0.001; I<sup>2</sup>=0%). Effect sizes were small. No  
25 effect on bodyweight (SMD = 0.16; 95% CI = -0.08, 0.41; Z = 1.34; P = 0.18; I<sup>2</sup> =  
26 67%) or any other outcome was found. No study was considered to have low risk of  
27 bias in all categories.

## 28 **Conclusion**

29 HMB, and supplements containing HMB, increased muscle mass and strength in a  
30 variety of clinical conditions, although the effect size was small. Given the bias  
31 associated with many of the included studies, further high quality studies should be  
32 undertaken to enable interpretation and translation into clinical practice.

33

34 **KEYWORDS:**  $\beta$ -hydroxy- $\beta$ -methylbutyrate, HMB, muscle, strength, nutrition,  
35 malnutrition, cancer cachexia, critical illness, sarcopenia

36

37

38

## 39 INTRODUCTION

40 Skeletal muscle wasting and weakness commonly occur with immobilisation and  
41 disuse (1), malnutrition (2), age-related sarcopenia (3), cancer cachexia (4) and  
42 during early critical illness (5). Furthermore, reduced muscle mass is associated  
43 with impaired physical function (6) and frailty (3), which drive morbidity (2, 7) and  
44 mortality (8-10). Interventions that can ameliorate, or even prevent, loss of muscle  
45 mass and improve physical function are a key clinical priority.

46 Depending on the technique used, skeletal muscle can be measured or estimated as  
47 either fat-free mass (FFM) or lean mass, although accuracy is variable (11). For  
48 example, bioelectrical impedance analysis (BIA) estimates FFM (sum of lean body  
49 mass and the bone mineral compartments) whereas DXA measures lean mass  
50 (body water, total body protein, carbohydrates, non-fat lipids, and soft tissue mineral)  
51 (11). Given the variety of techniques used to measure these body components, and  
52 that skeletal muscle is an important component of both fat free and lean mass, the  
53 term 'muscle mass' is used throughout this systematic review.

54 Muscle mass is maintained by a balance between muscle protein synthesis and  
55 muscle protein catabolism. Both resistance exercise and amino acid loading can  
56 enhance protein balance (12), however, resistance exercise is challenging, in  
57 particular during acute illness. This is compounded by insufficient protein intake in  
58 acute and chronic clinical patient populations (13-16) with anabolic resistance  
59 present in older patients (17, 18). For this reason, investigating novel interventions  
60 such as amino acids and their metabolites, which are not purely reliant on factors  
61 relating to appetite, are warranted.

62  $\beta$ -hydroxy- $\beta$ -methylbutyrate (HMB) is a metabolite of the amino acid, leucine, and its  
63 effects on skeletal muscle mass and strength has been investigated in athletes (19).  
64 HMB has several proposed mechanisms of action including stimulation of the  
65 mammalian target of rapamycin (mTOR) which leads to increased protein synthesis  
66 (20, 21) and attenuation of the proteasome pathways that lead to muscle protein  
67 catabolism (22, 23). Positive effects of HMB supplementation on maintenance of  
68 muscle mass in healthy older people undertaking bed rest, even in the absence of an  
69 exercise regimen, indicates this may be an efficacious nutrition intervention for  
70 immobile patients, such as during early critical illness (24). The effects of HMB  
71 supplementation have been studied in a variety of clinical conditions where muscle  
72 wasting is present, including critical illness (25), human immunodeficiency virus  
73 (HIV) (26) and cancer cachexia (27) with varying results.

74 The aim of the current study was to undertake a systematic review and meta-  
75 analysis to investigate the effects of HMB alone, and supplements containing HMB,  
76 on skeletal muscle mass and physical function in a variety of clinical conditions  
77 characterized by loss of muscle mass and skeletal muscle weakness.

## 78 **MATERIALS & METHODS**

79 This systematic review and meta-analysis was performed following guidelines from  
80 the Cochrane Handbook for Systematic Reviews of Interventions (28) and reported  
81 according to the Preferred Reporting Items for Systematic Reviews and Meta-  
82 Analysis (PRISMA) guidelines (29). The protocol was pre-registered on PROSPERO  
83 ([CRD42017058517](https://www.crd42017058517)).

84

### 85 **Search strategy**

86 A literature search for randomized controlled trials investigating the effect of HMB,  
87 and supplements containing HMB on muscle mass, strength or physical function in  
88 adult patients was conducted using electronic searching of four literature databases,  
89 two clinical trials databases, hand-searching of abstracts from three conference  
90 proceedings, back-searching of reference lists and discussions with key opinion  
91 leaders. One investigator (DEB) performed a database search (last search date 25  
92 September 2018) using MEDLINE, Web of Science, EMBASE and CINAHL using a  
93 pre-determined search strategy (**Supplemental Methods**). Limits were applied to  
94 the electronic search, restricting studies to those including adults and humans, and  
95 published in the English language only. No date range restrictions were applied. The  
96 international trial databases Clinical Trials ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) and ISRCTN  
97 ([www.ISRCTN.com](http://www.ISRCTN.com)), were also searched for completed, unpublished studies.  
98 Abstracts of the following conference proceedings were hand-searched to identify  
99 any potentially relevant studies: American Society for Parenteral and Enteral  
100 Nutrition, European Society of Clinical Nutrition and Metabolism, and the Society of  
101 Sarcopenia, Cachexia and Wasting Disorders. Reference lists of relevant studies  
102 and previous systematic reviews were manually reviewed for additional studies not  
103 identified in the electronic search.

#### 104 **Study selection**

105 After removing duplicates and non-relevant material, titles and abstracts identified  
106 from the search were screened independently by two investigators (DEB, AL).

107 Potentially eligible studies had their full texts screened for eligibility by the same two  
108 investigators (DEB, AL). Inclusion and exclusion criteria are shown in **Table 1**.

109 Data extraction was performed independently by two investigators (DEB, AL) using a  
110 pre-specified data collection form. Data were cross-checked for discrepancies and

111 corrected where appropriate. The data extracted included author details, year of  
112 publication, participant characteristics, details of intervention and control and results  
113 relating to the aim of the systematic review. Authors of the included studies were  
114 contacted to obtain missing data where required.

### 115 **Assessment of risk of bias**

116 Risk of bias was assessed independently by two investigators (DEB, AL) according  
117 to the Cochrane Collaboration Risk of Bias Tool (28). Studies were assessed at both  
118 the individual and study level and included the methods used to generate  
119 randomization, conceal allocation, blind participants and personnel along with  
120 assessing incomplete outcome data, selective reporting and other sources of bias.

121 Where disagreements between the two researchers (DEB, AL) on study eligibility,  
122 data extraction and risk of bias assessment were not resolved by consensus, a third  
123 investigator was available to arbitrate (LW).

### 124 **Data synthesis and statistical analysis**

125 The primary outcome of interest in this systematic review was the change in skeletal  
126 muscle mass. Secondary outcomes included the effect on body composition,  
127 strength, physical function and surrogate markers of muscle wasting.

128 Meta-analyses were performed on the extracted data, where appropriate, using a  
129 random effects model in Review Manager 5.3 (RevMan5, Copenhagen, Denmark).

130 Where studies included multiple time points, data from the end of the intervention  
131 was used in the overall meta-analysis. However, sub-analyses were also performed  
132 on outcome data measured in four-week epochs (0-4 weeks, 4-8 weeks, 8-12  
133 weeks, >12 weeks) in order to determine any effects of the duration of the  
134 intervention on outcome. For studies using multiple methods to measure muscle

135 mass or strength (e.g. both BIA and air displacement plethysmography or handgrip  
136 and leg extensor strength), data from the most frequently used method across the  
137 studies was used in the overall meta-analyses. However, sub-analyses were also  
138 performed on each method of measuring the outcome where adequate ( $\geq 3$ ) studies  
139 were available.

140 Given the varying methods used to measure muscle mass and strength, standard  
141 mean difference (SMD) with 95% confidence intervals (CI) were used to express  
142 effect size estimates. SMD values of 0.2, 0.5 and 0.8 were defined as small,  
143 moderate and large effect sizes respectively (30). One study did not present results  
144 for the overall group and therefore the results of the two subgroups were entered as  
145 independent groups in the meta-analyses (31).

146 As the reporting of outcome data varied across studies (absolute values at end of  
147 intervention, change scores), change scores were calculated from the available data  
148 and standard deviations imputed according to the Cochrane Handbook for  
149 Systematic Reviews of Interventions (28). Where two standard deviations were  
150 calculated, we imputed the largest to reduce the bias associated with this method.

151 Where change data were presented, but without mean and standard deviation or  
152 standard error, SMD was calculated from the reported p value. Studies reporting only  
153 median and interquartile range were converted to mean and standard deviations  
154 using a referenced formula (32). If more than half of studies included in a meta-  
155 analysis required imputation of standard deviations, rather than imputing change  
156 scores, the value at the end of the intervention period was used for that outcome.  
157 The meta-analysis for muscle strength was the only outcome where this was done.  
158 Sub-group analysis was undertaken where there were enough studies to explore the

159 effect of HMB presentation (e.g. alone, in ONS or combined with amino acids) on  
160 overall outcome.

