



Buckley, C., Austin, S., Corfe, B., Green, M. A., Johnstone, A., Stevenson, E., Williams, E., & Brunstrom, J. (2019). Protein valuation in food choice is positively associated with lean mass in older adults: Protein valuation and fat-free mass index. *Journal of Nutrition*, [nxz124]. <https://doi.org/10.1093/jn/nxz124>

Peer reviewed version

Link to published version (if available):  
[10.1093/jn/nxz124](https://doi.org/10.1093/jn/nxz124)

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PDF-document

This is the author accepted manuscript (AAM). The final published version (version of record) is available online via Oxford University Press at <https://academic.oup.com/jn/advance-article/doi/10.1093/jn/nxz124/5518966>. Please refer to any applicable terms of use of the publisher.

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Protein valuation in food choice is positively associated with lean mass in older adults.

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Running title: Protein valuation and fat-free mass index

Sources of support:

This project was funded by the Research Councils UK ‘Priming Food Partnerships’ initiative supported by BBSRC, MRC, EPSRC and ESRC. Project reference number – BB/P023886/1.

Conflict of Interest: All authors declare no conflicts of interest

Word count: 4245

Number of figures: 2

Number of tables: 5

Supplementary data submitted: 1 supplementary table

Abbreviations:

BMI: body mass index, DEBQ: Dutch Eating Behaviour Questionnaire,

FFMI: fat-free mass index, FFQ: food frequency questionnaire

## 1 Abstract

2

3 **Background:** Calorie-for-calorie, protein is more satiating than carbohydrate or fat. However,  
4 it remains unclear whether humans perceive calories derived from these macronutrients equally  
5 and whether lean mass is associated with a tendency to ‘value’ protein when dietary decisions  
6 are made.

7 **Objective:** This study aimed to determine the test-retest reliability of a novel method for  
8 quantifying macronutrient valuations in human volunteers and to determine whether ‘protein  
9 valuation’ is associated with a higher fat-free-mass index in older adults.

10 **Design:** A two-alternative, forced-choice task in which 25 foods were compared in 300 trials  
11 was undertaken in two studies. In study 1, participants (age range 19-71 years,  $n=92$ ) attended  
12 two test sessions, spaced one week apart. In study 2, older adults (age range 40-85 years;  $n=$   
13 91) completed the food-choice task and assessed the test foods for liking, expected satiety, and  
14 perceived healthiness. Body composition and habitual protein intake were assessed in both  
15 studies. Data was analyzed using individual binomial logistic regressions and multi-level  
16 binomial logistic regressions.

17 **Results:** In study 1, measures of macronutrient valuation showed excellent test-retest  
18 reliability; responses in the forced choice task were highly correlated (week 1 vs week 2;  
19 protein  $r=0.83$ ,  $P<0.001$ ; carbohydrate,  $r=0.90$ ,  $P<0.001$ ; fat  $r=0.90$ ,  $P<0.001$ ). Calorie-  
20 for-calorie, protein and carbohydrate were stronger predictors of choice than fat ( $P<0.001$ ). In  
21 study 2, protein was a stronger predictor than both carbohydrate ( $P=0.039$ ) and fat ( $P=0.003$ ),  
22 and a positive interaction was observed between protein valuation and fat-free mass index  
23 (OR=1.64; 95% CI: 1.38, 1.95;  $P<0.001$ ). This was the case after controlling for age, gender,  
24 liking for foods, and habitual protein consumption.

25 **Conclusions:** Together, these findings demonstrate that adult humans value calories derived  
26 from protein, carbohydrate, and fat differently, and that the tendency to value protein is  
27 associated with greater lean mass in older adults.

28

29 **Keywords:** protein valuation; sarcopenia; food choice; body composition; fat-free mass  
30 index; lean mass; ageing

31

32

### 33 **Introduction**

34 Many modern foods are energy dense (kcal/g) and the role this plays in promoting energy  
35 intake has been explored extensively [1]. Measures of energy density are useful because they  
36 provide a guide to the total energy in a fixed portion of food. However, humans do not detect  
37 energy-density directly and foods with equal energy density might differ in their fat, protein,  
38 and carbohydrate content, each of which is absorbed and utilized in different ways [2].

39         Studies have considered how chronically high intakes of fats and carbohydrates can  
40 promote obesity and cardio-metabolic disease [3, 4]. Conversely, lower intake of protein is a  
41 risk factor for sarcopenia – an age-associated decline of skeletal muscle tissue that can  
42 influence physical functioning and quality of life [5-7]. Intervention studies have  
43 demonstrated a causal association: muscle function is impaired when protein intake is  
44 reduced [8] and protein supplementation produces a corresponding improvement [9].

45         Additionally, variation in chronic macronutrient intake is considerable [10] and is  
46 influenced by individual dietary decisions which, in turn, are governed by environmental  
47 (e.g., food availability and cost) and subjective factors (e.g., expected satiety and perceived  
48 healthiness). However, as with other omnivores, humans also have an inherent ability to  
49 discriminate foods based on their macronutrient composition and do so using both sensory  
50 information [11, 12] and via learning [13]. For example, low-protein diets promote the  
51 ingestion of savory, high-protein, foods [14, 15] and sweet tastes (related to carbohydrate) are  
52 selected after physical activity [16]. These observations imply that acute changes in  
53 physiological state can affect the way that humans *value* and prioritize energy derived from  
54 different macronutrients.

