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Moderate adiposity levels counteract protein metabolism modifications associated with aging in rats

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► To cite this version:

Nathalie Atallah, Claire C. Gaudichon, Audrey Boulier, Alain Baniel, Dalila Azzout-Marniche, et al.. Moderate adiposity levels counteract protein metabolism modifications associated with aging in rats. European Journal of Nutrition, 2022, 10.1007/s00394-022-02881-4 . hal-03647308

HAL Id: hal-03647308

<https://hal.inrae.fr/hal-03647308>

Submitted on 13 Sep 2023

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18 **Abstract**

19 **Purpose:** Physiological parameters such as adiposity and age are likely to influence protein
20 digestion and utilization. The aim of this study was to evaluate the combined effects of age
21 and adiposity on casein protein and amino acid true digestibility and its postprandial utilization
22 in rats.

23 **Methods:** Four groups were included (n = 7/8): 2 months/normal adiposity, 2 months/high
24 adiposity, 11 months/normal adiposity and 11 months/high adiposity. Rats were given a
25 calibrated meal containing ¹⁵N-labeled casein (Ingredia, Arras, France) and were euthanized 6
26 h later. Digestive contents were collected to assess protein and amino acid digestibilities. ¹⁵N
27 enrichments were measured in plasma and urine to determine total body deamination.
28 Fractional protein synthesis rate (FSR) was determined in different organs using a flooding
29 dose of ¹³C valine.

30 **Results:** Nitrogen and amino acid true digestibility of casein was around 95-96% depending on
31 the group and was increased by 1% in high adiposity rats (P = 0.04). Higher adiposity levels
32 counteracted the increase in total body deamination (P = 0.03) that was associated with older
33 age. Significant effects of age (P = 0.006) and adiposity (P = 0.002) were observed in the muscle
34 FSR, with age decreasing it and adiposity increasing it.

35 **Conclusion:** This study revealed that a higher level of adiposity resulted in a slight increase in
36 protein and individual amino acid true digestibility values and seemed to compensate for the
37 metabolic postprandial protein alterations observed at older age.

38

39 **Keywords:** Aging, obesity, Protein metabolism, amino acid digestibility, rat model

40 **Introduction**

41 Protein quality can be defined as a protein's ability to satisfy the metabolic demand for
42 amino acids and nitrogen [1]. It not only depends on its composition in amino acids, but is
43 also conditioned by its digestibility value and the bioavailability of its individual amino acids.
44 To adapt protein intakes to meet the needs of the individual, factors such as age, health
45 status, physiological status, and energy requirements should also be considered when
46 addressing protein quality [2]. Factors influencing protein digestibility have not been entirely
47 elucidated.

48 In humans, the aging process has been associated with modulations to the gastro-intestinal
49 tract, notably with delayed gastric emptying, an increase in colon transit time, and
50 histological changes to the gut [3,4] as well as slower protein digestion [5]. Classic methods
51 to determine protein digestibility in humans are very invasive and not applicable to
52 vulnerable population. Hence, little data address the effect of aging on protein digestibility;
53 but, it was reported to be reduced in old rats compared to young rats, especially for the low
54 digestibility protein sources [6]. In contrast to digestion, muscle anabolism during aging is
55 well documented in both animal models and humans. For instance, a lower postprandial
56 protein synthesis rate was observed in older rats compared to adult rats [7,8] and similar
57 results were obtained in human trial [9] and cohort study [10].

58 Data associating body composition to protein digestion are scarce. However, studies related
59 to impact of overweight or obesity on protein metabolism and the resulting gut adaptations
60 have been conducted in animal models and humans. It was notably shown that diet-induced
61 obese mice underwent major intestinal adaptations, such as an increased intestinal
62 permeability, a delayed transit time, and an increased amino acid absorption alongside an

63 upregulation of some amino acid transporters [11]. Additionally, diet-induced obese mice
64 were shown to synthesize less skeletal muscle protein in response to nutrient ingestion [12]
65 and this result was confirmed in humans as a decline in muscle protein synthesis in response to
66 a high protein meal was found in overweight and obese adults compared to lean controls
67 [13].

68 Results of the aforementioned experiments suggest distinct effects of age and obesity on
69 protein digestion and metabolism, but studies linking both factors are rare and mostly focus
70 on their effect on muscle protein synthesis [14]. We hypothesized that age may decrease
71 protein digestibility and that both age and adiposity may reduce protein utilization and
72 synthesis. The aim of this study was to thus evaluate the combined effects of age and
73 adiposity on casein protein and amino acid true digestibility, nitrogen postprandial utilization
74 and fractional synthesis rates in different organs, after consumption of a ^{15}N -labeled casein
75 meal in a model of young and middle-aged rats with varying levels of adiposity.

76

77 **Materials and methods**

78 **Milk protein labeling**

79 The cow milk was labeled by Ingredia (Arras, France). Briefly, two cows were administered
80 50 g of ^{15}N ammonium sulfate [99%, $(^{15}\text{NH}_4)_2\text{SO}_4$] via drinking water twice a day for 9
81 consecutive days. The milk was collected every day starting from the fourth day. An isotopic
82 enrichment of 1.4 atom percent in the milk was obtained. ^{15}N native micellar casein was
83 then obtained by filtration (Prodiet[®] 85B, Ingredia, Arras, France).

84

85 **Experimental protocol**

86 The study was performed in agreement with the European Union directive 2010/63/EU for
87 animal experiments and approved by the ethics committee in animal experiments of INRAE
88 Jouy-en-Josas (Comethea, registration number: 18-14) and the French Ministry of Higher
89 Education and Research (APAFIS n°15907-2018061823133762 v1). Thirty male Wistar rats
90 aged 1 month (n = 15) and 10 months (n = 15) at their arrival were purchased from Envigo
91 (Horst, The Netherlands). They were housed in individual cages with wire bottoms to
92 prevent coprophagia. Temperature was controlled and rats were subjected to a normal
93 12h/12h dark-light cycle, then gradually switched to a reversed dark-light cycle (dark period
94 07:00 to 19:00) during the fourth week of the experiment. Rats had unlimited access to
95 water and chow for 7 days after arrival, before their respective experimental diets were
96 administered (**Table 1**). Rats were allowed *ad libitum* access to feed for twenty-eight days
97 (**Figure 1**) with either a standard diet or a Western diet in order to obtain rats of normal and
98 high adiposity levels, within each age. Four groups were constituted (n=7/8): 2
99 months/normal adiposity (2m-na), 2 months/high adiposity (2m-ha), 11 months/normal
100 adiposity (11m-na) and 11 months/high adiposity (11m-ha). Middle-aged rats were chosen in
101 order to evaluate the effect of age without confounding effects of old age.

102 Starting from the sixth week (**Figure 1**), all groups were fed the same diet that corresponds
103 to the standard diet composition but with casein (Prodiect® 85B, Ingredia, Arras, France) as
104 the sole source of protein. This 1-week diet standardization was to limit any potential effect
105 of the previous diet on postprandial tests. Rats were placed under a special protocol for a
106 period of one week to accustom them to consuming a calibrated meal at the beginning of
107 the dark phase, as described previously [15].

108 The day of euthanasia, rats consumed a calibrated meal of 4 g containing the ¹⁵N-labeled
109 casein protein. Blood was collected from the tail vein before meal ingestion and 1 h and 3 h
110 after, and plasma was then stored at -20°C. Thirty minutes before euthanasia, rats were
111 injected in the lateral tail vein with a flooding dose (150 μmol/100 g of body weight) of [1-
112 ¹³C] valine (Eurisotop, Saint Aubin, France) under gaseous anesthesia. Rats were euthanized
113 6 h after meal ingestion, after cardiac puncture under gaseous anesthesia. The luminal
114 contents of the different segments of the gastro-intestinal tract segments were rinsed with
115 NaCl (0.9%), collected, and stored at -20°C until freeze-drying, as described previously [15].
116 Feces were also collected under the cages during the postprandial period. The mucosae of
117 the duodenum, jejunum and ileum were scraped and muscle, liver, kidney, and a skin sample
118 were collected, frozen in liquid nitrogen and stored at -80°C. Urine was retrieved during the
119 postprandial period by placing an absorbent paper under the cages and by puncturing the
120 bladder after euthanasia. The absorbent paper was rinsed with distilled water, and the urine
121 eluates were stored at -20°C. Whole body composition was determined by dissection and
122 weighing of the main organs and tissues.

123

124 **Analytical methods**

125 Protein and amino acid true digestibility was assessed by respectively following the ¹⁵N
126 recovery and the ¹⁵N-amino acid recovery in the digestive contents, as described previously
127 [15]. *In vivo* protein synthesis rates in the gastrocnemius muscle, liver, kidney and skin were
128 estimated with the [¹³C]-valine flooding dose method as described previously [16]. Total urea
129 was determined in urine and plasma by the Urease-Berthelot method (Urea assay, Randox,
130 Crumlin, UK). Plasma separation of the nitrogen fraction (protein, free amino acid and urea)

131 was achieved as described previously [17]. ¹⁵N enrichment in plasma fraction and in urine
132 was measured as described previously [18].

133 The RNA expression of hormones [cholecystokinin (CCK), secretin] and the main peptide and
134 amino acid transporters in the intestinal mucosa was determined as described previously
135 [19]. The genes used are presented in **Supplementary Table 1**.

