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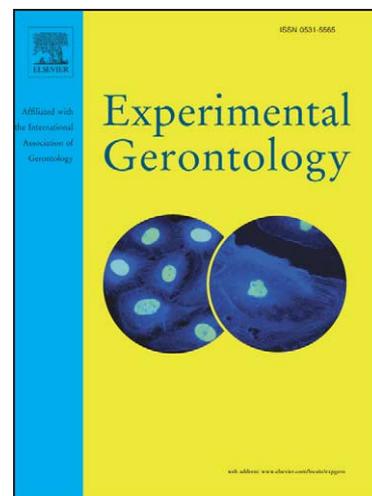
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1 **Amino Acid Supplementation Prevents the Loss of Appetite for Casein**

2 **in Old Lou/Cjall Rats**

3 Ghislaine Nzang Nguema, Elisabeth Debras *, Jean Grizard* and Josette Alliot ²

4
5 Laboratoire de Neuroendocrinologie du Vieillissement, Complexe Scientifique des Cézeaux,

6 Université Blaise Pascal, Clermont-Ferrand, France and * Institut National de la Recherche

7 Agronomique, Unité de Nutrition et Métabolisme Protéique, 63122 Ceyrat , France

8
9 Running Title: Protein Intake in Old Rats

10
11
12 Requests for reprints and correspondence should be addressed to:

13 Pr. Josette Alliot

14 Laboratoire de Neuroendocrinologie du Vieillissement,

15 Complexe Scientifique des Cézeaux, Université Blaise Pascal,

16 24 rue des landais

17 63177 Aubière Cedex, France

18 Tel: +33 4 73 40 74 76

19 Fax: +33 4 73 40 79 03

20 E .mail: josette.alliot@univ-bpclermont.fr

21
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24

25 **Abstract**

26 A deleterious reduction of casein intake occurring earlier in males than in females had been
27 previously observed in old Lou/Cjall rats. On the contrary, protein intake was observed to be
28 maintained in old males when they were offered whey protein. Present studies were designed to
29 investigate the effect of dietary casein modification on protein decrease. In two lifelong studies,
30 male and female Lou/Cjall rats were tested every four months in order to study protein intake
31 depending on the protein available: casein, whey protein or casein supplemented with an amino acid
32 mixture (SC). In subsequent cross-sectional studies, young, adult, middle-aged and old rats were
33 successively fed with casein, casein supplemented either with leucine or with alanine or with
34 glycine. Supplementing casein with an amino acid mixture both globally increased protein intake
35 and allowed old males to maintain a high rate of protein intake. In cross sectional experiments, no
36 effect of supplementation was seen in the young group. In older animals, the greatest effect was
37 seen when casein was supplemented with alanine or glycine, independently of sex and age. We
38 therefore, concluded that supplemented casein is more beneficial for old rats than casein alone,
39 probably by increasing amino acid availability. We hypothesize that alanine could act through its
40 effect on gluconeogenesis.

41

42 **Keywords:** Aging, casein, self-selection, amino acid supplementation, Lou/Cjall rats.

43 **1. Introduction**

44 Changes in macronutrient intake as a function of age and sex were highlighted by previous
45 studies carried out on Lou/Cjall rats, a lean strain which had been proposed as a model of healthy
46 aging (Alliot et al. 2002). As rats grew old, a shift in energetic nutrient preference from
47 carbohydrates to fat and a decrease in protein intake occurred (Veyrat-Durebex and Alliot 1997).
48 Moreover, a sexual dimorphic protein intake appears in old age. After 16 months of age, females
49 were shown to maintain the protein intake rate around 12% of total energy intake until 28 months,
50 while males dramatically decreased it. The reduction of protein intake observed in aged rats was
51 concurrent with body weight loss, skeletal muscle atrophy and decreased plasma concentrations of
52 IGF1 (Veyrat-Durebex et al., 1999).

53

54 Young and mature rats are capable of selecting a sufficient amount of protein to satisfy amino acid
55 requirements (White et al. 2000). We previously showed that this ability is manifested in old age:
56 old and senescent rats increased their protein intake in response to the specific needs induced by
57 protein deprivation (Boghossian et al., 2002) and by treatment with dexamethasone ((Nzang
58 Nguema et al., 2005). Therefore, it has been highlighted that a strong reduction of protein intake
59 with age cannot be attributed to a loss of regulation of protein intake with age.

60 So far, we used casein as the source of protein. The adequacy of casein for young rats had been
61 shown in numerous studies. Its composition specifically the ratio of essential to non-essential amino
62 acids is adequate to ensure protein synthesis in developing rats (James and Treloar, 1981;
63 Krajcovicova and al., 1993; Semon et al., 1987). However, aging may induce changes in amino acid
64 requirements in senescent animals. Moreover, studies on protein digestion rates and amino acid
65 absorption led to the concept of slow and fast dietary proteins (Boirie et al., 1997). Casein is a slow
66 dietary protein, which in young rats, results in a significant weight gain and in parallel assists
67 greatly in nitrogen retention when compared to the corresponding crystalline amino-acid mixture
68 (Daenzer et al., 2001). In elderly subjects, a fast protein such as whey protein might be more
69 beneficial than casein in order to limit protein loss (Dangin et al., 2003). Thus, casein may become

70 an inadequate source of protein for old animals, inducing an innate avoidance/aversion behaviour, a
71 protective mechanism against imbalanced food sources (Harper et al., 1970; Koehnle et al., 2003).
72 This hypothesis was supported by recent data; using various sources of proteins, we showed a
73 reduction of consumption of casein but not of whey protein in old rats (Nzang Nguema et al., 2004).
74 Consequently, the experiments presented here were designed to study the effect of dietary casein
75 modification. First, casein was supplemented with an amino acid mixture, in order to obtain a
76 composition close to that of whey protein. Two male and female cohorts were simultaneously
77 studied and the free choice of supplemented casein (SC) throughout life was compared to the free
78 choice of casein (C) and whey protein (W)
79 Second, using cross sectional studies, we studied the effect of supplementation of casein with
80 specific amino acids on casein intake, in order to identify specific amino acid requirement of old
81 animals.