55         This distinction between habitual macronutrient consumption (typically measured  
56 using a food frequency questionnaire) and *macronutrient valuation* (an underlying disposition

57 to select foods according to how macronutrients are prioritized) is important. For example, an  
58 individual's selection of a fried breakfast over oatmeal might reflect high *fat valuation*, or it  
59 might otherwise reflect habit or a general desire for a larger meal (the absolute difference in  
60 fat might be incidental). Instead, high fat valuation would be evidenced when fat influences  
61 choice even when foods with almost identical amounts of fat are compared – a small  
62 difference plays a role because calories from fat are still 'noticed' and influence choice.  
63 Similarly, a person with high carbohydrate valuation would be sensitive to small differences  
64 in carbohydrate and would select the more carbohydrate rich of two foods even when low-  
65 carbohydrate containing foods are compared.

66         Here, we describe a novel approach that enables researchers to quantify underlying  
67 macronutrient valuations. After controlling for expected satiety and perceived healthiness, we  
68 then used this approach to explore individual differences. Specifically, in a second study we  
69 predicted that people with high protein valuation will have greater fat-free mass and explored  
70 this relationship in a group of older adults.

71

## 72 **Subjects and Methods**

### 73 *Study objectives*

74 Study 1 sought to investigate the test-retest reliability of our measure of macronutrient  
75 valuation and to quantify differences in the valuation of fat, carbohydrate, and protein. Study  
76 2 aimed to explore the relationship between protein valuation and fat-free mass in a group of  
77 older participants.

### 78 *Study 1:*

#### 79 **Subjects**

80 Ninety-two participants were recruited into the study. This was based on an earlier  
81 unpublished study which observed a small-to-medium effect size ( $r= 0.3$ ) of macronutrient  
82 valuation in food choice [17]. We determined that a minimum sample size of 90 participants  
83 would be required with an alpha of 0.05 [18]. Participants were recruited from the population  
84 of staff and students at the University of Bristol (UK) and from the surrounding area via an  
85 existing volunteer database and newspaper advertisements. To enable participants to  
86 complete the food-choice measures, they were required to have English as a first language or  
87 an equivalent level of fluency, and were excluded if they were vegan or vegetarian, or if they  
88 reported a food allergy or intolerance.

#### 89 **Procedure**

90 An online questionnaire was used to collect demographic information (age, gender, and  
91 postcode) and responses to the Dutch Eating Behaviour Questionnaire (DEBQ) [19]. On a  
92 separate day, participants attended the Nutrition and Behavior Unit (University of Bristol) for  
93 the first of two test sessions, held at the same time of day and one week apart. Each session  
94 lasted approximately 30 minutes and they were scheduled at the same time of day between

95 09:00 and 17:00. On arrival, participants read an information sheet and signed a consent  
96 form. They then completed the two-alternative forced-choice task, followed by measures of  
97 expected satiety, liking, perceived healthiness, and familiarity. At the end of the second test  
98 session bodyweight and height were measured using standardized protocols. Participants  
99 were then debriefed and offered £15 in remuneration for their assistance.

## 100 *Study 2:*

### 101 *Subjects*

102 Participants completed either an online questionnaire or a short telephone interview to  
103 confirm eligibility. The same exclusion criteria were applied as in study 1. However,  
104 participants were also excluded if they were pregnant or breast-feeding, had diabetes, were  
105 taking any medications that might affect their appetite, had recently started taking a  
106 medication, were undergoing hospital treatment, had a significant current or past psychiatric  
107 illness (including Alzheimer's and dementia), or had a current or previous eating disorder.  
108 After screening, ninety-one participants were invited to attend the Nutrition and Behavior  
109 Unit for a single session that that was scheduled between 09.00 and 17.00 and that lasted  
110 approximately 90 minutes.

### 111 *Method*

112 The beginning of the test session was identical to study 1. However, after the computer-based  
113 measures and the DEBQ, participants also completed a Food Frequency Questionnaire  
114 (FFQ). The online FFQ comprised 149-items and was based on the European Prospective  
115 Investigation into Cancer and Nutrition (EPIC) [20] – a version modified to include  
116 wholegrain and to assess intake over seven days. The FFQ was automated to analyze the  
117 nutritional composition of the diet and provided an estimate of the proportion (%) of dietary  
118 energy intake derived from protein. Gender, postcode and height were recorded, and



119 bioelectrical impedance analysis (Tanita Corporation: Body Composition Analyzer, BC-418  
120 MA III) was used to measure body mass, fat mass, and fat-free mass. Measures of body mass  
121 index (BMI, kg/m<sup>2</sup>), body-fat percentage, and fat-free mass index (FFMI, kg/m<sup>2</sup>) were  
122 derived from these data. FFMI was calculated by dividing fat-free mass by height squared  
123 [21]. At the end of the session, participants were debriefed and offered £15 in remuneration  
124 for their assistance.

