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Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults: a randomized-controlled trial

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1 **Protein intake distribution pattern does not affect anabolic response, lean body**
2 **mass, muscle strength or function over 8 weeks in older adults: a randomized-**
3 **controlled trial**

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20 **Running head:** Protein intake pattern on function and protein kinetics

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24 **SUMMARY**

25 **Background & aims:** In our recent acute metabolic study, we found no differences in the
26 anabolic response to differing patterns of dietary protein intake. To confirm this in a
27 chronic study, we investigated the effects of protein distribution pattern on functional
28 outcomes and protein kinetics in older adults over 8 weeks. **Methods:** To determine
29 chronic effects of protein intake pattern at 1.1g protein/kg/day in mixed meals on lean
30 body mass (LBM), functional outcomes, whole body protein kinetics and muscle protein
31 fractional synthesis rate (MPS) over 8-week respective dietary intervention, fourteen
32 older subjects were randomly divided into either EVEN or UNEVEN group. The
33 UNEVEN group (n=7) consumed the majority of dietary protein with dinner (UNEVEN,
34 15/20/65%; breakfast, lunch, dinner), while the EVEN group (n=7) consumed dietary
35 protein evenly throughout the day (EVEN: 33/33/33%). **Results:** We found no significant
36 differences in LBM, muscle strength, and other functional outcomes between EVEN and
37 UNEVEN before and after 8-week intervention. Consistent with these functional
38 outcomes, we did not find significant differences in the 20-h integrated whole body
39 protein kinetics [net protein balance (NB), protein synthesis (PS), and breakdown (PB)]
40 above basal states and MPS between EVEN and UNEVEN intake patterns. **Conclusions:**
41 We conclude that over an 8-week intervention period, the protein intake distribution
42 pattern in mixed meals does not play an important role in determining anabolic response,
43 muscle strength, or functional outcomes. This trial is registered at
44 <https://ClinicalTrials.gov> as NCT02787889.

45 **Key words:** Sarcopenia, aging, stable isotope tracer, essential amino acids, protein
46 turnover

47 **Introduction**

48 The benefits of increased protein intake on the age-related loss of muscle mass and
49 strength, i.e., sarcopenia, and many related physiological functions is becoming
50 increasingly evident [1]. The NHANES data indicates that the average protein
51 consumption of both men and women over the age of 50 yrs is approximately 1.1 g/kg/d
52 [2], or 77g protein/d for 70kg adults. NHANES data also suggests that the American
53 pattern of dietary protein intake is typically skewed towards the evening meal, which
54 constitutes more than half of total daily protein intake [3]. The preponderance of
55 literature indicates that muscle protein synthesis (MPS) in resting conditions can be
56 maximally stimulated with approximately 20 - 35 g of protein or 0.25g – 0.43g/kg (based
57 on average body weight: 80 kg) [4-7], depending on protein quality and individual age.
58 These findings are consistent with the recent report by Moore et al. [8] showing that a
59 maximal MPS response is achieved with 0.24g/kg/meal and 0.4g/kg/meal for young and
60 older adults, respectively. The latter amount translates to the average protein intake
61 (1.1g/kg/d) of middle age and older American adults if an even distribution of protein
62 intake throughout the day is assumed [2]. Thus, with the traditional pattern of meal intake
63 (e.g., 15%/20%/65% of protein for breakfast, lunch, and dinner, respectively) in the
64 United States, a maximal stimulation of MPS would theoretically occur only at the dinner
65 meal of approximately 50g of protein (for a 70kg adult). In addition, this amount would
66 theoretically exceed the protein intake required to elicit the maximal anabolic effect by
67 ~80% (i.e., excess amount of 22g protein). This observation led to the promulgation of a
68 popular hypothesis that distributing total protein intake equally over three meals would
69 result in a more frequent stimulation of MPS as compared to the traditional intake pattern

70 [9]. Although recent acute metabolic studies in older individuals indicated no pattern
71 effect of dietary protein intake [10,11], it has been argued that acute studies may not
72 reflect functional changes over time [12]. Therefore, we hypothesized that 1) the 20-h
73 integrated whole-body net protein balance and MPS would be greater with even vs.
74 uneven distribution pattern of protein intake after the 8 week of dietary intervention; and
75 2) an even distribution pattern of dietary protein intake throughout the day would result in
76 greater gains in lean mass, strength, and function after 8 weeks of dietary intervention.