82

83

84 **2- Materials and Methods**

85

86 **2-1 Animals**

87 The rat facilities and protocol were approved by the appropriate ethics committee of the University.
88 Male and female rats of the inbred Lou/Cjall strain (Blaise Pascal University, France) were used.
89 The first Lou rats of Wistar origin (Bazin, 1990) were imported into the University of Louvain
90 (Belgium) as a single pair in 1937-1941. The Lou/Cjall rats were bred in our laboratory for 15
91 years. The biological and physiological changes occurring during aging have been previously
92 described (Alliot et al., 2002). Briefly, the most interesting characteristics of this strain are the
93 longevity (median life span is 29 months in males and 33-34 months in females), the absence of
94 obesity and the absence of severe pathologies. Basic phenomena of aging, expressed as body weight
95 loss and muscular atrophy as well as energy and protein intake declines, appear after 18 months of

96 age in males and are delayed in females. After 24 months of age, a markedly greater rate of body
97 and muscle mass loss seems to trigger the onset of senescence.

98 Lou/Cjall rats were housed in pairs in plastic cages (40/20/15 cm) and placed in a
99 temperature-controlled room ($22 \pm 1^\circ\text{C}$) on a 12:12h light dark cycle (lights off at 2000h). At the
100 time when rats were not employed for experiments, they were granted free access to both water and
101 standard pellets U.A.R. A04 (Usine d'Alimentation Rationnelle, Villemoisson, France). The energy
102 content of standard pellets was 2.9 kcal/g and consisted of 17.5% of soluble fish protein
103 concentrate, 3% of fat, and 49.8% of carbohydrate.

104

105 **2-2 Experimental Design**

106 Two separate experiments, including several stages, were successively conducted.

107

108 **2-2-1 Experiment 1**

109 The experimental design is depicted in Figure 1. 52 Lou/Cjall rats were employed for the
110 experiment. At the start of the experiment, the rats were 3 month-old for cohort 1 (12 males and 12
111 females) and 12 month-old for cohort 2 (14 males and 14 females). They were first adapted to the
112 powder diet and then habituated to the self-selection procedure. During the habituation periods,
113 casein (C: Louis François, Saint-Maur, France) and whey protein (W: Cofranlait, France) were
114 used.

115 **Habituation to Powder Diet:** All rats were maintained on a powdered chow diet for two weeks in
116 order to familiarise them to the experimental apparatus and to the new form of the diet. The chow
117 diet composition was closed to the U.A.R. A.04 composition (metabolisable energy was 3.31
118 Kcal/g). It consisted of 17% protein, 3% fat (lard), 70% carbohydrate (cornstarch: Cerestar 12018
119 Louis François Exploitation, France) 5% salt mixture (UAR, 205b³), 4% cellulose powder and 1%
120 vitamins (UAR, 200⁴). Food-filled (20 ± 2 g) glass cups anchored in the back of the cage with a
121 pierced stainless steel plate were used. To allow a habituation to the taste and odour of the two

122 different proteins, two powder diets differing only in their protein content (C or W) were
123 simultaneously available

124 At the end of the habituation, no spillage of food was seen.

125 ***Habituation to the Self-Selection Procedure:*** All rats were habituated to the self-selection
126 procedure during an 18-days period. Protein, fat and carbohydrates were placed in three separate
127 cups. The fat component (7.88 kcal/g) contained 91% lard and 2% sunflower oil. The carbohydrate
128 component (3.34 kcal/g) consisted of 85% cornstarch and 8% commercial grade sucrose. Salt
129 mixture (4 %), vitamins (1%) and cellulose powder (2%) were added to each macronutrient.

130 The offered protein change every 2 days according to 2 different patterns: *C, W or W, C*.

131 Then, a first test period (12 days) was introduced. Each protein was successively given for 6
132 consecutive days to each animal either in the order C, W or W, C.

133

134 Afterwards, when rats reached the age of 7, 11, 15, 19, 23, 27 for cohort 1 and the age of 16, 20, 24
135 and 28 months for cohort 2, they were tested again. They were submitted to the self-selection
136 procedure and to test period as described above. However, during the test, an additional period of 6
137 days was introduced at the end of the test. During this additional period, casein supplemented with
138 an amino acid mixture (15% of free amino-acids) was used as source of protein. Amino acid
139 mixture was used so that the supplemented casein (SC) mimicked whey protein composition (**Table**
140 **1**).

141

142 Body weight and macronutrient intakes were recorded daily during habituation and test periods.

143 At the age of 27-28 months, only 10 males (38.5%) and 15 females (61.5%) were successfully able
144 to survive. The first deaths occurred at 23 months (5 males and 2 females in cohort 1; 3 males and 3
145 females in cohort 2), as previously described.

146

147 **2-2-2 Experiment 2**

148 Experiment 2 was conducted to determine if old rats selected greater amount of casein, when it was
149 supplemented with individual amino acids. The experimental design is depicted in Figure 2. In
150 experiment 2a, casein was supplemented either with leucine or with alanine, used as isonitrogenous
151 control. Indeed, alanine can be considered as inert, and may be used as “placebo” (Chambon-
152 Savanovitch et al., 1999). In experiment 2b, the effect of supplementation of casein with alanine
153 was compared to supplementation with glucose and with glycine, taken as isonitrogenous control.

154 *Animals:*

155 44 male and 48 female Lou/Cjall rats were used. At the onset of the experiments, they were
156 distributed into four age groups: young [2 month-old; 14 males ($123\text{g} \pm 2.8$) and 12 females (104g
157 ± 1.5)], mature [7 month-old; 12 males ($265\text{g} \pm 3.4$) and 11 females ($178\text{g} \pm 3.1$)], middle-aged [16
158 month-old; 12 males ($314\text{g} \pm 8.2$) and 12 females ($199\text{g} \pm 4.2$)], old [24 month-old; 8 males (296.4g
159 ± 15.5) and 11 females ($190\text{g} \pm 3.8$)].

160 *Experiment 2a*

161 The total duration of the experiment 2a was 50 days, proceeding in three stages as already
162 described: habituation to the powder diet, habituation to the self-selection procedure and test
163 period. As only casein was used as the source of protein, only one chow diet was offered in the first
164 stage of habituation and only casein was given during habituation to self-selection. The last four
165 days of habituation were used as the basal period for casein intake.