125

### 126 *Ethics*

127 Both studies were conducted according to the ethical guidelines laid down in the Declaration  
128 of Helsinki and were approved by the University of Bristol Science Faculty Ethics  
129 Committee (approval codes: study 1: 52163, study 2:59121). Written informed consent was  
130 obtained from all participants. The aims and objectives of both studies were preregistered on  
131 the Open Science Framework [17, 22]. In each case, this incorporated pre-planned  
132 hypotheses as outlined in the introduction. No participant took part in both studies.

133

### 134 *Food evaluation tasks*

135 Images were taken of 25 different foods in 100-g portions. In a computerized two-alternative  
136 forced-choice task, images of two different foods were presented side-by-side on a computer  
137 screen. Every combination of food pairings was presented, rendering 300 binary-choice trials.  
138 The order of the trials was randomized (separately for each participant) and in each trial the  
139 relative position of each food (left or right) was allocated randomly. Participants were given  
140 the following instruction; “You will be shown two picnic foods, imagine this will be the only  
141 food you can eat between breakfast at 9am and dinner at 7pm and you must only pick one of  
142 the two foods.” Stimuli were carefully selected to include a range of foods that varied in

143 macronutrient composition and to minimize inter-correlations between sources of protein, fat,  
144 and carbohydrate. Respectively, correlations (Pearson  $r$ ) between calories derived from fat  
145 and protein, fat and carbohydrate, and protein and carbohydrate in the food images, were  
146 0.28, -0.33, and -0.36. Stimuli were also selected because they are referenced as foods that  
147 are commonly consumed in the United Kingdom [23]. **Table 1** includes a description of each  
148 food, together with its nutritional composition.

149         Expected satiety was measured by presenting an image of each test food alongside an  
150 image of a plate of rice. The portion of rice ranged in 20-kcal increments (20-800kcal) and  
151 participants adjusted the portion of rice until they were confident that both portions would  
152 reduce their hunger for the same amount of time. This and all other tasks were implemented  
153 using custom software written in Visual Basic (freely available on request).

154         Visual-analogue scales (VAS) were used to elicit ratings of healthiness and liking. For  
155 healthiness, the VAS was headed “How healthy is this food?” and anchored with “Not at all  
156 healthy” and “extremely healthy”. For liking, the VAS was headed “How much do you like  
157 the taste of this food?” and anchored with “I hate it” and “I love it”. In both cases, responses  
158 were assigned a value in the range 0 to 100. To assess familiarity, participants responded to  
159 the question “Have you eaten this food before?” with response options; “yes” or “no.”

160         In measures of expected satiety, liking, healthiness, and familiarity, each food was  
161 presented in turn and in a random order. The Dutch Eating Behavior Questionnaire (DEBQ)  
162 [19] was used to characterize trait dietary styles in our samples. Separate subscales assess  
163 restrained, emotional, and external eating. Participant postcodes were recorded, which were  
164 used to estimate ‘neighborhood deprivation’ – a proxy for socioeconomic status (SES) (Index  
165 of Multiple Deprivation; IMD, 2015).

166 *Statistical analysis*

167 All statistical analyses were conducted in the R environment [24] using the lme4 add-on  
168 package [25] and figures were created using the ggplot2 add-on package [26].

169 *Valuation of individual macronutrients*

170 In study 1, eight participants did not attend both sessions and were not included in the final  
171 analysis. Fifty-seven females and 27 males completed both test sessions. In study 2, data  
172 from one participant were excluded because of a computer error. Therefore, data from 23  
173 males and 68 females were analyzed. When a participant was unfamiliar with one of the  
174 foods then data from any associated trial were removed. On this basis, we excluded 3121  
175 (6.6%) trials in study 1 and 998 (4.2%) trials in study 2.

176 In the 2AFC task, for each participant and each trial, an ‘energy-density difference score’ was  
177 computed by subtracting the energy density (kcal/g) of the food presented on the left from the  
178 energy density of the food on the right. Separate difference scores were also calculated for  
179 calories derived from protein, fat, and carbohydrate, and for differences in expected satiety  
180 (kcal) and healthiness (mm). To enable direct comparison between expected satiety and  
181 healthiness, difference scores for these predictors were standardized within each participant.

182 Using binary logistic regression, we entered energy-density difference scores as predictors of  
183 choice. For study 1, a separate model was computed for each participant and each test session  
184 (84 x 2 models). For study 2, a single model was computed for each participant (91 models).

185 Using the same approach, we also generated models by entering differences in protein,  
186 carbohydrate, fat, expected satiety, and healthiness, as simultaneous predictors of choice (259  
187 models). In each model, every  $\beta$  coefficient was exponentiated to produce an odds ratio (OR)  
188 - an unbiased estimate of the relative contribution of each predictor as a determinant of  
189 choice. A protein OR refers to the odds of choosing the left-hand food when the left-hand

190 food contains 1 kcal/g more protein than the right-hand food. Similarly, an OR for  
191 carbohydrate or fat can be interpreted in the same way. Importantly, these three ORs quantify  
192 each macronutrient valuation. To determine whether fat, protein, and carbohydrate differ in  
193 valuation, a one-way ANOVA was used, with macronutrient type (protein, carbohydrate and  
194 fat) as a predictor of OR. Tukey-adjusted *post-hoc* tests were used to explore differences  
195 between individual macronutrients and *t*-tests were used to determine whether sets of ORs  
196 deviate from 1.0 (evidence that choice is influenced by a predictor).