77

78 **Materials and Methods**

79 **Subjects.** Nineteen healthy male and female older adults [51 – 69 yrs] with body mass
80 indexes between 25 and 30 kg/m² were enrolled in the study (February 2014 through
81 March 2015). Subject were excluded from the study participation if subjects had any of
82 the followings: type I or II diabetes mellitus, active malignancy within the past 6 months,
83 history of gastrointestinal bypass surgery, lactose intolerance or allergy to milk or milk
84 products, a chronic inflammatory or other chronic disease (e.g., HIV/AIDS), low
85 hematocrit or hemoglobin concentration, low platelets, current use of corticosteroids, any
86 unstable medical conditions. Also excluded were subjects who participated in regular
87 resistance exercise (> twice per week). All subjects actively signed written informed
88 consent, and the study was approved by the Institutional Review Board at the University
89 of Arkansas for Medical Sciences. Subjects were then randomly assigned to EVEN or
90 UNEVEN group. Sample size for the present study that has been estimated based on the
91 power analysis of muscle protein synthesis rate to detect effect sizes of 0.45 or larger
92 were sixteen older subjects (8 subjects per group). We included fourteen older adult

93 subjects [7 subjects per group; range of age: 51–69 yrs] for the final analyses (Table 1)
94 due to subject dropout (n=4) and screening failures (See CONSORT Diagram;
95 Supplemental figure 1).

96 **Experimental Design.** During the screening visit, body composition was determined by
97 dual-energy X-ray absorptiometry (DEXA, QDR-4500A; Hologic, Waltham, MA)
98 (**Table 1**) and was repeated at 8 weeks while subjects remained on their respective diets.
99 Eligible subjects were then randomly assigned by a study coordinator to one of two
100 dietary pattern groups in a permuted block randomization method using a sealed
101 envelope: UNEVEN group where subjects consumed 1.1g protein/kg body weight/day in
102 an uneven pattern (15/20/65% of total daily protein; breakfast/lunch/dinner, respectively);
103 or an EVEN group where subjects consumed the same amount of protein in an even
104 pattern (~33% of total protein with each meal) for an 8-week dietary intervention period.
105 After the screening, a 3-d dietary record and instruction were given to all subjects. The
106 Clinical Research Services Core (CRSC) research dietician used the information from
107 these dietary records to estimate their habitual food intake including the amount of
108 protein intake and food preferences. Diets were configured to provide adequate caloric
109 intake to maintain stable body weight over the 8-week intervention period using the
110 Harris-Benedict equation and their level of physical activity (range of physical activity
111 factor used = 1.38 – 1.83), and a daily vitamin/mineral supplement was included. The
112 study dietician prepared all diets in the Metabolic Kitchen at the CRSC (**Table 2**). Diets
113 were prepared to maximize protein intake from high quality protein sources including
114 egg, dairy, and beef (31.4 ± 0.3 % of EAA in the dietary protein). Individuals adhering to
115 a purely vegan diet were excluded from the study because of the difficulty in matching

116 the quality of protein with the other diets. Each distribution pattern was consumed for a
117 total of 8 weeks. Primary outcomes were studied before and after the 8-week dietary
118 intervention, and included body composition (lean body mass) and muscle strength and
119 functional outcomes (see Strength and functional tests). Secondary outcomes i.e., whole
120 body protein kinetics (protein synthesis, protein breakdown, and net balance) and MPS
121 were also determined at the beginning and end of the 8-week dietary intervention period.
122 Subjects obtained their meal allotment from the study coordinator at the Reynolds
123 Institute on Aging (RIOA) twice each week. Prior to dietary intervention, subjects were
124 provided a dietary record and point-and-shoot digital camera to record all the information
125 regarding their food intake including the time of meal consumption and the amount of
126 food leftover [10], which helped the study dietician ascertain calorie/protein intake as
127 well as study compliance. This trial is registered at <http://ClinicalTrials.gov> under
128 NCT02787889.