161 Heterogeneity of results between studies was determined by  $I^2$ , with values of 25-  
162 49.9% considered low, 50-74.9% considered moderate and 75-100% considered  
163 high heterogeneity. Test for overall effect (Z score) was regarded significant at  $P \leq$   
164 0.05. Effect sizes were also relied upon as these are of greater clinical relevance and  
165 recommended by both the American Statistical Association (33) and the Cochrane  
166 Handbook for Systematic Reviews of Interventions (28). Funnel plots were  
167 generated to assess for evidence of asymmetry and possible publication bias or  
168 effects due to the small size of some studies (34).

## 169 **RESULTS**

### 170 **Study Selection**

171 A total of 1426 results were generated from the search strategy (**Figure 1**). None  
172 were obtained from discussions with experts in the field or back-searching reference  
173 lists and hand-searching conference abstracts. After duplicates were removed, 840  
174 records were available for title and abstract screening. Of these, 21 records were  
175 retrieved for full-text screening with 15 RCTs being eligible for inclusion.

176

177

### 178 **Study characteristics**

179 The 15 eligible RCTs involved a total of 2137 adults (25-27, 31, 35-45). Authors of  
180 seven studies were contacted to provide additional data, of whom only one provided  
181 the requested data. All studies included patients who commonly experience muscle  
182 wasting as part of their clinical condition, including older care home residents

183 receiving tube feeding (n=1) (36), hospitalised older people with malnutrition /  
184 sarcopenia (n=2) (31, 43), hospitalised older people undergoing orthopaedic  
185 intervention (n=3) (38, 42, 44), critically ill (n=2) (25, 35), cancer cachexia (n=2) (27,  
186 41), HIV (n=1) (26), maintenance haemodialysis (n=1) (39), rheumatoid cachexia  
187 (n=1) (40), gastric bypass (n=1) (45) and bronchiectasis (n=1) (37). Study  
188 characteristics and intervention data are reported in **Table 2**.

189 Three of the studies used HMB as a single supplement (35, 36, 39), seven used  
190 HMB in combination with arginine and glutamine (HMB/ARG/GLN) (25-27, 38, 40,  
191 41, 45) and five used a high protein oral supplement containing HMB and other  
192 nutrients (31, 37, 42-44) (Table 2). All but one study (35) provided a dose of 3 g/d  
193 HMB. There was considerable variability in the duration of the intervention, ranging  
194 from seven days to six months (Table 2).

### 195 **Risk of bias**

196 No study was considered low risk of bias in all categories. Blinding of participants  
197 and personnel was uncommon, whilst selection (random sequence generation) and  
198 attrition bias had the lowest overall risk of bias (**Figure 2**).

199

200

### 201 **Skeletal muscle mass**

202 Ten of the 15 included studies reported measures of skeletal muscle mass (either  
203 FFM or lean mass) (26, 27, 31, 37-41, 44, 45) using DXA (31, 37, 39, 40, 45), air  
204 displacement plethysmography (Bod Pod) (26, 27), BIA (27, 37, 41, 44) or computed  
205 tomography (CT) (38) with measurement periods ranging from 4 weeks to 6 months  
206 (Table 2).

207 Meta-analysis was possible for 9 of these 10 studies (26, 27, 31, 37-40, 44, 45).  
208 Change scores were calculated from the available data and standard deviations  
209 imputed for four studies (37-39, 44). Median and interquartile range were converted  
210 to mean and standard deviation in one study (31). Some evidence was found to  
211 support supplementation with HMB alone, or supplements containing HMB, on  
212 increasing skeletal muscle mass compared with control, but the effect size was small  
213 (SMD = 0.25; 95% CI -0.00, 0.50; Z = 1.93; P = 0.05) (**Figure 3**). Moderate  
214 heterogeneity was present between studies ( $I^2 = 58%$ ,  $p = 0.01$ ).

215 Sub-group analyses according to the type of supplementation (HMB alone,  
216 combined with arginine and glutamine or within an ONS) revealed some evidence to  
217 support an increase in muscle mass when HMB/ARG/GLN was used, with a  
218 moderate effect size (SMD = 0.49, 95% CI -0.01, 0.99, Z = 1.93; P = 0.05;  $I^2 = 67%$ ,  
219  $p=0.02$ ). (Figure 3).

220 Four studies included muscle mass measurements at multiple time points (27, 31,  
221 37, 45). Therefore, sub-group analysis was performed according to duration of the  
222 intervention. No evidence was found to support improvements in muscle mass when  
223 the intervention was provided less than 4 weeks (27, 44, 45) (SMD = 0.34, 95% CI -  
224 0.21, 0.90, Z = 1.22; P = 0.22;  $I^2 = 69%$ ,  $p=0.04$ ), 4-8 weeks (26, 38, 45) (SMD =  
225 0.60, 95% CI -0.06, 1.25; Z = 1.79, P = 0.07;  $I^2 = 59%$ ,  $p=0.09$ ), 8-12 weeks (31, 37,  
226 40) (SMD = 0.16, 95% CI -0.28, 0.59, Z = 0.71, P = 0.48,  $I^2 = 76%$ ,  $p=0.005$ ) and  
227 greater than 12 weeks (27, 31, 37, 39) (SMD = 0.08, 95% CI = -0.12, 0.28, Z = 0.79,  
228 P = 0.43;  $I^2 = 0%$ ,  $p=0.62$ ).

229 The study by Berk et al (41) was excluded from meta-analysis as only percent  
230 change in muscle mass was reported and absolute change could not be determined

231 from the data reported. This study showed no difference in muscle mass in cancer  
232 cachexia following 8-weeks supplementation with HMB/ARG/GLN.

### 233 **Body weight and composition**

234 Thirteen studies measured body weight (26, 27, 31, 35-38, 40-45), six studies  
235 reported fat mass (27, 31, 37, 39, 40, 44), three studies reported mid-arm muscle  
236 circumference (36, 37, 42) three reported triceps skin fold (TSF) (26, 36, 41) and two  
237 reported thorax, calf, waist and hip circumference (36, 42).

238 Meta-analysis for change in bodyweight could be performed for 12 of the 13 studies  
239 (26, 27, 31, 35-38, 40, 42-45). One was excluded as only percent change in  
240 bodyweight was reported (41). Four of the included studies had change scores  
241 calculated from the available data and standard deviations imputed (37-39, 44).

242 Median and interquartile range were converted to mean and standard deviation in  
243 one study (31). There was no evidence to support the effect of HMB, or supplements  
244 containing HMB on change in bodyweight in the overall meta-analysis (SMD = 0.16;  
245 95% CI = -0.08, 0.41; Z = 1.34; P = 0.18) or in sub-group analysis according to  
246 supplement type (**Figure 4**). Heterogeneity was moderate ( $I^2 = 67%$ ,  $p=0.0003$ ).

247 All six studies reporting fat mass were included for meta-analysis (27, 31, 37, 39, 40,  
248 44). Four of the included studies had change scores calculated from the available  
249 data and standard deviations imputed (37-39, 44) and median and interquartile  
250 range were converted to mean and standard deviation in one study (31). Overall,  
251 there was no evidence to support a change in fat mass between patients receiving  
252 HMB and controls (SMD = 0.03; 95% CI -0.27, 0.34; Z = 0.21; P=0.83;  $I^2 = 58%$ ;  
253  $p=0.03$ ). Sub-group analysis was not undertaken due to the small number of studies.

254 Studies reporting other measures of body composition were unsuitable for meta-  
255 analysis. No difference in TSF was reported in three studies (36, 41, 42). Measures  
256 of arm or body area circumference were reported in three studies (36, 37, 42),  
257 however only Hsieh et al (36) reported a greater increase in waist circumference  
258 after 14 days of HMB alone vs. control ( $0.97 \pm 4.46\%$  vs  $-0.89 \pm 4.45\%$ ,  $p=0.026$ ),  
259 which continued to day 28 of supplementation ( $2.24 \pm 4.64\%$  vs  $-3.42 \pm 4.45\%$ ,  
260  $p<0.05$ ) with additional gains in calf circumference at this time point ( $2.57 \pm 5.02\%$  vs  
261  $-3.63 \pm 4.24\%$ ,  $p<0.05$ ). Baseline difference in BMI were controlled for in this study.

## 262 **Muscle strength**

263 Measures of strength were reported in seven studies (31, 37-40, 42, 44), specifically  
264 isokinetic knee extensor and elbow flexor strength and handgrip strength. Absolute  
265 strength at the end of the intervention period was used for this meta-analysis. Six  
266 studies (37-40, 42, 44) were included in the meta-analysis revealing strong evidence  
267 that HMB or supplements containing HMB improved muscle strength compared with  
268 controls, but with a small to moderate effect size (SMD = 0.31; 95% CI 0.12, 0.50; Z  
269 = 3.25; P = 0.001;  $I^2=0\%$ ) (**Figure 5**). Sub-group analysis according to supplement  
270 type demonstrated strong evidence to support the use of HMB alone (SMD = 0.26,  
271 95% CI -0.00, 0.53, Z = 1.95, P = 0.05) and ONS containing HMB (SMD = 0.37; 95%  
272 CI 0.06, 0.68; Z=2.31; P=0.02;  $I^2 = 0\%$ ) on improving muscle strength (Figure 6),  
273 although there was only one study providing HMB alone. Effect sizes were small to  
274 moderate.