136

137 **Calculations**

138 Estimation of dietary nitrogen or amino acids (mmol) in the different digestive contents was
139 determined using the following formula:

$$140 \quad N_{\text{diet digesta}} = N_{\text{tot}} \times \frac{APE_{\text{digesta}}}{APE_{\text{meal}}}$$

141 Where N_{tot} represents the total amount of nitrogen in the digesta sample (mmol) and APE is
142 the enrichment excess of ¹⁵N in the digesta and meal. APE is defined as the enrichment of
143 the sample in atom percent (AP) minus the natural abundance. Orocaecal digestibility was
144 determined as a proxy of ileal digestibility in rats [15,20] in order to have a sufficient amount
145 of digesta for isotopic enrichment determination. Collection of digesta 6 h after meal intake
146 allows for near complete digestion and minimal duration of fermentation of digesta in the
147 caecum. True orocaecal nitrogen digestibility (%) was calculated as follows:

$$148 \quad \text{True nitrogen orocaecal digestibility} = 100 \times \frac{N_{\text{ing}} - (N_{\text{diet ileum}} + N_{\text{diet caecum}})}{N_{\text{ing}}}$$

149 Where N_{ing} is the amount of nitrogen ingested by the rats (mmol). Because dietary nitrogen
150 was recovered in the stomach and thus did not enter into the digestive process, in all the
151 calculations, N_{ing} excluded this stomach residual amount. True orofecal nitrogen digestibility

152 was also determined considering nitrogen losses in the colon and feces, in addition to losses
153 in the ileum and caecum. Similarly to true oro-caecal nitrogen digestibility, true oro-caecal
154 digestibility for each individual amino acid was determined by estimating the dietary amino
155 acid not absorbed in the intestinal tract and recovered in the digesta, as described previously
156 [15].

157 The dietary nitrogen recovered in the urea body pool (mmol) was calculated assuming that
158 urea was uniformly distributed throughout the total body water, as described previously
159 [18]. The dietary nitrogen recovered in the urinary urea (mmol) was calculated assuming
160 that the urinary ¹⁵N-enrichment is a proxy of the urinary urea ¹⁵N-enrichment, as described
161 previously [18]. The total amount of dietary nitrogen transferred to urea ($N_{\text{diet urea total}}$, mmol)
162 is the sum of the dietary nitrogen excreted in urinary urea and the dietary nitrogen
163 recovered in the body urea pool. The dietary nitrogen recovered in proteins of organs or
164 plasma (mmol) was calculated as previously described [21]. In plasma proteins, it was
165 calculated assuming that plasma represented 3.5% of body weight [22]. The postprandial
166 fractional protein synthesis rate (FSR, expressed in %/day) in the organs and tissues was
167 calculated as previously described [21].

168

169 **Statistical analyses**

170 A power calculation was performed to determine the sample size required to detect
171 significant differences. According to Gilani study evaluating true protein digestibility in young
172 and old rats [23], an effect of age was observed for difference in digestibility > 3% for highly
173 digestible protein sources. In studies using ¹⁵N-labeled protein to determine digestibility,
174 interindividual variability ranged from 0.7 to 2.3%, with a mean around 1.5% [15,18,21,24].

175 Hence, according to these data and with a power set at 0.90 and α set at 0.05, the sample
176 size group was calculated to be 7 (G*Power 3.1).

177 All results are expressed as mean \pm SD and analyses were performed on GraphPad Prism
178 8.2.1. According to Quantile vs Quantile Plots and Shapiro Wilk tests [25], the true ileal
179 digestibility data were assumed to be normally distributed. Unpaired T-tests were performed
180 to evaluate differences in body composition between groups of the same age [26].

181 Differences between groups were tested using a two-way ANOVA with age and adiposity as
182 factors and post hoc Bonferroni tests were applied for pairwise comparisons [27]. Kinetic of
183 incorporation of dietary nitrogen in plasma proteins was analyzed using a mixed model with
184 a compound symmetry covariance matrix, and with the group as a fixed effect and time as a
185 repeated effect [28]. Differences were considered statistically significant with a P-value <
186 0.05.

187

188 **Results**

189 **Body weight and body composition**

190 The mean daily consumption of rats consuming the western diet was significantly higher
191 during the 4 weeks of diet in comparison to rats consuming the standard diet for both 2-
192 month ($P < 0.0001$; 60 ± 3 kJ/d and 73 ± 5 kJ/d) and 11-month ($P = 0.0063$; 72 ± 6 kJ/d and
193 101 ± 21 kJ/d) groups. During the 4 weeks of standard or Western diet, the 2-month rats
194 gained 172.0 ± 10.4 g and 184.8 ± 14.9 g for the normal and high adiposity groups,
195 respectively (**Supplementary Figure 1**). The 11-month rats gained 59.9 ± 17.2 g and $95.1 \pm$
196 38.6 g for the normal and high adiposity groups, respectively. A difference in weight gain was

197 noted between 11-month rats ($P = 0.0333$) and a trend for the 2-month rats ($P = 0.0791$). No
198 difference in final body weight between groups of the same age was observed, but the diets
199 administered resulted in differences in body composition (**Table 2**). A difference in adiposity
200 was noted between groups of the same age, with an increase of 45% in total adiposity in the
201 2-month rats ($P = 0.0008$) and 50% in the 11-month rats ($P = 0.0004$).

202

203 **Dietary nitrogen recovery and true digestibility of nitrogen and amino acids**

204 Rats consumed 3.8 ± 0.3 g of the test meal containing ^{15}N -labeled casein protein, which
205 represents 4.8 ± 0.3 mmol of dietary nitrogen. A majority of the dietary nitrogen was found
206 in the stomach and caecum, with a minimal amount in the small intestine, ileum and colon
207 (**Table 3**). More dietary nitrogen was recovered in the stomach and the small intestine in the
208 middle-aged compared to younger rat groups ($P = 0.0188$ and $P = 0.0401$, respectively).
209 Based on nitrogen recovery in the different segments of the gastrointestinal tract, we
210 calculated true nitrogen digestibility. True nitrogen digestibility of casein was high in all the
211 groups ($> 94\%$) and an effect of adiposity was found on both orofecal ($P = 0.0402$) and
212 oro-caecal digestibility ($P = 0.0491$) with the higher adiposity groups expressing higher
213 digestibility values. Indeed, for true nitrogen digestibility a difference of $\sim 1\%$ was observed
214 between groups of the same age. Similarly, higher adiposity levels were associated with an
215 increase of the mean true digestibility of individual amino acids ($P = 0.0370$; Table 3). Hence,
216 true oro-caecal digestibility differences were observed for some amino acids, such as lysine (P
217 $= 0.0426$), serine ($P = 0.0313$), aspartate/asparagine ($P = 0.0400$) and glutamate/glutamine (P
218 $= 0.0393$).

219

220 **Postprandial distribution of dietary nitrogen**

221 **Table 4** presents the percentage of dietary nitrogen retained in some organs and transferred
222 to body and urinary urea (deamination pool). Dietary nitrogen recovered in the body urea
223 pool as well as total deamination were increased in middle-aged rats with normal adiposity
224 in comparison to their young counterparts ($P = 0.0183$), whereas values were comparable in
225 high adiposity young and middle-aged rats. There was no effect of age or adiposity on the
226 incorporation of ^{15}N in the tissues and organs, except in the liver where a significant effect of
227 age was observed ($P = 0.0359$) with higher incorporation of dietary nitrogen in older rats.
228 When calculated with respect to body weight (**Supplementary Table 2**), a significant effect
229 of age was observed only in kidney ($P = 0.0007$) and skin ($P = 0.0066$), with lower
230 incorporation of dietary nitrogen in the kidney and skin of middle-aged rats.

231 **Figure 2** represents the incorporation of dietary nitrogen into plasma proteins over the
232 whole postprandial period according to the different groups. Overall, effects of group, time
233 and their interaction were observed ($P < 0.0001$). Three hours after the meal, middle-aged
234 rats with both normal and high adiposity presented a higher incorporation of dietary
235 nitrogen into plasma proteins compared to the young rats ($P < 0.05$). These differences
236 continued 6 h after the meal and at that time, the middle-aged rats with high adiposity also
237 presented higher incorporation of dietary nitrogen into plasma proteins than the middle-
238 aged normal adiposity rats ($P < 0.0001$). On the contrary, at this time point, the young rats
239 with high adiposity presented lower incorporation of dietary nitrogen than their lean
240 counterparts ($P = 0.0426$).

241

242 **Protein anabolism**

243 The postprandial FSR in the liver, gastrocnemius muscle, kidney and skin are presented in
244 **Figure 3**. No effect of age or adiposity was observed on the FSR per hour in the liver and
245 kidney. In the gastrocnemius muscle, age decreased the FSR ($P = 0.0063$) whereas adiposity
246 increased it ($P = 0.0018$). Hence, muscle FSR of the middle-aged group with normal adiposity
247 was significantly reduced compared to the young groups ($P = 0.0297$ and $P = 0.0005$ for
248 comparison with 2-month rats with normal and high adiposity, respectively) whereas muscle
249 FSR of the middle-aged group with high adiposity was not different from the young groups.
250 Furthermore, an effect of age on protein synthesis in the skin was noted ($P = 0.0066$) with
251 higher FSR per hour in the younger rat groups.

252

253 **Expression of hormones and peptide and amino acid transporters in the intestinal** 254 **mucosae**

255 For hormones, the expression of CCK was higher in older rats ($P = 0.0047$), with an increase
256 of 60 to 90% compared to young rats (**Table 5**). No effect of age, adiposity or their
257 interaction was observed in the expression of transporters in the duodenum and the
258 jejunum. In the ileum, an effect of adiposity was demonstrated for the expression of Slc6a19
259 ($P = 0.0339$), Slc38a2 ($P = 0.0075$) and Slc1a5 ($P = 0.0271$), with higher expression of these
260 transporters in normal adiposity groups compared to their higher adiposity counterparts.

