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Title:

Serum branched-chain amino acids, dietary factors, and sarcopenia risk in older adults with and without diabetes mellitus

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1 Abstract

2 **Background and Aims:** Branched-chain amino acids (BCAAs), primarily obtained from the
3 diet, can promote muscle protein synthesis, but excessive levels may lead to insulin resistance.

4 How circulating BCAA is associated with diet and sarcopenia risk in older adults with diabetes
5 mellitus (DM) remains unclear. This study aimed to examine the association of dietary factors
6 and serum BCAA levels, and their association with sarcopenia risk, stratified by diabetes status.

7 **Methods:** This cohort study included 2,994 community-dwelling older adults between 2001
8 and 2003. Serum BCAA levels (leucine, isoleucine and valine) were measured at baseline.
9 Dietary data were collected by a food frequency questionnaire. Sarcopenia was defined as low
10 muscle mass accompanied by low muscle strength and/or low physical performance at baseline
11 and 4-year follow-up. Associations of serum BCAA with dietary factors and sarcopenia risk
12 were examined using generalized linear and logistic regression models with restricted cubic
13 splines.

14 **Results:** There were 433 participants with DM and 2,561 without DM. In non-DM, dietary
15 protein, protein sources, and overall diet quality were significantly associated with serum
16 BCAA levels; in DM, only red meat and whole-grain intakes were associated. These
17 associations were attenuated after adjusting for sociodemographic, lifestyle and health-related
18 factors. Elevated serum BCAA levels were associated with lower sarcopenia risk in non-DM
19 (odd ratio [OR]: 0.72, 95% confidence interval [CI]: 0.64–0.81, per standard deviation [SD] of
20 log-BCAA). However, a U-shaped association was observed in DM (p -nonlinearity = 0.0006),
21 with the lowest risk at intermediate levels. Over 4 years of follow-up, higher BCAA levels were

22 association with reduced sarcopenia incidence in non-DM (OR: 0.79, 95% CI: 0.66–0.94), but
23 no association in DM. Similar patterns were found for individual BCAA.

24 **Conclusions:** Higher BCAA levels may reduce sarcopenia risk in older adults without DM, but
25 excessive BCAA levels may not offer muscle benefits in DM. Metabolic status should be
26 considered when evaluating BCAA in sarcopenia prevention strategies.

27 **Keywords:** Branched-chain amino acids; diabetes mellitus; sarcopenia; muscle health; older
28 adults

30 **Abbreviation**

31 AWGS, the Asian Working Group for Sarcopenia; ASMI, appendicular skeletal muscle mass
32 index; BCAA, branched-chain amino acids; BMI, body mass index; CI, confidence interval;
33 DM, diabetes mellitus; DQI-I, Diet Quality Index-International; DXA, dual-energy X-ray
34 absorptiometry; EAA, essential amino acids; eGFR, estimated glomerular filtration rate; FFQ,
35 food frequency questionnaire; IQR, interquartile range; LC-MS/MS, liquid chromatography-
36 tandem mass spectrometry; MPS, muscle protein synthesis; MPB, muscle protein breakdown;
37 mTOR, the mechanistic target of rapamycin; OR, odds ratio; PASE, Physical Activity Scale of
38 the Elderly; SD, standard deviation; T2DM, type 2 diabetes mellitus.

40 **1. Introduction**

41 Sarcopenia is characterized by progressive declines in muscle mass, strength, and physical
42 performance and is a common age-related condition affecting approximately 10–27% of older

43 adults [1]. Sarcopenia may lead to adverse health outcomes such as reduced quality of life,
44 hospitalization, disability, and premature death [2]. Maintaining skeletal muscle health relies
45 on amino acids, the basic constituents of muscle proteins. Of particular importance are essential
46 amino acids (EAAs). EAAs must be acquired through the diet, as the body cannot produce them
47 in adequate amounts. Among the nine EAAs, branched-chain amino acids (BCAAs), including
48 leucine, isoleucine and valine, play specific roles in regulating intracellular signaling pathways
49 involved in muscle growth and degradation [3, 4]. Meta-analyses of randomized controlled
50 trials suggest that BCAA supplementation, especially with leucine, can enhance muscle mass
51 and strength in older adults, especially those with sarcopenia [4, 5]. However, elevated BCAA
52 levels have been associated with insulin resistance and type 2 diabetes mellitus (T2DM), raising
53 questions about their potential contradictory role in muscle and metabolic health [6-8].

54
55 Insulin is a key regulator of amino acid metabolism. It can modulate affect the rate of
56 appearance and clearance of BCAA in circulation and the activity of catabolic enzymes [9].
57 Insulin resistance, a hallmark of T2DM, may impair this regulatory function. In individuals
58 with T2DM, the expression of the rate-limiting enzymes involved in BCAA catabolism is
59 reduced, leading to the accumulation of circulating BCAA [10]. Recent evidence suggests that
60 impaired BCAA catabolism may be the underlying mechanism of sarcopenia [11]. Therefore,
61 diabetes may influence how dietary intake affects circulating BCAA levels and how BCAA
62 relates to muscle outcomes. Despite this, few studies have investigated whether the associations
63 among dietary factors, serum BCAA levels, and sarcopenia risk differ between individuals with

64 and without diabetes. To our knowledge, only one prospective cohort study conducted in
65 Singapore involving 1,140 patients with T2DM investigated the association between circulating
66 BCAA levels and skeletal muscle mass [12]. The association between higher BCAA levels and
67 reduced odds of muscle mass loss was observed in patients younger than 60 years, but not in
68 those aged 60 and above [12]. However, this study did not include muscle function, which is
69 essential for identifying sarcopenia, nor did it stratify results by diabetes status to investigate
70 potential interaction between diabetes and BCAA-related muscle outcomes. Furthermore,
71 findings from randomized controlled trials on the effects of BCAA-related supplementation on
72 muscle health in older adults with T2DM remain inconsistent, with studies reporting either null
73 or modest benefits [13-16]. These gaps highlight the need for further studies to investigate the
74 role of BCAA in age-related sarcopenia within the context of diabetes.

75
76 Therefore, this study aimed to investigate the associations between dietary factors and serum
77 BCAA levels in Chinese older adults, stratified by diabetes status. Moreover, it aimed to
78 examine the association between serum BCAA levels and sarcopenia risk in older adults with
79 and without diabetes.

81 **2. Methods**

82 **2.1 Study participants**

83 Data were sourced from the Mr. OS and Ms. OS (Hong Kong) prospective cohort study,
84 designed to investigate musculoskeletal health in older adults [17]. Briefly, a total of 4,000

85 Chinese men and women were recruited from the local communities between August 2001 to
86 March 2003. Using a stratified sampling approach, participants were evenly distributed across
87 three age groups: 65 to 69, 70 to 74, and above 75 years old. The current study excluded
88 participants who did not have serum BCAA data (n=1,003) or who did not have dietary data
89 (n=3). Finally, 433 participants with DM and 2,561 without DM were included for the cross-
90 sectional analysis of the association of serum BCAA with dietary factors and sarcopenia risk.
91 After excluding those with baseline sarcopenia and those without follow-up data of sarcopenia,
92 358 participants with DM and 2,022 without DM were included for the prospective analyses of
93 incident sarcopenia over four years. The flowchart of study participants was shown in
94 Supplementary Figure S1. Ethical approval for this study was obtained from the Clinical
95 Research Ethics Committee of The Chinese University of Hong Kong. All participants were
96 informed of the study details and provided written consent before participation.

