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Plant protein can be as efficient as milk protein to maintain fat free mass in old rats, even when fat and sugar intakes are high

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Running title: Blended plant protein adequately replaces milk proteins

Abbreviations: AA: amino acids; AIN: American Institute of Nutrition; ANOVA; analysis of variance; ASR: absolute synthesis rates; FSR: fractional synthesis rates; IAA: indispensable amino acids; MHE: milk proteins, high energy; MSE: milk proteins, standard energy; PHE: plant proteins, high energy; PSE: plant proteins, standard energy; SE: standard error.

1 *Abstract*

2 Background: Alternative, sustainable and adequate sources of protein must be found
3 to meet global demand. Objective: The aim of this study was to assess the effect of a
4 plant-protein blend with a good balance of indispensable amino acids and high
5 contents of leucine, arginine and cysteine on the maintenance of muscle protein
6 mass and function during aging in comparison to milk proteins, and to determine if
7 this effect varied according to the quality of the background diet. Methods: Old male
8 Wistar rats (n = 96, 18 months old) were randomly allocated to four diet groups,
9 differing according to protein source (milk or plant blend) and energy content
10 (standard, with starch, or high, with saturated fat and sucrose), for 4 months. Plasma
11 biochemistry and body composition (echo MRI) were measured every two months.
12 Muscle functionality (Catwalk XT system) was assessed before and after the
13 experimental diet. After 4 months of diet, animals were injected with a flooding dose
14 of L-[1-13C] valine, and hind limb muscles, liver and heart were sampled and
15 weighed. Results: We found that the plant protein blend stimulated muscle protein
16 synthesis, maintained lean body mass, muscle mass, and muscle functionality during
17 aging as well as milk protein. Liver and heart weights were unchanged. The high fat
18 high sucrose diet increased body fat but, assessed by fasting plasma glucose and
19 insulin, had little impact on insulin sensitivity and related metabolisms. Therefore, we
20 could not test the hypothesis that in situations of higher insulin resistance, our plant
21 protein blend rich in leucine, arginine and cysteine may be better than milk protein.
22 Conclusion: Finally, the present findings offer significant proof of concept from the
23 nutritional standpoint that appropriately blended plant proteins can have high
24 nutritional value even in demanding situations such as ageing protein metabolism.

26 Keywords: plant proteins; aging; sarcopenia; obesity; muscle protein synthesis;
27 muscle functionality.

28

29 INTRODUCTION

30 By the year 2050, the combined increase in the world's population and living
31 standards in developing countries will lead to a very considerable rise in the demand
32 for dietary protein, especially from animal sources (1). This demand cannot be met
33 sustainably by increasing animal production, meaning that alternative sources of
34 protein must be developed. The simplest feasible option in the short term is to take
35 better advantage of the large amount and diversity of plant proteins already available.
36 The co-products of the oilseed and starch industry contain significant amounts of
37 proteins (15-50%). Their use in human nutrition is at present negligible and should be
38 developed (2).

39 Our objective falls in this context of diversifying protein resources for humans, with
40 the aim of tackling food insecurity while taking into account the sustainability of food
41 systems (exploitation of co-products). However, a key issue will be to use plant
42 proteins to develop protein-rich foods able to compete with animal products (meat,
43 eggs, milk), which are considered as providers of reference proteins in human
44 nutrition. Indeed, besides technical and organoleptic issues, one of the main
45 obstacles of using plant proteins in human nutrition is generally the low nutritive value
46 classically attributed to them, because of their unbalanced composition in
47 indispensable amino acids (IAA), and concerns regarding digestibility (3). Indeed,
48 amino acid (AA) bioavailability may be an issue for plant proteins, but when proteins
49 are extracted from their natural matrix and properly processed, their true ileal
50 digestibility is generally high (84-94%), and only slightly lower than that of animal
51 proteins (90-95%) (4). The unbalanced composition of individual plant proteins could
52 be readily solved by combining them. Finally, by taking advantage of a wider range of

53 available plant proteins and combining them, it should be possible to identify and
54 produce protein blends with tailored AA composition and high digestibility (5, 6).

55 This optimized AA composition can even be targeted for a specific population.

56 Undoubtedly, the most important problem to solve in the coming years is feeding the
57 elderly. Demographic trends in Western countries are characterized by the gradual
58 aging of the population and increased longevity (1/4 of the population in Europe and
59 North America will be over 65 in 2050 -

60 https://population.un.org/wpp/publications/files/wpp2019_highlights.pdf). Sarcopenia,
61 defined as the gradual decline in muscle mass and function, is a major feature of
62 aging. It is an inevitable physiological process, occurring even in healthy elderly
63 subjects, which increases the risk of loss of independence, falls and fractures, and
64 decreases resistance to nutritional, infectious or traumatic stresses (7). Muscle mass
65 is controlled by the balance between protein synthesis and degradation. It was
66 shown that the post-prandial stimulation of muscle protein synthesis and inhibition of
67 muscle protein degradation decreases during aging, with the development of
68 anabolic resistance (8). Thus, besides inactivity and protein undernutrition, other
69 factors like oxidative stress, inflammation (9), or insulin resistance (10) can
70 accelerate sarcopenia by worsening muscle anabolic resistance. Given these
71 phenomena, how can we optimize the AA composition of plant protein blends for
72 elderly subjects?

73 First, there is a risk of mismatch between requirements and intakes in the elderly,
74 because of an increased requirement and a decreased appetite (11). A significant
75 proportion of the elderly population may have marginal intake with regards to protein
76 requirements (12). In this specific context, the quality of protein intake in terms of IAA
77 supply is considered to be a key factor for optimal protein metabolism. In addition,

78 leucine is a potent stimulator of protein synthesis, and supplementation with this AA
79 was shown to restore muscle postprandial anabolism during aging (13, 14). Arginine,
80 as a precursor of NO, could play a major role in the provision of AA for muscles.

81 Indeed, the role of NO in the peripheral extraction of nutrients in response to insulin
82 has been established (15, 16). Supplementation with arginine was shown to increase
83 muscle perfusion in the elderly (17), although the effect on anabolism depends on the
84 level of supply of the other AAs. Cysteine is involved in the determination of the redox
85 status, mainly because of its presence in glutathione, the main molecular antioxidant
86 of the body. This redox status is altered with aging (18). The requirement for cysteine
87 seems to increase with aging because of changes in metabolic demand in relation
88 with oxidative stress, low grade inflammation, and increased medication use (19). In
89 line with this, long-term supplementation with cysteine was shown to delay loss of
90 muscle mass in aging (20, 21). Thus, we reasoned that a blend of plant protein with
91 an excellent IAA balance and particularly rich in leucine, arginine, and cysteine may
92 be an adequate diet for maintaining muscle mass and function during aging.

93 Using linear programming (5), we designed a plant protein blend with an excellent
94 IAA balance and high levels of leucine, arginine and cysteine, and used it as the
95 unique protein source in the diet of aging rats (final age 22 months) in a 4-month
96 study. The objective was to test if this blend could maintain lean body mass, muscle
97 mass and function as well as muscle protein synthesis rates, in comparison to protein
98 obtained from milk, recognized as an excellent protein source. This experiment was
99 performed in the context of a standard diet (22), and a high fat high sucrose diet
100 which better reflects western diets, with the expectation of increased insulin
101 resistance and related metabolic alterations. We showed that this blend of plant
102 proteins was as efficient as milk proteins to prevent sarcopenia during aging.