166 The test lasted for 18 days. At that time, animals were respectively 3 month-old for young group,
167 and 8, 17 and 25 month-old for respectively mature, middle-aged and old groups. Three proteins
168 were used: casein (C), casein supplemented with 4.5% of leucine (CLeu) and casein supplemented
169 with 4.5% of alanine (CAla). Each protein was given during 6 consecutive days, according to the
170 following schedule: C, CLeu, CAla for one third of animals; CLeu, CAla, C for the second third;
171 and CAla, C, CLeu, for the last third. Rats of all age groups were distributed into each pattern of
172 protein distribution.

173 *Experiment 2b*

174 Three months later, the adult rats used in experiment 2a were tested again. Animals were
175 maintained for 3 weeks in the self-selection procedure with casein as a source of protein before the
176 test began. The last four days of habituation were used as the basal period for casein intake.

177 The test lasted for 24 days. At that time, animals were 11, 20 and 28 month-old for respectively
178 mature, middle-aged and old groups. Four proteins were used: casein, casein supplemented with
179 4.5% of glucose (Cglu), casein supplemented with 4.5% of alanine (CAla) casein supplemented
180 with 4.5% of glycine (CGly). Each protein was given during 6 consecutive days, according to the
181 following schedule: C, Cglu, CAla, CGly for a quarter of animals; Cglu, CAla, CGly, C for the
182 second quarter; CAla, CGly, C, Cglu for the third quarter; and CGly, C, Cglu, CAla for the last
183 quarter. Animals of all age groups were distributed into each pattern of protein distribution.

184 Body weight and macronutrient intakes were recorded daily.

185

186 **2-3 Statistical Analyses**

187 All data are expressed as mean \pm SEM. As the rats were housed in pairs, the statistical unit
188 for food intake was generally the pair of rats except when a rat died because its counterpart
189 remained alone in the cage until the end of experiment.

190 **Experiment 1:** The data of the last four days of each test period were considered for statistical
191 analysis. A One-way ANOVA (effect of sex) for repeated measures (age and protein choices) with
192 significance at $p < 0.05$ was used. When a significant overall effect was detected, differences
193 between means were assessed with a mean least square test.

194 **Experiment 2:** The data of the last four days of habituation (basal intake) and of each test period
195 were considered for statistical analysis. A Two-way ANOVA (effect of age and sex) for repeated
196 measures with significance at $p < 0.05$ was performed to evaluate the effect of supplementation on
197 casein intake. ANOVA was followed by a mean least square test for a-posteriori means comparison.

198

199

200 **3- Results**

201

202 **3-1 Experiment 1: Life Long Studies**203 ***Body Weight and Caloric Intake (Table 2)***

204 Body weight and caloric intake were in agreement with previously obtained data (Alliot et al.
205 2002). In the two cohorts, a loss of body weight appeared in old males but not in females.
206 Concerning the age-related changes in caloric intake, females increased their caloric intake from the
207 age of 23 months onwards. Thus, the differences in caloric intake between males and females were
208 strongly reduced by 27 months of age.

209

210 ***Diet Selection and Effect of the Type of Protein (C or W)***

211 As rats grew old, they underwent a noticeable change in their diet composition (*cohort 1*: $F(12, 84)=16.4$; $p = 0.0001$; *cohort 2*: $F(8,48)=11.1$; $p = 0.0001$): rats consumed more fat and less
212 carbohydrates and protein (**Figure 3**).

214 The type of protein (C or W) did not induce significant changes either in total caloric intake or in fat
215 and CHO preference. However, it modified protein consumption. Overall, rats ate more casein than
216 whey protein but this preference appeared clearly only in the female group, mainly in the young age
217 (mean intake: $17.2\% \pm 1.4$ of casein versus $10.9\% \pm 0.5$ of whey protein. The pattern of protein
218 consumption differed as a function of protein and gender: (*protein X sex X age* ($F(6,42)=2.1$;
219 $p=0.07$). The preference for casein disappeared from 15-16 months of age in female groups
220 whereas a preference for whey protein appeared in males (10% versus 5% in the cohort 1). The
221 same results were observed in cohort 2: preference for whey protein in males, no preference for
222 casein in females from 16 months of age. Therefore, the reduction in protein intake seen in old male
223 groups after 15-16 months of age appeared only with casein and not with whey protein.

224

225 ***Effects of the Casein Supplementation***

226 Initially, when the rats had access to casein supplemented with an amino acid mixture (**Figure 4**),
227 they took a greater amount of protein, compared to casein alone ($F(1,7) \geq 48.4$; $p \geq 0.0001$; mean

228 intake *cohort 1*: 7.1 ± 0.4 kcal versus 5 ± 0.4 kcal; $20.3\% \pm 1$ versus $14.2\% \pm 1$; *cohort 2*: 4.4 ± 0.3
229 versus 2.1 ± 0.3 kcal; $12.1\% \pm 0.8$ versus $7.5\% \pm 0.8$). Secondly, the age-related changes in
230 protein intake underwent modification, mainly in males (*Interaction age x sex x protein* (F (5,44)=
231 2.9; p=0.03) in cohort 1; (F(3,26)= 3; p=0.04) in cohort 2). The consumption of SC was maintained
232 at a higher level than casein and remained constant from 15-16 months of age in males, at the level
233 of whey protein consumption. Females selected identical amounts of casein and SC except at the
234 age of 15 and 19 months in cohort 1. As was the case for casein alone, females preferred SC to
235 whey protein. This preference for SC versus whey protein disappeared at only 23-24 months in the
236 two cohorts (instead of 15-16 months for casein alone).
237 The nature of the protein did not influence the total energy intake (F (2,12) = 1.2 ns).

238

239 **3-2 Experiment 2.**

240 **3-2-1 Experiment 2a**

241 At the end of habituation, diet composition chosen by young rats was obviously different from that
242 chosen by other groups (**Table 3**). Young rats mainly selected CHO whereas all other groups
243 mainly chose fat as a source of energy. Large inter-groups variations in protein intake were
244 observed in females.

245 The basal intake of calories was dependent on age F (6,146)=72; p > 0.0001), being high in young
246 group and low in old groups.

247

248 *Effect of Supplementation on Total Protein Intake*

249 In the young groups, no effect of supplementation appeared (F (2,22) = 3 ns). However,
250 in the older groups, the amount of casein consumed was higher (F (2,56) = 35; p = 0.0001) when it
251 was supplemented. The greatest effect was seen with alanine as a supplement, independent of the
252 sex and age of animals (**Figure 5**).