98 **2.2 Serum BCAA measurement**

99 Fasting blood samples were collected following an overnight fast at baseline. Serum was
100 separated by centrifugation and stored at -80°C until analysis. Serum concentrations of leucine,
101 isoleucine, and valine were measured by liquid chromatography-tandem mass spectrometry
102 (LC-MS/MS) using a Shimadzu LC-20ADXR Prominence LC system (Kyoto, Japan) coupled
103 to a Sciex QTRAP5500 mass spectrometer with a Turbo V ion source and TurbolonSpray probe
104 (Framingham, MA, USA) as described previously [18]. Total BCAA concentration was
105 calculated as the sum of the circulating concentrations of individual BCAA (leucine, isoleucine,

106 and valine).

107

108 **2.3 Ascertainment of diabetes mellitus**

109 Diabetes mellitus (DM) was identified according to self-reported physician diagnosis and/or
110 the reported use of antidiabetic medications. Diabetes mellitus was diagnosed by physicians in
111 accordance with the diagnostic criteria established by the World Health Organization and
112 International Diabetes Federation. Use of antidiabetic medications among participants was
113 documented via clinical charts and electronic health records retrieved from the Hospital
114 Authority of Hong Kong.

115

116 **2.4 Dietary data**

117 Dietary intake was evaluated at baseline using a locally validated food frequency questionnaire
118 (FFQ) with 280 food items [19]. Participants were instructed to recall and report how often they
119 consumed various food items and the serving or portion sizes over the past 12 months. The
120 nutrient values of each food item were obtained from the Chinese Medical Sciences Institute
121 [20] and McCance and Widdowson [21]. Nutrient intake was estimated by multiplying the
122 reported consumption of each food item by its corresponding nutrient content, based on
123 standard portion sizes. To account for variations in energy intake, values were adjusted using
124 the residual method. Animal-based proteins included proteins from red and processed meats,
125 poultry, freshwater fish, seafood, eggs, dairy products, and other animal-derived foods. Plant-
126 based protein sources comprised whole and refined grains, vegetables, fruits, soy and soy

127 products, legumes, nuts, and additional plant-derived foods. Dietary quality was estimated by
128 Diet Quality Index-International (DQI-I), a comprehensive and validated tool to assess dietary
129 adequacy, moderation, variety, and balance [22].

130

131 **2.5 Muscle measurements and definition of sarcopenia**

132 Muscle measurements, including grip strength, gait speed, five-time chair stand test and muscle
133 mass, were conducted at baseline and the year four follow-up. Grip strength was assessed using
134 a dynamometer (JAMAR Hand Dynamometer 5030JO; Sammons Preston, Bolingbrook, IL,
135 USA). Two measurements were obtained from each hand, with the maximum value from each
136 side used in the final analysis. Gait speed was assessed by recording the time required for
137 participants to walk a 6-meter straight path at their usual walking pace. For the five-time chair
138 stand test, the time taken to complete the task was recorded. For both the gait speed and chair
139 stand tests, two measurements were obtained, and the better result from each was selected for
140 inclusion in the data analysis. Body composition analysis was performed using dual-energy X-
141 ray absorptiometry (DXA). The Hologic QDR 4500 W device (Waltham, MA, USA) was
142 utilized for the automatic analysis of fat mass, lean mass, and their respective percentages.
143 Appendicular skeletal muscle mass index (ASMI) was calculated as the sum of the muscle mass
144 in both arms and legs and then was divided by the square of height (kg/m^2).

145

146 Sarcopenia was identified at baseline and the year four follow-up according to the Asian
147 Working Group for Sarcopenia (AWGS) 2019 [1]. Sarcopenia was defined by low muscle mass

148 (ASMI $<7.0 \text{ kg/m}^2$ for men; $<5.4 \text{ kg/m}^2$ for women) accompanied by low muscle strength
149 (handgrip strength $<28 \text{ kg}$ for men; $<18 \text{ kg}$ for women) and/or low physical performance. Low
150 physical performance was determined as gait speed below 1.0 m/s or a time to complete the
151 five-time chair stand test of 12 seconds or longer for both men and women.

152

153 **2.6 Covariate assessment**

154 Demographic characteristics, lifestyle factors, medical history of chronic diseases, and
155 medication use were collected using standardized questionnaires at baseline. Physical activity
156 was estimated using the Physical Activity Scale of the Elderly (PASE) [23]. Chronic conditions
157 included cardiovascular diseases, hypertension, chronic obstructive pulmonary disease, cancer,
158 osteoporosis, arthritis, Parkinson's disease, glaucoma, and cataracts. The total number of self-
159 reported chronic diseases was categorized into three groups: no chronic diseases, one to two
160 chronic diseases, and three or more chronic diseases. Body weight was measured by the
161 Physician Beam Balance Scale with an accuracy of 0.1 kg . Height was measured by a
162 stadiometer with an accuracy of 0.1 cm . Body mass index (BMI) was calculated dividing body
163 weight (in kilograms) by the square of height (in meters), as kg/m^2 . Serum creatinine was
164 quantified using liquid chromatography-tandem mass spectrometry, as previously described.
165 The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney
166 Disease Epidemiology Collaboration (CKD-EPI) creatinine equation, incorporating serum
167 creatinine, age, and sex [24].

168

169 **2.7 Statistical analysis**

170 Baseline characteristics were compared between participants with DM and those without DM.
171 Normally distributed variables were analyzed using Student's *t*-test, nonparametric variables
172 using the Mann-Whitney *U* test, and categorical variables using Chi-square test. Descriptive
173 statistics were presented as mean with standard deviation (SD) or median with interquartile
174 range (IQR) for continuous variables, and percentages (%) for categorical variables. Serum
175 BCAA levels were log-transformed to obtain approximately normal distributions. Spearman
176 correlation coefficients between dietary factors and serum BCAA concentrations according to
177 the presence and absence of DM were estimated. Generalized linear models were used to further
178 examine these associations after adjusting for demographic and lifestyle factors. Dietary factors
179 were independent variables and serum BCAA concentrations were dependent variables.
180 Multiple covariates were included in the analysis, such as age, sex, educational attainment,
181 smoking status, current alcohol consumption, physical activity level, number of chronic
182 diseases, body mass index, and total energy intake. Multiple comparisons were corrected using
183 the Benjamini–Hochberg method to control the false discovery rate. Interactions between
184 dietary factors and diabetes status in relation to BCAA were assessed by including product
185 terms.