103

104 METHODS

105 *Animals and diet*

106 This experiment was conducted in accordance with institutional guidelines on animal
107 experimentation in France and was approved by the Ethics Committee in the Matter
108 of Animal Experiments of Auvergne (registration number: CE 56-12). Male 15-month-
109 old Wistar rats (n=96) (Janvier, France) were housed under controlled environmental
110 conditions (21°C, hygrometry 55%, 12-h dark period starting at 07:00) and were
111 allowed free access to water and standard pellets (UAR 04, UAR, France). After
112 three months, animals were randomly divided into four groups (n=24 per group) fed
113 four different diets: 1) MSE (milk protein, standard energy) rats were fed the AIN 93
114 diet (22) with milk proteins replacing casein; 2) PSE (plant proteins, standard energy)
115 rats were fed the AIN 93 diet with a mix of plant proteins replacing casein; 3) MHE
116 (milk proteins, high energy) rats were fed a diet with milk proteins and high fat and
117 sucrose content; 4) PHE (plant proteins, high energy) rats were fed a diet with the
118 same mix of plant protein as PSE rats and a high fat and sucrose content (see
119 detailed diet composition in Table 1). The plant protein mix contained 22.1% lupine
120 protein isolate (10600 Prolupin GmbH, Grimmen, Germany), 24.1% potato protein
121 (Protimex®, Tereos, France), 9.5% zein (f4400c-phg flozein®, Flo Chemical
122 Corporation, MA, USA), 37% rice protein hydrolysate (Meripro® Rice H, Tereos,
123 France), 7.3% rapeseed albumin (Napine, Supertein®, Burcon, Canada). These diets
124 were given *ad libitum* for 4 months, between the ages of 18 and 22 months, either as
125 pellets (MSE, PSE diets) or as a paste (MHE, PHE diets). Food intake and body
126 weight were measured once a week. Muscle functionality was assessed with the

127 CatWalk XT system (Noldus, Netherlands) before and at the end of the experimental
128 period. After 0, 2 and 4 months of experimental diets: 1) a blood sample was taken
129 from a lateral tail vein in the post-absorptive state; 2) body composition was
130 measured using magnetic resonance imaging (Echo MRI international, TX, USA). On
131 the 4th month of the experiment, *in vivo* muscle protein synthesis rates were
132 measured either in the post-absorptive state (PA - after overnight food deprivation,
133 with room lights on) for half of the rats or in the post-prandial state (PP - 2h after
134 feeding, with lights off) for the other half. Due to normal age-related mortality, the final
135 number of rats was 75, distributed as follows: MSE PA: n=10; MSE PP: n=9; PSE
136 PA: n=9; PSE PP: n=9; MHE PA: n=10; MHE PP: n=10; PHE PA: n=9; PHE PP: n=9.

137 *Measurements of in vivo protein synthesis, euthanasia, sampling*

138 Protein synthesis rates were measured using the flooding dose method as previously
139 reported (23). Briefly, twenty minutes before euthanasia, each rat was injected
140 intravenously with L-[1-¹³C] valine (Euriso-Top, France) (150 μ mol/100 g body
141 weight). Rats were then killed under 4% isoflurane anaesthesia by exsanguination
142 from the abdominal aorta. Posterior leg skeletal muscles (gastrocnemius, tibialis
143 anterior, extensor digitorum longus and soleus) were quickly excised, weighed, and
144 frozen in liquid nitrogen until further analysis. Liver and heart were weighed. Protein
145 synthesis rates were measured in the gastrocnemius muscle by measuring muscle
146 free and protein bound valine enrichments after grinding in liquid nitrogen in a ball
147 mill (Dangoumeau, Prolabo, France), and protein extraction with trichloroacetic acid.

148 *Calculations*

149 *In vivo* muscle fractional synthesis rates (FSR, %·d) were calculated as described
150 previously (24): $FSR = 100 \times (EP-EN)/(EA \times t)$ where t is the incorporation time

151 expressed in days, EP and EA (atom %) are the ^{13}C enrichments of protein-bound
152 valine and of muscle free valine, respectively. EN (atom %) is the natural ^{13}C
153 enrichment of protein-bound valine which was estimated in rats that were not injected
154 with the flooding dose (2 per group). Absolute synthesis rates (ASR) were calculated
155 from the product of FSR with protein content and expressed in milligrams per day.

156 *Plasma measurements*

157 Plasma glucose was determined by an enzymatic test using an automated system
158 (Olympus AU 400 analyzer, CRI biochemistry platform, France).

159 Plasma insulin concentrations were measured using a commercially available rat
160 insulin ELISA kit (80-INSRT-E01, Alpco Immunoassays; USA) at each time point,
161 optical densities were determined at 450 nm using a Tecan Infinite M200 Pro plate
162 reader (Tecan US, Inc., NC, USA).

163 *Statistical analysis*

164 Data were analysed using SAS® Studio (SAS Institute Inc) by variance analysis
165 (ANOVA) followed by the Tukey-Kramer test for post hoc comparison of means. We
166 used two class variables, “Protein” with two levels (milk and plant proteins), and
167 “energy content” with two levels (standard and high). When measurements were
168 repeated over time (body weight, food intake, plasma biochemistry, etc.), repeated
169 time ANOVA was performed. For muscle protein metabolism, we added “nutritional
170 state” as class variable with two levels (post absorptive, postprandial). For several
171 analyses, we also added covariates as factors in the variance analysis. To test
172 differences in death rates between groups, nonparametric estimates of the survivor
173 functions were computed to compare survival curves and perform a Log-Rank test.
174 Data are expressed as means \pm SE. The level of significance was set at $P < 0.05$.

175

176 RESULTS

177 *Survival, food intake, body weight, and body composition*

178 From randomization to euthanasia, the total number of deaths over 4 months was 2
179 in the MSE group, 3 in the PSE group, 2 in the MHE group, and 4 in the PHE group.

180 There was no significant difference in survival between groups ($P = 0.998$).

181 Spontaneous food intake (Figure 1) decreased over time, and this decline was more
182 marked in high energy fed rats. As expected, rats fed with these high energy diets
183 had a lower food intake (g) than rats fed with standard energy diets. There was no
184 difference in food intake between MHE rats and PHE rats, whereas in the first
185 months PSE rats ate significantly less than MSE rats. Energy intakes and protein
186 intakes followed the same pattern. Over the whole period, the energy intake was still
187 significantly higher in MHE rats (92.1 ± 1.6 kcal / day) and PHE rats (93.9 ± 1.7 kcal /
188 day) than in MSE rats (87.5 ± 1.6 kcal / day) and PSE rats (81.7 ± 1.7 kcal / day)($P =$
189 0.001). There was no difference between MHE and PHE rats, whereas the overall
190 energy intake was significantly lower in PSE rats than in MSE rats ($P = 0.01$). Thus,
191 the effect of protein type was different in rats fed the standard energy diet (lower
192 energy intake with plant proteins) and in rats fed the high-energy diet (no difference),
193 reflected by a significant protein x energy interaction ($P = 0.02$). Regarding protein
194 intake, as we anticipated a lower spontaneous food intake in high energy fed rats, we
195 chose to increase the protein content of these diets. Consequently, over the whole
196 period, protein intake was similar in all groups (MSE rats: 2.7 ± 0.1 g / day; PSE rats:
197 2.6 ± 0.1 g / day; MHE rats: 2.5 ± 0.1 g / day; PHE rats: 2.7 ± 0.1 g / day). However,
198 variance analysis detected one significant effect, protein x energy interaction

199 (P=0.02) due to the lower food intake with plant proteins specifically in standard
200 energy fed rats.

201 Weight slowly increased in all groups during the course of the experiment (P =
202 0.001), although it plateaued in the last month (Figure 2). Weight increased more in
203 high energy fed animals (P = 0.0001). Once again, due to the lower food intake with
204 plant proteins specifically in standard energy fed rats, there was also a significant
205 time x protein x energy interaction (P = 0.002). However, for a given energy level in
206 the diet, there was never a significant difference between the weight of milk protein
207 fed rats and plant protein fed rats.