253

254 **3-2-2 Experiment 2b**

255 Anova showed an effect of age ($F(2,28) = 6.4; p = 0.005$) and an effect of protein ($F(3,84) = 31; p$
256 $= 0.0001$). Total protein intake was greater in 11 month-old groups compared to older groups.
257 Casein supplemented with glucose had no effect on protein consumption whereas supplementation
258 with alanine and glycine increased casein consumption in the same way (**Figure 6**).

259

260 **4-General Discussion**

261 When casein was offered, the well-established age and sex-related changes in protein consumption
262 of Lou/Cjall rats (Alliot et al. 2002) were seen in the two cohorts. There was a clear gender
263 difference, with females maintaining their protein intake around 10-12% whereas the males showed
264 a strong decrease after 15 months of age. This late decrease was prevented by the use of whey
265 protein, according to previously obtained results (Nzang Nguema et al., 2004). When casein was
266 supplemented either with amino acid mixture or with a particular amino acid, mature and old rats
267 chose it in greater amount than casein alone. More specifically, experiment 1 showed that adding an
268 amino acid mixture to casein counteracted the strong decrease of casein consumption in males as
269 they grew old and maintained the preference for casein longer in the life in females. These data
270 suggest that supplemented casein, like whey protein, must be greatly beneficial for old rats than
271 casein alone and consequently, they support the hypothesis that casein may be an imbalanced or an
272 inadequate amino acids source for old rats, inducing an aversion behaviour.

273 As already mentioned, the adequacy of casein for young rats had been shown in numerous studies
274 but amino acid requirement is probably dependent on age (Young et al., 1994; Reeds, 2000). The
275 nutritional classification between dispensable, conditionally indispensable and indispensable amino
276 acids could be inaccurate for senescent organisms and the ability to synthesise the conditionally
277 essential amino can decrease with age.

278 However, as casein was supplemented with a mixture of free amino acid, both the amount and the
279 speed of absorption of amino acids were modified. We showed (Nzang Nguema 2005) that plasma
280 amino acid concentration rose as soon as within 15 minutes after an intake of a meal supplying CS.
281 Increasing amino acid availability via oral intake of mixed amino acid (Paddon-Jones et al., 2004;

282 Volpi et al., 1999) increased amino acids transport into muscle cells in elderly healthy humans and
283 stimulated protein synthesis. Therefore, supplementing casein can improve an efficient use of
284 exogenous amino acids by skeletal muscle. Volpi et al. (2003) showed that effective supplements
285 have to include essential amino acids. The analysis of the mixture added to the casein in experiment
286 1 suggested that leucine was the limiting amino acid. Indeed, the branched-chain amino acids
287 (BCAA) support numerous metabolic processes and the potential for BCAA to participate in each
288 of these metabolic processes appears to be proportional to their availability (Layman, 2003). More
289 specifically, leucine is the only amino acid able to reproduce the effect of a mixture of amino acids
290 on muscle protein synthesis. Moreover, old rats are less sensitive to its effect (Dardevet et al., 2000)
291 but an acute meal supplementation with leucine is sufficient to restore postprandial stimulation of
292 muscle protein synthesis (Dardevet et al., 2002). Since a small deficiency in a limiting amino acid
293 should be able to decrease protein selection (Hrupka et al., 1997), we hypothesised that leucine
294 supplement could restore the protein intake of old rats. Surprisingly, in experiment 2a, even though
295 leucine induced a slight stimulation of casein intake in adult and old groups, the greatest effect was
296 seen with alanine. Alanine is a dispensable amino acid, considered as being inert (Chambon-
297 Savanovitch et al., 1999). It has no effect on protein synthesis in old rats (Dardevet et al., 2002).
298 Nevertheless, it is possible that a greater intake of any amino acid resulting in a greater plasmatic
299 concentration may have stimulated a greater level of oxidation.

300 However, another possible mechanism can be evoked. On one hand, alanine is an important
301 precursor for hepatic gluconeogenesis (Ahlborg et al., 1974; Ruderman, 1975). It represents one of
302 the major amino acids released from skeletal muscle and converted to glucose in the liver (Garber et
303 al., 1976). Most amino acids are precursors for alanine synthesis in muscle (Garber, 1976),
304 specifically leucine (Holecek, 2001) and glycine (Garber et al., 1976; Chambon-Savanovitch, 1999;
305 Volpi et al., 2003). Glycine is also a precursor of serine, another gluconeogenic substrate. On the
306 other hand, glucose intolerance developed in aged animals. Spontaneous intake of carbohydrates is
307 strongly reduced in old rats compared to young ones, and fat plays a prominent role as an energetic
308 fuel in the diet. It is likely that there is an increased contribution of alanine to glycogen repletion

309 and that the source of alanine in the muscles of adult and old animals is mainly amino acids
310 precursors. However, in the present study, supplementing casein with glucose did not stimulate
311 casein intake. This last data could support a reduction in the glucose transport and utilisation with
312 aging.

313 Although casein supplementation with a particular amino acid was ineffective in young
314 rats, it had an identical stimulating effect in adult and old rats. A first explanation can lie in the use
315 of cross-sectional studies. As the three groups were compared on the basis of chronological age
316 whereas physiological aging may differ from those age cohorts, cross-sectional experiments are not
317 appropriate, compared to life long experiments, to study changes with advancing age. Secondly, in
318 behavioural as well as in metabolic studies, attempts to identify a unique amino acid requirement
319 are likely to be unsuccessful. Multiple results could be achieved for the same amino acid, depending
320 on the interaction of various factors such as the amount and the nature of protein and nonprotein
321 energy ingested (Wolfe, 2002). Moreover, there are interactions between individual amino acids,
322 which result in the concentrations of one amino acid influencing the oxidation rate and hence the
323 requirement of others (Millward and Rivers, 1988). Particularly, increased intake of leucine
324 increased plasma leucine concentration but decreased isoleucine and valine availability (Harper and
325 Benjamin, 1984).

326

327 In conclusion, the loss of appetite for protein observed in old age can be counteracted by
328 supplementation with a balanced mixture of amino acids, probably by increasing the amino acids
329 availability and followed by the ability to efficiently use them. Moreover, the unexpected
330 stimulating effect of supplementation with alanine suggests an age-related increase in
331 gluconeogenic substrates requirements. Nevertheless, further studies are needed to address this last
332 issue.