186
187 Associations between serum BCAA levels and the risk of sarcopenia in DM and non-DM were
188 analyzed using logistic regression models, based on both cross-sectional data at baseline and
189 incident sarcopenia identified during the 4-year follow-up. Serum BCAA levels were analyzed

190 both continuous variables (per SD of log-transformed serum BCAA levels) and categorically
191 based on tertiles. The tertile cut-offs were defined separately based on the distribution within
192 the DM and non-DM groups. This method was employed to account for baseline differences in
193 BCAA concentrations between the two groups and to evaluate the association of relative BCAA
194 levels with outcomes. The results were presented as odds ratios (OR) and their corresponding
195 95% confidence intervals (CI). Non-linear association between serum BCAA levels and
196 sarcopenia risk was estimated by restricted cubic spline (RCS) model with knots placed at the
197 10th, 50th, and 90th centiles of log-transformed serum BCAA. The median of serum BCAA was
198 used as the reference. Interactions between BCAA and diabetes status in relation to sarcopenia
199 risk were assessed by including product terms. To reduce residual confounding of medication
200 use and renal function, sensitivity analyses were also conducted by further adjusting for the use
201 of antidiabetic, lipid-lowering and antihypertensive drugs, and eGFR based on the
202 multivariable-adjusted Cox model. To examine whether the associations between BCAA levels
203 and sarcopenia risk differed by age, we tested for interactions by entering a multiplicative term
204 between BCAA levels and age (treated as a categorical variable based on the sampling strata:
205 65–69, 70–74, and ≥ 75 years) into the regression models. Stratified analyses were performed
206 to visualize potential differences across these age groups. Besides, associations between serum
207 BCAA levels with muscle measurements (ASMI, grip strength, gait speed and chair stand test)
208 in DM and non-DM were analyzed using multivariable-adjusted linear regression and RCS
209 models.

210

211 Statistical analyses were performed by STATA (StataCorp. 2021. Stata Statistical Software:
212 Release 17. College Station, TX: StataCorp LLC). The "corrplot" package in R (version 4.3.1,
213 R Development Core Team, Vienna, Austria) was used to generate heat map. Significance was
214 determined using a threshold of $p < 0.05$ (two-sided).

215

216 **3. Results**

217 **3.1 Participant characteristics**

218 Among 2,994 participants included in this study, the mean age was 71.9 (SD 4.9) years, and
219 women comprised 52.4% of the study population. The mean BMI was 23.7 (SD 3.2) kg/m².

220 Baseline characteristics between participants with and without DM are presented in Table 1.

221 Participants with DM had higher BCAA concentrations than those without DM (all $p < 0.001$).

222 Serum concentrations of BCAA, leucine, isoleucine, and valine in DM were 558.6 (IQR: 501.3–

223 616.1), 159.7 (IQR: 143.4–177.8), 86.2 (IQR: 76.3–98.6), 310.7 (IQR: 281.8–340.4) $\mu\text{mol/L}$,

224 respectively (Supplementary Figure S2). In non-DM, serum concentrations of BCAA, leucine,

225 isoleucine, and valine were 508.9 (IQR: 457.6–565.7), 144.8 (IQR: 129.5–161.7), 77.7 (IQR:

226 68.6–87.5), 285.6 (IQR: 256.5–318.9) $\mu\text{mol/L}$, respectively. Participants with DM were more

227 likely to have more chronic diseases, lower energy intake, higher BMI and WHR, but similar

228 lean mass percentage and fat mass percentage, and a lower rate of sarcopenia (13.2% vs 18.6%).

229 No differences in sex, age, education level and physical activity between DM and non-DM were

230 observed. Supplementary Table S1 shows the characteristics of dietary factors in DM and non-

231 DM. Participants with DM had higher intakes of total and animal protein, carbohydrate, fiber,

232 and fat (all $p < 0.001$), but similar plant protein intake, compared with those without DM
233 ($p = 0.812$). The DM group had higher intake of whole grains and lower intake of refined grains
234 than the non-DM group, while there was no difference in the intakes of other food groups.
235 Moreover, those with DM tended to have a lower diet quality index than those without DM
236 ($p = 0.024$).

237

238 **3.2 Association between dietary factors and serum BCAA in DM and non-DM**

239 The Spearman correlation coefficients between dietary factors and serum BCAA concentrations
240 are shown in Figure 1. Higher intake of animal protein, total fat, red meat, poultry, and lower
241 intakes of plant protein, fiber, plant-based foods, and lower DQI-I scores were associated with
242 increased serum BCAA levels in participants without DM. Among those with DM, only higher
243 red meat intake and lower whole grains intake were associated with serum BCAA levels.

244

245 No interactions between dietary factors and diabetes status in relation to serum BCAA levels
246 were found (Supplementary Table S2–S5). Higher intakes of animal protein, red meat, and
247 poultry, and lower intakes of plant protein, whole grains, and soy products, and lower DQI-I
248 scores were associated with higher levels of serum leucine among participants without DM in
249 multivariable-adjusted linear regression models. However, no associations between dietary
250 factors and serum BCAA levels were found among DM participants after adjusting for
251 sociodemographic, lifestyle, and health-related factors (Supplementary Table S2–S5).

252

253 3.3 Association between serum BCAA and sarcopenia risk in DM and non-DM

254 Table 2 and Figure 2 show the cross-sectional association between serum BCAA levels and
255 sarcopenia risk among participants with and without DM. There were significant interactions
256 between serum BCAA and valine with diabetes status in relation to sarcopenia risk (p -
257 interaction=0.009 for BCAA and 0.017 for valine), and borderline interactions were found for
258 leucine (p -interaction=0.070) and isoleucine (p -interaction=0.059). The ORs (95% CIs) of
259 sarcopenia across tertiles of serum total BCAA among participants with DM were 1.00, 0.25
260 (0.11–0.60) and 0.58 (0.29–1.19), respectively (p -trend=0.200), while in those without DM
261 were 1.00, 0.63 (0.48–0.81) and 0.48 (0.36–0.64), respectively (p -trend<0.001). Nonlinear
262 associations between log-transformed serum BCAA levels and sarcopenia risk were found in
263 participants with DM, following a U-shape curve (all p -nonlinearity<0.05). Compared with the
264 reference (median), moderately elevated BCAA levels were associated with a significantly
265 lower risk of sarcopenia (OR: 0.21, 95% CI: 0.09–0.47, per SD of log-transformed serum
266 BCAA levels) when level below median. However, higher BCAA levels were associated with
267 an increased risk (OR: 1.35, 95% CI: 0.62–2.94) when level exceeding median, though this
268 association was not statistically significant. Similar patterns were found for leucine, isoleucine,
269 and valine in relation to sarcopenia risk in DM. However, among participants without DM,
270 serum BCAA levels were inversely associated with sarcopenia risk. The ORs (95% CIs) of
271 sarcopenia for per SD increase in log-transformed serum levels were 0.72 (0.64–0.81) for
272 BCAA, 0.71 (0.62–0.80) for leucine, 0.78 (0.62–0.80) for isoleucine, and 0.73 (0.65–0.83) for
273 valine.

274

275 Table 3 and Figure 3 show the prospective association between serum BCAA and incident
276 sarcopenia risk over four years among participants with and without DM. The incidence of
277 sarcopenia over four years was 14.5% for DM and 9.3% for non-DM. There were no significant
278 interactions between serum BCAA levels and diabetes in relation to incident sarcopenia (all p -
279 interaction > 0.05). No significant associations between baseline serum BCAA levels and 4-year
280 incident sarcopenia were found in participants with DM. In contrast, among participants
281 without DM, higher serum BCAA levels were significantly associated with lower incident
282 sarcopenia. The ORs (95% CIs) of incident sarcopenia for per SD increase in log-transformed
283 serum levels were 0.79 (0.66–0.94) for BCAA, 0.81 (0.68–0.97) for leucine, 0.84 (0.70–0.99)
284 for isoleucine, and 0.78 (0.66–0.93) for valine.