261

262 **Discussion**

263 **A model of middle-aged rats with moderately high adiposity**

264 This study aimed to evaluate the effects of age and adiposity on the protein digestibility and
265 postprandial utilization of ¹⁵N-labeled native micellar casein in rats. Rat is an animal model
266 that has been extensively used to understand physiological and metabolic disorders
267 associated with obesity and/or aging [29]. In the context of protein digestibility studies, rat is
268 a suitable model [30] and good correlation exists between rats and humans digestibility
269 values [31]. Since evaluation of amino acid bioavailability in humans is currently very
270 invasive, expensive and complicated [32], rat model is instrumental in the investigation of
271 the effect of aging and adiposity on protein digestibility and metabolism. The rats included
272 in our study were young (2 month) and middle-aged (11 months) rats. To obtain different
273 levels of adiposity, half of the rats consumed a high fat/high sucrose diet (Western diet)
274 during 4 weeks. Despite no difference in final body weight, these rats presented significantly
275 higher adiposity compared to their lean counterparts of the same age. The relatively short
276 length of the nutritional intervention may explain the comparable final body weight within
277 age groups. Standard thresholds for obesity have not been developed in rats [33]. However,
278 adiposity levels varied from 8 to 32% in diet-induced obesity models in the literature [16,34-
279 37] suggesting that the 9.4% and 20.0% of fat mass in the young and middle-aged high
280 adiposity groups correspond to moderately high level of adiposity and thus, intermediate
281 obesity .

282

283 **Effect of age and adiposity on casein nitrogen and amino acid digestibility**

284 In our study, depending on the group, the true oro-caecal digestibility of micellar casein
285 ranged between 94.6% and 96.3%, and the mean true amino acid digestibility from 95.1% to
286 96.6%, as reported previously (96.5% for protein and amino acid digestibility [18,38]). We

287 observed no effect of age on protein or amino acid digestibility of casein, in contrast with
288 the study by Gilani & Sepehr [23]. They showed that 20-month-old rats presented a lower
289 fecal digestibility for many protein sources, including casein, compared to young rats. This
290 result suggests that in contrast to old rats, middle-aged rats may not present declined
291 digestibility. Nevertheless, in our study, gastric emptying rate seemed to be modified with
292 age since significant amount of dietary nitrogen was still in the stomach of middle-aged rats
293 6 h after meal. Studies conducted on young, adult, and old rats have demonstrated a
294 delayed gastric emptying that appeared in adult rats and increased gradually in old rats
295 [39,40]. In men, older age was also associated with a slower protein digestion [5]. The
296 mechanisms are not well elucidated but it might be due to reduced gastric contractile forces
297 [4]. Interestingly, we found a higher expression of CCK in the duodenum of the middle-aged
298 rats, a hormone that is known to slow down gastric emptying [41]. Whereas age did not alter
299 digestibility, we observed a significant effect of adiposity that increased true protein and
300 amino acid digestibility by around 1%. As usually observed for highly digestible protein, the
301 difference between groups was small and the effect of adiposity on digestibility has thus to
302 be confirmed with a low digestibility protein, for which higher differences may be observed
303 [32]. In a study performed on diet-induced obese mice, a higher nitrogen apparent
304 digestibility was reported together with higher expression of the Slc6a20a and Slc36a1
305 amino acid transporters in the small intestine [11]. We observed a slight decrease in gene
306 expression of some amino acid transporters in the ileum of the high adiposity rats and, while
307 the majority of peptide and amino acid absorption occurs in the upper small intestine, no
308 difference was observed in the duodenum or jejunum. Peptides and amino acids are mainly
309 absorbed via transporters, but they can also be absorbed through other mechanisms, such
310 as paracellular pathways [42]. Thus, the slightly improved digestibility found in the higher

311 adiposity groups might have been related to an increase in these transportation pathways
312 [43].

313

314 **Effect of age and adiposity on postprandial metabolic fate of casein nitrogen**

315 The use of ¹⁵N-labelled casein allowed us to evaluate the postprandial metabolic fate of
316 dietary nitrogen. We first found that postprandial total deamination 6 h after meal intake
317 was increased in middle-aged rats with normal adiposity, suggesting an increased protein
318 catabolism in older rats. We also measured dietary nitrogen incorporation into proteins and
319 found an increased uptake of dietary nitrogen in the liver and a higher incorporation of
320 dietary nitrogen in plasma proteins (that are mainly composed of exported liver proteins) in
321 middle-aged rats compared to young rats. This is in line with higher splanchnic sequestration
322 observed in the elderly [44,45], but may also be partly due to higher body (and liver) weight
323 of these older rats as this effect is not observed after adjustment for weight. The absence of
324 any effect on the liver anabolic rate despite higher splanchnic sequestration is not surprising
325 since the 11-month-rats are heavier than the 2-month-rats. Nevertheless, as all organs are
326 heavier in the middle-aged groups, our results show a differential postprandial dietary
327 nitrogen disposal in the tissues. The first-pass splanchnic metabolism, including deamination
328 and incorporation of dietary nitrogen into proteins, resulted in a proportionally smaller
329 amount of dietary nitrogen available for protein synthesis in peripheral tissues. Accordingly,
330 we reported a decrease in postprandial protein synthesis rates in the skin and muscle of
331 middle-aged rats. A gradual age-associated decline in protein synthesis rate in the skin (2-
332 fold decrease from 2-month to 6 or 10-month old rats) has been previously observed, due to
333 a general decrease in protein synthesis rate with age but also to the high protein turnover

334 and accretion in skin during rat growth [46,47]. Furthermore, the effect of age on
335 postprandial muscle protein synthesis has already been well demonstrated in rats [7,48-50]
336 and humans [9,10,51]. Taken together, we found that middle-aged rats of 11 months
337 displayed metabolic adaptations usually reported in aged rats, such as higher postprandial
338 deamination, increased splanchnic retention and decreased peripheral protein synthesis
339 rate. Interestingly, these alterations of protein metabolism were not all observed in the
340 middle-aged rats with high adiposity levels.

341 In addition to a slightly increased true protein and amino acid digestibility, middle-aged rats
342 with high adiposity levels had a postprandial deamination of casein comparable to younger
343 rats. However, dietary nitrogen retention in the liver and incorporation in plasma proteins
344 were comparable or even higher in middle-aged rats with high adiposity in comparison to
345 normal adiposity. These results suggest that high adiposity middle-aged rats may have age-
346 related higher nitrogen splanchnic sequestration, but that it was also accompanied by
347 reduced losses in dietary amino acids through deamination. Therefore, more dietary
348 nitrogen and amino acids may have been available for peripheral metabolism in these rats.
349 As the amount of substrate is a key factor in regulating protein synthesis, it could explain
350 why the protein synthesis rate in the muscle was higher in middle-aged rats with high
351 adiposity compared to normal adiposity. The impact of adiposity on muscle protein synthesis
352 has been previously observed in a study where muscle protein synthesis was increased at an
353 intermediate stage of obesity [37]. Moreover, the role of insulin, together with a high
354 availability of plasma amino acids, in muscle protein anabolism is now well established [52].
355 While insulin levels were not monitored in our study, we could suppose that a higher
356 adiposity was accompanied by higher insulin levels [53] which could have stimulated muscle
357 protein synthesis. Taken collectively, the moderately high levels of adiposity in our middle-

358 aged rats seemed to have counteracted the effect of aging on postprandial protein
359 metabolism such as the age-related increase in amino acid postprandial deamination and
360 decrease in muscle protein synthesis. The increased digestibility associated with higher
361 adiposity may have partly participated in counterbalancing this effect by means of a higher
362 absorption of dietary amino acids available for metabolic utilization. Due to the invasiveness
363 of the classical method to evaluate protein digestibility in humans, no data are available for
364 obese and/or aged people. However, a new minimally invasive method has been recently
365 developed [54]. It will thus make it possible to determine amino acid digestibility in
366 vulnerable people and extrapolate our results to humans.

367 **Conclusion**

368 In conclusion, this study provided the first data on the combined effects of adiposity levels
369 and age on micellar casein true protein and amino acid digestibility and postprandial
370 utilization in rats. A strength of our study is that we used ¹⁵N labeled proteins that allowed
371 us to track dietary nitrogen in the gastrointestinal tract, urea pools and in peripheral organ
372 and tissues. A higher level of adiposity resulted in a slight increase in protein and individual
373 amino acid digestibility values and seemed to compensate for the metabolic protein
374 alteration linked with aging, including reduced muscle anabolic response. Although our
375 results need to be confirmed in humans [55], our data suggest that moderate adiposity may
376 contribute to healthy aging in the context of protein metabolism.

377 **Acknowledgements**

378 We thank Antoine Verloin from GAEC des 3 chênes for labeling the cow milk and Aziz Smira
379 for contributing to sample analysis.

380

381 **Authorship**

382 The authors' responsibilities were as follows: C.G., J.C., A.Ba. and A.Bo. contributed to the
383 conception and design of the study; N.A., J.C. conducted the research; N.A., J.C., N.K., C.C.,
384 J.P., and M.C. contributed to data acquisition; N.A. and J.C. analyzed the data; N.A. wrote the
385 original draft of the paper; J.C., C.G., D.A.M, A.Ba., and A.Bo. reviewed and edited the
386 manuscript; C.G. administered the project; and all authors read and approved the final
387 manuscript.

388

389 **Statements and Declaration**

390 **Competing Interests and funding:**

391 C.G., D.A.M., N.K., C.C., J.P., M.C. and J.C. declare that they have no conflict of interest. N.A.,
392 A.Ba., and A.Bo. are employed by Ingredia.

393 The study was funded by Ingredia.

394 **Ethics approval:** All procedures involving animals were in compliance with the European Union
395 directive 2010/63/EU for animal experiments and approved by the ethics committee in animal
396 experiments of INRAe Jouy-en-Josas (Comethea, registration number: 18-14) and the French
397 Ministry of Higher Education and Research (APAFIS n°15907-2018061823133762 v1)

398 .