275 Three studies measured handgrip strength only (37, 42, 44), two studies measured  
276 leg strength only (38, 39), one study measured both handgrip and leg strength (31)  
277 and one study measured handgrip, leg and elbow strength (40). There was strong  
278 evidence to support an increase in handgrip strength (SMD = 0.38, 95% CI 0.10,

279 0.66;  $Z = 2.63$ ,  $P = 0.008$ ;  $I^2 = 0\%$ ,  $p=0.75$ ) (**Supplemental Figure 1**) and leg  
280 extensor strength (SMD = 0.28, 95% CI 0.08, 0.48,  $Z = 2.73$ ,  $P = 0.006$ ;  $I^2 = 0\%$ ,  
281  $p=0.85$ ) (**Supplemental Figure 2**) with the intervention. However, effect sizes were  
282 small to moderate.

283 The study by Cramer et al (31) was excluded from this meta-analysis as only data on  
284 change in handgrip and leg strength are provided. Leg strength was measured at 12  
285 and 24 weeks. There were no differences found between treatment groups for leg  
286 strength in the overall study population, but participants classified as having  
287 sarcopenia and normal grip strength displayed significantly greater increases in leg  
288 strength in the intervention compared with control group ( $p=0.032$ ).

### 289 **Physical Function**

290 Four studies reported measures of functional ability (31, 39, 40, 44), including gait  
291 speed (31, 39, 44), sit-to-stand (39, 40), shuttle walk (39) and the 8 foot up-and-go  
292 test (39). Meta-analysis could not be performed due to the nature of data reporting.  
293 None of the four studies reported between-group differences in any outcome of  
294 physical function, however two studies reported no significant changes in physical  
295 function in the HMB group (39, 44) and two studies reported within-group  
296 improvements in physical function over time in the HMB group (31, 40).

### 297 **Surrogate markers of muscle wasting**

298 Six studies reported surrogate markers of muscle wasting including blood urea  
299 nitrogen (BUN) (26, 27, 35, 36, 39), urinary urea nitrogen (UUN) (36), nitrogen  
300 balance and 3-methylhistidine (3-MH) excretion (25). Meta-analysis was not possible  
301 due to the reporting of data or an insufficient number of studies to include in the  
302 analysis.

303 Although BUN and UUN were reported in several studies, only two specifically  
304 investigated these as a surrogate marker of muscle wasting (35, 36) whilst the others  
305 measured these to investigate the safety of taking either HMB or a mixed amino acid  
306 supplement containing HMB (HMB/ARG/GLN) (26, 27, 39). Results from the studies  
307 were inconsistent. Only one study found a significant decrease in BUN in the  
308 intervention group at day 14, but not day 28 of the study period (36). In contrast, May  
309 et al (27) report a significant increase in BUN in the group receiving HMB/ARG/GLN  
310 compared with a decrease in the control which was significantly different between  
311 the two groups ( $P<0.05$ ). Clark et al (26) also report an increase in BUN in the  
312 HMB/ARG/GLN group, but overall results were not displayed. Lastly, Fitschen et al  
313 (39), report that BUN differences between the groups were not significant.

314 In the one study reporting UUN as a surrogate measure of muscle wasting, the  
315 change in 24-hour UUN excretion was significantly lower in the group receiving HMB  
316 compared with control participants at both 14 days (-12.5% vs 29.7%,  $p=0.02$ ) and  
317 28 days (-30.7% vs 15.7%,  $p<0.001$ ) (36). Kuhls et al (25) reported no difference in  
318 UUN excretion between the three groups (HMB alone, HMB/ARG/GLN or placebo),  
319 but nitrogen balance was significantly improved following HMB alone compared with  
320 the HMB/ARG/GLN from day 1 to day 7 and from day 8 to day 14 ( $p<0.05$ ). The  
321 control group were also in greater negative nitrogen balance compared to the HMB  
322 alone, but this was not significant ( $p<0.08$ ). Muscle proteolysis as measured by 3-MH  
323 was not different between the three groups.

#### 324 **Other clinical outcomes**

325 Other clinical outcomes included inflammation (35) or infections (SIRS score) (25),  
326 degree of sepsis (25), mortality (43), hospital and ICU length of stay (25, 43) and  
327 hospital readmissions (43) and vitamin D status (31, 43, 44).

328 Kuhls et al (25), found a decreased incidence of patients with SIRS score 3 or 4 on  
329 days 3 and 7 in the HMB group, but no difference in other clinical outcomes. The use  
330 of an ONS containing HMB compared to placebo reduced 90-day mortality in one  
331 study (4.8% vs. 9.7%,  $p=0.018$ ), but this was a secondary outcome (43). No other  
332 differences were reported as significant for the remaining clinical outcomes.

### 333 **Adverse events**

334 Five studies reported absolute numbers or percentages of adverse events (31, 36,  
335 40, 41, 43). Marcora et al (40), reported significantly lower proportion of participants  
336 with gastrointestinal discomfort in those receiving HMB/ARG/GLN compared with  
337 placebo (28% vs. 67%  $p = 0.02$ ). Hsieh et al (36), reported that some patients  
338 dropped out due to the development of scabs, but numbers and groups were  
339 unclear. The other three studies reported similar numbers of adverse events  
340 between groups.

### 341 **Publication bias**

342 Visual inspection of the funnel plots for all outcomes did not reveal substantial  
343 asymmetry and therefore publication bias (**Supplemental Figures 3-5**). Statistical  
344 tests to explore funnel plot asymmetry were not undertaken due to the use of SMD in  
345 the meta-analysis as per the recommendations of the Cochrane Handbook for  
346 Systematic Reviews of Interventions (28).

347

## 348 **DISCUSSION**

349 This systematic review and meta-analysis aimed to investigate the effects of HMB,  
350 and supplements containing HMB, on skeletal muscle mass and physical function in  
351 a variety of clinical conditions characterized by loss of muscle mass and skeletal

352 muscle weakness, including ageing and critical illness. We found some evidence to  
353 support a positive effect of HMB on the change in muscle mass and strong evidence  
354 to support an increase in absolute muscle strength. However, the effect size was  
355 small in both instances. Sub-group analysis indicated that participants receiving  
356 HMB and supplements containing HMB were significantly stronger compared with  
357 the control group, but with only a small to moderate effect size. These findings may  
358 have important clinical implications given the well-documented detrimental effects of  
359 low muscle mass and skeletal muscle wasting in a number of clinical conditions (6-  
360 10). Of major clinical relevance, is that HMB alone, and supplements containing  
361 HMB, have a strong safety profile without increased incidence of adverse events  
362 compared to placebo or standard care groups used across this range of clinical  
363 conditions.

364 To our knowledge, this is the first meta-analysis investigating the effect of HMB on  
365 strength in a variety of clinical conditions. As it is common for studies to use multiple  
366 measurements for each outcome, we also performed separate meta-analysis for the  
367 outcomes of handgrip strength and leg extensor strength and observed that  
368 evidence in support of this remained strong providing further support for the use of  
369 HMB and supplements containing HMB.

370 Of interest, none of the studies included in the meta-analysis for strength reported a  
371 between group difference in absolute muscle mass following the intervention. This is  
372 perhaps unsurprising given the complex relationship between nutritional status and  
373 muscle mass, muscle quality, muscle strength and physical function that is non-  
374 linear. Indeed, an improvement in muscle quantity may not translate to a proportional  
375 improvement in volitional, or non-volitional, force production (46), and in turn a  
376 commensurate change in performance of physical function, which additionally

377 requires coordination of cognitive and executive function. Furthermore, establishing  
378 a true baseline of physical functional status is challenging in the absence of robust  
379 markers to determine pre-morbid ability. Careful consideration should be given to  
380 selection of these outcomes in such trials (47).

381 Despite including a potentially heterogeneous group of adult patients, the results of  
382 the current study provide some evidence to support the beneficial effect of HMB on  
383 muscle mass and strong evidence to support the effect on strength. This is in line  
384 with previous systematic reviews that have reported the effect of HMB in older  
385 people (48) and athletes (19). By only including studies in adults with a clinical  
386 condition associated with skeletal muscle wasting, the current systematic review and  
387 meta-analysis is unique and of major clinical importance as it highlights the potential  
388 of using HMB, and supplements containing HMB, in the management of a variety of  
389 clinical conditions characterised by skeletal muscle wasting and weakness.

390 Interestingly, the increase in muscle mass reported in this current study was  
391 observed without changes in either body weight or FFM, although this may be more  
392 reflective of methodological differences in the included studies rather than any  
393 mechanistic effect of HMB itself and should be interpreted with caution.

394 Many conditions, such as critical illness and cancer cachexia, result in reduced  
395 muscle mass (4, 5), strength and physical function (2, 3, 6). Whilst the shared  
396 common pathway is an imbalance between muscle protein synthesis and muscle  
397 protein breakdown, the specific intra-cellular proximal signalling pathways leading to  
398 this differ between conditions (5, 49). Older patients also display anabolic resistance  
399 whereby muscle protein synthesis is resistant to stimuli such as resistance training  
400 and amino acid loading (17, 18), a serious clinical consideration with an increasing  
401 ageing population. Moreover, concurrent inflammation (50) and immobilisation, a

402 hallmark of critical illness (1, 51) and other acute clinical conditions, could further  
403 contribute to skeletal muscle wasting. Interventions that target muscle protein  
404 turnover, in terms of reducing muscle protein breakdown and enhancing muscle  
405 protein synthesis as well as reducing inflammation (52), such as HMB, are of  
406 increasing clinical interest.