285

286 No statistically significant interactions were observed between age group (65–69, 70–74, ≥ 75
287 years) and serum BCAA levels in relation to sarcopenia risk in either cross-sectional or
288 prospective analyses (all p for interaction > 0.05 ; Supplementary Figures S3 and S4). However,
289 among participants with diabetes, the U-shaped association between BCAA levels and
290 sarcopenia risk appeared more pronounced in those aged ≥ 75 years compared to the 65–69 age
291 group in RCS analyses. In the prospective analysis, although RCS plots suggested a potential
292 U-shaped pattern in individuals aged ≥ 75 years, stratified analyses did not observe significant
293 associations within any age subgroup. In sensitivity analysis, the cross-sectional and
294 prospective associations of serum BCAA levels and sarcopenia risk remained unchanged after

295 adjusting for the use of antidiabetic, lipid-lowering and antihypertensive drugs in both DM and
296 non-DM (Supplementary Table S6 and S7, Figure S5 and S7). However, after further
297 adjustment for eGFR, the longitudinal associations of serum BCAA levels, especially leucine
298 and isoleucine, with incident sarcopenia were attenuated in participants without DM
299 (Supplementary Table S7 and Figure S8), although the cross-sectional associations were not
300 altered appreciably (Supplementary Table S6 and Figure S6).

301
302 Supplementary Table S8 and Figure S9–S12 show association between serum BCAA levels
303 with muscle measurements (ASMI, grip strength, gait speed and chair stand test) in DM and
304 non-DM. In multivariable-adjusted linear regression models, serum total and individual BCAA
305 levels were positively associated with ASMI and grip strength in non-diabetic participants (all
306 $p < 0.05$). These associations remained consistent in RCS models, indicating a linear relationship
307 across the range of BCAA concentrations in non-DM. Among participants with diabetes, RCS
308 models suggested a potential nonlinear association between serum BCAA levels with ASMI
309 and grip strength. When BCAA levels were below the median, higher BCAA levels were
310 associated with increased ASMI and grip strength, using the median as reference. However,
311 when BCAA levels exceeded the median, the associations plateaued and became non-
312 significant. No significant associations were observed between serum BCAA levels and gait
313 speed or chair stand test in either group.

314

315 **4. Discussion**

316 This cohort study of community-dwelling older adults found that dietary protein, protein
317 sources, and overall diet quality were significantly associated with serum BCAA levels in
318 individuals without DM, but only red meat and whole grain intake were found to be associated
319 with serum BCAA levels in those with DM. However, the associations between dietary factors
320 and serum BCAA levels were attenuated after adjustment for sociodemographic, lifestyle, and
321 health-related factors. Furthermore, among non-diabetic individuals, higher serum BCAA
322 levels were associated with lower sarcopenia risk in both cross-sectional and prospective
323 analyses. However, among individuals with DM, a U-shaped association between serum BCAA
324 levels and sarcopenia risk was observed in the cross-sectional analysis, but no significant
325 prospective association was found.

326

327 BCAAs (including leucine, isoleucine, and valine) are essential amino acids that must be
328 provided in the diet, as they cannot be produced in sufficient quantities to fulfill physiological
329 requirements in the body [25]. The primary dietary sources of BCAA are red meat, poultry, fish,
330 egg, dairy products, and soy and soy products [25]. Moreover, dietary intakes of whole grains
331 and fiber, and overall diet quality have also been associated with circulating BCAA levels,
332 suggesting that dietary context may influence BCAA metabolism independently of absolute
333 BCAA intake [26]. In this study, higher serum BCAA levels were associated with greater
334 intakes of animal protein, total fat, red meat, and poultry, and associated with lower intakes of
335 plant protein, dietary fiber, and plant-based foods among individuals without DM in unadjusted

336 models. Among participants with DM, only higher red meat intake and lower whole grain intake
337 showed significant associations with serum BCAA levels. However, these associations were
338 substantially attenuated after adjustment for sociodemographic and lifestyle factors, BMI, and
339 number of chronic diseases. This suggests that the observed association between dietary intakes
340 and circulating BCAA levels may be partially modulated by underlying metabolic or lifestyle
341 factors. Similar results were found in several previous studies, which reported null or weak
342 associations between dietary and circulating BCAA levels [27, 28]. In addition, findings from
343 a large cohort of US women indicated that BMI, more than diet, is related to plasma BCAA
344 concentrations [29]. Circulating BCAA levels result from a complex interplay among dietary
345 intake, intestinal absorption, and catabolic processes, and are therefore influenced by multiple
346 physiological mechanisms. Notably, metabolic dysfunction, such as obesity, insulin resistance,
347 type 2 diabetes, and gut microbiota dysbiosis, has been associated with elevated BCAA levels,
348 supporting the notion that BCAA may serve as a biomarker of metabolic dysregulation [30].
349 Consistent with this framework, our study found that individuals with DM had significantly
350 higher serum BCAA levels compared to those without DM (Table 1). Future research should
351 further investigate the mechanisms linking diet, BCAA metabolism, and metabolic dysfunction,
352 particularly in populations with varying metabolic health profiles.

353

354 The significant association between higher serum BCAA levels and reduced sarcopenia risk
355 was observed among individuals without DM in both cross-sectional and prospective analyses
356 in this study. This finding was consistent with two cross-sectional studies reporting that low

357 BCAA levels were associated with increased risk of sarcopenia, reduced muscle mass and
358 function in community-dwelling older adults [31, 32]. In addition, evidence from the UK
359 Biobank further supports this association, showing that higher circulating BCAA levels are
360 positively correlated with greater muscle mass and strength [33]. Furthermore, a systematic
361 review and meta-analysis of 35 randomized controlled trials found that BCAA-rich
362 supplements significantly improved handgrip strength and muscle mass in older adults
363 compared to placebo [5]. Moreover, an umbrella review of 15 systematic reviews and meta-
364 analyses identified leucine supplementation as having the strongest evidence for enhancing
365 muscle mass in elderly individuals with sarcopenia [4]. BCAA, particularly leucine, are key
366 regulators of muscle protein metabolism. BCAA can activate the mechanistic target of
367 rapamycin (mTOR) signaling pathway, enhancing translation initiation and promoting muscle
368 protein synthesis (MPS) [3]. In addition, BCAA provision can reduce indices whole-body
369 protein breakdown and muscle protein breakdown (MPB) [3]. However, since BCAA cannot
370 provide the full complement of EAAs required to build muscle, their ability to sustain and
371 maximize MPS is limited [4, 5]. The anabolic response triggered by BCAA is therefore less
372 effective than that induced by complete protein sources containing all indispensable amino
373 acids [3, 5]. Therefore, combining BCAA supplementation with a well-balanced diet enhances
374 its effectiveness in supporting optimal muscle health.