208 *Body composition and tissue weight*

209 Before the start of the experiment, body composition was homogenous between rats,
210 and there was no significant difference between groups. The overall mean initial fat
211 mass was 109.3 ± 3.1 g, and the overall mean initial lean mass was 457.4 ± 3.7 g. As
212 shown in figure 3, during the first two months, the fat mass of all rats increased
213 significantly. Animals fed the high-energy diet almost doubled their fat mass. The
214 gain in fat mass was much lower for the standard-energy diet fed rats, but there was
215 a significant difference between MSE rats and PSE rats, with a lower gain for the
216 latter. Thus, the significant effects of variance analysis were energy (P = 0.0001) and
217 protein x energy (P = 0.04). During the same period, lean mass was preserved in
218 high-energy diet fed rats whereas the loss of lean mass of the rats fed the standard-
219 energy diet was significant. Thus, the high-energy diet induced increased lean mass
220 expressed in percent of initial value (P = 0.0003). There was no significant effect of
221 protein type on the evolution of lean mass.

222 During the last two months, the evolution of body composition was similar in all
223 groups of rats. All groups except PSE rats gained fat mass (+8-10%). Indeed, fat
224 mass, expressed in percent of value at 2 months, was significantly lower in PSE rats
225 than in MSE rats and PHE rats, as reflected by protein x energy interaction ($P =$
226 0.05). Regarding lean mass loss, it was significant for all the groups of rats, ~4%,
227 with no differences between groups.

228 At the end of the experiment, after 4 months of experimental diet, there was no
229 significant difference between the groups regarding muscle mass (Table 2) or muscle
230 total protein mass (data not shown). There was no negative impact of the high-
231 energy diet. In addition, plant proteins allowed the maintenance of muscle mass. In
232 particular, despite a slightly lower food intake in animals fed the standard energy diet,
233 muscle mass was well maintained in PSE rats compared with all the other groups.

234 Heart weight (Table 2) increased significantly in response to the high-energy diet, as
235 shown by variance analysis. There was no difference in liver weight between groups
236 (Table 2).

237 *Muscle functionality*

238 Among the >300 parameters of footfalls and locomotion characteristics assessed by
239 the CatWalk XT system, we selected simple integrative parameters, which are
240 related to muscle functionality and easy to interpret (Table 3). Among these
241 parameters, there was a significant effect of time only for the number of steps to
242 perform the run, cadence (steps / s) and swing speed (cm / sec). Cadence
243 significantly decreased with time. It could reflect a deleterious effect of aging.
244 However, the number of steps and swing speed improved with time. The high-energy
245 diet significantly reduced this gain with time in the number of steps and swing speed

246 (significant interaction time and energy). There were no other significant effects of the
247 high-energy diet. Regarding the protein effect, there was a significant time x protein
248 interaction for run duration, average speed, and step cycle. Compared to plant
249 protein, milk protein improved the performances of rats regarding run duration and
250 step cycle, but reduced their performances regarding average speed. There was also
251 a significant triple interaction (time x protein x energy) for the number of steps and
252 stride length. It was related to lower values in the PHE group.

253 In addition, we tested the impact of body weight using multivariate models (data not
254 shown). Body weight significantly and independently influenced swing speed and run
255 duration, without affecting the other significant effects. Swing speed and run duration
256 increased when body weight increased. Body weight was also significantly
257 associated with the number of steps, with a size effect similar to that of the effect of
258 the high-energy diet. When body weight was included as a covariate in the statistical
259 model, the interaction time x energy level was no longer significant. Thus, it is likely
260 that the significant effect of a higher energy level on the number of steps was mostly
261 mediated by increases in body weight.

262 *Gastrocnemius muscle protein synthesis*

263 Absolute protein synthesis rates (ASR) were measured in the gastrocnemius muscle
264 in each group, either in the post absorptive or in the postprandial state. The only
265 significant effect was nutritional state (Figure 4). Overall, feeding increased by 13%
266 ASR in the gastrocnemius muscle. ASR could be maintained at the same level
267 whatever the protein type or energy level. Regarding fractional synthesis rates (FSR),
268 there was no significant effect. However, when using a covariate such as
269 gastrocnemius muscle mass or lean body mass, both significantly correlated with

270 FSR, a significant increase in FSR, independent of feeding, was detected in rats fed
271 plant proteins ($P < 0.001$). Adjusted means \pm SE for FSR were 6.5 ± 0.2 % / h for
272 milk proteins and 7.6 ± 0.2 % / h for plant proteins.

273 *Plasma biochemistry*

274 Overall, plasma glucose (Figure 5A) declined over time irrespective of energy level or
275 protein type, even if after two months the energy level tended to increase fasting
276 glycaemia.

277 There was no clear trend regarding plasma insulin (Figure 5B), probably in relation
278 with heterogeneous values in animals, even before the start of the experiment.

279 Overall, we found a significant time x protein x energy interaction ($P = 0.02$) when
280 analyzing global values, and at 4 months we found lower plasma insulin after feeding
281 with plant proteins compared to milk protein ($P = 0.04$).

282

283 DISCUSSION

284 The aim of this study was to assess the capacity of a mix of purified plant proteins
285 rich in all the indispensable amino acids (IAA) and with relatively high leucine,
286 arginine, and cysteine contents to limit sarcopenia, compared to milk protein. Using
287 aging rats fed a normal diet or a diet rich in fat and sugar for 4 months as a model,
288 we focused our analyses on postprandial protein metabolism and lean muscle mass
289 and function.

290 We showed that despite a slightly lower food intake in rats fed with the standard
291 energy plant protein diet, lean mass, muscle mass and functionality were maintained

292 in all the groups of rats fed plant proteins to the same extent as in rats fed milk
293 proteins.

294 Likewise, the response of muscle protein synthesis to feeding was also similar
295 between plant protein and milk protein. This is a key point because we showed that
296 during healthy aging, a defect in the stimulation of muscle protein synthesis during
297 feeding is thought to drive the slow erosion of muscle mass (25). To overcome this
298 resistance of muscle anabolism, one strategy is to improve peripheral amino acid
299 (AA) or leucine availability after feeding (25): 1) Leucine is a key AA for optimizing the
300 stimulation of muscle protein synthesis after feeding in old age (26); 2) Arginine, as a
301 precursor of NO, can increase muscle perfusion and thus the peripheral extraction of
302 nutrients in response to insulin (15, 17). Another strategy would be to mitigate
303 oxidative stress, inflammation, and insulin resistance that increase during aging (9,
304 27). Because of its presence in glutathione, cysteine can help to prevent the
305 alteration of the redox status observed during aging (18, 19), while long-term
306 supplementation with cysteine was shown to delay loss of muscle mass in aging (20).
307 This was the rationale for designing a protein blend with relatively high amounts of
308 leucine, arginine and cysteine contents and testing its effect in both standard and
309 high fat high sucrose diets.

310 The possibility that our plant protein blend could stimulate muscle protein synthesis
311 independently of feeding, as suggested by our results on adjusted FSR, was a
312 surprise. The fact that muscle mass was not increased implies a corresponding
313 increase in muscle proteolysis, and thus a higher turnover. To the best of our
314 knowledge, such a result has not been described previously.

315 Currently, numerous studies are performed to assess the suitability of plant proteins
316 to partially replace animal proteins. Single proteins and mixtures of proteins are

317 tested, as are crude food products (lentils for instance) and purified fractions. Plant
318 crude products with a high protein content are more likely to have low digestibility
319 (presence of anti-nutritional factors), and to be marginally deficient in certain IAA
320 (lysine in cereals, sulfur AA in legumes).