333

334

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336

337

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458

LEGENDS OF FIGURES

459

460

461 **Figure 1:**462 *Experiment 1- Procedure*

463 A: 3 month-old (cohort 1) and 12 month-old (cohort 2) male and female rats were submitted to
464 habituation periods (habituation to powdered complete diet and to self selection procedure (18
465 jours). During test, casein (C) and whey protein.(W) were successively available.

466 B: Four months later, and after, each four months, all rats were submitted to self selection
467 procedure. An additional test period was introduced, during which supplemented casein was given.

468

469 **Figure 2**470 *Experiment 2- Procedure*

471 *Experiment 2A:* Four groups of age (2, 7, 16 and 24 months) were habituated to powdered diet and
472 to self selection procedure. During test, casein alone and casein supplemented either with leucine
473 (Cleu) or with Alanine (Cala) were successively available.

474 *Experiment 2B:* 3 months later, adult, middle-aged and old rats were tested again. During test,
475 casein alone and casein supplemented either with glucose (CGlu) either with alanine (CAla) or with
476 glycine (Cgly) were successively and randomly proposed.

477

478

479 **Figure 3:**

480 Age-related changes in diet composition as a function of advancing age and of dietary protein in
481 cohort 1(upper panels) and cohort 2 (lower panels)

482 During test, casein and whey protein are successively proposed during six days. Each point
483 represents the mean percentage of total caloric intake for protein (P), fat, and carbohydrates (CHO)
484 during the last four days of the test periods.

485 *a posteriori*-tests: a: $p < 0.05$ vs 3 months b: $p < 0.05$ vs 7 months;
 486 c: $p < 0.05$ vs 11-12 months d: $p < 0.05$ vs 15-16 months
 487 * $p < 0.05$ vs casein $\neq p < 0.05$ vs male group

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490 **Figure 4** : Protein intake of male and female Lou/Cjall rats as a function of advancing age and
 491 protein available.

492 Data points indicate the mean 24-h protein selection in Kcal (mean \pm SEM) when rats were given
 493 C, W or SC.

494 *a posteriori*-tests: b: $p < 0.05$ vs 7 months; c: $p < 0.05$ vs 11-12 months
 495 d: $p < 0.05$ vs 15-16 months e: $p < 0.05$ vs 19-20 months
 496 * $p < 0.05$ vs casein \$ $p < 0.05$ vs whey

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500 **Figure 5**: Experiment 2a: Casein intake during the test period in young rats (upper panel) and in
 501 adult, middle-aged and old rats (lower panel).

502 Data points represent mean (\pm SEM) caloric intake of casein during the last four days of habituation
 503 (C_{basal}) or during the last four days of presentation of each protein (C, C_{Leu} and C_{Ala}) during the
 504 test. The intake of casein alone was similar during the test compared to basal period.

505 *a posteriori* tests: a: $p < 0.05$ vs 8 month-old; b: $p < 0.05$ vs 17 month-old;
 506 * $p < 0.05$ vs casein \$ $p < 0.05$ vs Cleu

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511 **Figure 6**: Experiment 2b: Casein intake during the test period.

512 Data points represent mean (\pm SEM) caloric intake of casein during the last four days of habituation
 513 (C_{basal}) or during the last four day of presentation of each protein (C, C_{glu}, C_{Gly} and C_{Ala}) during
 514 the test period. The intake of casein alone was similar during the test compared to basal period.

515 *a posteriori* tests: a: $p < 0.05$ vs 11 month-old; b: $p < 0.05$ vs 20 month-old;
 516 * $p < 0.05$ vs casein $\mu p < 0.05$ vs C_{glu}



TABLE 1 :
Amino acid composition of the mixture, whey protein, casein¹ and SC²

	% of amino-acid in mixture	% of amino acid in protein diet		
		Whey Protein ¹	Casein ¹	Supplemented casein (SC) ²
<i>Essential amino-acids (%)</i>				
Histidine	1.26	1.9	2.9	2.8
Isoleucine	3.38	5.0	5.1	4.8
Leucine	30.00	12.3	9.2	12.3
Lysine	6.55	9.7	8.0	7.8
Methionine	1.34	2.0	2.9	2.7
Phenylalanine	2.43	3.6	5.1	4.9
Threonine	3.56	5.3	4.3	4.2
Valine	3.25	4.8	6.6	6.5
Tryptophane	7.97	2.1	1.1	2.1
<i>Nonessential amino-acids (%)</i>				
Alanine	3.12	4.6	3.0	3
Arginine	1.91	2.8	3.6	3.9
Aspartate	4.60	6.8	7.1	6.5
Asparagine	3.08	4.6	4.9	4.6
Glutamate		10.9	23.7	20.1
Glutamine	3.95	5.9	6.2	5.9
Glycine	1.34	2.0	1.8	1.7
Serine	3.30	4.9	5.7	5.3
Tyrosine	2.34	3.5	5.6	5.1
Proline		4.1	10.3	8.7
Cysteine	16.60	2.9	0.5	2.9

¹Amino-acid composition of whey protein and casein were obtained from INRA.

²SC was composed of 85 % casein and 15 % amino-acid mixture

TABLE 2 :

Body weight and caloric intake of male and female LOU/Cjall before the start of experimental periods in experiment 1