399 **References**

400 1. Consultation JWFUE (2007) Protein and amino acid requirements in human nutrition. World
401 Health Organ Tech Rep Ser. Switzerland

402 2. Millward DJ, Layman DK, Tome D, Schaafsma G (2008) Protein quality assessment: impact of
403 expanding understanding of protein and amino acid needs for optimal health. *The American journal of*
404 *clinical nutrition* 87 (5):1576S-1581S. doi:10.1093/ajcn/87.5.1576S

405 3. Bhutto A, Morley JE (2008) The clinical significance of gastrointestinal changes with aging.
406 *Current opinion in clinical nutrition and metabolic care* 11 (5):651-660.
407 doi:10.1097/MCO.0b013e32830b5d37

408 4. Salles N (2007) Basic mechanisms of the aging gastrointestinal tract. *Digestive diseases* 25
409 (2):112-117. doi:10.1159/000099474

410 5. Gorissen SHM, Trommelen J, Kouw IWK, Holwerda AM, Pennings B, Groen BBL, Wall BT,
411 Churchward-Venne TA, Horstman AMH, Koopman R, Burd NA, Fuchs CJ, Dirks ML, Res PT, Senden JMG,
412 Steijns J, de Groot L, Verdijk LB, van Loon LJC (2020) Protein Type, Protein Dose, and Age Modulate
413 Dietary Protein Digestion and Phenylalanine Absorption Kinetics and Plasma Phenylalanine Availability
414 in Humans. *The Journal of nutrition* 150 (8):2041-2050. doi:10.1093/jn/nxaa024

415 6. Gilani GS, Cockell KA, Sepehr E (2005) Effects of antinutritional factors on protein digestibility
416 and amino acid availability in foods. *Journal of AOAC International* 88 (3):967-987

417 7. Dardevet D, Sornet C, Balage M, Grizard J (2000) Stimulation of in vitro rat muscle protein
418 synthesis by leucine decreases with age. *The Journal of nutrition* 130 (11):2630-2635.
419 doi:10.1093/jn/130.11.2630

420 8. Dardevet D, Sornet C, Bayle G, Prugnaud J, Pouyet C, Grizard J (2002) Postprandial stimulation
421 of muscle protein synthesis in old rats can be restored by a leucine-supplemented meal. *The Journal*
422 *of nutrition* 132 (1):95-100. doi:10.1093/jn/132.1.95

423 9. Wall BT, Gorissen SH, Pennings B, Koopman R, Groen BB, Verdijk LB, van Loon LJ (2015) Aging
424 Is Accompanied by a Blunted Muscle Protein Synthetic Response to Protein Ingestion. *PLoS one* 10
425 (11):e0140903. doi:10.1371/journal.pone.0140903

426 10. Markofski MM, Dickinson JM, Drummond MJ, Fry CS, Fujita S, Gundermann DM, Glynn EL,
427 Jennings K, Paddon-Jones D, Reidy PT, Sheffield-Moore M, Timmerman KL, Rasmussen BB, Volpi E
428 (2015) Effect of age on basal muscle protein synthesis and mTORC1 signaling in a large cohort of young
429 and older men and women. *Experimental gerontology* 65:1-7. doi:10.1016/j.exger.2015.02.015

430 11. Do TT, Hindlet P, Waligora-Dupriet AJ, Kapel N, Neveux N, Mignon V, Delomenie C, Farinotti R,
431 Feve B, Buyse M (2014) Disturbed intestinal nitrogen homeostasis in a mouse model of high-fat diet-
432 induced obesity and glucose intolerance. *American journal of physiology Endocrinology and*
433 *metabolism* 306 (6):E668-680. doi:10.1152/ajpendo.00437.2013

434 12. Anderson SR, Gilge DA, Steiber AL, Previs SF (2008) Diet-induced obesity alters protein
435 synthesis: tissue-specific effects in fasted versus fed mice. *Metabolism: clinical and experimental* 57
436 (3):347-354. doi:10.1016/j.metabol.2007.10.009

437 13. Beals JW, Sukiennik RA, Nallabelli J, Emmons RS, van Vliet S, Young JR, Ulanov AV, Li Z, Paluska
438 SA, De Lisio M, Burd NA (2016) Anabolic sensitivity of postprandial muscle protein synthesis to the
439 ingestion of a protein-dense food is reduced in overweight and obese young adults. *The American*
440 *journal of clinical nutrition* 104 (4):1014-1022. doi:10.3945/ajcn.116.130385

441 14. Zhu S, Tian Z, Torigoe D, Zhao J, Xie P, Sugizaki T, Sato M, Horiguchi H, Terada K, Kadomatsu T,
442 Miyata K, Oike Y (2019) Aging- and obesity-related peri-muscular adipose tissue accelerates muscle
443 atrophy. *PLoS one* 14 (8):e0221366. doi:10.1371/journal.pone.0221366

444 15. Tessier R, Khodorova N, Calvez J, Kapel R, Quinsac A, Piedcoq J, Tome D, Gaudichon C (2020)
445 15N and (2)H Intrinsic Labeling Demonstrate That Real Digestibility in Rats of Proteins and Amino Acids
446 from Sunflower Protein Isolate Is Almost as High as That of Goat Whey. *The Journal of nutrition* 150
447 (3):450-457. doi:10.1093/jn/nxz279

- 448 16. Chevalier L, Bos C, Azzout-Marniche D, Fromentin G, Mosoni L, Hafnaoui N, Piedcoq J, Tome D,
449 Gaudichon C (2013) Energy restriction only slightly influences protein metabolism in obese rats,
450 whatever the level of protein and its source in the diet. *International journal of obesity* 37 (2):263-271.
451 doi:10.1038/ijo.2012.19
- 452 17. Bos C, Stoll B, Fouillet H, Gaudichon C, Guan X, Grusak MA, Reeds PJ, Burrin DG, Tome D (2005)
453 Postprandial intestinal and whole body nitrogen kinetics and distribution in piglets fed a single meal.
454 *American journal of physiology Endocrinology and metabolism* 288 (2):E436-446.
455 doi:10.1152/ajpendo.00263.2004
- 456 18. Lacroix M, Leonil J, Bos C, Henry G, Airinei G, Fauquant J, Tome D, Gaudichon C (2006) Heat
457 markers and quality indexes of industrially heat-treated [15N] milk protein measured in rats. *Journal*
458 *of agricultural and food chemistry* 54 (4):1508-1517. doi:10.1021/jf051304d
- 459 19. Moro J, Chaumontet C, Even PC, Blais A, Piedcoq J, Gaudichon C, Tome D, Azzout-Marniche D
460 (2021) Severe protein deficiency induces hepatic expression and systemic level of FGF21 but inhibits
461 its hypothalamic expression in growing rats. *Scientific reports* 11 (1):12436. doi:10.1038/s41598-021-
462 91274-4
- 463 20. Guillin FM, Gaudichon C, Guerin-Deremaux L, Lefranc-Millot C, Azzout-Marniche D, Khodorova
464 N, Calvez J (2021) Multi-criteria assessment of pea protein quality in rats: a comparison between
465 casein, gluten and pea protein alone or supplemented with methionine. *The British journal of nutrition*
466 125 (4):389-397. doi:10.1017/S0007114520002883
- 467 21. Tessier R, Ribeiro-Parenti L, Bruneau O, Khodorova N, Cavin JB, Bado A, Azzout-Marniche D,
468 Calvez J, Le Gall M, Gaudichon C (2019) Effect of different bariatric surgeries on dietary protein
469 bioavailability in rats. *American journal of physiology Gastrointestinal and liver physiology* 317
470 (5):G592-G601. doi:10.1152/ajpgi.00142.2019
- 471 22. Waynforth HB, Flecknell PA (1992) *Experimental and surgical technique in the rat*. Academic
472 Press, San Diego
- 473 23. Gilani GS, Sepehr E (2003) Protein digestibility and quality in products containing
474 antinutritional factors are adversely affected by old age in rats. *The Journal of nutrition* 133 (1):220-
475 225. doi:10.1093/jn/133.1.220
- 476 24. Oberli M, Lan A, Khodorova N, Sante-Lhoutellier V, Walker F, Piedcoq J, Davila AM, Blachier F,
477 Tome D, Fromentin G, Gaudichon C (2016) Compared with Raw Bovine Meat, Boiling but Not Grilling,
478 Barbecuing, or Roasting Decreases Protein Digestibility without Any Major Consequences for Intestinal
479 Mucosa in Rats, although the Daily Ingestion of Bovine Meat Induces Histologic Modifications in the
480 Colon. *The Journal of nutrition* 146 (8):1506-1513. doi:10.3945/jn.116.230839
- 481 25. Ghasemi A, Zahediasl S (2012) Normality tests for statistical analysis: a guide for non-
482 statisticians. *International journal of endocrinology and metabolism* 10 (2):486-489.
483 doi:10.5812/ijem.3505
- 484 26. Whitley E, Ball J (2002) Statistics review 5: Comparison of means. *Critical care* 6 (5):424-428.
485 doi:10.1186/cc1548
- 486 27. Mishra P, Singh U, Pandey CM, Mishra P, Pandey G (2019) Application of student's t-test,
487 analysis of variance, and covariance. *Annals of cardiac anaesthesia* 22 (4):407-411.
488 doi:10.4103/aca.ACA_94_19
- 489 28. Gałeczki A, Burzykowski T (2013) Linear Mixed-Effects Model. In: *Linear Mixed-Effects Models*
490 *Using R: A Step-by-Step Approach*. Springer New York, New York, NY, pp 245-273. doi:10.1007/978-1-
491 4614-3900-4_13
- 492 29. Chalvon-Demersay T, Blachier F, Tome D, Blais A (2017) Animal Models for the Study of the
493 Relationships between Diet and Obesity: A Focus on Dietary Protein and Estrogen Deficiency. *Frontiers*
494 *in nutrition* 4:5. doi:10.3389/fnut.2017.00005
- 495 30. FAO (2013) Dietary protein quality evaluation in human nutrition. Report of an FAO Expert
496 Consultation. Food and Nutrition Paper n°92, Rome
- 497 31. Deglaire A, Moughan PJ (2012) Animal models for determining amino acid digestibility in
498 humans - a review. *The British journal of nutrition* 108 Suppl 2:S273-281.
499 doi:10.1017/S0007114512002346