#### 197 *Test-retest reliability*

198 For study 1, test-retest reliability was assessed by evaluating the association between  
199 participant odds ratios for the two test sessions. Separate Pearson's coefficients were  
200 computed for energy density and for each macronutrient.

#### 201 *Relationship between fat-free mass index and protein valuation*

202 Due to machine error, fat-free-mass was not recorded for seven participants and their data  
203 were excluded from this analysis. The remaining data comprised 24,201 trials from 84  
204 participants. To account for the intra-class correlation between individual participant  
205 responses [27] a multi-level (rather than a standard GLM) binary-logistic modelling approach  
206 was adopted.

207 *Basic model:* For study 2, Our objective was to explore the extent to which protein valuation  
208 is associated with a higher fat-free mass index (FFMI). For each trial, the difference between  
209 the protein content (standardized kcal/g) of the two foods was entered as a predictor of  
210 choice. The associated OR from the model provides a measure of protein valuation across  
211 participants. We also specified the interaction between protein difference and FFMI. A  
212 positive interaction indicates that protein valuation is stronger in participants with greater

213 muscle mass. In addition, ‘participant’ was entered as a random factor, and age and gender  
214 were included as covariates.

215 *Extended model:* In an extended model, we specified an identical model that also included  
216 liking difference scores and their interaction with protein-difference scores. The model also  
217 incorporated habitual protein intake (% energy in diet) and the interaction between habitual  
218 protein intake and protein-difference scores. Note that the main effect of habitual protein  
219 intake was not expected to predict choice (*i.e.*, make participants preferentially choose the left  
220 option), but was included to properly assess the interaction with protein difference. As above,  
221 a positive interaction between FFMI and protein difference indicates that protein valuation is  
222 stronger in individuals with a higher fat-free mass, and that this is independent of liking for  
223 high-protein foods or habitual protein consumption. For this model, one participant was  
224 excluded due to missing responses on the food frequency questionnaire. An exploratory  
225 analysis was also conducted, extending the extended model to add an interaction term between  
226 gender, protein difference and FFMI.

227 For both multilevel models, odds ratios, confidence intervals, and *p*-values are reported, and a  
228 main or interaction effect was regarded as a significant predictor of choice if the 95%  
229 confidence interval for an odds ratio failed to cross 1.0. To enable a direct comparison of  
230 their relative importance, all variables were standardized before entering them into the food-  
231 choice models. Unless specified otherwise, data are presented as means  $\pm$  SDs.

232

233

234 Results

235 *Participant demographics*

236 Ninety-two participants completed study 1 (68% female). Their ages ranged from 19 to 71  
237 years ( $24.9 \pm 7.30$  years) and their mean BMI was  $23.0 \pm 3.9$  kg/m<sup>2</sup>. Ninety-one participants  
238 completed study 2 (75% female). They had mean BMI of  $26.2 \pm 4.3$  kg/m<sup>2</sup> and their ages  
239 ranged from aged 40 to 85 years ( $60.6 \pm 12.2$  years). **Table 2** provides additional  
240 demographic information about the participants in both studies.

241

242 *Study 1: Test retest reliability*

243 **Figure 1** shows relationships between odds ratios obtained from separate participants in  
244 session 1 and session 2. Respectively, panels, A, B, C and D show associations for fat,  
245 carbohydrate, protein, and overall energy density. In each case, we observed strong positive  
246 relationships, indicating excellent test-retest reliability across sessions; protein  $r= 0.71$ ,  $P<$   
247  $0.001$ , carbohydrate  $r= 0.97$ ,  $P< 0.001$ , fat  $r= 0.90$ ,  $P< 0.001$ , energy  $r= 0.86$ ,  $P< 0.001$ .  
248 Inspection of Figure 1 also shows considerable individual variability in the relative  
249 importance of food characteristics as predictors of choice and that this variability is quite  
250 stable over a one-week period.

251 *Liking and familiarity*

252 The foods were well liked (study 1:  $67.9 \pm 10.6$  mm, range = 39 – 93 mm; study 2:  $66.1 \pm$   
253  $11.2$  mm, range = 42 -77 mm) and familiar (study 1,  $96.5 \pm 4.1\%$ , range = 82 – 100%; study  
254 2,  $98\% \pm 2.8\%$ , range = 88 – 100%). **Table 3** shows descriptive statistics (means and SDs)  
255 for individual foods and for each study, separately.

256

257 *Energy density as a predictor of choice*

258 In study 1, as anticipated, energy density was a positive predictor of choice in both session 1  
259 (OR= 1.08; 95% CI: 1.06, 1.10;  $P < 0.001$ ) and session 2 (OR= 1.05; 95% CI: 1.03, 1.07;  $P <$   
260  $0.001$ ). These odds ratios show that when two foods differ in energy density by 1 kcal/g then  
261 the more energy dense food was 8% more likely to be selected in session 1 and 5% more  
262 likely to be chosen in session 2. These effects are small, but statistically significant. By  
263 contrast, energy density (kcal/g) was a non-significant predictor (OR did not deviate from  
264 1.0) of choice in study 2 (OR= 1.02; 95% CI: 0.93, 1.11;  $P=0.642$ ).