		Males		Females	
	Age (months)	BW (g)	Caloric intake (Kcal/24h)	BW (g)	Caloric intake (kcal/24h)
Cohort 1	3	180.9 ± 3.6	49.1 ± 1.3	119.0 ± 2.2 [#]	34.9 ± 1.2 [#]
	7	277.4 ± 4.0 ^a	45.6 ± 1.9	170.3 ± 3.4 ^{a#}	33.0 ± 1.6 [#]
	11	301.7 ± 8.4 ^{ab}	42.6 ± 1.7	189.8 ± 2.9 ^{a#}	31.7 ± 1.5 [#]
	15	301.0 ± 12.0 ^{ab}	42.2 ± 1.3	193.2 ± 4.1 ^{ab#}	32.1 ± 0.8 [#]
	19	297.9 ± 18.9 ^{ab}	41.5 ± 0.9 ^a	203.2 ± 4.1 ^{ab#}	30.5 ± 0.9 [#]
	23	302.3 ± 28.9 ^{ab}	40.0 ± 1.5 ^a	193.1 ± 8.6 ^{ab#}	33.4 ± 1.6 [#]
	27	285.2 ± 11.1 ^{acdef}	44.1 ± 2.0	189.8 ± 4.1 ^{ab#}	34.8 ± 1.8 [#]
Cohort 2	12	284.7 ± 7.2	44.0 ± 1.4	178.9 ± 3.4 [#]	30.4 ± 1.5 [#]
	16	296.3 ± 7.6	41.1 ± 1.4	184.9 ± 3.5 [#]	30.2 ± 1.2 [#]
	20	286.8 ± 12.7	41.4 ± 3.1	182.3 ± 4.7 [#]	34.3 ± 2.3 [#]
	24	278.0 ± 11.0 ^{cdc}	43.9 ± 3.2	178.0 ± 7.5 [#]	35.2 ± 2.9 [#]
	28	267.1 ± 19.2 ^{cdef}	40.6 ± 3.4	185.7 ± 6.0 [#]	36.2 ± 5.8 ^{cd}

[#] p<0.05 vs male group;

^a p<0.05 vs 3 –month-old ; ^b p<0.05 vs 7 –month-old ; ^c p<0.05 vs 11-12 months

^d p<0.05 vs 15-16 months; ^e p<0.05 vs 19-20 months; ^f p<0.05 vs 23-24 months. (a-posteriori tests)

A



TABLE 3:

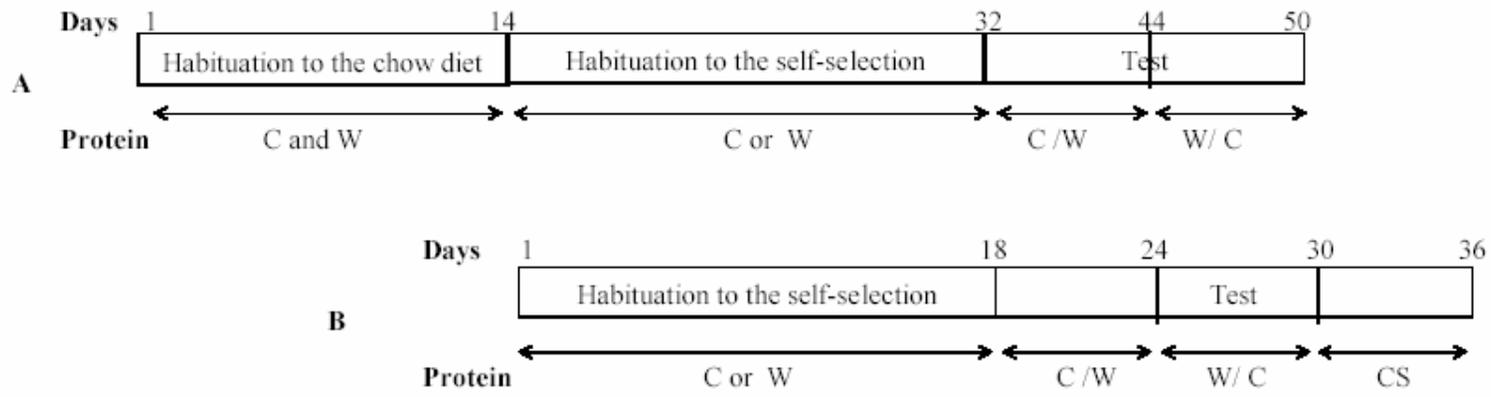
Percentage of macronutrients and total caloric intake (Kcal/24h) before test in experiment 2a .

	<i>Males</i>				<i>Females</i>			
	Young	Adult	Middle-aged	Old	Young	Adult	Midde-aged	Old
%Casein	24.3 ± 0.7	13.0 ± 1.7 ^a	13.5 ± 3.1 ^a	9.7 ± 1.5 ^a	13.9 ± 1.8 [#]	23.0 ± 3.4 ^{a#}	14.8 ± 2.4	18.0 ± 3.8 ^b
% Fat	16.0 ± 1.9	66.0 ± 1.7 ^a	60.0 ± 2.7 ^a	60.3 ± 3.7 ^a	18.1 ± 2.7	47.0 ± 4.0 ^{a#}	63.2 ± 3.5 ^{ab}	60.0 ± 4.7 ^{ab}
% carbohydrate	60.2 ± 2.2	21.0 ± 2.2 ^a	26.5 ± 2.4 ^a	30.0 ± 5.1 ^a	68.7 ± 2.5	30.0 ± 6.5 ^a	22.0 ± 2.7 ^a	22.0 ± 4.5 ^a
Total caloric intake	48.7 ± 0.5	41.3 ± 1.6	44.3 ± 1.0	39.0 ± 2.1 ^c	36.0 ± 1.7 [#]	34.8 ± 1.8 [#]	31.8 ± 1.2 [#]	31.1 ± 1.3 [#]

A posteriori tests: [#] p<0.05 vs male group; ^a p<0.05 vs young ; ^b p<0.05 vs adult; ^c p<0.05 vs middle-aged



Nzang et al EXG- FIGURE 1



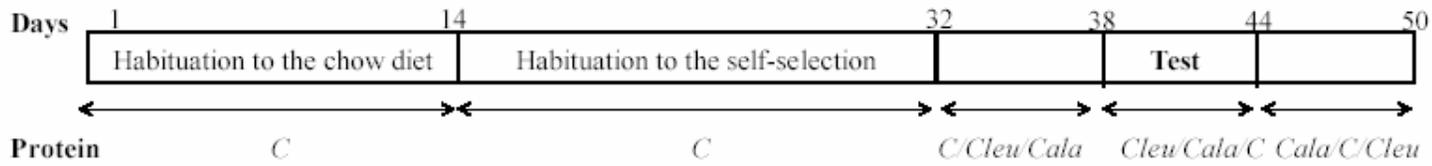
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ACCE