129

130 **Strength and functional tests.** One repetition maximum (1RM) for knee extension [13]
131 and handgrip strength (dominant hand) were determined. Subjects also performed a
132 battery of functional tests. For the 5-repetition sit-to-stand test, subjects were asked to
133 start seated with arms crossed over chest and were timed from start until seated for 5th
134 time. For the stair ascent/descent power test, subjects were asked to start at bottom or at
135 top until both feet touch upper landing or lower landing, respectively [14]. For the 10-
136 meter maximal gait speed test, subjects were asked to walk for 20 meter distance as fast
137 as possible while the middle 10 meter was timed. All the strength and functional tests
138 were performed several days before the initiation of dietary intervention and several days

139 before the second infusion studies. During the second strength and functional tests,
140 subjects were on their respective interventional diets.
141
142 **Stable Isotope tracer infusion protocol.** A metabolic study was performed before and
143 after the 8-week dietary intervention. Following a 3-day dietary control, subjects reported
144 to the RIOA after an overnight (after 2200 h) fast for the first metabolic infusion study
145 during which subjects consumed meal for their respective intake pattern. The same
146 metabolic study was repeated after the 8-week dietary intervention. The 23-h stable
147 isotope tracer infusion protocol is depicted in **Figure 1**. During each metabolic study, a
148 IV catheter was inserted into each lower arm; one for the tracer infusion and the other for
149 the sampling of "arterialized" blood [15]. To determine background isotopic enrichments
150 and blood chemistry, a blood sample was collected prior to the initiation of the tracer
151 infusion. Then, primed continuous infusions of L-[²H₅]phenylalanine (prime, 4.60
152 μmol/kg; rate, 3.92 μmol/kg/h) and L-[²H₂]tyrosine (prime, 0.95 μmol/kg; rate, 1.57
153 μmol/kg/h) were performed to determine in vivo whole body protein kinetics. A priming
154 dose of L-[²H₄]tyrosine was also administered (prime: 0.33 μmol/kg) for achieving an
155 isotopic steady-state of L-[²H₄]-tyrosine enrichment derived from L-[²H₅]phenylalanine
156 tracer infused (Cambridge Isotope Laboratories, Andover, MA). For measurements of
157 tracer enrichment and blood concentrations of glucose, insulin and leucine, blood
158 samples were taken at 0, 120, 150, 180, 210, 240, 270, 300, 330, 360, 480, 510, 540, 570,
159 630, 660, 720, 750, 780, 810, 840, 900, 960, 1020, 1080, 1140, 1200, 1260, 1320, 1380
160 min (total: approx. 180 mL). Meals were provided at 180, 450, and 720 min. To reduce
161 the catabolic effects of 23 h of bed rest during the infusion of tracers, subjects walked on

162 a treadmill for 15 min at 3.22 km/h starting at 660 min of the metabolic study
163 immediately before the dinner meal. The first muscle biopsy from the vastus lateralis was
164 taken 2.5 h (at 150 min) after the initiation of tracer infusion. The second muscle biopsy
165 was taken at the end of the metabolic study (at 1380 min).

166

167 **Analytic methods.** Tracer enrichments and leucine concentrations were determined by
168 gas-chromatography mass spectrometry (GCMS: Models 7890A/5975, Agilent
169 Technologies, Inc. Santa Clara CA) as previously described [10,16]. Plasma glucose
170 concentrations were measured spectrophotometrically on a Cobas c 111 analyzer (Roche,
171 F. Hoffman-La Roche Ltd, Basel, Switzerland). Plasma insulin concentrations were
172 measured by using commercially available human insulin ELISA kit (Alpco Diagnostics,
173 Salem, USA).