407 Interventions varied in the studies in this systematic review and included HMB alone,  
408 HMB in combination with the amino acids arginine and glutamine and HMB in ONS  
409 with high protein and vitamin D. However, there was relative consistency in the  
410 doses used across studies, being 3 g/d for all but one study, which used 4 g/d (36),  
411 and any future studies should consider a minimum dose of 3g/d. Regarding the  
412 combined preparations of HMB that have been used (HMB/ARG/GLN, HMB in  
413 ONS), although such an approach may be potentially beneficial, these distract from  
414 our understanding of the mechanism of action of HMB alone on skeletal muscle  
415 anabolism and catabolism as protein and vitamin D impact muscle mass gain and  
416 muscle strength but through differing mechanisms (12, 20, 21, 53).

#### 417 ***Strengths and limitations***

418 This systematic review and meta-analysis has several strengths related to the robust  
419 methodological approach. The protocol was pre-registered on PROSPERO and all  
420 eligibility screening, data extraction and risk of bias assessment was undertaken  
421 independently in duplicate, with a third person available for arbitration if required,  
422 thus limiting the potential for error and bias.

423 The limitations of this systematic review relate to the quality of the design and  
424 reporting of the included studies. First, although we contacted seven authors to  
425 obtain missing data, only one responded despite several follow-up attempts, this

426 combined with inconsistent reporting of data across studies, required some standard  
427 deviations to be imputed (28). No study was considered at low risk of bias across all  
428 domains. Most prominent was the lack of blinding and compliance with the  
429 intervention was a significant issue in several of the studies.

430 The studies included utilised varying measures of body composition analysis  
431 including dual energy X-ray absorptiometry, air displacement plethysmography (Bod  
432 Pod), CT and BIA, with some studies using more than one method. It is possible that  
433 HMB may influence different compartments of FFM and lean mass differently and  
434 therefore these techniques may provide different results depending upon the  
435 compartments measured and the choice of measurement techniques for strength  
436 and physical function may influence results (e.g. handgrip vs. leg-extensor strength).  
437 Thus, techniques to measure both muscle mass and strength should be carefully  
438 considered in future trials of HMB.

439 Although we also included surrogate markers of muscle wasting as an outcome  
440 measure, these results are more difficult to interpret. The rationale for BUN and UUN  
441 as surrogate markers for muscle wasting is controversial and measuring nitrogen  
442 balance in the critically ill population has several limitations (54). Future studies  
443 should investigate muscle protein turnover using stable isotopes to ensure the  
444 reporting of more precise indications of the effect of HMB on muscle wasting.

## 445 **Conclusion**

446 Investigating interventions that reduce skeletal muscle wasting and maintain or  
447 improve muscle mass are a clinical priority. This systematic review and meta-  
448 analysis of RCTs found that HMB alone, and supplements containing HMB, improve  
449 muscle mass and muscle strength in a variety of clinical groups, although the effect

450 size was small to moderate. Furthermore, sub-group analysis revealed strong  
451 evidence to support the use of HMB to increase muscle strength. However, given the  
452 bias associated with many of the included studies, further, high quality RCTs should  
453 be undertaken with greater methodological rigor.

454

#### 455 **Conflicts of interest**

456 DEB reports receiving advisory board fees, speaker fees and conference attendance  
457 support from Nutricia, Nestle Nutrition, BBraun, Baxter healthcare, Fresenius Kabi  
458 and Abbott Nutrition. LW reports conference attendance support from Fresenius  
459 Kabi. AL, ED, SDR, NH, BC and KW report no conflicts of interest.

460

#### 461 **Statement of authorship**

462 DEB, KW, BC, NH, SDRH designed the research; DB, AL, ED, LW, KW conducted  
463 research; DB, ED analyzed data; DB drafted the manuscript; DB had primary  
464 responsibility for final content. All authors interpreted the data and contributed to,  
465 read and approved the final manuscript.

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**Table 1** - Inclusion and exclusion criteria used to evaluate studies for the systematic review

	Inclusion criteria	Exclusion criteria
Study design	Randomized controlled trial	Non-randomized controlled trial
Population	Adult ( $\geq 18$ years) with a primary clinical diagnosis (eg. chronic obstructive pulmonary disease, cancer, malnutrition) In- or outpatient setting	Children, athletes, healthy elderly, animals
Intervention	Minimum 1.5 g/d $\beta$ -hydroxy- $\beta$ -methylbutyrate either alone or in combination with other nutrients of any duration	<1.5 g/d $\beta$ -hydroxy- $\beta$ -methylbutyrate
Comparator	Placebo or usual care	Nil
Outcome measure	Muscle mass (measured by any means), body composition (measured by any means), strength, physical function	Specified outcome measures not investigated (e.g. clinical indices only)

**Table 2** - Study characteristics of included trials.

Study	Patient population	Age (years)	BMI (kg/m <sup>2</sup> )	Intervention	Control	Duration of Intervention	Outcome and outcome relevant to review	Other outcomes
Hsieh (35)	Mechanically ventilated COPD	I: 78.8 (9.7) C: 78.3 (7.4)	I: 21.1 (3.72) C: 18.69 (3.33)	HMB 3 g (2 x 1.5.g doses / d)	Usual care	7 days	Inflammation (CRP) Protein metabolism (BUN) Body composition (Body weight)	Pulmonary function
Hsieh (36)	Tube fed older people	I: 72.5 (11.8) C: 70.8 (9.8)	I: 19.2 (4) C: 21.6 (3.1)	HMB 4 g (2 x 2 g doses / d)	Usual care	14 days plus a subgroup for 28 days	Body composition (thorax, waist, hip, calf circumference, MAMC, TSF) Protein metabolism (BUN)	Nil
Fitschen (39)	Maintenance haemodialysis	I: 57 (8) C: 53 (13)	I: 31.9 (7) C: 30.8 (6.4)	HMB 3 g (3 x 1 g doses / d)	Non-nutritive placebo	6 months	Body composition (DXA, body weight) Muscle strength (knee extension and flexion isokinetic muscle torque) Physical function (shuttle walk, sit-to-stand, up-and-go)	Nil
Clark (26)	HIV	I: 40.9 (1.4) <sup>3,6</sup> C: 40.2 (1.3) <sup>3,6</sup>	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g  (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Maltodextrin	8 weeks	Body composition (body weight, forearm, upper arm and thigh circumference, TSF, Bod Pod, CT thigh)	Blood chemistry (liver function tests, blood lipids, hematologic parameters, HIV viral load, BUN)

May (27)	Cancer Cachexia (stage IV, advanced solid tumours)	I: 66 (2.3) <sup>3,6</sup> C:66 (2.1) <sup>3,6</sup>	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g  (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Iso-nitrogenous control with non-essential amino acids (L-alanine 11 g, L-glutamic acid 1.75 g, L-glycine 6.10 g, L-serine 4.22 g)	24 weeks	Body composition (body weight, BIA, Bod Pod)	Quality of life (SF-36) Dietary Intake
Marcora (40)	Rheumatoid arthritis	I: 54 (10) C: 57 (8)	I: 25.2 (4.1) C: 27.2 (4.8)	HMB 3 g L-arginine 14 g L-glutamine 14 g  (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Iso-nitrogenous control with non-essential amino acids (L-alanine 11 g, L-glutamic acid 1.75 g, L-glycine 6.10 g, L-serine 4.22 g)	12 weeks	Body composition (DXA, BIA) Physical function (sit-to-stand, modified HAQ, advanced ADLs scale, habitual physical activity)	Dietary intake Disease activity index
Kuhls (25)	Mechanically ventilated trauma	I: 36 (3.2) <sup>6</sup> ; 41 (3.2) <sup>6</sup> C: 37 (3.3) <sup>6</sup>	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g  (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)  and  3 g HMB (2 x 1.5 g doses / d) <sup>1</sup>	Iso-nitrogenous control with non-essential amino acids (30.6 g of hydrolyzed gelatin, 7.8 g L-alanine, 4.2 g L-glycine, 3.0 g L-serine, and 1.2 g L-glutamic acid)	14 days	Protein metabolism (nitrogen balance, 3-MH excretion) Inflammation (SIRS score)	Pre-albumin
Berk (41)	Cancer Cachexia	I: 67 (23,91) <sup>4</sup> C: 65 (35,90) <sup>4</sup>	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g  (2 x HMB 1.5 g, L-arginine 7 g, L-	Iso-nitrogenous control with non-essential amino acids (30.52 g gelatin, L-alanine 7.72 g,	8 weeks	Body composition (body weight, BIA, upper arm, forearm, chest, hip and thigh circumference, 7 site)	Fatigue (Schwartz fatigue score) Quality of life (Spritzer quality of life index)

				glutamine 7 g doses / d)	L-glutamic acid 1.23 g, L-glycine 4.28 g, L-serine 2.96 g)		skinfold thickness, Bod Pod)	
Clements (45)	Gastric bypass	I: 47.9 (9.6) C: 46 (7.5)	I: 42.9 (4.1) C: 43.6 (4.2)	HMB 3 g L-arginine 14 g L-glutamine 14 g  (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Usual care	8 weeks	Body composition (body weight, DXA) Resting metabolic rate	Nil
Olveira (37)	Bronchiectasis	I: 58.4 (12.9) C: 53.7 (13.1)	I: 25.9 (3.4) C: 27.3 (5.8)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D  (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	Usual care	24 weeks	Body composition (body weight, BIA phase-angle; DXA, MAMC) Strength (handgrip dynamometry)	Quality of life Dietary intake Myostatin, Somatomedin C, Insulin
Ekinci (42)	Older females with hip fracture	I: 82.19 (7.28) C: 83.07 (7.08)	I: 21.83 (2.11) C: 22.25 (2.7)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D  (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	Usual care	30 days	Body composition (body weight, calf and arm circumference, TSF, MAMC) Muscle strength (handgrip dynamometry)	Immobilisation period Wound healing CRP
Deutz (43)	Hospitalised, older people with malnutrition <sup>2</sup>	I: 77.7 (8.2) C: 78.1 (8.6)	I: 24.3 (5.2) C: 23.9 (5)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D	Placebo containing 48 kcal, 12 g CHO, 10mg Vitamin C	90 days	Post-discharge incidence of death or non-elective readmission Length of stay	SGA class Vitamin D level ADLs

