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Ghislaine Nzang Nguema, Elisabeth Debras, Jean Grizard, Josette Alliot. Amino Acid Supplementation Prevents the Loss of Appetite for Casein in Old Lou/Cjall Rats. *Experimental Gerontology*, 2007, 42 (7), pp.652. 10.1016/j.exger.2007.02.001 . hal-00499009

HAL Id: hal-00499009

<https://hal.science/hal-00499009>

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Accepted Manuscript

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PII: S0531-5565(07)00031-9
DOI: [10.1016/j.exger.2007.02.001](https://doi.org/10.1016/j.exger.2007.02.001)
Reference: EXG 8309

To appear in: *Experimental Gerontology*

Received Date: 8 September 2006
Revised Date: 1 February 2007
Accepted Date: 2 February 2007

Please cite this article as: Nguema, G.N., Debras, E., Grizard, J., Alliot, J., Amino Acid Supplementation Prevents the Loss of Appetite for Casein in Old Lou/Cjall Rats, *Experimental Gerontology* (2007), doi: [10.1016/j.exger.2007.02.001](https://doi.org/10.1016/j.exger.2007.02.001)

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Amino Acid Supplementation Prevents the Loss of Appetite for Casein**in Old Lou/Cjall Rats**

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This work was supported by research grant (ATC Vieillissement) from the *Institut National de la
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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.

1. Introduction

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

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

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

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

2- Materials and Methods

2-1 Animals

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

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

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

2-2 Experimental Design

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

2-2-1 Experiment 1

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

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

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

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

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

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

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

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

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

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

2-2-2 Experiment 2

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

Animals:

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

Experiment 2a

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

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

Experiment 2b

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

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

Body weight and macronutrient intakes were recorded daily.

2-3 Statistical Analyses

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

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

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

3- Results

3-1 Experiment 1: Life Long Studies

Body Weight and Caloric Intake (Table 2)

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

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

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 carbohydrates and protein (**Figure 3**).

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

Effects of the Casein Supplementation

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

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 versus 2.1 ± 0.3 kcal; $12.1\% \pm 0.8$ versus $7.5\% \pm 0.8$). Secondly, the age-related changes in protein intake underwent modification, mainly in males (*Interaction age x sex x protein* (F (5,44)= 2.9; p=0.03) in cohort 1; (F(3,26)= 3; p=0.04) in cohort 2). The consumption of SC was maintained at a higher level than casein and remained constant from 15-16 months of age in males, at the level of whey protein consumption. Females selected identical amounts of casein and SC except at the age of 15 and 19 months in cohort 1. As was the case for casein alone, females preferred SC to whey protein. This preference for SC versus whey protein disappeared at only 23-24 months in the two cohorts (instead of 15-16 months for casein alone).

The nature of the protein did not influence the total energy intake (F (2,12) = 1.2 ns).

3-2 Experiment 2.

3-2-1 Experiment 2a

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

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

Effect of Supplementation on Total Protein Intake

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

3-2-2 Experiment 2b

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

4-General Discussion

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

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

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

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

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

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

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

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

Acknowledgements

We thank Dr R. Taylor and Dr D. Bechet for proofreading the English version of this article.

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LEGENDS OF FIGURES

Figure 1:*Experiment 1- Procedure*

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

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

Figure 2*Experiment 2- Procedure*

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

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

Figure 3:

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

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

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

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

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

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

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

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

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

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

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

a posteriori tests: a: $p < 0.05$ vs 11 month-old; b: $p < 0.05$ vs 20 month-old;
* $p < 0.05$ 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 ^{cde}	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)

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 ^{a b}	60.0 ± 4.7 ^{a b}
% 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

Experiment 2A

Experiment 2B

Figure 4

Figure 5

Figure 6