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175 **Calculations of protein kinetics.** Whole body protein kinetics [protein synthesis (PS),
176 protein breakdown (PB), and net protein balance (NB), $\text{g protein} \cdot 1200 \text{ min}^{-1}$] were
177 calculated as previously described [10,16]. For the estimation of the amount of
178 exogenous protein (g) that are appearing as amino acids in the circulation as a result of
179 the exogenous protein digestion, we accounted for digestibility (65%) of amino acids in
180 older adults [17].

181

182 **Statistical analysis**

183 Paired t-tests were performed to assess the time effect (pre vs. post) within each protein
184 distribution pattern group for each outcomes measure. Independent t-tests were

185 performed to compare differences in macronutrient consumptions between distribution
186 pattern groups. A one-factor analysis of covariance (ANCOVA) model was used to
187 evaluate the effect for protein distribution pattern (Even vs. Uneven) for each outcomes
188 measure. Baseline values were included as covariates to adjust for each subject's starting
189 values. Statistical significance was declared when P-values are less than 0.05. In cases
190 where multiple testing was necessary, Hommel's method was used to adjust the p-values.
191 All data were analyzed using PROC GLM in SAS (SAS Institute Inc., Cary, NC).

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208 **Results**

209 **Changes in body composition and functional outcomes.** LBM, muscle strength, and
210 functional outcomes were presented in Table 3. There were no changes from pre- to post-
211 dietary intervention (for all, $p > 0.05$) and no differences between patterns in LBM, sit-to-
212 stand speed, 10 m gait speed, handgrip strength, 1 RM knee extension, stair ascent power,
213 or stair descent power (for all, $p > 0.05$).

214 **Protein kinetics at whole body and muscle levels** The 20-h integrated whole-body
215 kinetics (180 – 1,380 min) were calculated as changes from the fasted state to the fed
216 state (i.e., fed minus fasted kinetic values) (**Figure 2**). Positive NB (PS – PB) was
217 achieved with UNEVEN and EVEN before and after the 8-week dietary intervention. The
218 positive NB in both patterns was achieved entirely through reductions in PB from the
219 fasted states, as PS in both patterns was decreased below the fasted states before and after
220 the intervention. There were no effects of pattern and time (post vs. pre-intervention) on
221 NB, PS, and PB (for all, $p > 0.05$). Consistent with the whole body protein kinetics in the
222 present study, the 8-week dietary intervention did not change MPS. There were no
223 differences from pre- to post- dietary intervention ($p > 0.05$) and no differences in pattern
224 ($p = 0.980$) on MPS (**Figure 3**).

225 **Plasma concentrations** Plasma responses of glucose, insulin, and leucine are presented
226 in **Figure 4**. Plasma responses of glucose and insulin are expressed as area under the
227 curves (AUC). There were no differences from pre- to post- dietary intervention and no
228 differences in pattern on glucose, insulin and leucine AUC responses (for all, $p > 0.05$).

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230

231 **Discussion**

232 In our previous acute metabolic study, we demonstrated that even distribution of
233 protein intake did not further increase the anabolic responses to dietary protein at either
234 the whole body or muscle levels [10] when compared to an uneven pattern of protein
235 intake. To confirm whether this relation occurs in a longitudinal outcomes study, we
236 investigated effects of protein intake pattern on LBM, muscle strength, and functional
237 outcomes over 8 weeks. As predicted by the results of our acute metabolic study [10], we
238 found no differences in LBM, strength, or functional outcomes between patterns
239 following 8 weeks of either dietary paradigm. The improvement in 1RM knee extension
240 is inconsistent with other outcomes in both EVEN and UNEVEN after 8-weeks and is
241 most likely a learning effect. The 1RM test was performed only once at the baseline,
242 while the other tests were familiarized and performed 2-3 times, which average scores
243 reported. In retrospect, an extra 1RM familiarization would most likely have eliminated
244 this incongruent finding and been consistent with the fact that no differences were
245 detected between groups in any of the functional tests.