				(ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)			Body composition (Body weight)	
Cramer (31)	Older people with malnutrition and sarcopenia	I: 77 (71,81) <sup>5</sup> C: 77 (71,81) <sup>5</sup>	I: 25 (23,29) <sup>5</sup> C: 26 (24,29) <sup>5</sup>	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D  (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	ONS containing 330 kcal, 14 g pro, 147 IU Vitamin D <sub>3</sub>	24 weeks	Body composition (body weight, DXA) Strength (grip strength, leg strength) Physical function (gait speed)	Nil
Nishizaki (38)	Older people following knee arthroplasty	I: 71.1 (NR) C: 69.8 (NR)	NR	HMB 3 g L-arginine 14 g L-glutamine 14 g  (2 x HMB 1.5 g, L-arginine 7 g, L-glutamine 7 g doses / d)	Orange juice containing 113 kcal and 140 mg pro	5 days before and 28 days after surgery	Body composition (body weight, CT of RFcsa) Strength (knee extensor)	Nil
Malafarina (44)	Older people with hip fracture	I: 85.7 (6.5) C: 84.7 (6.3)	I: 24.9 (4.4) C: 26 (5.4)	HMB 3 g in ONS with 660 kcal, 36 g protein, 2000 IU Vitamin D  (ONS with 1.5 g HMB, 330 kcal, 18 g protein, 1000 IU Vitamin D twice daily)	Usual care	Duration of rehabilitation unit stay	Body composition (body weight, BIA) Strength (hand grip dynamometry) Physical function (gait speed)	Plasma Vitamin D

<sup>1</sup>30.6 g of hydrolyzed gelatin, 7.8 g L-alanine, 4.2 g L-glycine, 3.0 g L-serine, and 1.2 g L-glutamic acid added to HMB alone supplement to make isonitrogenous.

<sup>2</sup>Patients with congestive heart failure, acute myocardial infarction, pneumonia or chronic obstructive pulmonary disease

<sup>3</sup>Completed patients; <sup>4</sup>Median (range); <sup>5</sup>Median (interquartile range); <sup>6</sup>Mean (SE)

ADLs = activities of daily living; BF = breakfast; BIA = bioelectrical impedance analysis; BMI = body mass index; BUN = blood urea nitrogen; C = control; CHO = carbohydrate; CRP = C-Reactive Protein; CT = computed tomography; D = dinner; HAQ = health assessment questionnaire; HIV =

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Human Immunodeficiency Virus; I = intervention; IU = international units; L = lunch; MAMC = mid-arm muscle circumference; NR = not reported; ONS = oral nutrition supplement; Pro = protein; RFcsa = rectus femoris cross sectional area; SGA = subjective global assessment; SF-36 = short-form 36; TSF = triceps skin fold; 3-MH = 3 methyl-histidine

## Figure titles and legends

**Figure 1:** Flow diagram of study selection process

**Figure 2:** Risk of bias summary for all studies and outcomes

**Figure 3:** Forest plot for the effect of HMB or supplements containing HMB on change in muscle mass

Forest plot of a random effects meta-analysis of nine studies for change in muscle mass. Results are presented as standardized mean difference with 95% confidence intervals. Sub-group analysis are included for studies including HMB alone, HMB in combination with glutamine and arginine (HMB/ARG/GLN) and HMB incorporated in an oral nutrition supplement (HMB in ONS).

**Figure 4:** Forest plot showing the effect of HMB or supplements containing HMB on change in body weight.

Forest plot of a random effects meta-analysis of twelve studies for change in body weight. Results are presented as standardized mean difference with 95% confidence intervals. Sub-group analysis are included for studies including HMB alone, HMB in combination with glutamine and arginine (HMB/ARG/GLN) and HMB incorporated in an oral nutrition supplement (HMB in ONS).

**Figure 5:** Forest plot showing the effect of HMB or supplements containing HMB on absolute strength.

Forest plot of a random effects meta-analysis of six studies for absolute strength. Results are presented as standardized mean difference with 95% confidence intervals. Sub-group analysis are included for studies including HMB alone, HMB in combination

with glutamine and arginine (HMB/ARG/GLN) and HMB incorporated in an oral nutrition supplement (HMB in ONS).

Figure 1 -

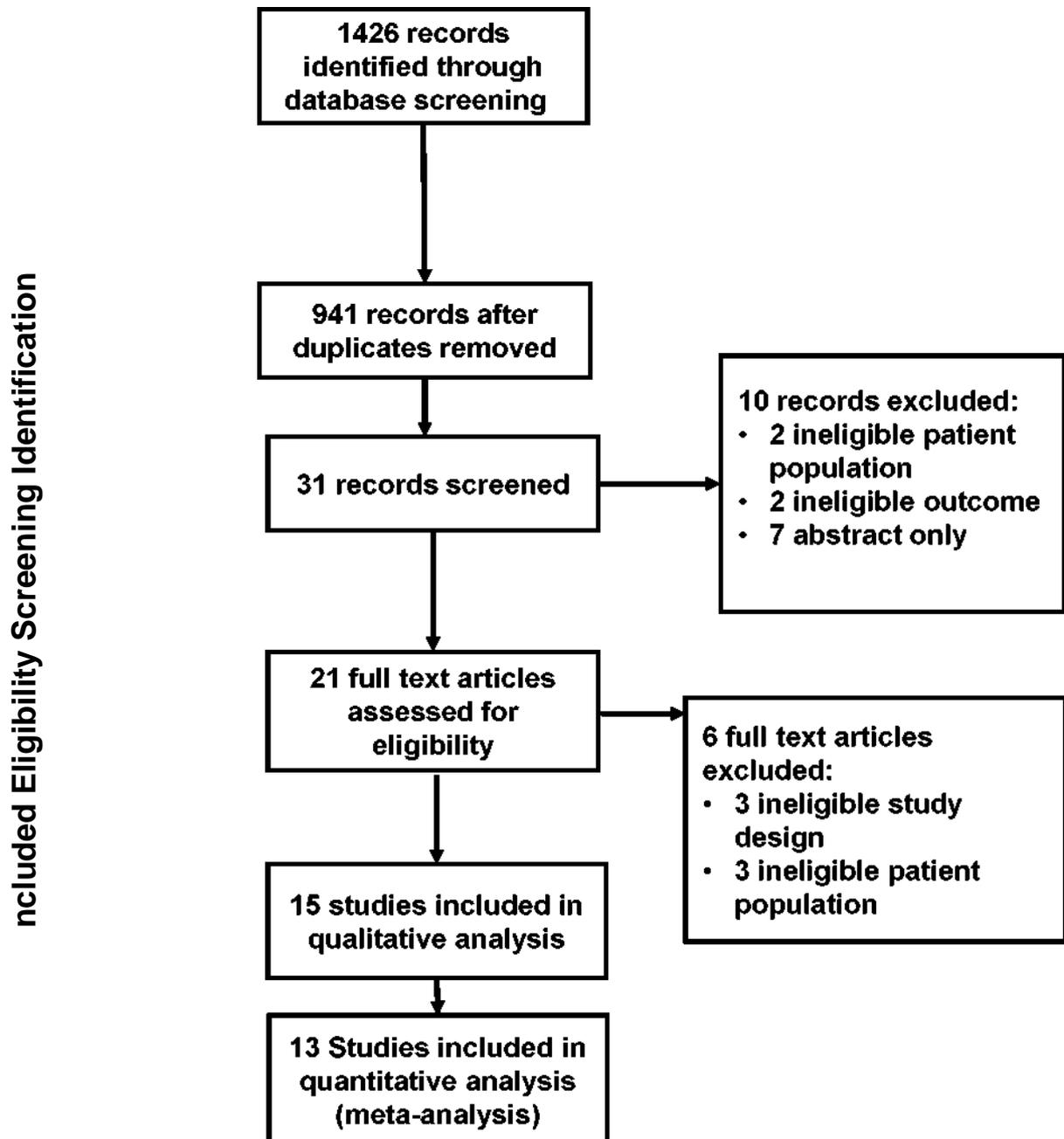
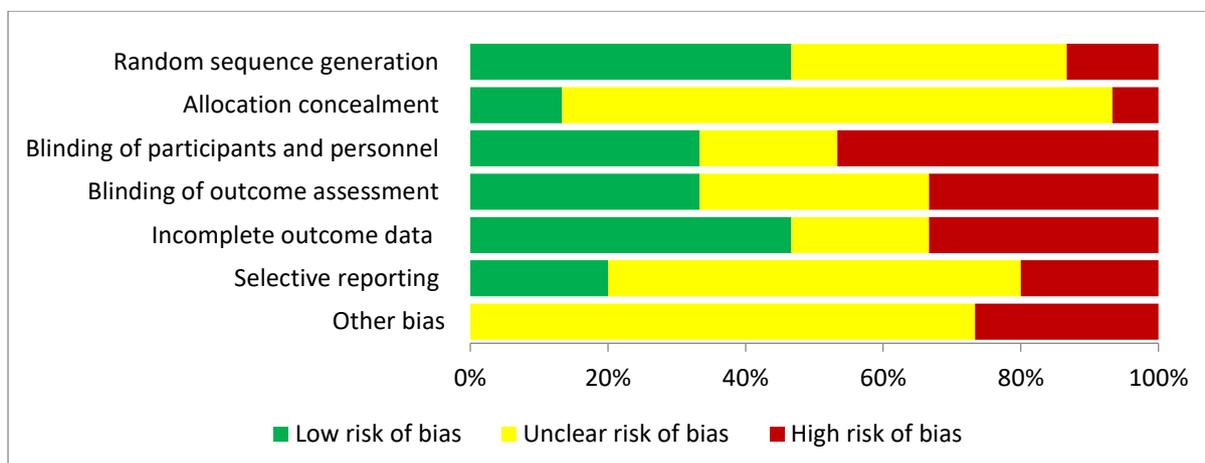