375

376 This study found a U-shaped association between serum BCAA levels and sarcopenia risk in
377 older adults with DM. Higher BCAA levels were significantly associated with lower

378 sarcopenia risk when serum BCAA level was below the median; however, BCAA may not
379 provide protective effects on muscle when above the median. Moreover, no significant
380 association between BCAA levels and incident sarcopenia risk over 4 years was found in those
381 with DM. Consistent with our findings, no significant association between dietary BCAA intake
382 and skeletal muscle mass was observed in participants with obesity, but the beneficial effects
383 of BCAA intake on skeletal muscle mass were found in the non-obese group in a cross-sectional
384 study of 3,966 adults aged 50–64 years in Korea [34]. In contrast, a prospective cohort study
385 involving 1,140 patients with T2DM and a mean age of 56.6 (SD 10.6) years in Singapore found
386 that higher BCAA levels were associated with greater muscle mass and reduced odds of muscle
387 mass loss over seven years of follow-up [12]. However, this association was observed in
388 patients younger than 60 years, but not in those aged 60 and above [12]. Our study specifically
389 targeted individuals aged 65 years and above and similarly did not observe a significant
390 association between BCAA levels and incident sarcopenia in DM. The potential muscular
391 benefits of BCAA in older adults with DM may be attenuated by a combination of impaired
392 BCAA metabolism, particularly driven by insulin resistance. Insulin resistance has been
393 consistently associated with impaired BCAA catabolism in Mendelian randomization studies
394 [35, 36]. A potential mechanism may be the reduced expression of BCAA-related catabolic
395 enzymes such as branched-chain α -ketoacid dehydrogenase (BCKD) in T2DM and obesity [10,
396 37]. BCKD catalyzes the rate-limiting step of BCAA catabolism and is subject to activation by
397 the mitochondrial phosphatase encoded by the *PPMIK* gene. In response to a glucose challenge
398 (e.g., oral glucose tolerance test, OGTT), *PPMIK* expression is typically upregulated in skeletal

399 muscle. However, this response is attenuated in individuals with T2DM [10, 35]. Recent multi-
400 omics analyses by Zuo et al. identified that impaired BCAA catabolism may serve as a
401 molecular hallmark in sarcopenia [11]. The accumulation of BCAA was associated with
402 reduced muscle mass and strength, and experimental validation in mouse models confirmed
403 that defective BCAA metabolism compromises muscle mass and strength by dysregulated
404 mTOR signaling [11]. Although BCAA accumulation may enhance protein synthesis via
405 mTOR activation, sustained mTOR signaling impairs insulin sensitivity, blocks autophagy, and
406 disrupts mitochondria, thereby accelerating muscle loss [11, 38, 39]. Together, insulin
407 resistance and impaired BCAA catabolism may establish a vicious cycle [40], exacerbating
408 both metabolic dysfunction and muscle degradation. Interestingly, while older adults have a
409 reduced mTOR response to anabolic stimuli such as nutrition or exercise, commonly referred
410 to as anabolic resistance [41], this does not preclude the presence of chronic mTOR activation
411 in older adults with diabetes. The paradox highlights the differential regulation of mTOR
412 signaling, where acute responsiveness to anabolic stimuli is impaired and it limits muscle
413 protein synthesis, yet sustained activation, driven by insulin resistance, contributes to muscle
414 degradation. Collectively, these findings suggest that in older adults with diabetes, the
415 accumulation of BCAA due to impaired catabolism may not confer muscular benefits. Instead,
416 excessive BCAA levels may exacerbate muscle deterioration, thereby explaining the lack of
417 association, or even a paradoxical relationship, between BCAA levels and sarcopenia risk in
418 this population.

419

420 Notably, the U-shaped association was more pronounced in adults aged ≥ 75 years with diabetes
421 (Supplementary Figure S3 and S4). The increased sarcopenia risk at low BCAA levels may
422 represent exacerbated age-related anabolic resistance, as skeletal muscle responsiveness to
423 amino acid stimulation declines with age [41]. This implies that older adults may require a
424 higher baseline amino acid threshold to initiate muscle protein synthesis. In contrast, the risk
425 associated with elevated BCAA levels points to the worsening cycle of insulin resistance and
426 impaired BCAA catabolism [11, 40]. Aging may amplify this vicious cycle, as supported by
427 transcriptomic data and previous studies showing age-related downregulation of genes
428 encoding BCAA-catabolic enzymes [11, 42, 43]. Therefore, in older adults with diabetes,
429 maintaining BCAA levels within a specific intermediate range may be critical for preserving
430 muscle mass and strength while avoiding metabolic accumulation.

431
432 The observed discrepancy between the U-shaped cross-sectional association and the lack of a
433 significant prospective association among individuals with DM may be explained by the role
434 of BCAAs as a biomarker of current metabolic state related to muscle health, rather than a
435 predictor of muscle decline in DM. BCAA has been regarded as a biomarker of cardiometabolic
436 diseases [44, 45]. In cross-sectional analysis, both high BCAA levels may reflect underlying
437 metabolic dysfunction, such as insulin resistance, that are concurrently associated with reduced
438 muscle mass or strength. However, baseline BCAA levels may not capture dynamic changes in
439 metabolic status over time, which can be influenced by lifestyle, dietary interventions, or
440 medical treatment during follow-up. In addition, individuals with extreme BCAA levels may

441 have higher risks of dropout or mortality, leading to attenuating the prospective association.
442 Monitoring and modulating BCAA levels, potentially through personalized nutritional
443 interventions, may represent a key strategy for metabolism and muscle health in older adults
444 with diabetes. However, their predictive utility of muscle loss over time appears limited. Future
445 studies with repeated BCAA measurements, larger cohorts, and extended follow-up are needed
446 to examine the temporal and causal roles of BCAA homeostasis in the development of
447 sarcopenia in DM.

448
449 In cross-sectional analyses, all three BCAAs showed associations with prevalent sarcopenia in
450 both DM and non-DM. However, in prospective analyses, only valine remained independently
451 associated with incident sarcopenia in non-diabetic participants, while leucine showed a
452 borderline association and isoleucine was not significant. Notably, additional adjustment for
453 renal function (eGFR) attenuated the associations for leucine and isoleucine, while the valine
454 association remained robust (Supplementary Table S7 and Figure S8). This suggests that
455 differences across individual BCAAs may partly reflect confounding or mediation by renal
456 function and the broader metabolic milieu captured by eGFR, such as inflammation, insulin
457 resistance, and comorbidity burden, all of which are also linked to muscle loss. Individual
458 BCAAs have different roles in muscle and metabolic regulation. Leucine is a well-known
459 activator of the mTOR pathway, promoting muscle protein synthesis [3]. However, its
460 supplementation appears more effective in individuals with sarcopenia than in healthy older
461 adults [4], potentially explaining its weaker association in our prospective analysis. In contrast,

462 valine and isoleucine are more involved in glucose metabolism and energy homeostasis [7].
463 Experimental studies have shown that dietary restriction of isoleucine reprograms hepatic and
464 adipose metabolism, enhances insulin sensitivity, and increases energy expenditure via
465 activation of the FGF21-UCP1 axis [46]. Valine restriction induces similar but less pronounced
466 effects. Moreover, valine has been linked to regulating glucose uptake and lipid metabolism,
467 suggesting a more direct role in metabolic signaling relevant to muscle maintenance [47, 48].
468 Whether these differences of three individual BCAAs reflect independent mechanisms or
469 shared pathways modulated by renal or metabolic factors warrants further investigation.