321 Our approach was to mix numerous highly purified plant proteins, using industrial co-
322 products when possible, thus lowering the risk of poor digestibility, mainly through the
323 inactivation of trypsin inhibitors.

324 Blending sources also offer the opportunity to tailor AA composition. It was easy to
325 find a blend with an excellent AA balance (5). A deficiency in one AA, especially in
326 the long term, can affect tissue protein anabolism. However, another additional effect
327 could occur. Indeed, studies using soy and wheat as protein sources observed an
328 increase in urea synthesis (28). This was attributed to poor utilization of dietary AA in
329 the intestine, leading in turn to a marked increase in free AA content in the portal
330 vein, which could constitute a signal for the liver to increase urea synthesis, thus
331 further limiting the availability of dietary AA for peripheral protein synthesis (28). The
332 reason for the poor utilization of dietary AA after the ingestion of wheat or soy is not
333 completely clear. It could result from a suboptimal IAA balance (for instance lysine in
334 wheat) and / or in the lower proportion of total IAA among total AA (38% for soy vs
335 43% for milk proteins). It was also proposed that this phenomenon could result from
336 modifications in digestion kinetics (29). Thus, it was shown that soy proteins could
337 not stimulate muscle proteins in old rats to the same extent as whey (30) .

338 In our plant blend, the content of all the IAA was similar to or even slightly higher than
339 that of milk proteins, except for lysine. Lysine content was 56% of milk protein
340 content but 163% of wheat protein content and allowed a lysine intake much higher
341 than the requirement of mature rats (31). However, the proportion of IAA was 38% in

342 our plant protein blend, likewise for soy. Given the excellent results we obtained with
343 our plant blend regarding the long-term effect on muscle mass and the short-term
344 effect on muscle protein synthesis, this suggests that the stimulation of urea
345 synthesis observed with soy (29) is not related to the slightly lower IAA proportion but
346 to possible specific digestion kinetics issues.

347 To the best of our knowledge, no similar plant protein mix has been studied in the
348 long term in the context of muscle aging. In previous studies, combinations of cereals
349 and legumes were studied, showing most of the time that protein retention was better
350 with the mix than with cereals or legumes alone, but still lower than with animal
351 proteins (32). Recently, Salles et al. (33) concluded that nitrogen balance, lean body
352 mass, muscle mass and post-absorptive protein synthesis could be maintained
353 during aging at the same level after two months of feeding with purified pea proteins
354 compared with casein or whey. The same team compared casein, whey and legume
355 enriched pasta (lentil, split pea, and faba bean), and showed that after 6 weeks in 22
356 month-old rats, despite lower digestibility and leucine content, legume enriched pasta
357 allowed maintaining lean body mass, nitrogen balance and post-absorptive muscle
358 protein synthesis (34). The authors argued that higher post-prandial insulin secretion
359 and high glycine and arginine content could have been contributive factors. Our plant
360 protein mix had similar glycine and arginine levels to that of legume enriched pasta
361 (34), and higher levels of leucine and cysteine than pea proteins (33). If the results
362 on muscle mass and function during aging were indeed mediated by these AA, our
363 findings would confirm those of Salles et al. (33).

364 Another unexpected result of our study was that the high-energy diet (rich in fat and
365 sucrose) seemed to have protective effects regarding lean body mass, independently
366 of the type of ingested protein. Indeed, the high-energy diet prevented a decrease in

367 lean body mass during the first two months of the experiment. Our initial objective
368 was to challenge the metabolism of aging rats by inducing insulin resistance to test
369 the capacity of our plant protein mix with high arginine and cysteine contents to
370 counteract the potential deleterious effects of the high-energy diet. It turned out that
371 insulin and glucose levels were not affected by energy, although there was a marked
372 gain in body weight and fat mass. Previously, we showed that replacing all starch by
373 sucrose induced insulin resistance in old rats (27). However, such changes are not
374 always obtained after high fat high sucrose diets in rats (23). In the present study,
375 food intake remained moderate and decreased during aging, in particular in rats fed
376 the high-energy diet. This may have attenuated the negative impacts of the high-
377 energy diet, in particular on the regulation of lean body mass.

378 In situations where higher energy intake does not lower insulin action, it is considered
379 favorable to protein metabolism, through classical sparing effects on intermediary
380 metabolism (35). In addition, the high-energy diet increased the weight of animals,
381 which then had to use more force to move in the cage, perhaps also explaining the
382 better maintenance of lean body mass during the first two months of the experiment.

383 A similar or slightly higher lean mass was also observed in other animal studies using
384 high fat diets during aging (36-38). In humans, obesity is often associated with a
385 higher lean body mass, and it was suggested that “carrying greater weight might
386 have physical training-like effects” (39).

387 However, these “training effects” on lean body mass did not translate into
388 improvements in muscle functionality. Indeed, two measurements obtained with the
389 CatWalk XT system, the number of steps to perform the run and the swing speed,
390 improved with time, but the high-energy diet significantly reduced this improvement.

391 It was surprising to observe an improvement with time in muscle functionality.
392 Recently, comparing 6 and 24-month-old Wistar rats, Le Bacquer et al. (40) obtained
393 a higher average speed, lower step number, and higher cadence in adult rats
394 compared to older rats. This is what could be expected in response to the deleterious
395 effect of aging. For the same parameters, we saw no direct effect of time on average
396 speed, a contrary result for step number, and a similar result for cadence. Our
397 studies are different because there was a large age difference in the study by Le
398 Bacquer et al. (40), while in our study the measurements were repeated for the same
399 animals. That is why we postulate that the improvements that we saw with time may
400 have been due to habituation. Animals were more familiar with the task they were
401 required to perform the second time. It makes sense that, despite habituation, high-
402 energy fed animals would have greater difficulty in performing the task due to the fact
403 that they were heavier the second time.

404 Besides energy, we found other significant effects of the type of protein with the
405 CatWalk XT system. Compared to milk protein, plant proteins reduced the
406 performances of rats regarding run duration and step cycle but improved their
407 performances regarding average speed. These results are difficult to interpret,
408 because 1) there was no effect of time alone; 2) they cannot be clearly linked to
409 differences in body weight, lean body mass or muscle mass; 3) the same type of
410 protein had positive and negative effects. Analyzing footfalls and locomotion
411 characteristics by the CatWalk XT system is a complex process, probably involving
412 not only neuro-muscular function but also brain function (memory, learning capacity,
413 confidence). It is possible that these differences observed between animal and plant
414 proteins could result from effects on brain function. Indeed, the type of protein
415 ingested can affect brain function by changing the blood concentration of large

416 neutral AA and hence serotonine and catecholamine synthesis. Specific peptides
417 generated during protein digestion could also influence brain function and some are
418 found in dairy products (41). However, the tryptophan, tyrosine, and branched chain
419 AA content was similar between milk proteins and our mix of plant proteins, and the
420 amount and nature of bioactive peptides generated after the ingestion of milk
421 proteins or our blend of plant protein remains poorly understood.

422 In conclusion, in this aging rat model, we showed that a tailored blend of plant protein
423 can adequately replace milk proteins in the long term, as it resulted in no difference in
424 the response of muscle protein synthesis to feeding, or in the evolution of lean body
425 mass and muscle mass during aging. We could not test the capacity of these plant
426 proteins to protect muscle in a situation of altered insulin sensitivity because the high
427 fat high sucrose diet only induced a marked increase in body fat without obvious
428 deleterious effects on insulin sensitivity. Many other aspects of substituting animal
429 proteins with plant protein need to be examined in other studies, but the present
430 findings offer an important proof of concept from the nutritional standpoint that
431 appropriately blended plant proteins can have a high nutritional value even in
432 demanding situations such as protein metabolism and sarcopenia in the elderly.