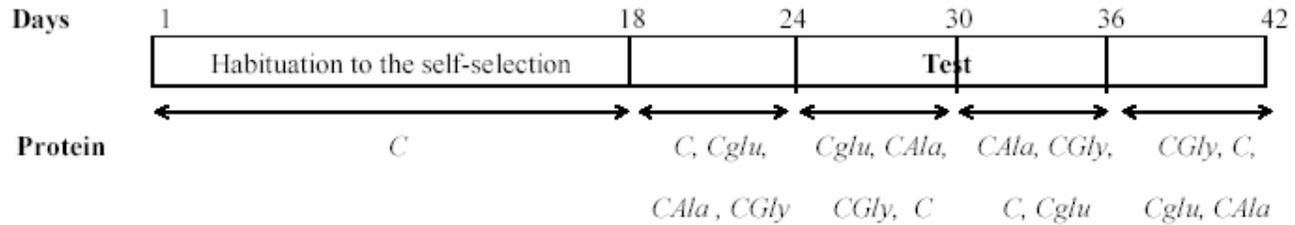


Nzang et al EXG Figure 2

Experiment 2A



Experiment 2B



AC



Figure 3

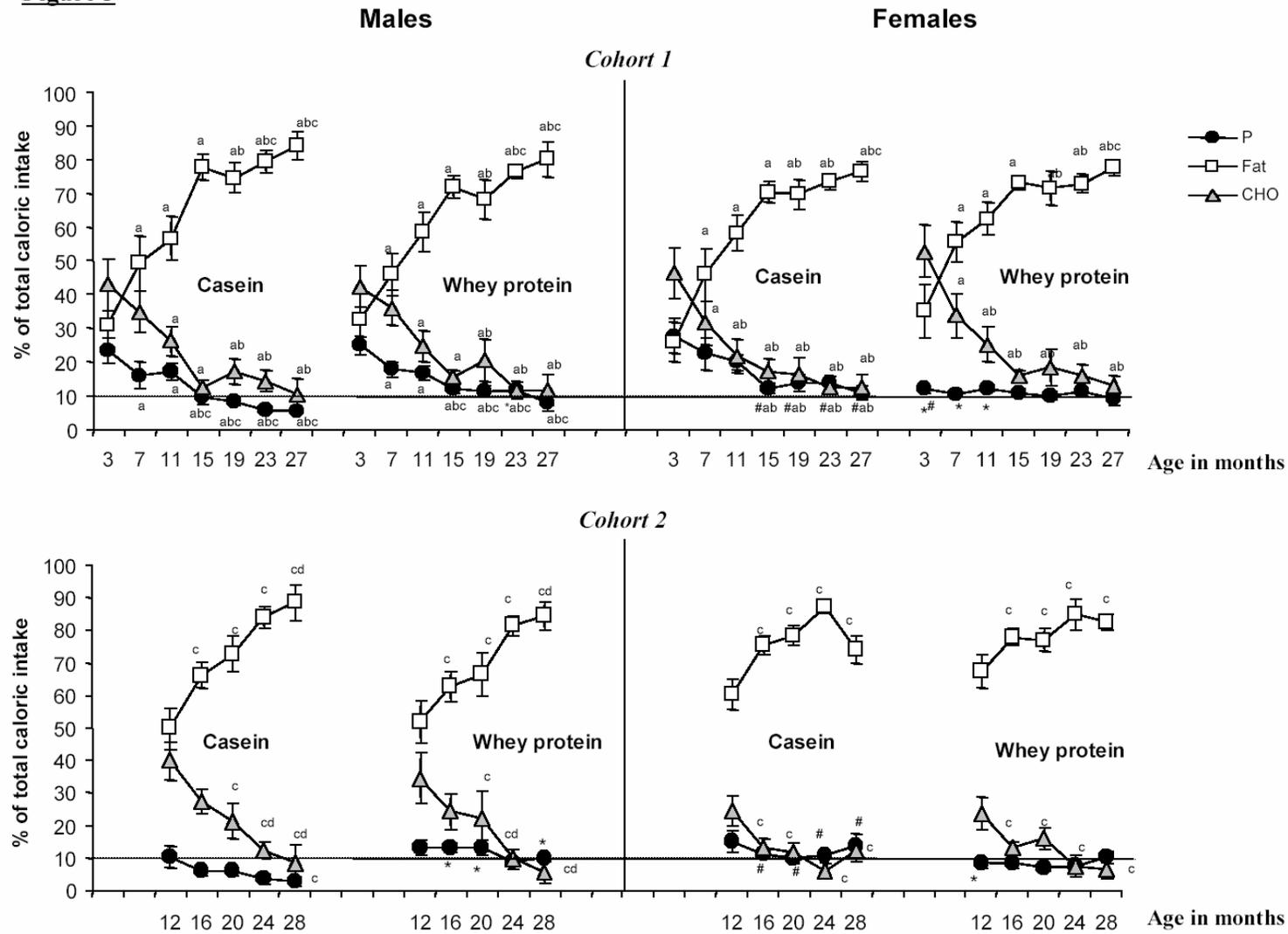