265

266 *Individual macronutrients and psychological variables as predictors of choice*

267 **Figure 2** shows the extent to which protein, carbohydrate, fat, expected satiety, and  
268 healthiness played a role in food choice. In each case, separate odds ratios are provided for  
269 study 1 and study 2. Because we observed very good test-retest reliability (see Figure 1), for  
270 each participant, we averaged separate odds ratios across sessions in study 1. Odds ratios for  
271 protein, carbohydrate and expected satiety (but not fat or healthiness) were significantly  
272 larger than 1, suggesting they independently influence food choice. Associated statistics are  
273 summarized in **Supplementary Table 1**. One-way ANOVA confirmed that average odds

274 ratios also differed across macronutrients,  $P < 0.001$ . Tukey-adjusted *post-hoc* tests showed  
275 that carbohydrate ( $P < 0.001$ ) and protein ( $P < 0.001$ ) were stronger predictors of choice than  
276 fat. There was no difference in odds ratios for protein compared to carbohydrate ( $P = 0.775$ )  
277 and the difference between expected satiety and healthiness was marginal ( $P = 0.055$ ).

278

279 Mean odds ratios for protein, carbohydrate, and fat also differed in study 2  $P = 0.003$ . Tukey-  
280 adjusted *post-hoc* tests demonstrated that protein had a stronger influence on choice than  
281 carbohydrate ( $P = 0.039$ ) or fat ( $P = 0.003$ ), and there was no difference between odds ratios for  
282 carbohydrate compared to fat ( $P = 0.654$ ). Odds ratios for carbohydrate and fat did not differ  
283 ( $P = .654$ ) and healthiness was a stronger predictor of choice than expected satiety ( $P < 0.001$ ).



284 *How do individual differences in protein valuation interact with body composition to predict*  
285 *choice?*

286 **Table 4** and **Table 5** summarize the basic and extended models used to explore the  
287 interaction between body composition and protein valuation. The basic model showed a  
288 positive interaction between protein valuation and fat-free mass index as a predictor of food  
289 choice. A difference in protein (kcal/g) is a stronger predictor of choice in individuals with a  
290 higher fat-free mass index, after controlling for age and gender ( $P<0.001$ ). The extended  
291 model indicates that this interaction is also observed after controlling for liking and habitual  
292 protein consumption. In this model, for an individual with a higher fat-free mass index (+1  
293 SD), a 1 kcal/g (standardized) difference in protein content is associated with increased odds  
294 of 64% for choosing that food. ( $P<0.001$ )

295

296

## 297 Discussion

298 Numerous studies have explored the relationship between food energy density, food intake,  
299 and food preference [28]. Here, we introduce a novel method that quantifies the underlying  
300 value that humans place on a calorie derived from fat, carbohydrate, and protein. Study 1  
301 shows that protein and carbohydrate tend to be valued more than fat (compared calorie for  
302 calorie). However, we also observed considerable variability across individuals. Indeed, these  
303 differences showed excellent test-retest reliability across two sessions, held one week apart.  
304 In study 2, protein was valued more than carbohydrate and fat and, again, we observed the  
305 same variability across individuals. In study 2 we also found that individuals with a higher  
306 fat-free mass index show greater protein valuation. Since body composition can be influenced  
307 by protein consumption [8] this correspondence with protein valuation further validates our  
308 approach.

309 Note that the relationship between FFMI and protein valuation was observed after  
310 controlling for age, gender, liking for foods, and habitual protein consumption. In other  
311 words, protein valuation appears to be associated with FFMI and this occurs even after  
312 controlling for an estimate of protein consumption obtained from a widely used FFQ.  
313 Following other work [29] we transformed the OR (1.64) for this interaction term into a  
314 Cohens D. The associated effect size ( $D = 0.27$ ) indicates that the effect of differences in  
315 protein valuation is small but could be important at a population level.

316 In relation to the above, the interaction between habitual protein consumption  
317 (measured by FFQ) and ‘protein difference’ (Table 4) also merits careful consideration. A  
318 significant interaction would indicate that people who report consuming a high protein diet  
319 are especially sensitive to small differences in the protein content of food pairs in the choice  
320 task, and selected foods on this basis. This interaction was not observed, suggesting that

321 protein valuation is not governed exclusively by differences in self-reported protein intake.  
322 Again, to clarify this distinction, protein valuation refers to an underlying sensitivity to small  
323 differences in protein, which biases *all* food choices. By contrast, food-frequency  
324 questionnaires provide an estimate of habitual protein intake, which, in turn, will also be  
325 governed by cost, availability, liking and so on [30]. This distinction between protein  
326 valuation and habitual protein intake is important - high valuation will promote greater  
327 protein intake, but this relationship is not axiomatic – a person might have high valuation, but  
328 low protein intake due to food availability. Conversely, a high protein intake might be  
329 reported (perhaps governed by family shopping habits) even in someone with low valuation.  
330 In other words, there might be multiple interacting determinants of total protein intake  
331 including both opportunity (the environment) and valuation.