246 Since stimulation of net anabolic response is the metabolic basis for maintaining
247 or increasing muscle mass [18], strength, and function [13], we also determined protein
248 kinetics at muscle and whole body levels before and after a 8-week of each dietary
249 intervention. Consistent with our previous findings [10] and extrapolated to the functional
250 outcomes of the present study, we found no pattern effects of protein intake on whole
251 body net protein balance and MPS before and after the 8-week dietary intervention. In
252 agreement with our findings, Murphy et al. [11] demonstrated that MPS responses were
253 not different in older adults subjected to UNEVEN (7/17/72/4% B/L/D, and pre-bed

254 snack) or UNEVEN (25% per each) diets at 1.3g protein/kg/day. However, Mamerow et
255 al. in relatively younger adults ($37 \pm 3y$) [19] showed that MPS responses were greater
256 with EVEN (33% per meal) compared to the UNEVEN (11/17/72% for breakfast, lunch,
257 and dinner) at 1.2g protein/kg/day. An age-associated anabolic resistance to protein-
258 containing meals [8,20,21] may be a potential explanation for the discordant findings. For
259 example, in the study by Mamerow et al. [19], the average amount of protein eaten per
260 meal ($\sim 0.39g$ protein/kg per meal) in the EVEN groups was greater than the “optimal”
261 protein dose ($0.24g/kg/meal$) that is effective for maximal MPS responses in young
262 subjects [22]. Thus, in the Mamerow work, the EVEN group achieved a maximal MPS
263 response every meal intake, while the UNEVEN group achieved a maximal MPS
264 response only with the dinner meal. In the present study, although similar amounts of
265 protein intake ($0.37g/kg/meal$ on average) in the EVEN group approaches the optimal
266 protein dose (i.e., $0.40 g/kg/meal$) for maximal MPS in older adults, it may be slightly
267 “suboptimal” to account for the majority of older subjects (i.e., $< 0.59g/kg/meal$) [22].
268 Moreover, it is likely that in the context of mixed meal, greater protein intake is required
269 to achieve similar aminoacidemia (particularly leucine) to trigger maximal MPS
270 responses. For example, it has been shown that peak leucine concentrations were 300%
271 of basal concentrations following only 7 g of EEA intake [21]. However, in the present
272 study, it was only 140% of the basal concentrations in EVEN groups with a far greater
273 amount of protein intake ($>14g$ EAA). Consistent with this finding, the pattern effect was
274 not discernable following mixed meal intake with higher protein in our previous study
275 [10]. Furthermore, a recent study supports our assertion by demonstrating a positive
276 association between protein intake, leg lean mass, and strength at a level of $\sim 45g$

277 protein/meal, or 0.55g protein/kg/meal [23]. Lastly, it may be argued that the null effect
278 would be due to sex differences with respect to MPS (i.e., lower anabolic response to
279 protein or amino acid in older women) [24]. If true, it would manifest as an increased
280 opportunity to observe a greater anabolic response in the EVEN pattern, as more women
281 were in the EVEN. Furthermore, as in our previous study [10], we found that both older
282 men and women responded similarly to mixed meals containing varying amounts of
283 dietary protein.

284 It is possible that the null finding may be due to an insufficient duration of the
285 intervention. However, the evidence for this hypothesis is not strong. A recent study
286 demonstrated that a 12-week EAA supplementation (1,260g EAA over 12 weeks)
287 resulted in a 1.7 kg gain of LBM in older women [25]. In the present study, the subjects
288 consumed approximately 448 g EAAs (32% EAA in the proteins) from the dietary
289 protein over the 8-week intervention period. Thus, if a pattern effect existed, we would
290 have expected an increase in LBM of approximately 0.60 kg, which did not occur.

