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The LOU/c/jall Rat as an Animal Model of Healthy Aging?

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We propose the LOU/c/jall rat as a possible model for research into aging. Physiological and behavioral data have been collected over the past 5 years, using lifelong and cross-sectional studies. The median life span of the rats was 29 months in males and 33–34 months in females. A low level of body fat throughout life was observed in both sexes. Basic phenomena of aging such as body weight loss, decrease in caloric intake, and dramatic drop in protein selection were noted from the age of 18 months in males and 28 months in females. A decline in muscle mass, depending on the sex and the type of muscle, was seen. These data allowed us to demonstrate physiological aging in male and female LOU/c/jall rats. The most interesting characteristics of this strain of rat for aging studies are longevity, and the absence of obesity and of severe pathologies. Further studies are required in order to confirm this last point.

AGING has been defined as a general decline in body functions, associated with a decrease in the ability to maintain homeostasis (1,2). The decline in body functions with age is obvious in the deterioration of tissue and cell functions, muscle atrophy, loss of bone, and changes in organs such as the kidney, liver, and pancreas. These age-related modifications probably involve genetic, endocrine, and environmental factors.

Because of their ready availability and short life span, rodents have been used extensively in studies on aging. However, the frequency and the extent of disease in some strains of rat are so severe that most of the recorded age-related physiological changes are likely to be more a consequence of disease than a consequence of aging. Moreover, laboratory rodents develop an age-related obesity that may increase the incidence of pathologies and decrease life span. In addition, obese rats become hard to handle and to study.

It is crucial for researchers to have animal models showing successful aging to facilitate the study of the specific events causing normal aging (3). Therefore, we present what we believe is a new model of aging, the LOU/c/jall rat. It has the two advantages of not developing obesity with age, and of having a spontaneous average to long life span.

Methods

Animals

Animals were inbred albino LOU (Louvain)/c rats. Ancestors of the LOU rats were imported from an unknown place around 1937 or 1941, probably from a unique couple (4). The rats were considered to be of Wistar origin. Bazin and Bekers started breeding rats, and they bred 28 different and distinct lines in which they observed a high tumor incidence in the ileocecal area. The line representing the highest incidence was called LOU/c (for review, see 5).

Our colony (LOU/c/jall) was initially obtained from the Bazin and Bekers laboratory, by means of the Institut National de la Recherche Agronomique research center (France). This colony has been breeding in our laboratory for more than 12 years. LOU/c/jall rats are docile, easy to handle, and easy to breed. The litter size of our colony, based on 150 gestations, is 6.5.

Housing

Animals were raised under conventional conditions. They were housed in pairs in cages (18 cm large, 20 cm high, and 40 cm long) in a temperature-controlled (22 ± 1°C) and humidity-controlled (50 ± 10%) room with a 12 hour:12 hour light–dark cycle (lights off at 8 PM). The rats were handled regularly from birth in order to eliminate the stress of manipulation. The absence of stress was confirmed by the fact that rats did not emit ultrasonic emissions (measured with a Mini 3 bat detector, Ultra Sound Advice, London, UK).

Feeding

Apart from self-selection studies, rats had free access to a standard diet, with the following composition in grams: 17.5% of protein, 3% of fat, and 49.8% of carbohydrate (A04 Usine d'alimentation rationnelle, Villemoise, France). When body weight (BW) and energy intake were recorded,

rats and diet were weighed twice daily (8 AM and 7 PM) for at least 3 weeks.

Details of the self-selection procedure have already been reported (6). Animals had a simultaneous choice between separate sources of three macronutrients: protein (93% casein, 3.12 kcal/g), fat (91% lard, 2% sunflower oil, 7.88 kcal/g), and carbohydrate (85% starch, 8% sucrose, 3.34 kcal/g). Cellulose, vitamins, and mineral supplements were added to each of the macronutrients.

Study of Aging: Experimental Procedure

The data presented here have been collected over a period of 5 years. More than 350 male and female rats that were conceived, born, and bred in the laboratory were used. Two cohorts who lived their whole life in the animal house under standard conditions were used to determine longevity. The first cohort was born in 1995, and the second one was born in 1997.