332         The temporal direction of the association between protein valuation and FFMI is  
333 currently unclear. One possibility is that muscle mass plays a causal role in food choice –  
334 higher protein valuation reflects a bias that serves to ensure that a biologically determined  
335 level of muscle mass is preserved. Alternatively, differences in protein valuation may occur  
336 for other reasons and, over the life span, they have a secondary and incidental effect on  
337 muscle mass. We suspect the latter is more likely because there is little evidence that  
338 sarcopenia is associated with an increased preference for protein (indeed, the converse seems  
339 more likely) [7] Indeed, individuals with reduced protein valuation may be particularly  
340 vulnerable to sarcopenia as they age. If correct, then our methods might be applied to identify  
341 individuals for targeted dietary advice, before age-related muscle deterioration occurs. This is  
342 important because a 30-40% decrease in muscle mass occurs between the ages of 40 and 80  
343 [31], which suggests that interventions should occur in the fourth decade of life [6].

344         In future, studies might incorporate a measure of physical activity, which is known to  
345 influence muscle synthesis after protein intake [32] and a major factor influencing muscle

346 wastage is low physical activity during ageing [33]. Indeed, the combined effects of low  
347 protein valuation and low physical activity might place an individual at an even greater risk  
348 of muscle loss during ageing. Other determinants of protein intake such as socioeconomic  
349 status may be used alongside our method to clarify the relationship between protein  
350 valuation, protein intake and physical activity, particularly as risk factors for protein  
351 undernutrition and sarcopenia. A second future question relates to whether humans  
352 discriminate the protein quality (amino acid profile) of different sources of protein and show  
353 differential protein valuation on this basis. Animal and plant-based sources might be  
354 compared, addressing both fundamental questions and broader concerns about food security,  
355 the environment, and health [6].

356         We also observed a three-way interaction between gender, FFMI, and difference in  
357 protein content. This was not an *a priori* prediction and therefore the study was not powered  
358 to investigate gender-related differences in protein valuation and their relation to FFMI.  
359 However, it is worth noting that sarcopenia develops at a different rate in men and women  
360 [34]. In our healthy community dwelling sample, we saw little evidence that protein valuation  
361 changes markedly with age. Again, in an appropriately powered sample this might be  
362 investigated. A further step would be to administer this task to people with sarcopenia to test  
363 the prediction that extreme muscle deterioration is associated with especially low protein  
364 valuation. Note that although FFMI has been used to assess sarcopenia previously [35]  
365 assessments of muscle strength might also be incorporated in this context.

366         In addition, we see opportunities to apply our methods to address fundamental  
367 questions about human appetite control. Various sources indicate that omnivores adapt their  
368 dietary behavior in response to periods consuming a low protein-containing diet [36]. Some  
369 indicate a strategic orientation towards high protein foods [14, 15] and others suggest a more  
370 general adaptation whereby overall intake is increased to mitigate a shortfall in protein [37].

371 However, in both cases the evidence is mixed [38] and is limited in humans. Typically, a  
372 selective preference for protein is measured by direct observation of food choices over a short  
373 period [39]. One possibility is that a shift in macronutrient prioritization is manifest as a  
374 ‘nudge’ towards the selection of protein across all foods in the diet rather than a selective  
375 preference for specific foods that have high protein content. As we have already noted, food  
376 choice may be governed largely by habit and by a more general desire to consume alternative  
377 foods after a monotonous (low protein) diet [40]. Hence, observations of food intake may  
378 lack the sensitivity that is needed to detect subtle strategic changes in protein prioritization. If  
379 correct, our measure of protein valuation might be particularly useful alongside more  
380 traditional forms of assessment [30].

381 In summary, previous methods for assessing macronutrient intake have tended to rely  
382 on self-report (FFQ and diet diaries). Here, we approach the problem of quantifying  
383 macronutrient prioritization from a very different perspective – specifically, we introduce a  
384 method that quantifies and focuses on *sensitivity* to differences in macronutrient composition  
385 rather than overall macronutrient intake. Our novel methods capture aspects of behavior that  
386 are orthogonal to these traditional approaches, show excellent test-retest reliability, and that  
387 are associated with a measure of muscle mass. We have highlighted areas where our  
388 approach might be refined, and we see exciting opportunities for its application, both in  
389 clinical and fundamental research.

390

## Acknowledgements

### Author contributions:

The authors responsibilities were as follows – CB and SA collected the data; CB analyzed the data; CB and JB wrote the manuscript; all authors designed the study, interpreted the data and critically revised the manuscript. All authors read and approved the final manuscript. The authors have no conflicts of interest to declare.