291 It is primarily the stimulation of protein synthesis that leads to a greater net
292 protein balance following an ingestion of pure EAA or protein alone [21,26]. However, in
293 the context of mixed meal, the main driving force for the improved net protein balance at
294 the whole-body level, and potentially at muscle (though not measured), appears to be a
295 suppression of protein breakdown. In this present study, we presented whole body protein
296 kinetic data as changes from the fasted to the fed states (i.e., fed – fasted states), as these
297 metabolic changes would account for physiological outcomes. We found that 1.1 g/kg/d
298 of protein intake, regardless of the intake pattern, resulted in a positive protein balance
299 attributable to reductions in protein breakdown. In fact, protein synthesis was actually

300 reduced from the fasted to fed state. It is likely the insulin response to the mixed meal
301 was at least in part responsible for the decrease in protein synthesis [27]. Since the total
302 availability of intracellular EAA (i.e., from breakdown and inward transport from
303 plasma) is a primary determinant of protein synthesis [18] and total plasma leucine
304 responses were not different between patterns, it is likely that the reduction in protein
305 synthesis in response to a meal ingestion in both groups was an indirect suppressive
306 effect of insulin on protein breakdown. However, it is unlikely a major factor that
307 explains differences in the net anabolic response, since the insulin response to the two
308 intake patterns were similar.

309 Although our current results do not support the anabolic impact of an even pattern
310 of protein intake, increasing protein intake at each meal would promote protein intake
311 above RDA and realize the demonstrated effects in older adults [1,10,23]. In an older
312 population, protein intake is often confounded by a myriad of issues ranging from cost to
313 their ability to chew. Since the consolidated message of our work indicates that, with
314 regard to the anabolic response, the pattern of intake is of minimal importance as
315 compared to the amount of protein eaten [10], it is reasonable to recommend that daily
316 protein intake be distributed in a manner most convenient to an older population.

317 In conclusion, in older adults consuming an average amount of protein, the
318 distribution pattern of protein intake in mixed meals does not affect changes in lean body
319 mass, muscle strength or muscle function, nor result in changes in whole body net protein
320 balance or MPS after 8 weeks of dietary intervention.

321

322

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332 **Authors contributions:** I.-Y.K., A.A.F., and R.R.W. analyzed data and interpreted
333 results of experiments; I.-Y.K. performed calculations of protein kinetics. AS and HS
334 performed statistical analysis; I.-Y.K. prepared figures and drafted manuscript; I.-Y.K.,
335 and S.E.S. performed experiments; G.A. provided medical supervision; I.-Y.K., A.A.F.,
336 and R.R.W. research conception and design of experiments. All authors read and
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464 **TABLE LEGENDS**

465 **Table 1.** Values are expressed as means \pm SEM; M/F is the no. of male and female
466 subjects in each group; BMI, body mass index; LBM, lean body mass.

467 **Table 2.** Values are expressed as means \pm SEM; B, breakfast; L, lunch; D, Dinner.
468 Independent student t-test revealed no differences in daily total intakes of energy, protein,
469 fat, carbohydrate, and fiber. Significant differences in B, L, or D existed between the
470 EVEN and the UNEVEN as the study was designed for; * $p < 0.05$, ** $p < 0.01$, and
471 *** $p < 0.001$

472 **Table 3.** Values are expressed as means \pm SEM; 1RM, 1 repetition maximum. There
473 were no differences in any of the functional outcomes between the EVEN and the
474 UNEVEN (for all, $p > 0.05$). Power was calculated as body weight (kg) \times $9.8 \text{ m/s}^2 \times$
475 vertical height of the stairs (meters) \div time (sec.) [14].

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487 **FIGURE LEGENDS**

488 **Figure 1.** Infusion protocol utilized for each metabolic study before and after the 8 weeks
489 of each nutritional intervention.

490 **Figure 2.** Changes in the rates of 20-h whole body protein synthesis (PS), protein
491 breakdown (PB), and net protein balance (NB) from the fasted state before and after 8
492 weeks of each dietary intervention. Values are expressed as means \pm SEM.