500 32. Gaudichon C, Calvez J (2021) Determinants of amino acid bioavailability from ingested protein
501 in relation to gut health. *Current opinion in clinical nutrition and metabolic care* 24 (1):55-61.
502 doi:10.1097/MCO.0000000000000708

503 33. de Moura EDM, Dos Reis SA, da Conceicao LL, Sediyaama C, Pereira SS, de Oliveira LL, Gouveia
504 Peluzio MDC, Martinez JA, Milagro FI (2021) Diet-induced obesity in animal models: points to consider
505 and influence on metabolic markers. *Diabetology & metabolic syndrome* 13 (1):32.
506 doi:10.1186/s13098-021-00647-2

507 34. Hira T, Suto R, Kishimoto Y, Kanahori S, Hara H (2018) Resistant maltodextrin or
508 fructooligosaccharides promotes GLP-1 production in male rats fed a high-fat and high-sucrose diet,
509 and partially reduces energy intake and adiposity. *European journal of nutrition* 57 (3):965-979.
510 doi:10.1007/s00394-017-1381-7

511 35. Matias AM, Estevam WM, Coelho PM, Haese D, Kobi J, Lima-Leopoldo AP, Leopoldo AS (2018)
512 Differential Effects of High Sugar, High Lard or a Combination of Both on Nutritional, Hormonal and
513 Cardiovascular Metabolic Profiles of Rodents. *Nutrients* 10 (8). doi:10.3390/nu10081071

514 36. Miranda J, Eseberri I, Lasa A, Portillo MP (2018) Lipid metabolism in adipose tissue and liver
515 from diet-induced obese rats: a comparison between Wistar and Sprague-Dawley strains. *Journal of*
516 *physiology and biochemistry* 74 (4):655-666. doi:10.1007/s13105-018-0654-9

517 37. Masgrau A, Mishellany-Dutour A, Murakami H, Beaufrere AM, Walrand S, Giraudet C, Migne
518 C, Gerbaix M, Metz L, Courteix D, Guillet C, Boirie Y (2012) Time-course changes of muscle protein
519 synthesis associated with obesity-induced lipotoxicity. *The Journal of physiology* 590 (20):5199-5210.
520 doi:10.1113/jphysiol.2012.238576

521 38. Rutherfurd SM, Moughan PJ (1998) The digestible amino acid composition of several milk
522 proteins: application of a new bioassay. *Journal of dairy science* 81 (4):909-917. doi:10.3168/jds.S0022-
523 0302(98)75650-4

524 39. Horiuchi A, Tanaka N, Sakai R, Kawamata Y (2014) Effect of Age and Elemental Diets on Gastric
525 Emptying in Rats. *Journal of Gastroenterology and Hepatology Research* 3:1340-1343

526 40. Smits GJ, Lefebvre RA (1996) Influence of aging on gastric emptying of liquids, small intestine
527 transit, and fecal output in rats. *Experimental gerontology* 31 (5):589-596. doi:10.1016/0531-
528 5565(96)00029-0

529 41. Rehfeld JF (2017) Cholecystokinin-From Local Gut Hormone to Ubiquitous Messenger.
530 *Frontiers in endocrinology* 8:47. doi:10.3389/fendo.2017.00047

531 42. Miner-Williams WM, Stevens BR, Moughan PJ (2014) Are intact peptides absorbed from the
532 healthy gut in the adult human? *Nutrition research reviews* 27 (2):308-329.
533 doi:10.1017/S0954422414000225

534 43. Rohr MW, Narasimhulu CA, Rudeski-Rohr TA, Parthasarathy S (2020) Negative Effects of a
535 High-Fat Diet on Intestinal Permeability: A Review. *Advances in nutrition* 11 (1):77-91.
536 doi:10.1093/advances/nmz061

537 44. Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrere B (1997) Slow and fast dietary
538 proteins differently modulate postprandial protein accretion. *Proceedings of the National Academy of*
539 *Sciences of the United States of America* 94 (26):14930-14935. doi:10.1073/pnas.94.26.14930

540 45. Jourdan M, Deutz NE, Cynober L, Aussel C (2011) Features, causes and consequences of
541 splanchnic sequestration of amino acid in old rats. *PLoS one* 6 (11):e27002.
542 doi:10.1371/journal.pone.0027002

543 46. Mays PK, McAnulty RJ, Laurent GJ (1991) Age-related changes in rates of protein synthesis and
544 degradation in rat tissues. *Mechanisms of ageing and development* 59 (3):229-241. doi:10.1016/0047-
545 6374(91)90134-I

546 47. Obled C, Arnal M (1992) Contribution of skin to whole-body protein synthesis in rats at
547 different stages of maturity. *The Journal of nutrition* 122 (11):2167-2173. doi:10.1093/jn/122.11.2167

548 48. Holloszy JO, Chen M, Cartee GD, Young JC (1991) Skeletal muscle atrophy in old rats:
549 differential changes in the three fiber types. *Mechanisms of ageing and development* 60 (2):199-213.
550 doi:10.1016/0047-6374(91)90131-I

- 551 49. Mosoni L, Patureau Mirand P, Houlier ML, Arnal M (1993) Age-related changes in protein
552 synthesis measured in vivo in rat liver and gastrocnemius muscle. *Mechanisms of ageing and*
553 *development* 68 (1-3):209-220. doi:10.1016/0047-6374(93)90152-h
- 554 50. Mosoni L, Valluy MC, Serrurier B, Prugnaud J, Obled C, Guezennec CY, Mirand PP (1995) Altered
555 response of protein synthesis to nutritional state and endurance training in old rats. *The American*
556 *journal of physiology* 268 (2 Pt 1):E328-335. doi:10.1152/ajpendo.1995.268.2.E328
- 557 51. Rasmussen BB, Fujita S, Wolfe RR, Mittendorfer B, Roy M, Rowe VL, Volpi E (2006) Insulin
558 resistance of muscle protein metabolism in aging. *FASEB journal : official publication of the Federation*
559 *of American Societies for Experimental Biology* 20 (6):768-769. doi:10.1096/fj.05-4607fje
- 560 52. Wolfe RR (2000) Effects of insulin on muscle tissue. *Current opinion in clinical nutrition and*
561 *metabolic care* 3 (1):67-71. doi:10.1097/00075197-200001000-00011
- 562 53. Gloy VL, Lutz TA, Langhans W, Geary N, Hillebrand JJ (2010) Basal plasma levels of insulin,
563 leptin, ghrelin, and amylin do not signal adiposity in rats recovering from forced overweight.
564 *Endocrinology* 151 (9):4280-4288. doi:10.1210/en.2010-0439
- 565 54. Devi S, Varkey A, Sheshshayee MS, Preston T, Kurpad AV (2018) Measurement of protein
566 digestibility in humans by a dual-tracer method. *The American journal of clinical nutrition* 107 (6):984-
567 991. doi:10.1093/ajcn/nqy062
- 568 55. Pes GM, Licheri G, Soro S, Longo NP, Salis R, Tomassini G, Niolu C, Errigo A, Dore MP (2019)
569 Overweight: A Protective Factor against Comorbidity in the Elderly. *International journal of*
570 *environmental research and public health* 16 (19). doi:10.3390/ijerph16193656

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573 **Figure legends**

574

575 **Fig. 1** Experimental protocol of the study. 2m-na, 2 months/normal adiposity; 2m-ha, 2
576 months/high adiposity; 11m-na, 11 months/normal adiposity; 11m-ha: 11 months/high
577 adiposity.

578

579 **Fig. 2** Incorporation of dietary nitrogen to plasma proteins over the 6-h postprandial period.
580 Values are mean \pm SD, n = 7 - 8 rats. [£] indicating a significant difference (P < 0.05) between
581 the 2m (-na and -ha) and 11m (-na and -ha) groups and [§] indicating a significant difference (P
582 < 0.05) between every groups. 2m-na, 2 months/normal adiposity; 2m-ha, 2 months/high
583 adiposity; 11m-na, 11 months/normal adiposity; 11m-ha: 11 months/high adiposity.

584

585 **Fig. 3** Fractional synthesis rate (FSR) in the gastrocnemius muscle (A), skin (B), liver (C), kidney
586 (D) 6 h after meal ingestion. Values are mean \pm SD, n = 7 - 8 rats. The effects of age and
587 adiposity and their interaction were tested with a two-way ANOVA model. Values with
588 different letters within a graph are statistically different. n.s, not significant. 2m-na, 2
589 months/normal adiposity; 2m-ha, 2 months/high adiposity; 11m-na, 11 months/normal
590 adiposity; 11m-ha: 11 months/high adiposity.