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Table 1. Nutritional composition of test foods in study 1 and 2

Food	Energy density (kcal/g)	kcal per 100 g		
		Protein	Carbohydrate	Fat
Apple	0.5	2.0	48.0	4.5
Avocado	2.0	7.6	7.6	175.5
Bacon	2.3	103.2	4.0	124.2
Bagel	2.6	41.2	195.6	11.7
Baked beans	0.8	18.8	51.6	1.8
Banana	1.0	4.8	92.0	4.5
Blueberries	0.5	3.6	36.4	4.5
Broccoli	0.4	17.2	12.4	5.4
Cheddar cheese	3.3	113.2	8.4	203.4
Chicken	1.1	95.6	2.0	14.4
Coleslaw	1.8	3.2	21.6	153.0
Crumpets	2.1	26.4	170.4	9.9
Egg	1.4	56.4	2.0	86.4
Grapes	0.7	2.0	61.6	4.5
Ham	1.1	76.0	6.8	20.7
Mediterranean vegetables	0.6	4.4	31.6	15.3
Mushrooms	0.2	7.2	2.0	4.5
Pasta	1.6	20.4	130	6.3
Potato salad	1.4	4.0	42.4	91.8
Potato waffle	1.8	10.0	88.0	78.3
Prawns	0.6	56.4	2.0	4.5
Sausage	2.5	52.0	26.8	168.3
Smoked salmon	1.9	80.4	13.2	92.7
Sweet potato	0.9	4.4	75.6	4.5
Tuna	1.1	108	2.0	4.5

Table 2. Participant demographic information for men and women aged 19-85 y in study 1 and 2<sup>1</sup>

	Study 1 n= 84 (female =57)	Study 2 n= 91 (female= 68)
% female	67.8	74.7
Age (years)	25.1 ± 8.4 (19-71)	60.6 ± 12.3 (40-85)
BMI (kg/m <sup>2</sup> )	23.0 ± 4.2 (14.7-29.7)	26.2 ± 4.3 (18.2-37.9)
FFMI (kg/m <sup>2</sup> )	Not measured	17.3 ± 2.5 (12.4-24.3)
Habitual protein consumption (% of total energy)		14.3 ± 2.5 (9.1-21.5)
Index of multiple deprivation	14.2 ± 9.3 (2.6-46.7)	16.7 ± 12.1 (2.6-53.3)
DEBQ emotional	2.4 ± 0.8 (1.0-4.6)	2.2 ± 0.8 (1.0-3.8)
DEBQ external	3.3 ± 0.6 (1.9-3.9)	3.0 ± 0.6 (1.3-4.9)
DEBQ restraint	2.4 ± 0.7 (1.0-3.9)	2.9 ± 0.8 (1.1-4.8)

<sup>1</sup>Values are means ± SDs (ranges) or percentages

Note: BMI: Body Mass Index; FFMI: Fat-free mass index; DEBQ: Dutch Eating Behaviour Questionnaire; Fat-free mass index.

Table 3. Ratings for liking, healthiness, expected satiety and familiarity from men and women aged 19-85 y in study 1 and 2. <sup>1</sup>

Food	Study 1				Study 2			
	Liking	Healthiness	Expected Satiety	Familiarity	Liking	Healthiness	Expected Satiety	Familiarity
Apple	74.5 ±18.8	87.6±10.5	146.8±109.8	100	78.6±19.4	90.1±9.7	183.3±108.5	100
Avocado	63.2±30.9	83.6±13.0	198.1±107.3	94	66.2±32.1	80.1±19.2	226.2±105.4	98
Bacon	71.5±25.9	16.3±17.4	282.4±128.4	98	70.9±24.7	23.6±19.5	302.6±133.9	100
Bagel	68.8±21.3	32.1±16.9	266.4±131.3	98	48.1±25.8	32.5±17.0	264.0±120.1	92
Baked beans	55.5±26.1	48.6±21.3	178.6±118.4	95	62.9±24.1	65.9±20.2	216.0±108.5	100
Banana	92.7±18.5	85.7±12.7	165.4±86.9	99	77.8±22.9	83.8±13.9	223.7±93.7	99
Blueberries	75.3±22.3	90.9±11.9	142.4±102.5	98	74.6±26.0	90.9±10.3	174.1±100.8	97
Broccoli	65.6±26.2	95.0±6.2	137.4±73.1	98	68.0±26.2	91.3±8.7	173.4±98.0	97
Cheddar Cheese	73.1±23.9	30.9±19.2	227.1±113.7	100	77.1±21.1	45.6±21.4	293.6±139.6	99
Chicken	77.3±17.1	71.7±18.8	218.8±119.7	99	79.9±17.2	77.1±16.7	255.4±110.8	100
Coleslaw	38.8±29.7	30.9±19.2	148.7±113.4	82	48.3±26.9	40.0±19.5	178.7±92.1	97
Crumpets	66.1±22.2	30.5±18.1	239.5±98.6	92	60.2±25.3	28.7±15.9	257.4±119.7	100
Egg	66.5±26.9	76.4±14.6	183.5±90.8	99	71.4±24.6	75.5±15.9	210.5±100.9	100
Grapes	81.1±18.3	83.7±15.3	99.8±75.9	99	82.6±15.3	85.2±13.4	150.3±127.8	100
Ham	54.8±24.2	38.1±24.9	200.5±98.7	99	58.7±24.5	32.6±22.2	260.4±123.5	100
Mushrooms	63.4±27.3	83.8±14.0	142.4±103.2	99	74.2±23.9	80.5±16.7	177.8±125.8	99
Pasta	70.1±21.9	44.7±17.9	231.3±105.1	100	59.5±23.8	52.2±19.7	240.2±101.3	98
Potato salad	52.9±24.9	32.7±17.8	169.2±91.2	95	48.1±27.0	37.2±16.5	184.4±84.7	97
Prawns	63.4±25.8	21.1±13.9	214.4±96.7	90	68.5±28.5	73.3±18.8	215.4±97.2	97
Potato waffle	67.4±26.7	70.1±17.3	193.2±79.7	96	42.3±28.9	20.0±14.9	239.8±108.1	88
Sausage	69.3±25.0	20.9±15.4	218.8±98.6	100	57.6±28.2	21.0±17.1	240.7±108.9	99
Smoked Salmon	73.6±25.1	71.0±17.0	204.7±106.9	94	67.4±31.8	69.6±20.5	221.5±121.8	96