Figure 2 -

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Hsieh 2006	-	-	-	?	?	?	?
Hsieh 2010	?	?	-	?	?	?	?
Fitschen 2016	?	?	?	?	-	+	?
Clark 2000	+	?	?	?	+	?	?
May 2002	+	?	+	+	-	?	?
Marcora 2005	+	+	+	+	+	?	-
Kuhls 2007	+	?	+	+	-	+	?
Berk 2008	?	?	?	?	-	?	-
Clements 2011	?	?	-	-	+	?	?
Olivera 2015	+	?	-	-	+	-	?
Ekinci 2016	-	?	-	-	+	?	-
Deutz 2017	+	+	+	+	+	-	-
Cramer 2016	?	?	+	+	-	+	?
Malafarina 2017	+	?	-	-	?	-	?
Nishizaki 2015	?	?	-	-	+	?	?



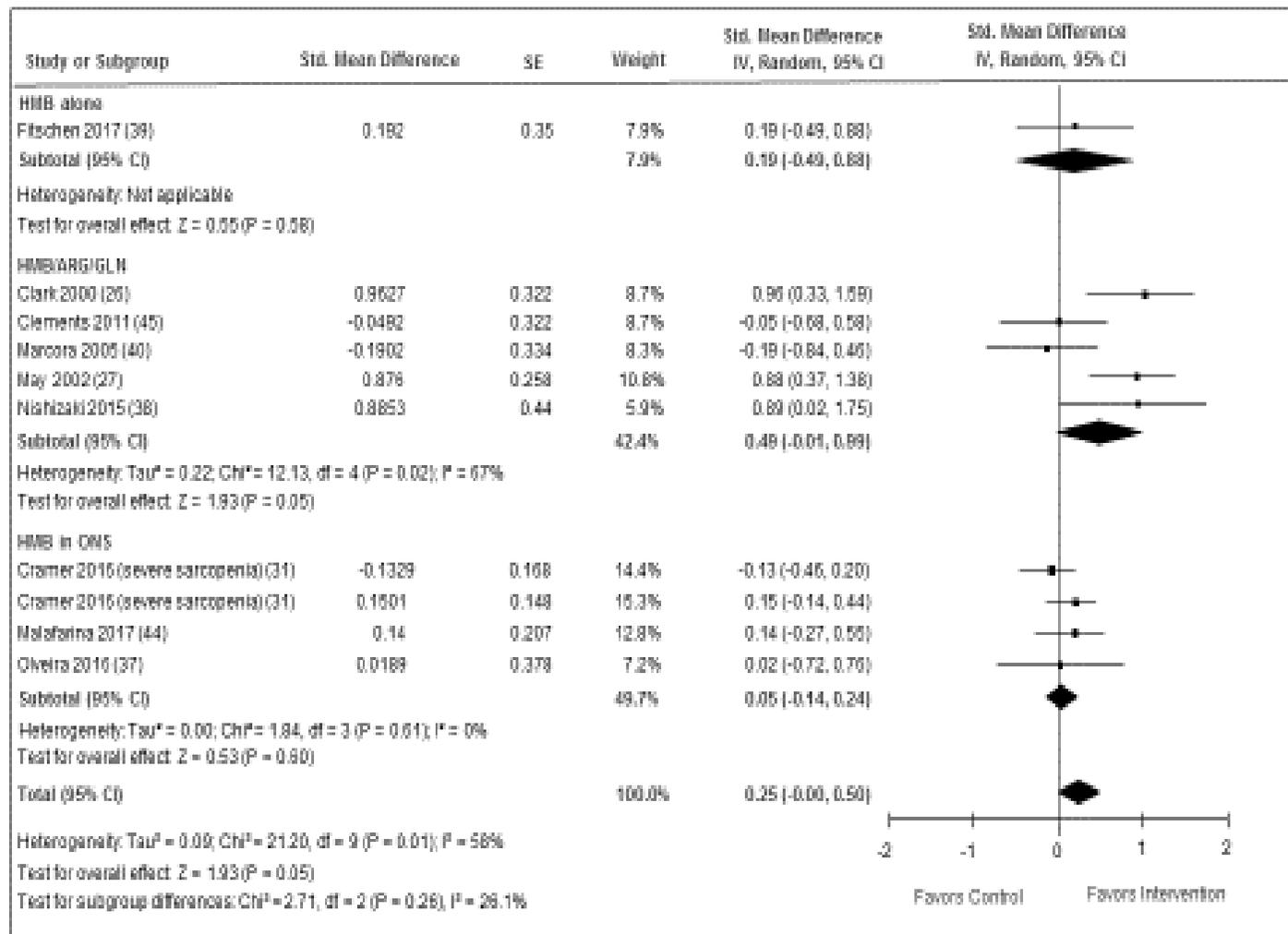


Figure 3 -



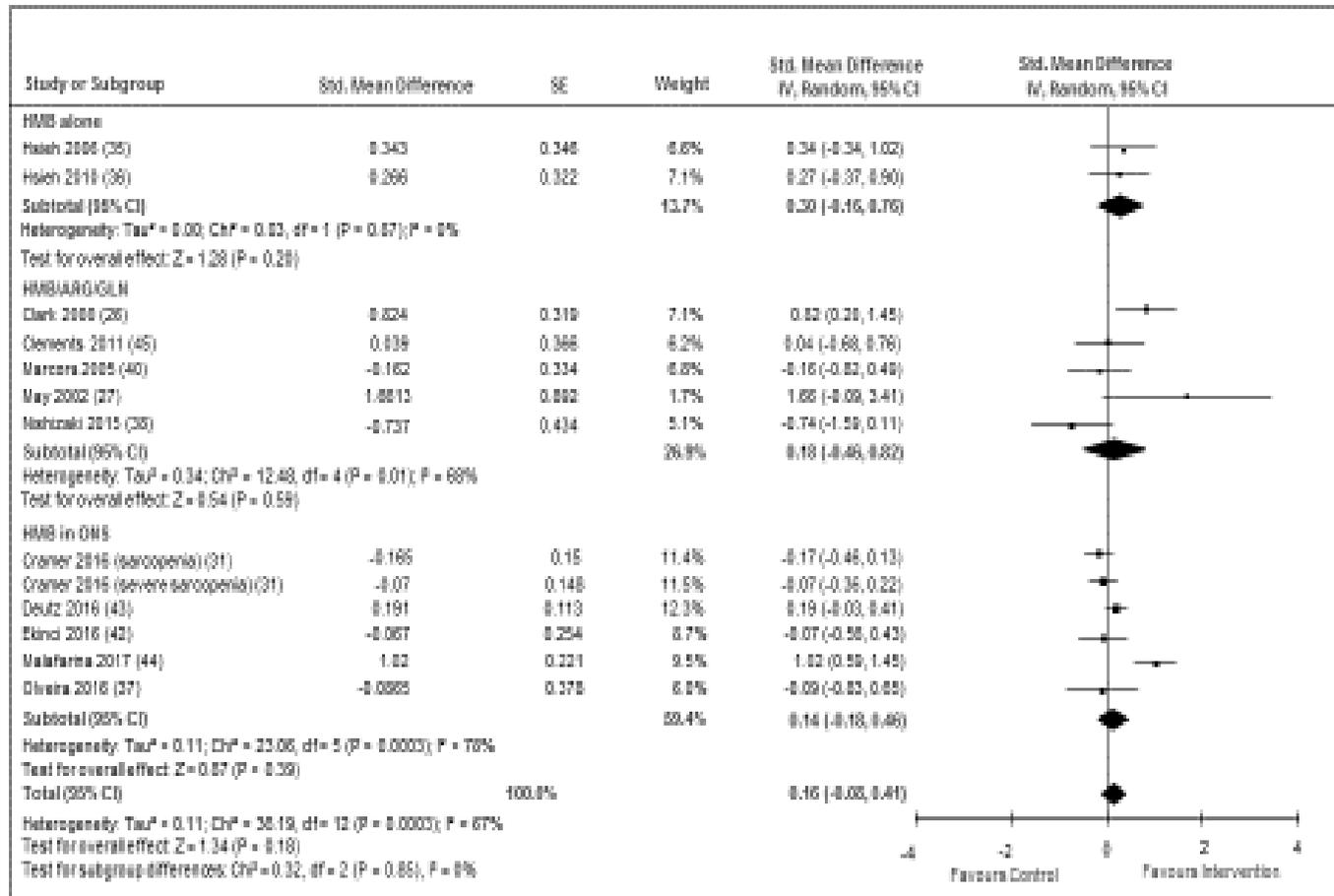


Figure 4 -



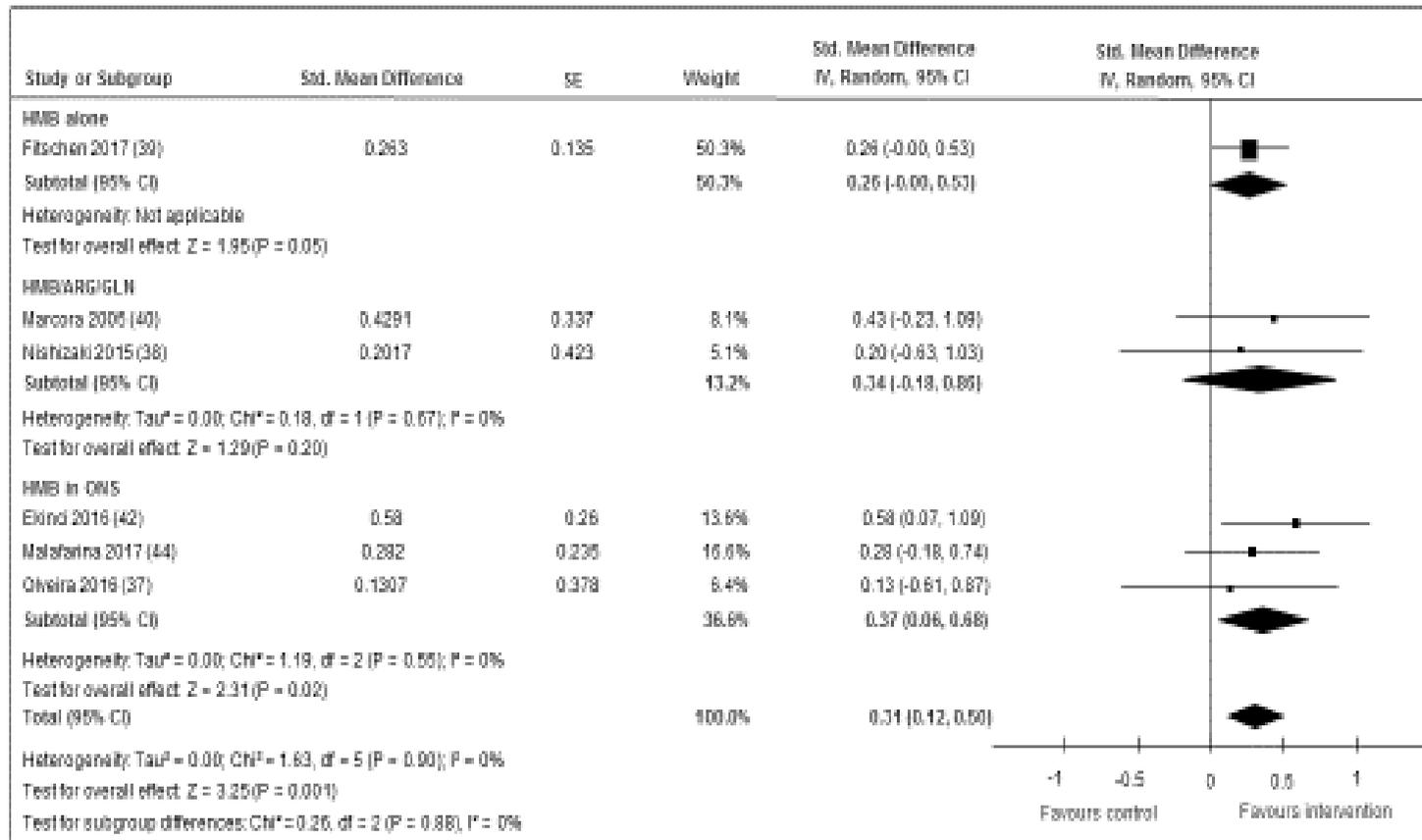


Figure 5 -

## Supplemental Methods

### Example search strategy

Database: Epub Ahead of Print, In-Process & Other Non-Indexed Citations, Ovid MEDLINE(R)

Daily and Ovid MEDLINE(R) <1946 to Present>

Search Strategy:

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1 HMB.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (2626)

2 beta-hydroxy-beta-methylbutyrate.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (262)

3 'beta hydroxy beta methylbutyrate'.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (262)

4 1 or 2 or 3 (2674)

5 muscle wasting.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (4399)

6 muscle loss.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (1448)