433

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437 LD, FM, DR, DH, LM designed the research. LD, JL, VM, MJ, LM conducted the
438 research. LD, CLB, FM provided essential reagents. LD, FM, DR, LM analyzed data
439 or performed statistical analysis. LD, FM, LM wrote the paper. LM had primary
440 responsibility for the final content. All the authors have read and approved the final

441 manuscript. The data described in the manuscript will be made available upon
442 request.

443

444

Figure Legends

445 **Figure 1** Evolution of food intake in MSE, PSE, MHE and PHE rats.

446 18-month-old rats were fed for 4 months with a diet containing as protein source
447 either milk proteins or a plant protein mix combined with either standard energy
448 content or high energy content with a high level of fat and sucrose: MSE = milk
449 protein standard energy, PSE = plant protein standard energy, MHE = milk protein
450 high energy, PHE = plant protein high energy. Rats ate *ad libitum*. Significant effects
451 (SE) of repeated time variance analysis are given and the results showed that the
452 time effect was significant, as were all the interactions (time x protein, time x energy,
453 time x protein x energy). *: at this time point, there was a significant difference
454 between MSE rats and PSE rats, $P < 0.05$. #: at this time point there was a significant
455 difference between rats fed standard energy diets and rats fed high energy diets, $P <$
456 0.05 . Values are means \pm SE (n=18-20 per group).

457 **Figure 2** Evolution of body weight in MSE, PSE, MHE and PHE rats.

458 See figure 1 for group description. Significant effects of repeated time variance
459 analysis are given and the results showed that the time effect was significant, as
460 were time x energy and time x protein x energy interactions. # : at this time point,
461 there was a significant difference between rats fed standard energy diets and rats fed
462 high energy diets, $P < 0.05$. Values are means \pm SE (n=18-20 per group)

463 **Figure 3** Evolution of body composition in MSE, PSE, MHE and PHE rats.

464 See figure 1 for group description. Body composition was measured before and after
465 2 and 4 months of the experimental diet. Fat mass and lean mass measurements are
466 expressed in percent of the initial measurement for the first 2 months, and in percent
467 of the value at 2 months for the last 2 months. Significant effects (SE) of variance
468 analysis are given. a, b: means assigned with the same letter were not significantly
469 different. *: significantly different from 0 ($P < 0.05$). Values are means \pm SE (n=18-20)

470 **Figure 4** Absolute synthesis rates (ASR) in the gastrocnemius muscle after 4 months
471 of experimental diets.

472 See Figure 1 for group description. Absolute protein synthesis rates were measured
473 in the gastrocnemius muscle either in the post absorptive state (PA) or in the post-
474 prandial state (PP) in MSE, PSE, MHE and PHE rats, and are expressed in mg
475 protein synthesized per day. Bigger red dots represent mean values, and smaller blue
476 dots individual values (n= 5-9 per group). SE: variance analysis significant effects.
477 Only nutritional state had a significant effect ($P = 0.02$).

478 **Figure 5** Evolution of glycemia and insulinemia in MSE, PSE, MHE and PHE rats.

479 See figure 1 for group description. Plasma glucose (A) and insulin (B) levels were
480 measured in the post- absorptive state before and after 2 and 4 months of
481 experimental diets. SE: significant effect of repeated time variance analysis. *:
482 Variance analysis performed at this time point detected a significant effect of energy
483 ($P = 0.01$); \$: Variance analysis performed at this time point detected a significant
484 effect of protein ($P = 0.04$).

References

1. Henchion M, Hayes M, Mullen AM, Fenelon M, Tiwari B. Future Protein Supply and Demand: Strategies and Factors Influencing a Sustainable Equilibrium. *Foods* 2017;6(7). doi: 10.3390/foods6070053.
2. Pojić M, Mišan A, Tiwari B. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends in Food Science & Technology* 2018;75:93-104. doi: <https://doi.org/10.1016/j.tifs.2018.03.010>.
3. Dietary protein quality evaluation in human nutrition. Report of an FAQ Expert Consultation. *FAO Food Nutr Pap* 2013;92:1-66.
4. Mariotti F. 35 - Plant Protein, Animal Protein, and Protein Quality. Edtion ed. In: Mariotti F, ed. *Vegetarian and Plant-Based Diets in Health and Disease Prevention*: Academic Press, 2017:621-42.
5. Dimina L, Rémond D, Huneau J-F, Mariotti F. Combining Plant Proteins to Achieve Amino Acid Profiles Adapted to Various Nutritional Objectives—An Exploratory Analysis Using Linear Programming. *Frontiers in Nutrition* 2022;8. doi: 10.3389/fnut.2021.809685.
6. Gorissen SHM, Crombag JJR, Senden JMG, Waterval WAH, Bierau J, Verdijk LB, et al. Protein content and amino acid composition of commercially available plant-based protein isolates. *Amino Acids* 2018;50(12):1685-95. doi: 10.1007/s00726-018-2640-5.
7. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. *Lancet* 2019;393(10191):2636-46. doi: 10.1016/s0140-6736(19)31138-9.
8. Dardevet D, Rémond D, Peyron M-A, Papet I, Savary-Auzeloux I, Mosoni L. Muscle Wasting and Resistance of Muscle Anabolism: The “Anabolic Threshold Concept” for Adapted Nutritional Strategies during Sarcopenia. *The Scientific World Journal* 2012;2012:1-6. doi: 10.1100/2012/269531.
9. Balage M, Averous J, Remond D, Bos C, Pujos-Guillot E, Papet I, et al. Presence of low-grade inflammation impaired postprandial stimulation of muscle protein synthesis in old rats. *J Nutr Biochem* 2010;21(4):325-31. doi: 10.1016/j.jnutbio.2009.01.005.
10. Gatineau E, Savary-Auzeloux I, Migne C, Polakof S, Dardevet D, Mosoni L. Chronic Intake of Sucrose Accelerates Sarcopenia in Older Male Rats through Alterations in Insulin Sensitivity and Muscle Protein Synthesis. *J Nutr* 2015;145(5):923-30. doi: 10.3945/jn.114.205583.
11. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. *Clin Nutr* 2008;27(5):675-84. doi: 10.1016/j.clnu.2008.06.008.
12. Mathewson SL, Azevedo PS, Gordon AL, Phillips BE, Greig CA. Overcoming protein-energy malnutrition in older adults in the residential care setting: A narrative review of causes and interventions. *Ageing Res Rev* 2021;70:101401. doi: 10.1016/j.arr.2021.101401.
13. Rieu I, Balage M, Sornet C, Giraudet C, Pujos E, Grizard J, et al. Leucine supplementation improves muscle protein synthesis in elderly men independently of hyperaminoacidaemia. *J Physiol* 2006;575(Pt 1):305-15. doi: 10.1113/jphysiol.2006.110742.
14. Atherton PJ, Kumar V, Selby AL, Rankin D, Hildebrandt W, Phillips BE, et al. Enriching a protein drink with leucine augments muscle protein synthesis after resistance exercise in young and older men. *Clin Nutr* 2017;36(3):888-95. doi: 10.1016/j.clnu.2016.04.025.
15. Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, et al. Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. *Cell Metab* 2011;13(3):294-307. doi: 10.1016/j.cmet.2011.01.018.
16. Mariotti F. Arginine supplementation and cardiometabolic risk. *Curr Opin Clin Nutr Metab Care* 2020;23(1):29-34. doi: 10.1097/MCO.0000000000000612.
17. Mitchell WK, Phillips BE, Wilkinson DJ, Williams JP, Rankin D, Lund JN, et al. Supplementing essential amino acids with the nitric oxide precursor, L-arginine, enhances skeletal muscle