591 **Table 1** Composition of the experimental diets

	Standard diet (g/kg)	Western diet (g/kg)	Casein diet (g/kg)
Protein ¹	140	160	120
Starch	622	291	642
Sucrose	100	291	100
Soya bean oil	40	40	40
Lard	0	120	0
Mineral mix	35	35	35
Vitamin mix	10	10	10
Cellulose	50	50	50
Choline	3	3	3
Protein (%Energy)	14	14	14
Carbohydrates (%Energy)	75	51	75
Lipids (%Energy)	11	35	11
Energy (kJ/g)	15.0	18.1	15.5

592 ¹Total milk protein for Standard and Western diet and casein Prodiel[®] 85B (Ingredia, Arras, France) for Casein
593 diet

594

595 **Table 2** Body weight and body composition in rats according to age and adiposity levels

Age Group	2 months			11 months		
	2m-na	2m-ha	T-test	11m-na	11m-ha	T-test
Body weight (g)	315.5 ± 18.9	323.2 ± 12.1	<i>n.s.</i>	536.0 ± 32.1	574.1 ± 52.0	<i>n.s.</i>
Total adiposity (%)	6.5 ± 0.9	9.4 ± 1.5 *	<i>0.0008</i>	13.3 ± 3.9	20.0 ± 1.2 *	<i>0.0004</i>
Total lean mass (%)	73.3 ± 2.3	72.5 ± 1.5	<i>n.s.</i>	70.5 ± 7.3	67.1 ± 1.0	<i>n.s.</i>
Subcutaneous AT (%)	3.5 ± 0.6	3.8 ± 0.4	<i>n.s.</i>	5.8 ± 2.6	9.5 ± 1.1 *	<i>0.0025</i>
Epididymal AT (%)	1.6 ± 0.4	1.7 ± 0.3	<i>n.s.</i>	2.4 ± 0.5	4.0 ± 0.8 *	<i>0.0005</i>
Retroperitoneal AT (%)	1.6 ± 0.3	1.8 ± 0.4	<i>n.s.</i>	2.7 ± 0.8	4.5 ± 0.6 *	<i>0.0003</i>
Mesenteric AT (%)	1.3 ± 0.1	1.8 ± 1.2	<i>n.s.</i>	1.9 ± 0.6	2.4 ± 0.6 *	<i>0.0002</i>
Brown AT (%)	0.2 ± 0.02	0.2 ± 0.03	<i>n.s.</i>	0.1 ± 0.03	0.2 ± 0.03 *	<i>0.0001</i>
Liver (g)	8.3 ± 0.7	8.5 ± 0.6	<i>n.s.</i>	10.9 ± 0.9	12.1 ± 1.4	<i>n.s.</i>
Gastrocnemius muscle (g)	1.6 ± 0.3	1.6 ± 0.1	<i>n.s.</i>	2.3 ± 0.2	2.5 ± 0.4	<i>n.s.</i>
Kidney (g)	1.7 ± 0.3	1.9 ± 0.1	<i>n.s.</i>	2.2 ± 0.1	2.3 ± 0.3	<i>n.s.</i>

596 Values are mean ± SD, n = 7 - 8 rats. Body weight and body composition were determined at the end of
597 experiment. The differences in body composition were tested by an unpaired student t-test between groups of
598 the same age. * Significantly different from normal adiposity group within same age. AT, adipose tissue. 2m-na,
599 2 months/normal adiposity; 2m-ha, 2 months/high adiposity; 11m-na, 11 months/normal adiposity; 11m-ha: 11
600 months/high adiposity.

601 **Table 3** Dietary nitrogen recovered in gastrointestinal segment 6 h after ingestion of the test meal
 602 containing ¹⁵N-labelled casein protein and true digestibility of nitrogen and individual amino acid
 603 according to age and adiposity levels

Age Group	2 months		11 months		<i>P-value</i>		
	2m-na	2m-ha	11m-na	11m-ha	<i>Age effect</i>	<i>Adiposity effect</i>	<i>Age x Adiposity</i>
Dietary nitrogen recovery (% of ingested)							
Stomach	2.1 ± 5.5 ^a	5.5 ± 7.9 ^{ab}	7.3 ± 8.1 ^{ab}	14.8 ± 9.3 ^b	0.0188	n.s.	n.s.
Proximal intestine	0.6 ± 0.7	0.5 ± 0.3	0.9 ± 0.6	1.1 ± 0.7	0.0401	n.s.	n.s.
Ileum	0.06 ± 0.03	0.03 ± 0.02	0.03 ± 0.03	0.03 ± 0.01	n.s.	n.s.	n.s.
Caecum	3.9 ± 1.3	3.4 ± 1.3	4.1 ± 1.4	2.5 ± 0.4	n.s.	0.0261	n.s.
Colon + feces	0.7 ± 1.1	0.5 ± 0.4	0.4 ± 0.3	0.4 ± 0.3	n.s.	n.s.	n.s.
True nitrogen digestibility (% of ingested)							
Orofecal digestibility	94.1 ± 1.1	95.2 ± 1.7	94.5 ± 2.2	95.8 ± 0.7	n.s.	0.0402	n.s.
Orocaecal digestibility	94.8 ± 1.6	95.7 ± 1.5	95.0 ± 2.2	96.3 ± 0.4	n.s.	0.0491	n.s.
True amino acid orocaecal digestibility (% of ingested)							
Alanine	95.7 ± 1.3	95.7 ± 1.5	94.8 ± 1.4	96.0 ± 1.0	n.s.	n.s.	n.s.
Aspartate + asparagine	95.5 ± 1.4	95.8 ± 1.4	94.6 ± 1.7	96.4 ± 0.9	n.s.	0.0400	n.s.
Glycine	95.7 ± 1.4	96.0 ± 1.3	94.5 ± 1.6	96.1 ± 1.2	n.s.	n.s.	n.s.
Glutamate + glutamine	94.2 ± 1.7	94.9 ± 1.6	93.2 ± 2.1	95.3 ± 1.2	n.s.	0.0393	n.s.
Isoleucine	93.1 ± 2.1	93.6 ± 2.3	91.3 ± 2.7	93.6 ± 1.4	n.s.	n.s.	n.s.
Leucine	98.2 ± 0.5	98.3 ± 0.5	98.0 ± 0.5	98.6 ± 0.4	n.s.	n.s.	n.s.
Lysine	98.2 ± 0.6	98.3 ± 0.6	98.0 ± 0.6	98.7 ± 0.3	n.s.	0.0426	n.s.
Methionine	96.5 ± 1.1	96.4 ± 1.4	95.8 ± 1.5	97.0 ± 0.8	n.s.	n.s.	n.s.
Phenylalanine	99.2 ± 0.3	99.2 ± 0.2	99.1 ± 0.3	99.4 ± 0.3	n.s.	n.s.	n.s.
Proline	98.5 ± 0.4	98.5 ± 0.5	98.3 ± 0.5	98.8 ± 0.3	n.s.	n.s.	n.s.
Serine	87.1 ± 3.8	88.4 ± 3.5	83.8 ± 5.8	89.9 ± 3.8	n.s.	0.0313	n.s.
Threonine	96.6 ± 1.0	96.0 ± 2.6	96.0 ± 2.6	96.8 ± 1.0	n.s.	n.s.	n.s.
Valine	95.7 ± 1.3	95.8 ± 1.3	94.8 ± 1.5	96.4 ± 0.9	n.s.	n.s.	n.s.
Mean all AA	95.8 ± 1.1	96.2 ± 1.1	95.1 ± 1.4	96.6 ± 0.8	n.s.	0.0370	n.s.

604 Values are mean ± SD, n = 7 - 8 rats. The effect of the group was tested with a two-way ANOVA model. Values
 605 with different letters within the same row are statistically different. AA, amino acid; n.s., not significant. 2m-na,
 606 2 months/normal adiposity; 2m-ha, 2 months/high adiposity; 11m-na, 11 months/normal adiposity; 11m-ha: 11
 607 months/high adiposity.

608 **Table 4** Transfer of dietary nitrogen to the urea pool and to the liver, gastrocnemius muscle, kidney,
 609 and skin 6 h after ingestion of the experimental meal (expressed as % of ingested nitrogen)

Age Group	2 months		11 months		P-value		
	2m-na	2m-ha	11m-na	11m-ha	Age effect	Adiposity effect	Age x Adiposity
Body urea pool	3.9 ± 2.1 ^a	5.3 ± 2.2 ^{ab}	8.3 ± 1.9 ^b	5.4 ± 3.1 ^{ab}	0.0221	n.s.	0.0296
Urinary urea pool	2.4 ± 1.4	4.0 ± 2.1	4.1 ± 2.3	3.6 ± 2.1	n.s.	n.s.	n.s.
Total deamination	6.3 ± 3.1 ^a	9.3 ± 3.8 ^{ab}	12.4 ± 3.5 ^b	9.0 ± 2.4 ^{ab}	0.0303	n.s.	0.0170
Liver	8.6 ± 2.7	9.6 ± 3.8	11.9 ± 6.7	15.3 ± 7.3	0.0359	n.s.	n.s.
Muscle	0.8 ± 0.5	0.7 ± 0.7	1.0 ± 0.8	0.8 ± 0.8	n.s.	n.s.	n.s.
Kidney	1.7 ± 0.7	1.7 ± 0.4	2.0 ± 0.3	1.8 ± 0.3	n.s.	n.s.	n.s.
Skin	12.4 ± 3.8	14.0 ± 5.0	15.2 ± 3.7	16.8 ± 4.3	n.s.	n.s.	n.s.

610 Values are mean ± SD, n = 5 - 7 rats. The effects of age and adiposity and their interaction were tested with a
 611 two-way ANOVA model. Values with different letters within the same row are statistically different. n.s, not
 612 significant. 2m-na, 2 months/normal adiposity; 2m-ha, 2 months/high adiposity; 11m-na, 11 months/normal
 613 adiposity; 11m-ha: 11 months/high adiposity.