470

471 This study has several strengths. It includes a relatively large sample size of community-
472 dwelling older adult population, which allows for the detection of potential non-linear
473 associations between serum BCAA levels and sarcopenia risk. Stratified analyses by diabetes
474 status suggest a modifying role of metabolic health in this association. However, there are
475 several limitations in this study. First, serum BCAA levels were measured only at baseline and
476 not during follow-up, preventing the assessment of how changes in BCAA levels may influence
477 the development of sarcopenia. Second, the identification of diabetes was primarily based on
478 self-reports and medication use, which may have led to undiagnosed cases. This bias may
479 underestimate the observed associations of BCAA and sarcopenia risk in diabetes. In addition,
480 data on diabetes duration, complications, and glycemic control, such as HbA1c, were not
481 collected, potentially affecting the outcomes. Although the lack of HbA1c data prevents us from
482 examining whether the observed associations were confounded or mediated by glucose

483 homeostasis, we were able to adjust for several other key confounders using accurate
484 measurements. These included gold-standard DXA measures for fat mass, the use of
485 antidiabetic, lipid-lowering, and antihypertensive medications, renal function, and dietary
486 intake via a validated FFQ. Third, selection bias may exist. Participants tended to have higher
487 education levels, greater health awareness, and higher physical activity levels, compared with
488 the general older population in Hong Kong. Therefore, the generalizability of these findings to
489 other populations may be limited. Fourth, some potential confounders influencing the results,
490 such as lipid metabolism and gut microbiota, were not assessed.

491

492 **5. Conclusion**

493 In conclusion, dietary factors showed only a weak association with serum BCAA levels in older
494 adults, which was further attenuated after adjusting for sociodemographic, lifestyle, and health-
495 related variables. Older adults with DM had higher serum BCAA levels compared to those
496 without DM. While elevated serum BCAA levels may reduce sarcopenia risk in older adults
497 without DM, excessive BCAA levels may not offer muscle benefits in individuals with DM.
498 These findings may suggest that metabolic status may modify the effects of BCAA on muscle
499 health and should be considered in sarcopenia prevention strategies. Further studies are needed
500 to determine whether targeted modulation of BCAA levels can improve muscle outcomes in
501 older adults with DM or other cardiometabolic diseases.

502

503

504 Author contributions

505 Timothy Kwok and Shu-Yi Li contributed to the conception and design of this study. Timothy
506 Kwok, Ting Zhang, Jason Leung and Shu-Yi Li contributed to acquisition of data. Data analysis
507 was performed by Shu-Yi Li. The first draft of the manuscript was written by Shu-Yi Li, and
508 all authors commented on previous versions of the manuscript. All authors read and approved
509 the final version of this manuscript. Timothy Kwok supervised this work. Timothy Kwok and
510 Shu-Yi Li had full access to all the data in the study and takes responsibility for the integrity of
511 the data and the accuracy of the data analysis. All individuals who meet the criteria for
512 authorship have been included in the list of authors. No one eligible for authorship has been
513 excluded.

514

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518

519 Conflict of interest

520 The authors declare that they have no conflict of interest.

521

522 Ethics approval and consent to participate

523 The study was conducted according to the guidelines of the Declaration of Helsinki, and
524 approved by the Joint Chinese University of Hong Kong—New Territories East Cluster Clinical

525 Research Ethics Committee (protocol code is CRE-2003.102 and it was renewed on 27 August
526 2005). Written informed consent was obtained from each participant in this study.

527

528 **Data sharing statement**

529 The data that support the findings of this study are available from the corresponding author
530 upon reasonable request.

531

532 **Declaration of Generative AI and AI-assisted technologies in the writing process**

533 During the preparation of this work, the author(s) used ChatGPT-4o, in order to check grammar
534 and improve readability. After using this tool/service, the author(s) reviewed and edited the
535 content as needed and take(s) full responsibility for the content of the published article.

536

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541

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697 Figure legend

698

699 Figure 1. Heat map of Spearman correlation coefficients between serum BCAA concentrations
700 and dietary factors in DM and non-DM.

701 Abbreviation: BCAA, Branched-chain amino acid; DM, diabetes mellitus; DQI-I, Diet Quality Index-International. *p*-values
702 were FDR adjusted. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

703

704 Figure 2. Cross-sectional association between serum BCAA levels and sarcopenia risk in DM
705 and non-DM. (A) for BCAA; (B) for leucine; (C) for isoleucine; (D) for valine. The median was
706 used as the reference value with knots placed at 10th, 50th and 90th centiles of log-transformed serum BCAA
707 levels. In restricted cubic spline logistic regression analysis, confounding factors included age, sex,
708 educational attainment, smoking status, current alcohol consumption, physical activity level, number of
709 chronic diseases, fat mass, total energy intake and total protein intake. *p*-interactions between serum BCAA,
710 leucine, isoleucine and valine with DM in relation to sarcopenia risk were 0.009, 0.070, 0.059, and 0.017,
711 respectively.

712

713 Figure 3. Prospective association between serum BCAA levels and incident sarcopenia in DM
714 and non-DM. (A) for BCAA; (B) for leucine; (C) for isoleucine; (D) for valine. The median was
715 used as the reference with knots placed at 10th, 50th and 90th centiles of log-transformed serum BCAA levels.
716 In restricted cubic spline logistic regression model, confounding factors included age, sex, educational
717 attainment, smoking status, current alcohol consumption, physical activity level, number of chronic diseases,
718 fat mass, total energy intake and total protein intake. *p*-interactions between serum BCAA, leucine, isoleucine,
719 and valine with DM in relation to sarcopenia risk were 0.090, 0.122, 0.332, and 0.073, respectively.

Table 1. Baseline characteristics of participants stratified according to the presence or absence of DM.

	DM (n=433)	Non-DM (n=2,561)	<i>p</i>
BCAA, $\mu\text{mol/L}$	558.6 (501.3–616.1)	508.9 (457.6–565.7)	<0.001
Leucine, $\mu\text{mol/L}$	159.7 (143.4–177.8)	144.8 (129.5–161.7)	<0.001
Isoleucine, $\mu\text{mol/L}$	86.2 (76.3–98.6)	77.7 (68.6–87.5)	<0.001
Valine, $\mu\text{mol/L}$	310.7 (281.8–340.4)	285.6 (256.5–318.9)	<0.001
Age, y	71 (68–76)	71 (68–75)	0.191
Male, %	48.3	47.4	0.750
Education, %			0.765
No education	21.9	20.7	
Primary or below	48.5	50.2	
Secondary or above	29.6	29.1	
Smoking status, %			0.233
Never	67.0	65.8	
Past smoking	28.9	27.9	
Current smoking	4.2	6.2	
Current alcohol drinking, %	9.0	13.7	0.007
Number of chronic diseases, %			<0.001
0	9.7	23.0	
1-2	58.7	56.4	
≥ 3	31.6	20.6	
Antihypertensive drugs, %	55.0	27.0	<0.001
Antidiabetic drugs, %	73.7	0.0	<0.001
Lipid-lowering drugs, %	32.9	13.1	<0.001
Weight, kg	60.4 \pm 9.7	58.2 \pm 9.6	<0.001
BMI, kg/m^2	24.5 \pm 3.1	23.6 \pm 3.3	<0.001
ASMI, kg/m^2	6.8 \pm 1.0	6.6 \pm 1.0	<0.001
FMI, kg/m^2	7.3 \pm 2.2	7.1 \pm 2.4	0.069
Handgrip strength, kg	27.7 \pm 8.1	28.4 \pm 8.2	0.110
Gait speed, m/s	1.01 \pm 0.24	1.03 \pm 0.22	0.072
Chair stand test, s	13.3 \pm 5.2	12.7 \pm 4.2	0.007
Sarcopenia, %	13.2	18.6	0.006
PASE score	93.1 \pm 44.3	93.1 \pm 42.6	0.850
Energy intake, kcal/day	1727.0 \pm 535.8	1860.6 \pm 586.6	<0.001
eGFR, mL/min/1.73 m^2	80.3 \pm 15.3	81.3 \pm 13.7	0.214

Abbreviation: DM, diabetes mellitus; BCAA, Branched-chain amino acid; BMI, body mass index; ASMI, appendicular skeletal muscle mass index; FMI, fat mass index; PASE, Physical Activity Scale for the Elderly; eGFR, estimated glomerular filtration rate.