- perfusion without impacting anabolism in older men. *Clin Nutr* 2017;36(6):1573-9. doi: 10.1016/j.clnu.2016.09.031.
18. Go YM, Jones DP. Redox theory of aging: implications for health and disease. *Clin Sci (Lond)* 2017;131(14):1669-88. doi: 10.1042/CS20160897.
 19. Mast C, Pourpe C, Voyard G, Remond D, Migne C, Centeno D, et al. Dietary supplementation with cysteine prevents adverse metabolic outcomes of repeated cures with paracetamol in old rats. *Br J Nutr* 2017;118(11):889-96. doi: 10.1017/S0007114517002847.
 20. Sinha-Hikim I, Sinha-Hikim AP, Parveen M, Shen R, Goswami R, Tran P, et al. Long-term supplementation with a cystine-based antioxidant delays loss of muscle mass in aging. *J Gerontol A Biol Sci Med Sci* 2013;68(7):749-59. doi: 10.1093/gerona/gls334.
 21. Karelis AD, Messier V, Suppere C, Briand P, Rabasa-Lhoret R. Effect of cysteine-rich whey protein (immunocal(R)) supplementation in combination with resistance training on muscle strength and lean body mass in non-frail elderly subjects: a randomized, double-blind controlled study. *J Nutr Health Aging* 2015;19(5):531-6. doi: 10.1007/s12603-015-0442-y.
 22. Reeves PG, Nielsen FH, Fahey GC, Jr. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J Nutr* 1993;123(11):1939-51.
 23. Adechian S, Giardina S, Remond D, Papet I, Buonocore D, Gaudichon C, et al. Excessive energy intake does not modify fed-state tissue protein synthesis rates in adult rats. *Obesity (Silver Spring)* 2009;17(7):1348-55. doi: 10.1038/oby.2009.35.
 24. Mosoni L, Valluy MC, Serrurier B, Prugnaud J, Obled C, Guezennec CY, et al. Altered Response of Protein-Synthesis to Nutritional State and Endurance Training in Old Rats. *American Journal of Physiology-Endocrinology and Metabolism* 1995;268(2):E328-E35.
 25. Dardevet D, Remond D, Peyron MA, Papet I, Savary-Auzeloux I, Mosoni L. Muscle Wasting and Resistance of Muscle Anabolism: The "Anabolic Threshold Concept" for Adapted Nutritional Strategies during Sarcopenia. *Scientific World Journal* 2012. doi: Artn 269531 10.1100/2012/269531.
 26. Dardevet D, Rieu I, Fafournoux P, Sornet C, Combaret L, Bruhat A, et al. Leucine: a key amino acid in ageing-associated sarcopenia? *Nutr Res Rev* 2003;16(1):61-70. doi: 10.1079/NRR200252.
 27. Gatineau E, Savary-Auzeloux I, Migne C, Polakof S, Dardevet D, Mosoni L. Chronic Intake of Sucrose Accelerates Sarcopenia in Older Male Rats through Alterations in Insulin Sensitivity and Muscle Protein Synthesis. *J Nutr* 2015;145(5):923-30. doi: 10.3945/jn.114.205583.
 28. van Vliet S, Burd NA, van Loon LJ. The Skeletal Muscle Anabolic Response to Plant- versus Animal-Based Protein Consumption. *J Nutr* 2015;145(9):1981-91. doi: 10.3945/jn.114.204305.
 29. Fouillet H, Mariotti F, Gaudichon C, Bos C, Tome D. Peripheral and splanchnic metabolism of dietary nitrogen are differently affected by the protein source in humans as assessed by compartmental modeling. *J Nutr* 2002;132(1):125-33. doi: 10.1093/jn/132.1.125.
 30. Jarzaguet M, Polakof S, David J, Migne C, Joubrel G, Efstathiou T, et al. A meal with mixed soy/whey proteins is as efficient as a whey meal in counteracting the age-related muscle anabolic resistance only if the protein content and leucine levels are increased. *Food Funct* 2018;9(12):6526-34. doi: 10.1039/c8fo01903g.
 31. National Research Council Subcommittee on Laboratory Animal N. Edtion ed. *Nutrient Requirements of Laboratory Animals: Fourth Revised Edition, 1995*. Washington (DC): National Academies Press (US) © 1995 by the National Academy of Sciences. All rights reserved., 1995.
 32. Berrazaga I, Micard V, Gueugneau M, Walrand S. The Role of the Anabolic Properties of Plant- versus Animal-Based Protein Sources in Supporting Muscle Mass Maintenance: A Critical Review. *Nutrients* 2019;11(8). doi: 10.3390/nu11081825.

33. Salles J, Guillet C, Le Bacquer O, Malnero-Fernandez C, Giraudet C, Patrac V, et al. Pea Proteins Have Anabolic Effects Comparable to Milk Proteins on Whole Body Protein Retention and Muscle Protein Metabolism in Old Rats. *Nutrients* 2021;13(12). doi: 10.3390/nu13124234.
34. Berrazaga I, Salles J, Laleg K, Guillet C, Patrac V, Giraudet C, et al. Anabolic Properties of Mixed Wheat-Legume Pasta Products in Old Rats: Impact on Whole-Body Protein Retention and Skeletal Muscle Protein Synthesis. *Nutrients* 2020;12(6). doi: 10.3390/nu12061596.
35. Millward DJ. The use of protein:energy ratios for defining protein requirements, allowances and dietary protein contents. *Public Health Nutr* 2013;16(5):763-8. doi: 10.1017/s1368980013000396.
36. Judge MK, Zhang J, Tumer N, Carter C, Daniels MJ, Scarpace PJ. Prolonged hyperphagia with high-fat feeding contributes to exacerbated weight gain in rats with adult-onset obesity. *Am J Physiol Regul Integr Comp Physiol* 2008;295(3):R773-80. doi: 10.1152/ajpregu.00727.2007.
37. Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraegen EW. Long-term high-fat feeding leads to severe insulin resistance but not diabetes in Wistar rats. *Am J Physiol Endocrinol Metab* 2002;282(6):E1231-8. doi: 10.1152/ajpendo.00173.2001.
38. McDonald RB, Carlson K, Day C, Stern JS, Horwitz BA. Effect of gender on the response to a high fat diet in aging Fischer 344 rats. *J Nutr* 1989;119(10):1472-7. doi: 10.1093/jn/119.10.1472.
39. Jura M, Kozak LP. Obesity and related consequences to ageing. *Age (Dordr)* 2016;38(1):23. doi: 10.1007/s11357-016-9884-3.
40. Le Bacquer O, Salles J, Piscitelli F, Sanchez P, Martin V, Montaurier C, et al. Alterations of the endocannabinoid system and circulating and peripheral tissue levels of endocannabinoids in sarcopenic rats. *J Cachexia Sarcopenia Muscle* 2021. doi: 10.1002/jcsm.12855.
41. Camfield DA, Owen L, Scholey AB, Pipingas A, Stough C. Dairy constituents and neurocognitive health in ageing. *Br J Nutr* 2011;106(2):159-74. doi: 10.1017/S0007114511000158.