Eight cohorts of rats fed ad libitum with standard diet allowed us to study lifelong body weight and energy intake. Each cohort was composed of males ($11 < n < 16$) and females ($10 < n < 15$), born in the same week and having identical postnatal experiences. The first longitudinal study started in 1995, and the last started in 1998. Three cohorts born in 1993 and 1994 were studied only after 18 months of age. The study of the cohorts was stopped between 27 and 34 months of age when senescent animals were introduced in one of the experimental studies being done in the laboratory.

Since 1995, changes in daily macronutrient intakes with increasing age have been extensively studied in our group. Results obtained in a lifelong study using self-selection procedures are presented here.

Biological and physiological changes during aging were established by using several cross-sectional studies. Each cross-sectional study included from five to nine age groups, and all animals were fed ad libitum with a standard diet.

Biochemical blood analyses were performed on plasma samples of 4- to 26-month-old males and females. Only a few samples (females $n = 4$, males $n = 1$) were obtained from 35-month-old rats. After animals were killed by decapitation between 8:30 and 10:30 AM, blood samples were centrifuged. Plasma was immediately frozen in dry ice and stored at −30°C. Concentrations of urea, creatinine, glucose, protein, sodium (Na), potassium (K), chloride (Cl), calcium (Ca), phosphate (P), and magnesium (Mg) were measured on a Hitachi 911 (Boehringer, Meylan, France).

For body composition study, rats were anesthetized intraperitoneally with pentobarbital (30 mg/kg). Whole body lean mass, fat mass, and bone mineral concentration were determined by dual-energy x-ray absorptiometry on a hologic QDR-4500A x-ray densitometer (Hologic France, Massy, France). In a first experiment, LOU/c/jall groups of increasing age were studied. A second experiment aimed at comparing the body composition of 3-, 12-, and 19-month-old Wistar and LOU/c/jall rats. In this latter study, rats were fasted during the night preceding the measure.

For the study of organ and muscle mass, rats were killed by decapitation between 8:30 and 10:30 AM. Each day of sacrifice included six to eight animals paired by sex and age. Liver, heart, brain, pituitary gland, and, in most groups, kidneys and adrenals were removed and weighed. Three muscles, the soleus, extensor digitorum longus, and gastrocnemius medialis, were excised and weighed.

Some behavioral parameters were recorded. Total motor activity and locomotion (Experiment 1), and locomotion across time (Experiment 2), were studied. Eight rats from each age group were individually placed for 20 minutes in a new box (25 cm × 25 cm × 25 cm). We used an automatic system (videotrack, VIEWPOINT, Lyon, France) consisting of a camera set above the cage illuminated with normal light. The position of the animal's center of gravity (24 points/min) was analyzed in real time by means of computerized image processing.

The study of mechanical sensitivity was carried out on eight rats of each age group. Rats were individually placed in a plastic cage with a wire mesh bottom for 20 minutes (until the animals remained still). Mechanical threshold was measured by applying Von Frey hair (Semmes–Weinstien set) to the midplantar hind paw until paw withdrawal was elicited. The Von Frey hair was applied serially to the paw with logarithmically incremental stiffness (0.172–20.9 g; numbers 3.22–5.46). The Von Frey hair was presented five times, in ascending intensity of application, at a right angle to the plantar surface with sufficient force to cause slight bending. Threshold was defined as the minimum pressure required to elicit the paw withdrawal.

Statistical Analysis

Except for the longevity curve, all data were expressed as means ± standard error. In each longitudinal study, the effect of age and sex was determined by using an analysis of variance (ANOVA) with repeated measures, followed by a mean least-squares test for a posteriori multiple means comparison.

In cross-sectional studies, an ANOVA followed by a Fischer protected least significant difference test for a posteriori means comparison was performed. In the mechanical sensitivity study, the nonparametric Kruskal–Wallis test was performed on the data. Significance at $p < .05$ was considered to evaluate the differences.

RESULTS

Characteristics of the Strain

When they were compared with Wistar male rats purchased from the Charles River breeding center (Table 1), young mature LOU/c/jall male rats had a lighter BW and their daily caloric intake was lower. However, their diet consumption expressed in grams of BW was significantly higher (standard diet), or identical (self-selection procedure). The total number of meals (measured as presence in the food cup area with an automatic system videotrack, VIEWPOINT) was lower in LOU than in Wistar rats, particularly during the nocturnal period.