Mean \pm SD or Median (IQR) for continuous variables and percentage (%) for categorical variables were presented.

Table 2. Cross-sectional association between serum BCAA levels and sarcopenia risk according to the presence or absence of DM.

	Tertiles of serum BCAA, OR (95% CI)				Per SD of log-transformed serum BCAA	<i>p</i>
	T1	T2	T3	<i>p</i> -trend		
DM (n=433)						
No. of sarcopenia	26	9	22		57	
BCAA						
Model 1	1.00 (Ref)	0.27 (0.12–0.60)	0.62 (0.32–1.21)	0.210	0.74 (0.55–1.01)	0.060
Model 2	1.00 (Ref)	0.25 (0.11–0.60)	0.58 (0.29–1.19)	0.200	0.73 (0.53–1.02)	0.070
Leucine						
Model 1	1.00 (Ref)	0.51 (0.24–1.06)	0.69 (0.35–1.37)	0.328	0.77 (0.57–1.05)	0.096
Model 2	1.00 (Ref)	0.51 (0.23–1.11)	0.70 (0.34–1.45)	0.389	0.76 (0.54–1.06)	0.102
Isoleucine						
Model 1	1.00 (Ref)	0.56 (0.27–1.16)	0.68 (0.34–1.35)	0.308	0.76 (0.57–1.03)	0.081
Model 2	1.00 (Ref)	0.64 (0.29–1.40)	0.69 (0.33–1.45)	0.374	0.75 (0.54–1.04)	0.083
Valine						
Model 1	1.00 (Ref)	0.36 (0.17–0.78)	0.63 (0.32–1.22)	0.194	0.74 (0.54–1.01)	0.055
Model 2	1.00 (Ref)	0.37 (0.16–0.82)	0.61 (0.30–1.25)	0.218	0.74 (0.53–1.03)	0.073
Non-DM (n=2,561)						
No. of sarcopenia	189	155	132		476	
BCAA						
Model 1	1.00 (Ref)	0.60 (0.47–0.77)	0.43 (0.33–0.56)	<0.001	0.69 (0.61–0.77)	<0.001
Model 2	1.00 (Ref)	0.63 (0.48–0.81)	0.48 (0.36–0.64)	<0.001	0.72 (0.64–0.81)	<0.001
Leucine						
Model 1	1.00 (Ref)	0.66 (0.51–0.85)	0.45 (0.34–0.59)	<0.001	0.67 (0.60–0.75)	<0.001
Model 2	1.00 (Ref)	0.73 (0.56–0.95)	0.51 (0.39–0.69)	<0.001	0.71 (0.62–0.80)	<0.001
Isoleucine						
Model 1	1.00 (Ref)	0.63 (0.49–0.82)	0.53 (0.41–0.69)	<0.001	0.75 (0.67–0.83)	<0.001
Model 2	1.00 (Ref)	0.67 (0.51–0.87)	0.58 (0.44–0.77)	<0.001	0.78 (0.69–0.88)	<0.001
Valine						
Model 1	1.00 (Ref)	0.71 (0.55–0.91)	0.47 (0.36–0.62)	<0.001	0.70 (0.62–0.78)	<0.001
Model 2	1.00 (Ref)	0.74 (0.57–0.96)	0.54 (0.40–0.71)	<0.001	0.73 (0.65–0.83)	<0.001

Abbreviation: BCAA, Branched-chain amino acid; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; Ref, reference.

Model 1: adjusted for age and sex. Model 2: additionally adjusted for educational attainment, smoking status, current alcohol consumption, physical activity level, number of chronic diseases, fat mass, total energy intake and total protein intake.

Table 3. Prospective association between serum BCAA levels and incident sarcopenia according to presence or absence of DM.