7 muscle mass.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (14504)

8 skeletal muscle.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (107133)

9 sarcopenia.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (5143)

10 cache\*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (11284)

11 physical fitness.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (31143)

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16 grip strength.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (9374)

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19 lean body mass.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (7378)

20 main\*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier] (47521)

21 exp Muscle, Skeletal/ or exp Cachexia/ or exp Muscular Atrophy/ or exp Muscles/ (715992)

22 exp Body Composition/ or exp Sarcopenia/ (53752)

23 exp Muscle Strength/ or exp Body Weight/ (475694)

24 exp Hand Strength/ or exp Muscle Strength Dynamometer/ (14388)

25 exp Physical Fitness/ (27735)

26 exp "Activities of Daily Living"/ (66805)

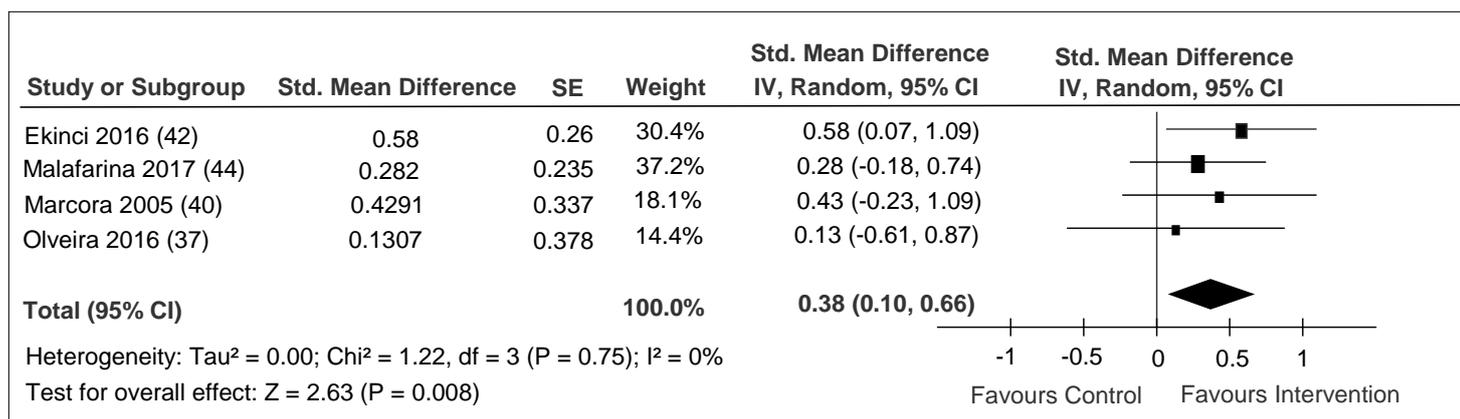
27 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 (1576685)

28 4 and 27 (311)

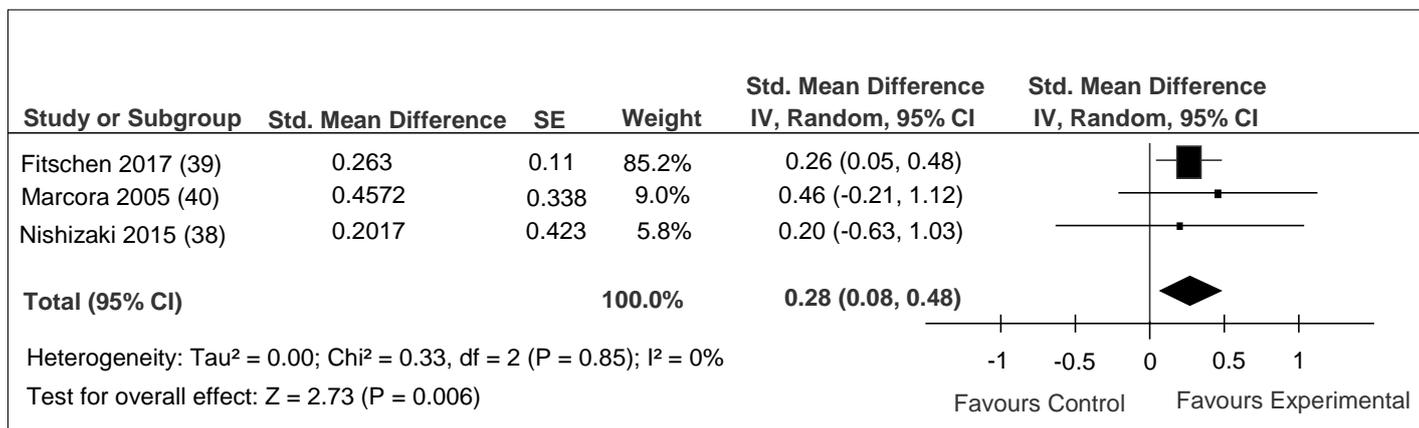
29 exp animals/ not humans.sh. (4860425)