The study of macronutrient choice showed that young mature Wistar and LOU/c/jall male rats have a similar regimen composition, except that LOU/c/jall rats choose more protein.

Longevity of Male and Female Cohorts

Figure 1 shows the mean survival rate of the male and female cohorts. In males, the first deaths were observed at 24

Table 1. BW and Daily Food Intake of LOU/c/jall Versus Wistar Rats

Parameter	Wistar	LOU/c/jall
BW (g)	428 ± 20	251 ± 11.1***
Standard diet		
Food intake (kcal/d)	80.2 ± 4.1	40.4 ± 4.5**
kcal/100 g BW/d	14.4 ± 0.8	16.7 ± 1.0*
Number of meals/d	9.0 ± 0.9	5.6 ± 0.5**
Weight gain/w (g)	9.3 ± 1.6	3.3 ± 0.5**
Self-selection regimen		
Food intake (kcal/d)	85.5 ± 1.3	37.3 ± 1.7***
kcal/100 g BW	13.7 ± 0.4	15.3 ± 1.7 (NS)
Macronutrient choice (g)		
% proteins	38.5 ± 6.8	55.4 ± 1.3*
% fat	20.9 ± 0.9	15.0 ± 3.3 (NS)
% carbohydrates	40.6 ± 5.9	29.6 ± 3.0 (NS)

Notes: Comparison is for 4-month-old male LOU/c/jall and Wistar rats. Rats were studied on standard and self-selection diets given ad libitum. BW = body weight; NS = not significant.

*$p < .05$; **$p < .01$; ***$p < .0001$ (Student's *t* test).

months (Cohort 1) and 12 months (Cohort 2), and the median life span (50% of survivors) was 28 and 30 months in Cohort 1 and Cohort 2, respectively. In females, the first death was observed at 21 months and 24 months, and the median life span was 33 and 34 months in Cohort 1 and Cohort 2, respectively. The maximum life span of males was 35 months. Females of Cohort 1 were sacrificed at 37 months of age. Survivor females of Cohort 2 died at 40 months.

Age-Related Changes in BW and in Food and Macronutrient Intakes (Longitudinal Studies)

BW.—Figure 2 gives the BW of male and female cohorts followed in eight longitudinal surveys. Small variations were seen between the cohorts, and the highest BW was around 320 g for males and 190 g for females. The growth period was followed until they were 6 months old. After this age, male rats continued to gain weight slowly until they were 15 months old. BW decreased from 16 to 18 months of age onward. The loss of BW accelerated considerably from 22 months onward, when larger intergroup and interindividual variations appeared, showing that BW loss depended on physiological but not on chronological aging. BW remained stable during the major part of life in females. A slight decrease was observed from 24 to 28 months of age onward.

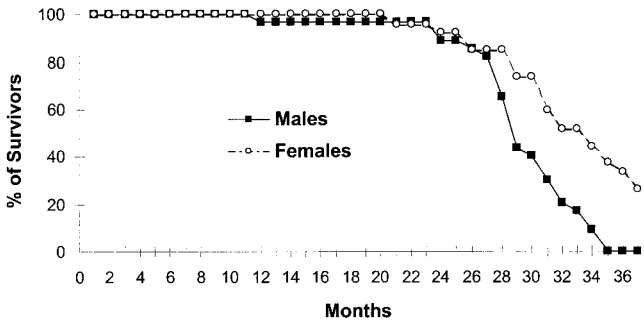

Figure 1. Mean survival rate of LOU/c/jall rats. To establish this survival curve, cohorts fed ad libitum with a standard chow were followed—Cohort 1 (12 males, 11 females) and Cohort 2 (15 males, 15 females). At 24 months, more than 90% of the males and females were still alive (for details, see Results). A significant difference appeared in the male group survival rate from 28 months of age versus 24-month-old males and versus females.