493 **Figure 3.** Muscle protein fractional synthesis rate (MPS) with EVEN or UNEVEN before
494 and after 8 weeks of each dietary intervention. Values are expressed as means \pm SEM.

495 **Figure 4.** Area under the curve (AUC) for plasma glucose and insulin before and after 8
496 weeks of respective dietary intervention. Values are expressed as means \pm SEM.

497 **Figure S.1.** Plasma enrichments of infused tracers in the fasted states (A: d₅-
498 Phenylalanine; B: d₂-Tyrosine) before and after respective dietary intervention over 8
499 weeks. Values are expressed as means \pm SE. TTR, tracer-to-tracee ratio.

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Table 1. Group characteristics before and after 8-week dietary intervention

Groups	EVEN		UNEVEN	
	Pre	Post	Pre	Post
N (M/F)	7 (4/3)		7 (2/5)	
Age, yrs	58.1 ± 2.4		60.3 ± 2.4	
Height, cm	170.6 ± 3.3		170.9 ± 4.5	
Total mass, kg	80.4 ± 2.4	78.9 ± 2.1	79.7 ± 4.7	76.9 ± 4.8
BMI, kg/m ²	27.7 ± 0.6	27.2 ± 0.7	27.2 ± 0.7	26.3 ± 0.9
LBM, kg	50.5 ± 2.7	50.3 ± 3.1	47.7 ± 4.2	46.9 ± 4.1
Body fat mass, %	31.8 ± 2.6	31.5 ± 2.9	35.3 ± 2.0	34.3 ± 2.4

Table 2. Interventional diet during the entire study period

Groups		EVEN	UNEVEN
Daily energy intake, kcal	Total	2390 ± 139	2194 ± 162
	Per kg	29.7 ± 1.4	27.5 ± 0.9
Protein, g	B	29.3 ± 0.9	13.1 ± 0.8 ^{***}
	L	29.3 ± 0.8	17.7 ± 1.1 ^{***}
	D	29.2 ± 0.9	55.6 ± 3.6 ^{***}
	Total	87.8 ± 2.6	86.4 ± 4.9
Fat, g	B	25.8 ± 1.5	12.2 ± 0.8 ^{***}
	L	38.0 ± 2.1	25.1 ± 2.0 ^{***}
	D	29.0 ± 2.7	46.4 ± 4.0 ^{**}
	Total	92.8 ± 6.3	83.6 ± 6.6
Carbohydrate, g	B	100.0 ± 6.6	88.5 ± 5.2
	L	99.1 ± 3.1	84.9 ± 6.0
	D	109.4 ± 10.8	106.9 ± 11.5
	Total	308.5 ± 19.6	280.3 ± 21.8
Fiber, g	B	5.7 ± 0.4	5.2 ± 0.3
	L	9.8 ± 0.4	8.0 ± 0.6 [*]
	D	10.1 ± 0.7	9.9 ± 0.8
	Total	25.6 ± 1.3	23.0 ± 1.5

Table 3. Changes in muscle strength and function before and after 8-week dietary intervention

Groups	EVEN		UNEVEN	
	Pre	Post	Pre	Post
1RM. Knee Extension, kg	59.2 ± 5.6	73.1 ± 7.4*	45.8 ± 6.1	52.3 ± 8.7
Sit-Stand Speed, sec	10.4 ± 0.9	8.0 ± 1.0	10.5 ± 1.7	9.7 ± 1.2
10 m Gait Speed, sec	5.6 ± 0.6	5.0 ± 0.4	6.2 ± 0.6	6.7 ± 0.6
Handgrip strength, kg	37.5 ± 3.8	40.7 ± 4.5	33.0 ± 4.6	32.9 ± 3.9
Stair ascend power, Nm/s	347.9 ± 15.7	360.3 ± 30.3	290.6 ± 46.6	282.7 ± 46.6
Stair descend power, Nm/s	363.9 ± 16.8	401.1 ± 32.7	300.8 ± 53.7	304.6 ± 58.1