614

615 **Table 5** Expression of the main peptides and amino acids transporters and hormones in the mucosa of
 616 the different segments of the small intestine according to age and adiposity levels

Age	2 months		11 months		P-value		
Group	2m-na	2m-ha	11m-na	11m-ha	Age effect	Adiposity effect	Age x Adiposity
Duodenum							
PEPT1	4.4 ± 3.9	4.5 ± 3.1	6.3 ± 3.5	4.4 ± 2.6	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
CCK	2.2 ± 1.1	2.2 ± 1.1	3.5 ± 1.5	4.2 ± 1.6	<i>0.0047</i>	<i>n.s.</i>	<i>n.s.</i>
Secretin	4.7 ± 3.2	6.6 ± 5.4	5.0 ± 1.1	6.9 ± 6.9	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Jejunum							
PEPT1	1.2 ± 0.4	0.9 ± 0.3	1.0 ± 0.7	0.9 ± 0.3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc6a19	2.5 ± 1.8	1.8 ± 0.5	1.7 ± 0.7	1.4 ± 1	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc38a2	1.2 ± 0.6	1.1 ± 0.3	1.5 ± 0.6	0.8 ± 0.2	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc1a1	2.7 ± 3.7	1.1 ± 0.7	1.5 ± 0.7	1.2 ± 1.4	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc6a14	3.8 ± 2.6	4.3 ± 3.4	7.0 ± 3.2	3.5 ± 2.0	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc36a1	2.2 ± 0.7	1.7 ± 0.4	2.1 ± 1.2	1.8 ± 0.7	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slca31	3.0 ± 1.3	2.4 ± 2.7	3.4 ± 1.4	2.8 ± 1.8	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc1a5	1.1 ± 0.8	1.0 ± 0.5	1.4 ± 0.9	0.8 ± 0.2	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Ileum							
PEPT1	3.7 ± 1.4	4.0 ± 1.1	4.4 ± 1.6	3.4 ± 0.8	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc6a19	1.5 ± 1.0	1.0 ± 0.2	1.4 ± 0.5	1.0 ± 0.3	<i>n.s.</i>	<i>0.0339</i>	<i>n.s.</i>
Slc38a2	5.9 ± 1.7 ^a	4.5 ± 0.9 ^{ab}	6.9 ± 4.2 ^{ab}	4.0 ± 1.1 ^b	<i>n.s.</i>	<i>0.0075</i>	<i>n.s.</i>
Slc1a1	1.8 ± 0.6	1.4 ± 0.4	1.6 ± 0.6	1.4 ± 0.4	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc6a14	6.3 ± 2.8	5.4 ± 1.9	7.6 ± 4.7	7.8 ± 5.8	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc36a1	4.0 ± 0.5	3.8 ± 0.7	5.8 ± 3.4	4.3 ± 0.8	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slca31	8.5 ± 2.9	8.2 ± 2.3	9.4 ± 4.1	7.1 ± 1.3	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Slc1a5	5.1 ± 2.9	4.1 ± 1.9	8.2 ± 5.0	3.7 ± 1.5	<i>n.s.</i>	<i>0.0271</i>	<i>n.s.</i>

617 Values are mean ± SD, n = 7 - 8 rats. The results are expressed in arbitrary units. The effects of age and adiposity
 618 and their interaction were tested with a two-way ANOVA model. Values with different letters within the same
 619 row are statistically different. CCK, cholecystokinin; PEPT1, Peptide transporter 1; Slc, solute carrier; *n.s.*, not
 620 significant. 2m-na, 2 months/normal adiposity; 2m-ha, 2 months/high adiposity; 11m-na, 11 months/normal
 621 adiposity; 11m-ha: 11 months/high adiposity.

FIGURE 1

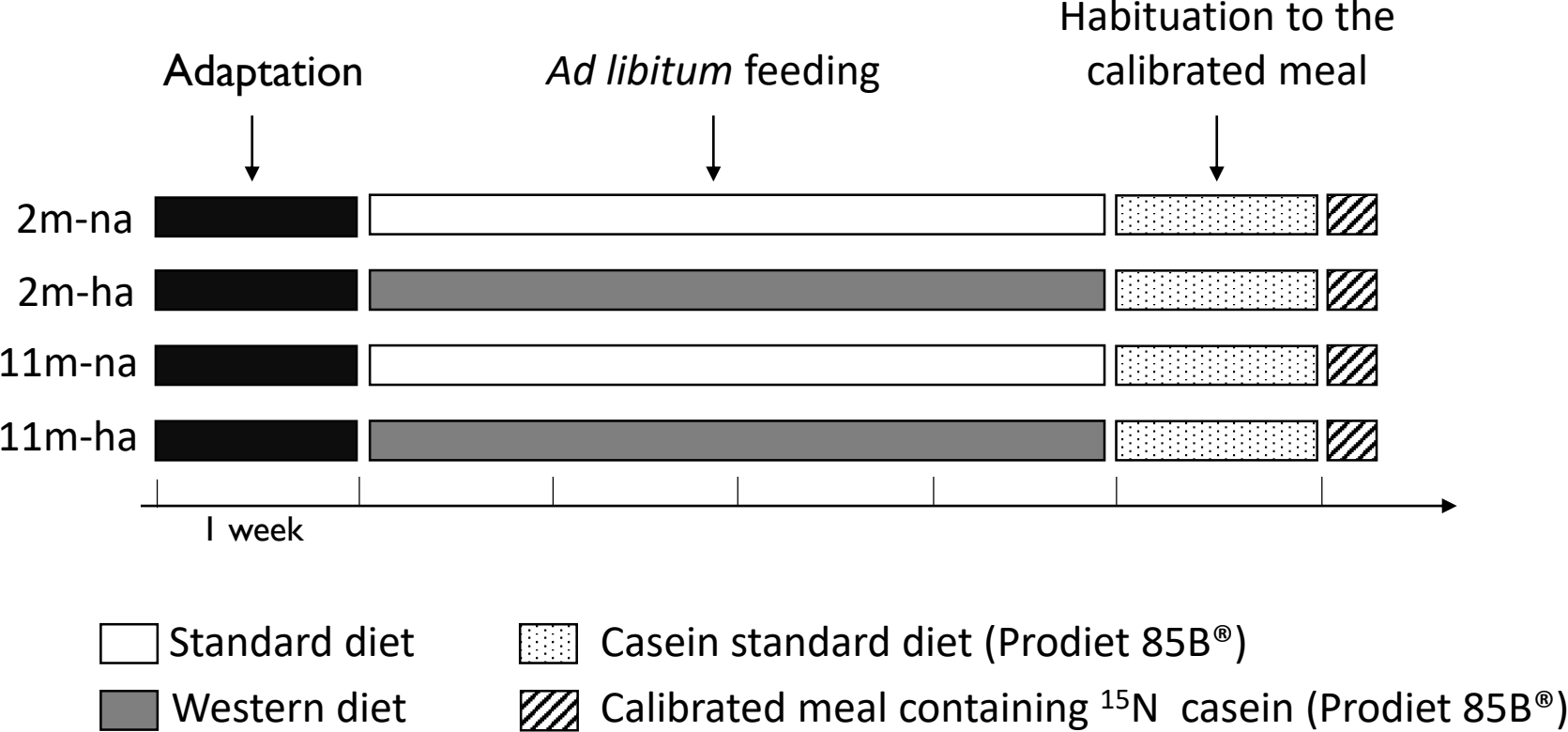


Figure 2

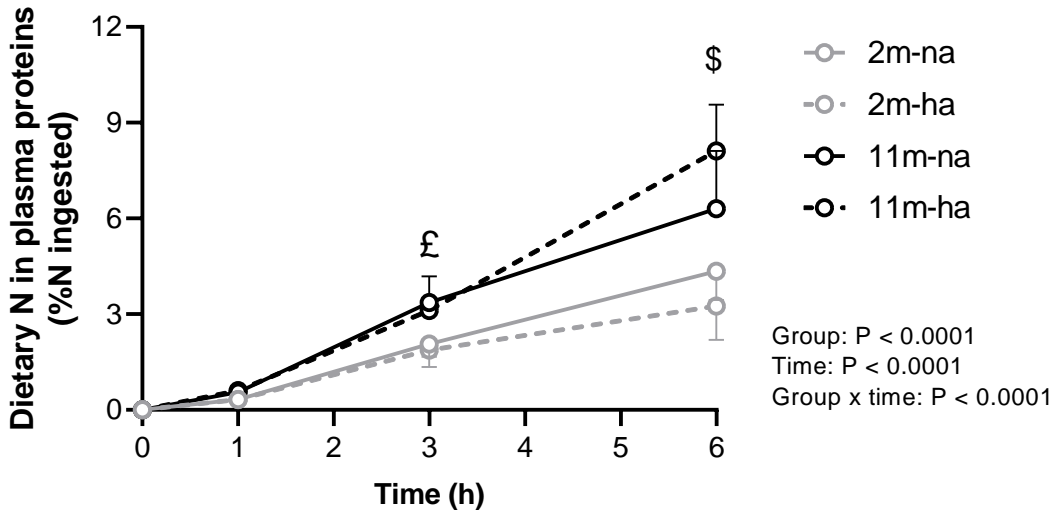
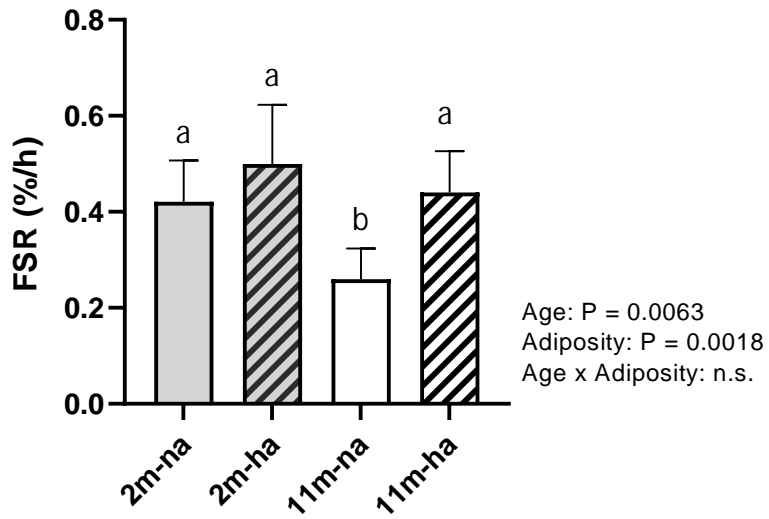
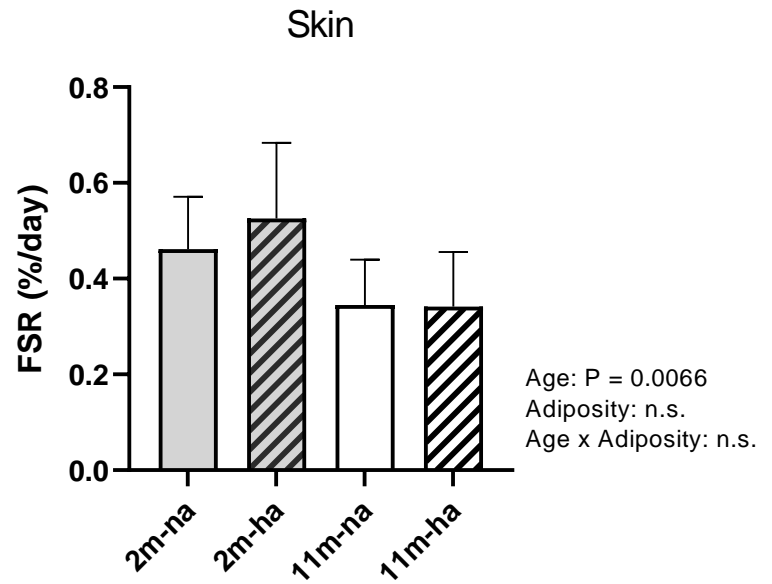