Figure 4

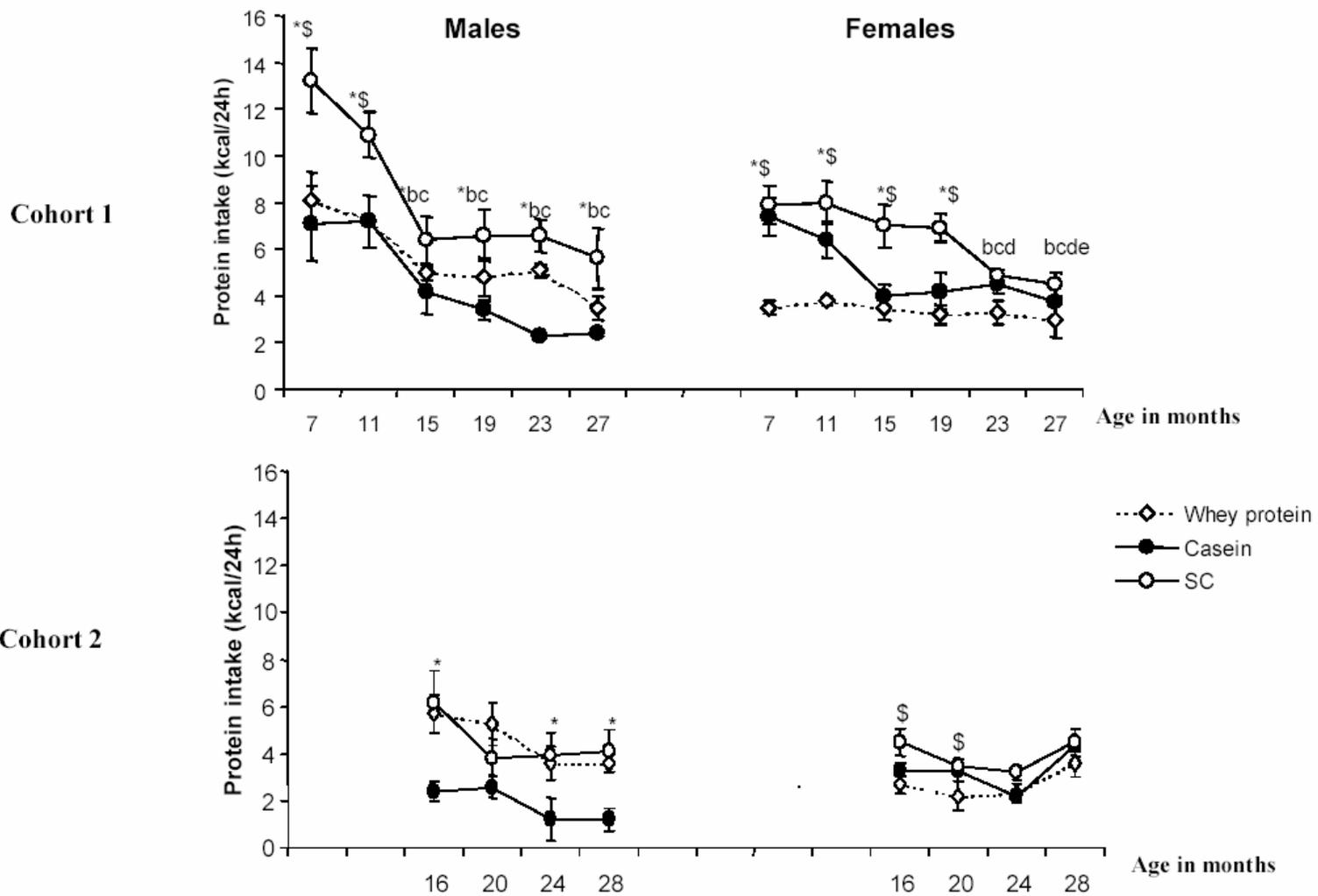




Figure 5

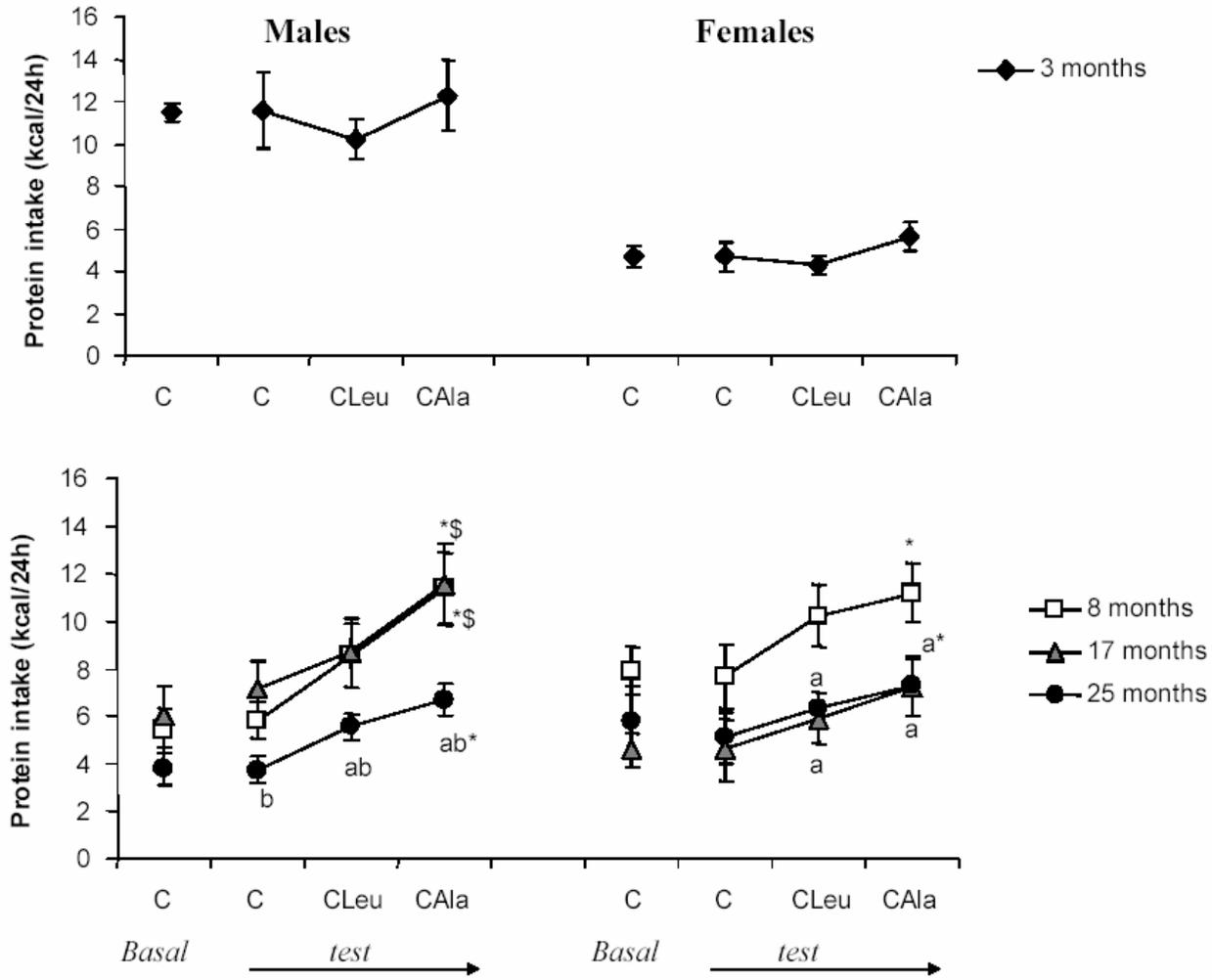


Figure 6