Sweet potato	77.9±19.5	74.0±18.0	232.0±95.3	96	67.4±27.2	79.9±16.0	239.8±89.0	99
Tuna	60.7±26.0	73.4±16.8	211.3±95.1	94	61.5±29.8	76.9±19.4	249.7±111.6	96
Vegetables	74.4±20.7	83.4±13.7	149.9±82.0	99	79.7±20.5	81.3±15.1	194.7±85.2	100

<sup>1</sup> Values are means ± SDs or percentages, liking and healthiness are measured on a 0-100 scale and expected satiety is measured in kcal. Familiarity is the proportion (%) of participants that indicated they were familiar with the food.

Table 4. Summary of fixed parts of two hierarchical multilevel binomial logistic regressions predicting food choice from protein content, fat-free mass index, habitual protein consumption and liking for men and women aged 19-85 y in study 1 and 2.<sup>1</sup>

Fixed parts	Basic model		Extended model	
	OR (95% CI)	<i>P</i>	OR (95% CI)	<i>P</i>
(Intercept)	1.02 (0.87,1.20)	0.780	0.97 (0.79,1.19)	0.775
Protein difference	1.13 (0.99,1.30)	0.790	0.86 (0.72,1.02)	0.075
Protein difference x FFMI	1.47 (1.28, 1.70)	<0.001	1.64 (1.38,1.95)	<0.001
Protein difference x Age	1.00 (1.00,1.00)	0.815	1.00 (1.00,1.01)	<0.001
Protein difference x Gender <sup>a</sup>	1.26 (1.15,1.39)	<0.001	1.37 (1.22,1.53)	<0.001
FFMI x Gender <sup>a</sup>	1.03 (0.94,1.12)	0.518	1.09 (0.98, 1.22)	0.100
Protein difference x FFMI x Age	1.00 (0.99,1.00)	0.001	0.99 (0.99, 1.00)	<0.001
Protein difference x FFMI x Gender <sup>2</sup>	0.84 (0.78,0.90)	0.001	0.78 (0.71,0.85)	<0.001
Liking difference			5.54 (5.28,5.82)	<0.001
Protein difference x Habitual protein consumption %			1.02 (0.99,1.05)	0.214
Protein difference x Liking difference			1.05 (1.00,1.10)	0.065

<sup>1</sup>age and gender are covariates in the model

<sup>2</sup>reference group =female.

Note: In a separate analysis, carbohydrate and fat difference scores were added to the extended model as an interaction term with FFMI.

Carbohydrate difference negatively interacted with FFMI to predict food choice (OR= 0.86; 95% CI: 0.83,0.91; *P*<0.001) and fat difference did not interact with FFMI to predict food choice (OR= 1.00; 95% CI: 0.97, 1.04; *P*=0.901).

*Table 5. Summary of random parts of two hierarchical multilevel binomial logistic regressions predicting food choice*

Random parts	Basic Model	Extended model
$\tau_{00}$ , Participant	0.005	0.009
$N_{\text{Participant}}$	84	83
$ICC_{\text{Participant}}$	0.002	0.003
Observations	24201	23925
Tjur's D	0.010	0.320
Deviance	33269.496	24258.752

Note: ICC: Intra class correlation

Figure 1. Relationships between odds ratios obtained for men and women aged 19-71 y in session 1 and 2 in study 1.<sup>1</sup>

<sup>1</sup>Separate panels show associations for A) fat, B) carbohydrate, c) protein and d) energy

Note: minor dashed lines represent OR= 1 (no significant effect on food choice). Major dashed lines show the correlation (shaded  $\pm 1$  95% CI) between participant odds ratios between sessions. Each data point shows two odds ratios, obtained from a single participant (total  $n= 84$ ) tested on two occasions, separated by a one-week interval.



Figure 2. Box and whisker plots describing odds ratios for predictors of choice for men and women aged 19-81 y in study 1 and study 2.<sup>1</sup>

<sup>1</sup>Separate panels show ORs for macronutrients in Study 1 (a) and study 2 (B) and psychological predictors (expected satiety and healthiness) for Study 1 (C) and Study 2 (D).

Note: Dashed line indicates no effect on choice. In cases where a 95% confidence interval fails to cross this line then the associated variable has a non-random effect on choice. Odds ratios for study 1 were averaged across test sessions. For all figures, black triangle indicates mean odds ratio, black line represents the median, the upper edge of the box represents the 75% quartile, and the lower edge represents the 25% quartile. Black dots represent outliers.