Table 1: Diet composition

Ingredients and dietary composition Per kg of diet	Standard energy Milk protein	Standard energy Plant mix	High energy Milk protein	High energy Plant mix
Milk proteins (g)	140	0	168	186.69
Plant protein mix (g)	0	155.8	0	0
L-cystine (g)	0.69	0.69	0.83	0.83
Wheatstarch (g)	495	479	144	125
Sucrose (g)	127	127	230	230
Lactose (g)	100	100	60	60
Canola oil (g)	20	20	20	20
Sunflower oil (g)	2	2	2	2
Peanut oil (g)	18	18	18	18
Lard (g)	0	0	260	260
Cellulose (g)	50	50	50	50
Mineral mix (ain-93g-mx) (g)	35	35	35	35
Vitamin mix (ain-93-vx) (g)	10	10	10	10
Choline bitartrate (41.1% choline) (g)	2.5	2.5	2.5	2.5
Energy (kcal)	3624.07	3657.62	4889.76	4929.68
% as protein	13.54	14.55	12.66	13.55
% as CHO	76.40	71.41	35.15	33.71
% as fat	10.30	10.39	50.14	50.50
Amino acids (g)	121.97	128.13	146.36	153.53
Alanine	3.72	7.40	4.47	8.86
<u>Arginine</u>	<u>4.14</u>	<u>9.39</u>	<u>4.97</u>	<u>11.26</u>
Asparagine/aspartate	8.62	11.94	10.35	14.30
<u>Cystine</u>	<u>1.82</u>	<u>2.49</u>	<u>2.18</u>	<u>2.98</u>
Glutamine/glutamate	24.78	22.68	29.74	27.17
Glycine	2.20	5.22	2.64	6.26
Histidine	3.14	3.02	3.76	3.62
Isoleucine	5.74	5.70	6.89	6.83
<u>Leucine</u>	<u>11.21</u>	<u>12.30</u>	<u>13.46</u>	<u>14.74</u>
Lysine	10.47	5.86	12.57	7.02
Methionine	3.19	2.49	3.83	2.98
Phenylalanine	5.60	6.53	6.72	7.82
Proline	10.99	7.04	13.19	8.44
Serine	6.36	6.69	7.63	8.01
Threonine	5.03	5.20	6.03	6.23
Tryptophan	1.68	1.60	2.02	1.92
Tyrosine	6.58	6.11	7.90	7.32
Valine	6.69	6.49	8.03	7.78

Table 2 : Tissue weight after 4 months of experimental diet in MSE, PSE, MHE and PHE rats

Groups		MSE	PSE	MHE	PHE	ANOVA SE
Muscles						
<i>Gastrocnemius</i>	g	2.12 ± 0.11	2.22 ± 0.09	1.95 ± 0.09	2.24 ± 0.14	ns
<i>Tib anterior</i>	g	0.65 ± 0.03	0.65 ± 0.03	0.57 ± 0.03	0.65 ± 0.05	ns
<i>Soleus</i>	mg	215 ± 11	226 ± 8	219 ± 14	243 ± 13	ns
<i>EDL</i>	mg	198 ± 9	215 ± 8	190 ± 7	202 ± 12	ns
Heart	g	1.65 ^a ± 0.03	1.75 ^{ab} ± 0.06	1.81 ^{ab} ± 0.05	1.85 ^b ± 0.06	E (P=0.01)
Liver	g	14.6 ± 0.5	13.8 ± 0.6	14.3 ± 0.5	15.1 ± 0.7	ns
Body weight	g	657 ^{ab} ± 17	629 ^a ± 21	710 ^{ab} ± 26	737 ^b ± 28	E (P=0.001)
Lean mass	g	424 ± 9	433 ± 11	440 ± 10	447 ± 13	ns

See figure 1 for group description. Body weight and lean mass are given again here for readers that prefer calculating tissue weight in proportion of body weight or lean mass. MSE = milk protein standard energy; PSE = plant protein standard energy; MHE = milk protein high energy; PHE = plant protein high energy; ANOVA SE: significant effects of variance analysis; Tib = tibialis; EDL = Extensor Digitorum Longus muscle; E = energy; ns = not significant. a, b = means affected by the same letter were not significantly different. Values are means ± SE (n=18-20).

Table 3: Evolution of muscle functionality before and after nutritional treatments

Parameter	Time	MSE	PSE	MHE	PHE	RSE	ANOVA SE
Run duration (s)	Before	4,00	4,12	4,38	3,98	0,85	T x P (P=0.02)
	After	4,02	4,32	3,98	4,49		
Average speed (cm / s)	Before	30,9	30,5	28,5	31,3	6,00	T x P (P=0.04)
	After	30,3	28,7	30,0	27,6		
Number of steps	Before	62,4	64,4	66,5	62,4	6,03	T (P=0.01), T x E (P=0.04), T x P x E (P=0.01)
	After	59,6	58,1	61,8	65,7		
Cadence (steps / s)	Before	8,05	8,13	7,91	8,08	1,07	T (P=0.01)
	After	7,70	7,17	7,86	7,65		
Stride length (cm)	Before	14,6	14,4	13,9	14,9	1,37	T x P x E (P=0.01)
	After	14,2	14,7	14,3	13,8		
Step cycle (s)	Before	0,47	0,46	0,48	0,48	0,06	T x P (P=0.02)
	After	0,45	0,49	0,46	0,48		
Swing speed (cm / s)	Before	104,7	99,3	97,4	99,8	13,70	T (P=0.001), T x E (P=0.001)
	After	78,8	75,2	88,7	86,0		

See figure 1 for group description. We give means and residual standard errors (RSE) before and after nutritional treatments in each group (n=17-18). We performed repeated time variance covariance analysis. MSE: milk protein standard energy; PSE: plant protein standard energy; MHE: milk protein high energy; PHE: plant protein high energy; RSE: residual standard error; ANOVA SE: significant effects of variance analysis. T = time; E = energy.

Figure 1:

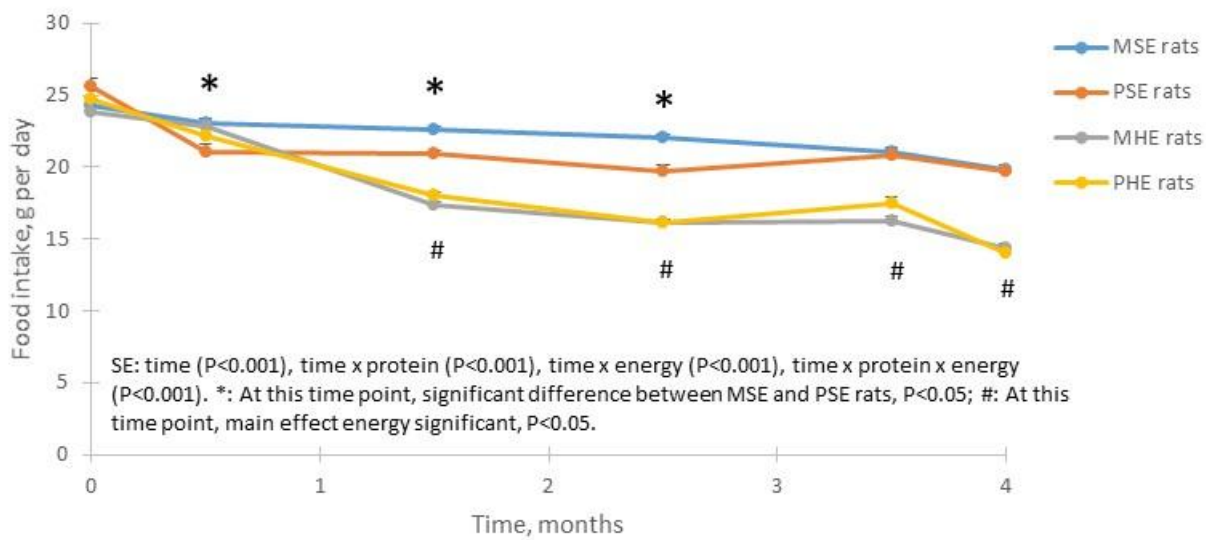


Figure 2 :