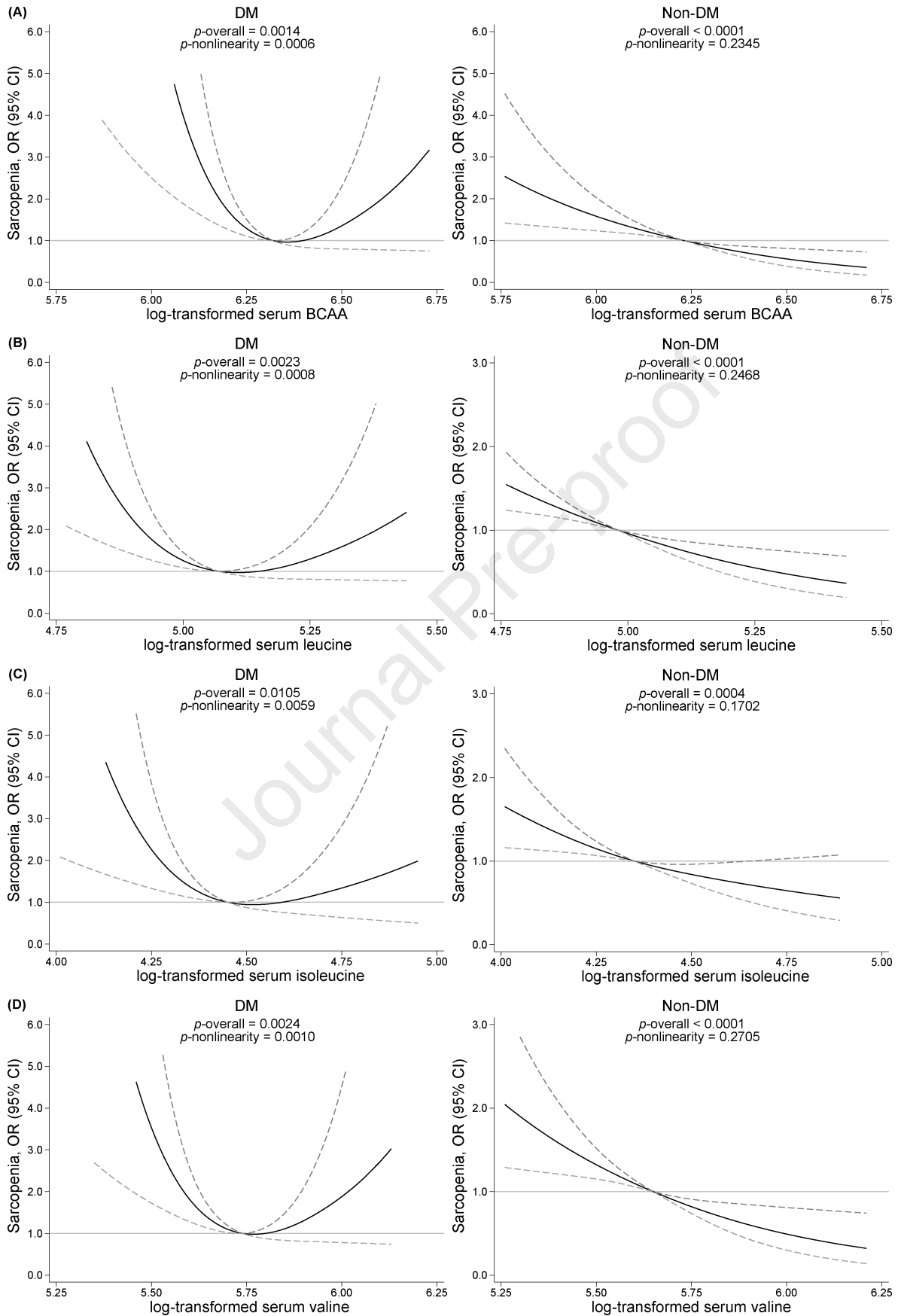
	Tertiles of serum BCAA, OR (95% CI)				Per SD of log-transformed serum BCAA	<i>p</i>
	T1	T2	T3	<i>p</i> -trend		
DM (n=358)						
No. of sarcopenia	16	15	21		21	
BCAA						
Model 1	1.00 (Ref)	0.86 (0.40–1.85)	1.16 (0.55–2.45)	0.644	1.09 (0.78–1.53)	0.623
Model 2	1.00 (Ref)	0.89 (0.40–2.01)	1.22 (0.55–2.70)	0.585	1.16 (0.81–1.68)	0.415
Leucine						
Model 1	1.00 (Ref)	0.78 (0.36–1.70)	1.06 (0.51–2.22)	0.814	1.14 (0.81–1.59)	0.453
Model 2	1.00 (Ref)	0.89 (0.39–2.05)	1.15 (0.53–2.53)	0.682	1.21 (0.84–1.74)	0.303
Isoleucine						
Model 1	1.00 (Ref)	0.94 (0.44–2.02)	1.07 (0.50–2.27)	0.839	1.06 (0.77–1.45)	0.735
Model 2	1.00 (Ref)	1.10 (0.48–2.51)	1.17 (0.53–2.61)	0.698	1.10 (0.78–1.56)	0.583
Valine						
Model 1	1.00 (Ref)	1.12 (0.53–2.36)	0.96 (0.45–2.05)	0.904	1.06 (0.75–1.49)	0.737
Model 2	1.00 (Ref)	1.11 (0.51–2.44)	1.02 (0.46–2.27)	0.972	1.14 (0.79–1.64)	0.495
Non-DM (n=2,022)						
No. of sarcopenia	80	59	50		189	
BCAA						
Model 1	1.00 (Ref)	0.60 (0.41–0.87)	0.46 (0.31–0.70)	<0.001	0.75 (0.64–0.89)	0.001
Model 2	1.00 (Ref)	0.62 (0.42–0.91)	0.51 (0.33–0.77)	0.002	0.79 (0.66–0.94)	0.010
Leucine						
Model 1	1.00 (Ref)	0.75 (0.52–1.09)	0.56 (0.37–0.84)	0.005	0.77 (0.65–0.92)	0.003
Model 2	1.00 (Ref)	0.79 (0.54–1.16)	0.63 (0.41–0.95)	0.029	0.81 (0.68–0.97)	0.025
Isoleucine						
Model 1	1.00 (Ref)	0.58 (0.40–0.85)	0.55 (0.37–0.81)	0.003	0.81 (0.68–0.95)	0.012
Model 2	1.00 (Ref)	0.60 (0.41–0.89)	0.59 (0.39–0.89)	0.013	0.84 (0.70–0.99)	0.050
Valine						
Model 1	1.00 (Ref)	0.59 (0.40–0.85)	0.43 (0.29–0.64)	<0.001	0.75 (0.63–0.88)	0.001
Model 2	1.00 (Ref)	0.61 (0.42–0.90)	0.48 (0.32–0.73)	0.001	0.78 (0.66–0.93)	0.007

Abbreviation: BCAA, Branched-chain amino acid; DM, diabetes mellitus; OR, odds ratio; CI, confidence interval; Ref, reference.

Model 1: adjusted for age and sex. Model 2: additionally adjusted for educational attainment, smoking status, current alcohol consumption, physical activity level, number of chronic diseases, fat mass, total energy intake and total protein intake.

	DM				Non-DM				
	BCAA	Leucine	Isoleucine	Valine	BCAA	Leucine	Isoleucine	Valine	value
Total protein	0.020	0.041	0.011	0.005	0.012	0.018	0.005	0.010	
Animal protein	0.088	0.114	0.083	0.066	0.049*	0.064**	0.045*	0.041	
Plant protein	-0.111	-0.121	-0.102	-0.098	-0.084***	-0.101***	-0.090***	-0.070**	
Carbohydrate	-0.088	-0.123	-0.098	-0.060	-0.060**	-0.075***	-0.063**	-0.048*	
Fiber	-0.124	-0.116	-0.117	-0.121	-0.114***	-0.119***	-0.121***	-0.103***	
Total fat	0.084	0.108	0.098	0.062	0.067**	0.082***	0.068**	0.056*	
Red meat	0.146*	0.157*	0.126	0.139*	0.082***	0.097***	0.087***	0.067**	
Processed meat	0.028	0.029	-0.004	0.033	0.032	0.039	0.022	0.031	
Poultry	0.018	0.031	0.015	0.002	0.065**	0.071***	0.068**	0.059**	
Freshwater fish	0.038	0.051	0.017	0.038	0.011	0.019	-0.001	0.011	
Seafood	0.040	0.025	0.050	0.040	-0.014	-0.006	-0.015	-0.016	
Dairy products	-0.056	-0.052	-0.063	-0.050	-0.074***	-0.063**	-0.062**	-0.079***	
Eggs	-0.039	-0.045	-0.056	-0.026	-0.015	-0.024	0.002	-0.017	
Whole grains	-0.158*	-0.174**	-0.153*	-0.136*	-0.120***	-0.135***	-0.128***	-0.104***	
Refined grains	-0.011	-0.050	-0.045	0.022	0.013	-0.001	0.013	0.020	
Vegetables	0.002	0.003	-0.005	0.002	-0.075***	-0.086***	-0.084***	-0.061**	
Fruits	-0.027	-0.029	-0.037	-0.015	-0.059**	-0.048*	-0.061**	-0.061**	
Soy and soy products	-0.018	-0.020	-0.007	-0.021	-0.056**	-0.078***	-0.047*	-0.045*	
Legumes	0.005	-0.026	0.021	0.013	-0.068**	-0.081***	-0.079***	-0.054*	
Nuts	0.028	0.037	0.020	0.021	-0.027	-0.011	-0.031	-0.030	
DQH	-0.093	-0.104	-0.098	-0.078	-0.085***	-0.097***	-0.095***	-0.071**	

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