Figure 3

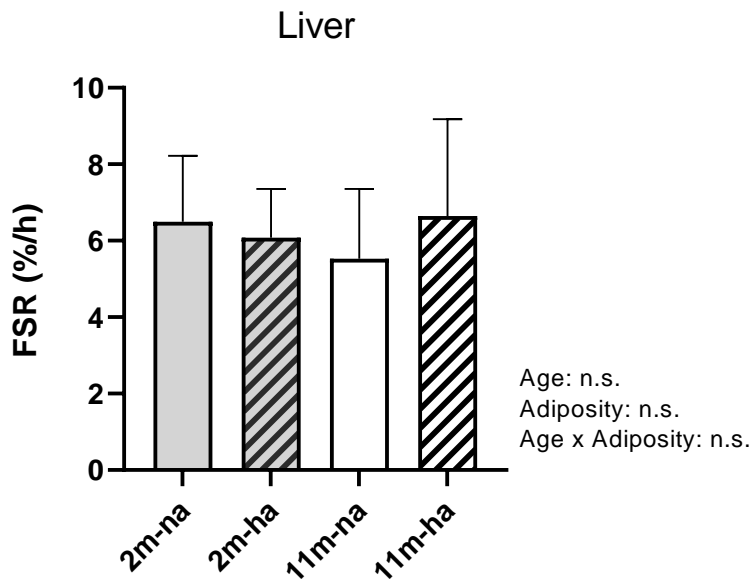
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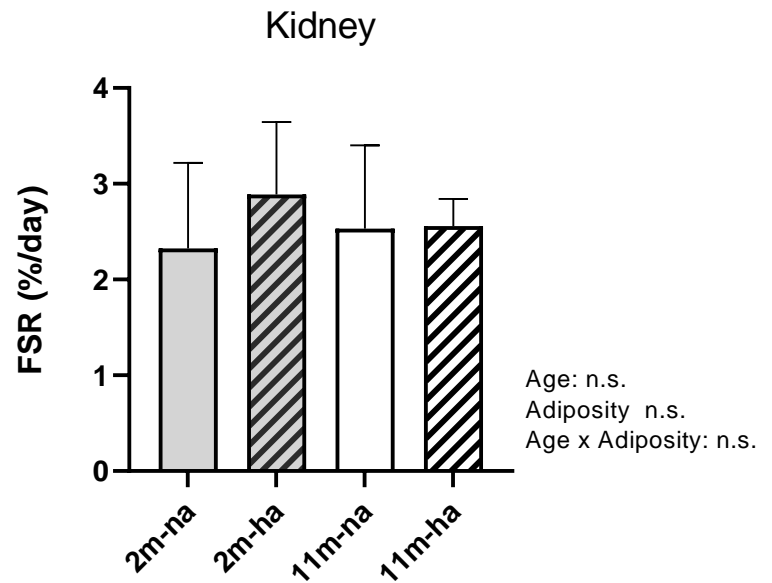
B



C



D



**Moderate adiposity levels counteract protein metabolism modifications associated with
aging in rats**

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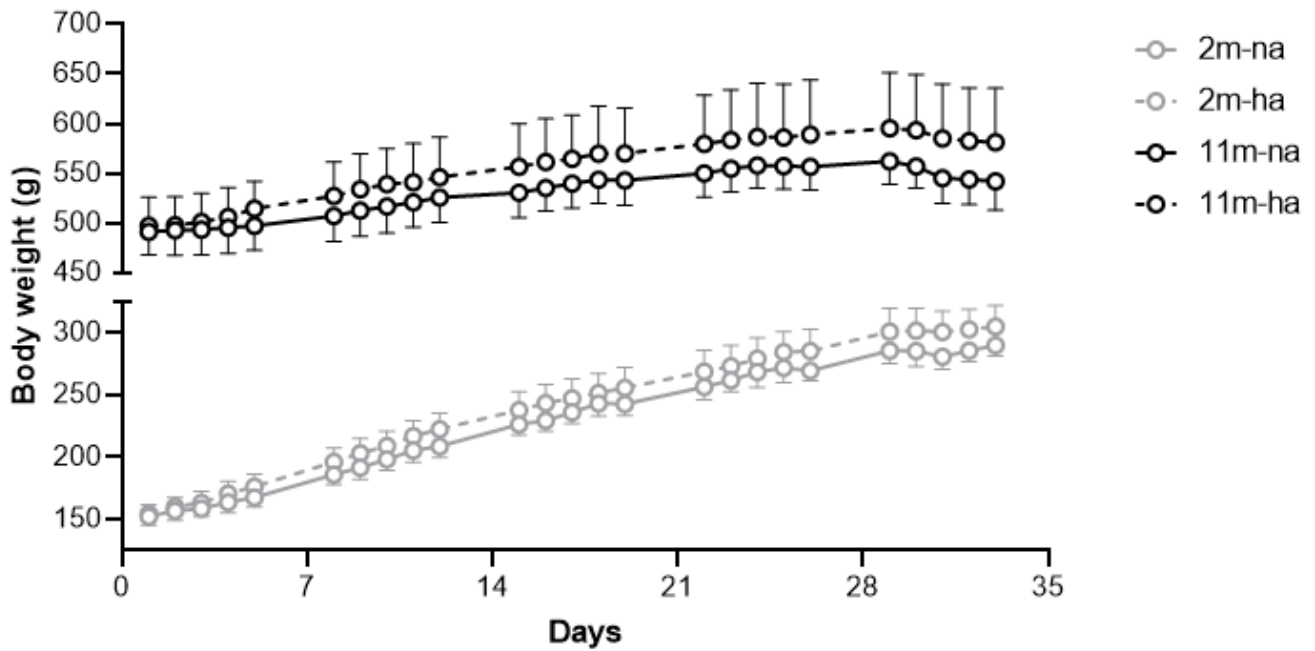
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Supplemental data



Supplementary Figure 1. Body weight of the 2-month and 11-month rats from their arrival to the sixth week of experiment. Values are expressed as mean \pm SD (n = 7 - 8). Within each age group, the two-way ANOVA revealed a significant effect of time and interaction of time and group ($P < 0.001$), without any group effect. No post hoc difference was observed between the different adiposity level groups in young or middle-aged rats. 2m-na, 2 months/normal adiposity; 2m-ha, 2 months/high adiposity; 11m-na, 11 months/normal adiposity; 11m-ha: 11 months/high adiposity.

Supplementary Table 1. Primer sequences designed with Prime Express software and used for duodenum, jejunum and ileum mRNA analysis

Gene	Froward Primer (5' to 3')	Reverse primer (3' to 5')
β-actin	GCTTCTTTGCAGCTCCTTCGT	AGCGCAGCGATATCGTCAT
CCK	CAGGTCCGCAAAGCTCCTT	TCCAGGCTCTGCAGGTTCT
Seceretin	TGCTGCTCTCAAGTTCTTTCGT	CGTCCCCTCCGAGTGTCT
PEPT1	GGCTGAGGCAGGCCACTT	AGCAAGGAGGCGAACAGAAC
Slc6a19	CCGAGCGCTTTGATGACTGT	GGCAGGTCTGAACCCATTG
Slc38a2	TCTGCAGGCGGACATTAACC	ACGCGGCAGGCAGATG
Slc1a1	GTCCTGAGTGGGCTTGCAAT	CCGCACGACTATGAAATAGATCAGT
Slc6a14	TCTGTGTGACTCAGGCTGGAA	CCCATCCAGCACAGAAGTGA
Slc36a1	TTCTGCTGCGTCTACTTTGTGTTT	TGGCTGCCTCTATCACCTGTT
Slca31	GCGGTCCATGACAAAGGTTTAA	TCGATTGGAACCAAGGATGTTT
Slc1a5	TCCAATCTGGTGTCTGCTTCTG	TGGTCCATGGTTGCATTGC

Supplementary Table 2. Transfer of dietary nitrogen to the liver, gastrocnemius muscle, kidney, and skin 6 h after ingestion of the experimental meal (expressed as % of ingested nitrogen per 100 g of body weight)

Age Group	2 months		11 months		P-value		
	2m-na	2m-ha	11m-na	11m-ha	Age effect	Adiposity effect	Age x Adiposity
Liver	2.7 ± 0.8	3.0 ± 1.2	2.2 ± 1.3	2.6 ± 1.2	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Muscle	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Kidney	0.6 ± 0.2 ^a	0.5 ± 0.1 ^a	0.4 ± 0.1 ^{ab}	0.3 ± 0.1 ^b	0.0007	<i>n.s.</i>	<i>n.s.</i>
Skin	3.9 ± 1.2	4.4 ± 1.6	2.8 ± 0.7	3.0 ± 0.8	0.0066	<i>n.s.</i>	<i>n.s.</i>

Values are mean ± SD, n = 7 - 8 rats. The effects of age and adiposity and their interaction were tested with a two-way ANOVA model. Values with different letters within the same row are statistically different. *n.s.*, not significant. 2m-na, 2 months/normal adiposity; 2m-ha, 2 months/high adiposity; 11m-na, 11 months/normal adiposity; 11m-ha: 11 months/high adiposity.