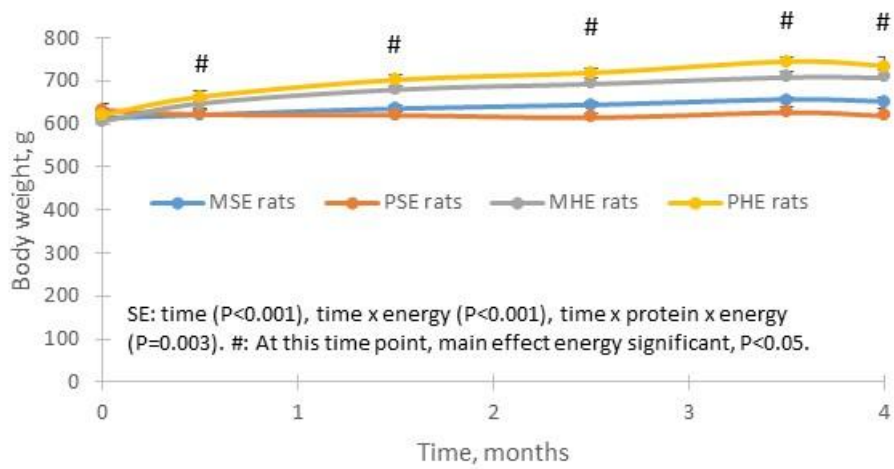
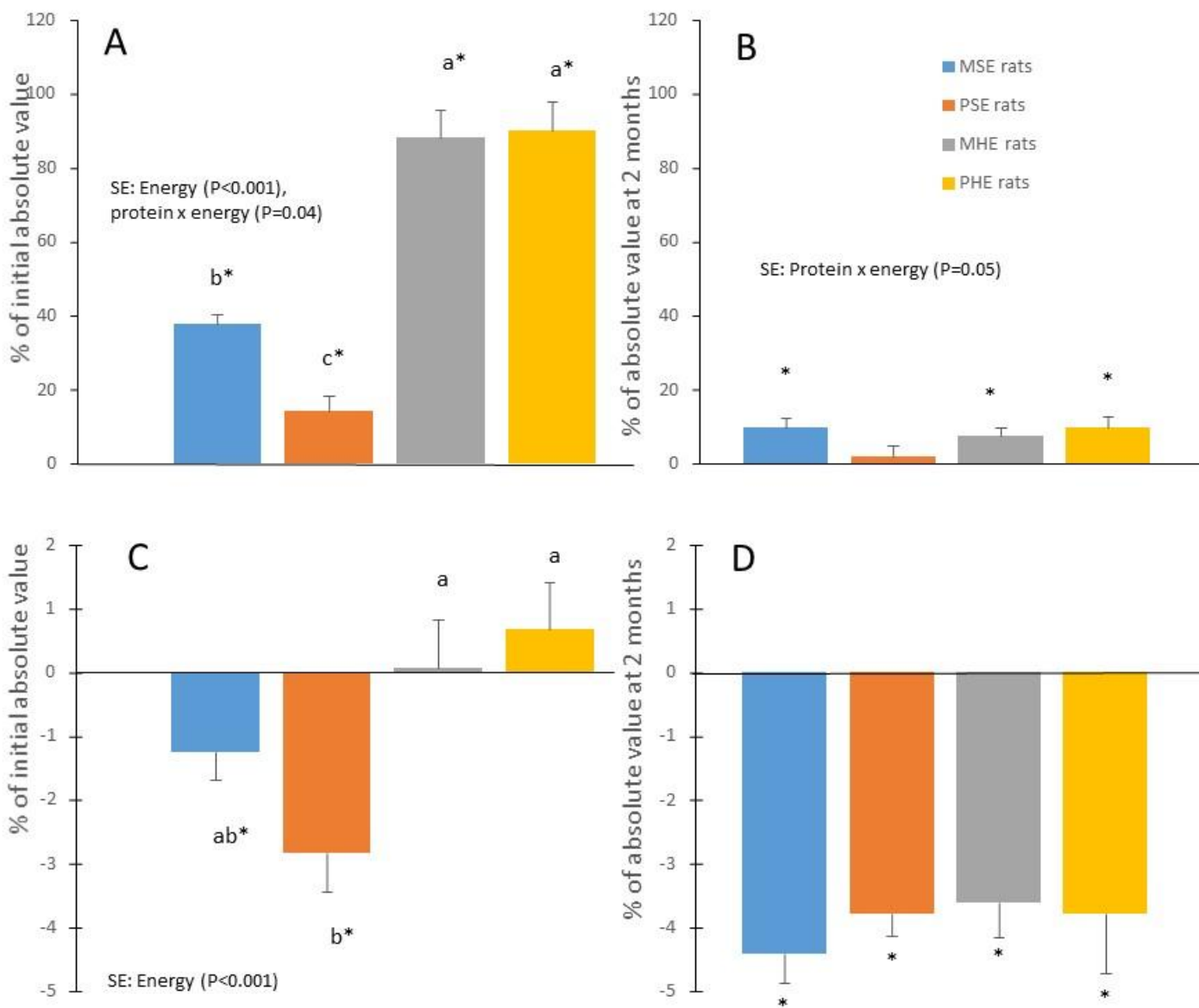


Figure 3:



a,b,...: labeled means without a common letter differ, $P < 0.05$; *: significantly different from 0, $P < 0.05$.

Figure 4:

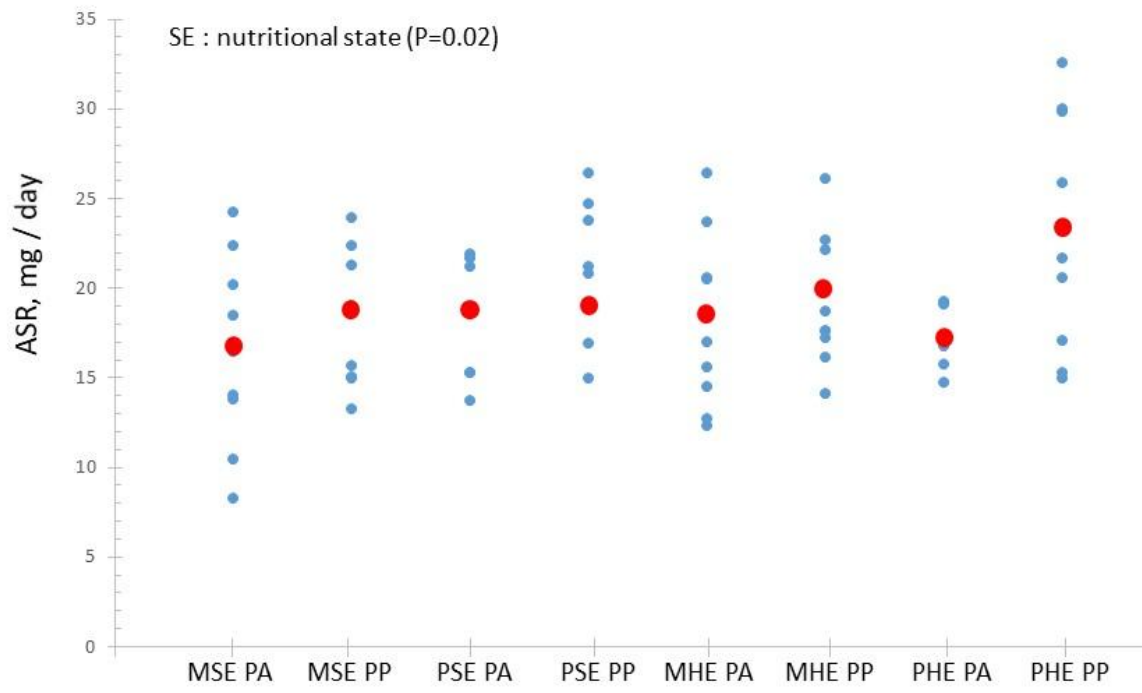


Figure 5 :