30 28 not 29 (236)

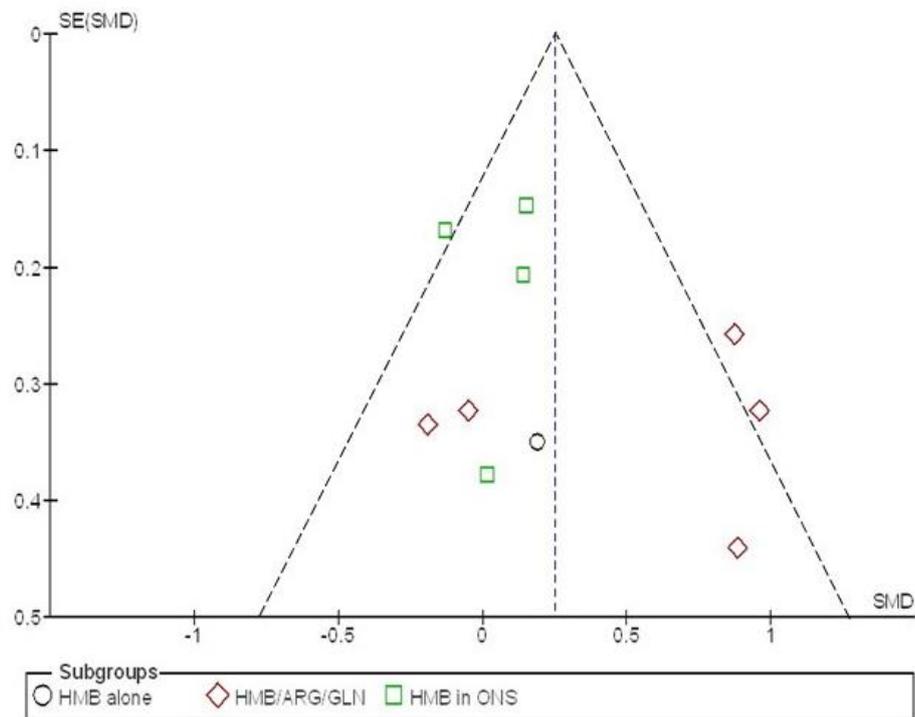
### Supplemental Results



**Supplemental Figure 1:** Forest plot of a random effects meta-analysis of four studies for handgrip strength. Results are presented as standardized mean difference with 95% confidence intervals.

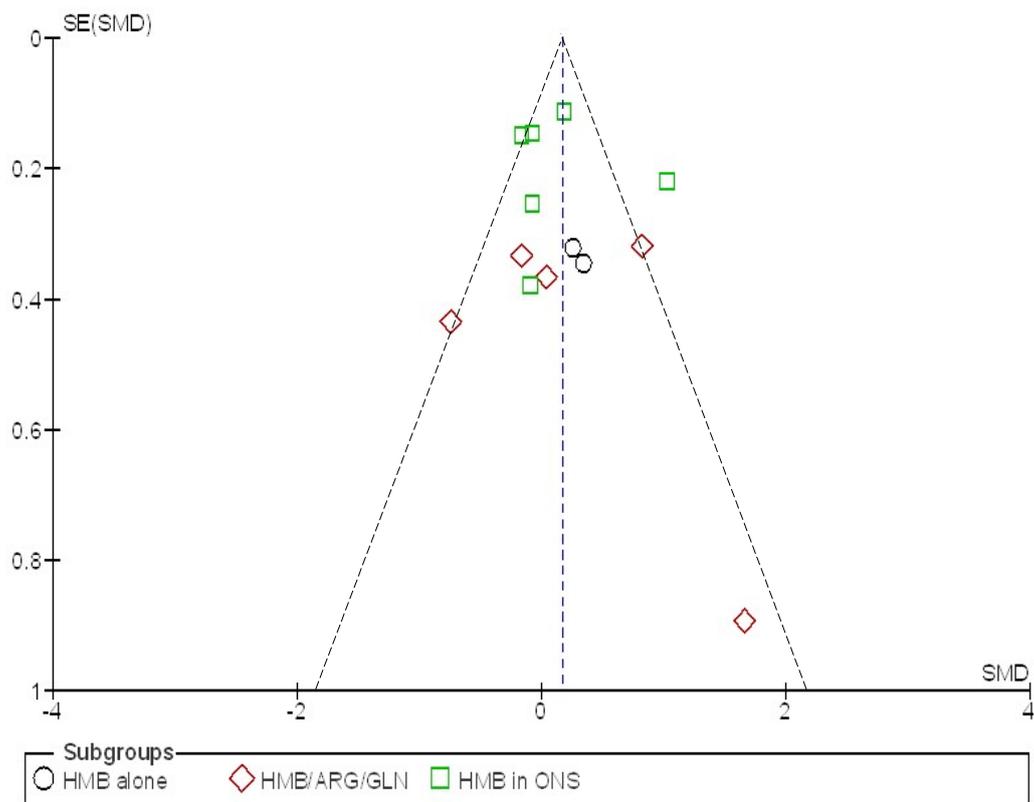


**Supplemental Figure 2:** Forest plot of a random effects model of three studies for leg strength. Results are presented as standardized mean difference with 95% confidence intervals.



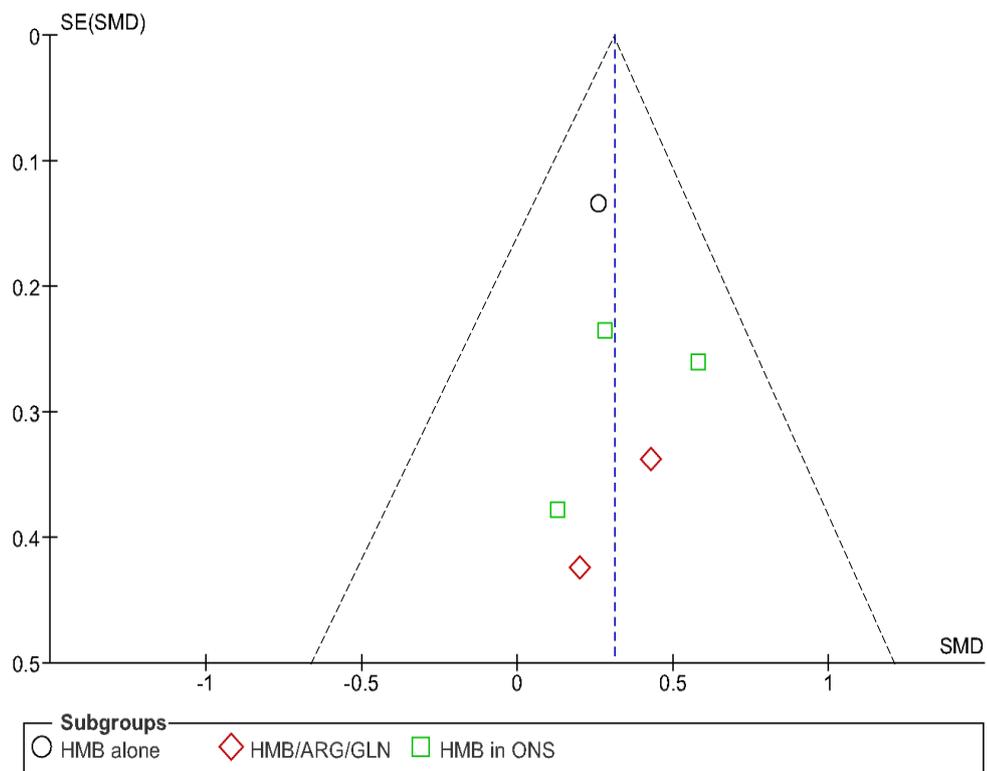
**Supplemental Figure 3** – Funnel plot of change in muscle mass.

Funnel plot describing the degree of publication bias. The standardized mean difference (SMD) of change in muscle mass is plotted on the x axis and the standard error (SE) of the SMD is plotted on the y axis. The vertical dotted line indicated the mean value of the standardized mean differences reported by the 9 included trials. Visual inspection of the funnel plot does not reveal any substantial asymmetry and hence publication bias.



**Supplemental Figure 4 – Funnel plot of change in body weight.**

Funnel plot describing the degree of publication bias. The standardized mean difference (SMD) of change in body weight is plotted on the x axis and the standard error (SE) of the SMD is plotted on the y axis. The vertical dotted line indicated the mean value of the standardized mean differences reported by the 12 included trials.



**Supplemental Figure 5 – Funnel plot of change in absolute strength.**

Funnel plot describing the degree of publication bias. The standardized mean difference (SMD) of absolute strength is plotted on the x axis and the standard error (SE) of the SMD is plotted on the y axis. The vertical dotted line indicated the mean value of the standardized mean differences reported by the six included trials.