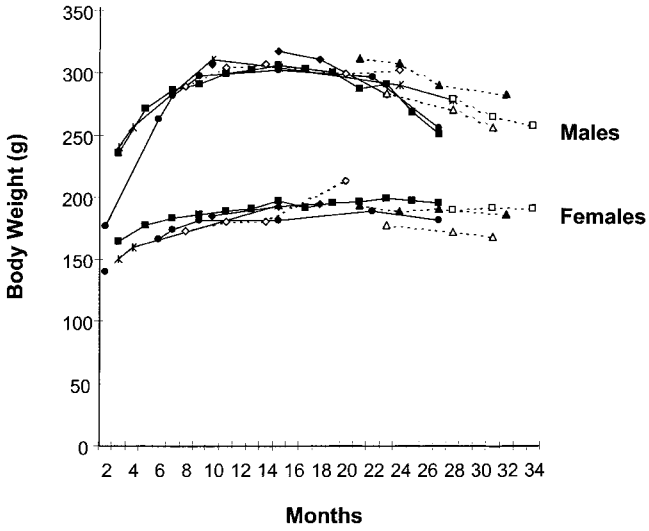

Figure 2. Age-related changes in male and female body weight (BW) as studied in eight long-life surveys using LOU/c/jall rats. Rats were fed ad libitum with a standard chow. The higher BW was observed around 14–15 months of age. In males, the rate of loss increased from 22 months of age while larger intergroup and interindividual variations appeared. Females showed a remarkable stability of BW during most of their life.

Caloric intake.—The age-related changes in caloric intake (Figure 3) as measured in longitudinal studies can be summarized as follows: females increased their caloric intake (from 32 ± 1.7 kcal to 41 ± 4 kcal) from the age of 24

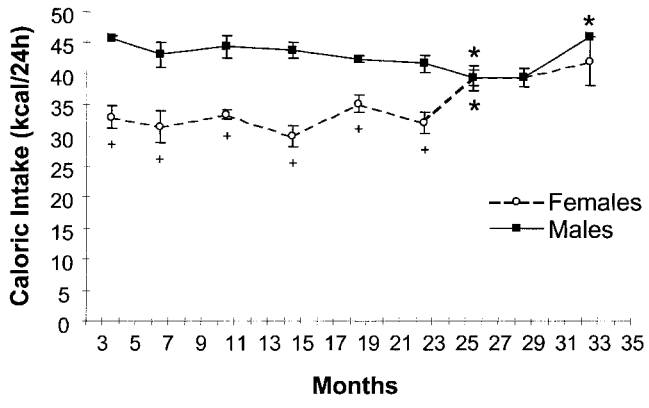

Figure 3. Age-related changes in caloric intake. Each point was the average of caloric intake recorded during the long-life surveys of eight cohorts of LOU/c/jall rats fed with a standard chow. An increase of caloric intake in females and a decrease in males (also significant when we analyze each longitudinal study) was observed from 24 months of age. From 28 months of age, females and males showed no difference in daily energy intake. It is noteworthy that 34-month-old male survivors, as did females, showed an increase in daily caloric consumption. *$p < .05$ versus adjacent age group; $^{+}p < .05$ versus male group.

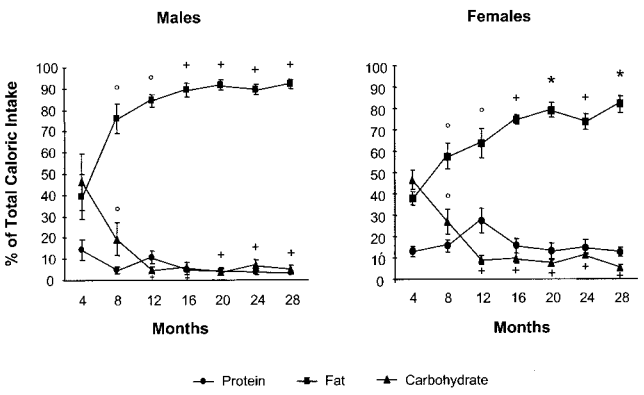

Figure 4. Age-related changes in percent concentration scores of the three macronutrients across daily energy intake, obtained in the third week of self-selection diets for male (n = 10) and female (n = 10) LOU/c/jall rats, using a long-life study. An analysis of variance showed an effect of age and sex. Compared to 4-month-old rats, increased lipid consumption with a concomitant decrease of carbohydrate intakes ($p < .05$) was seen in males and females. A decrease in protein intake occurred for males, but not for females, after 16 months. $^{\circ}p < .05$ versus 4 months; $^{+}p < .05$ versus 4 and 8 months; $^{*}p < .05$ versus 4, 8, and 12 months. Adapted from Veyrat-Durebex et al., 1998 (7).

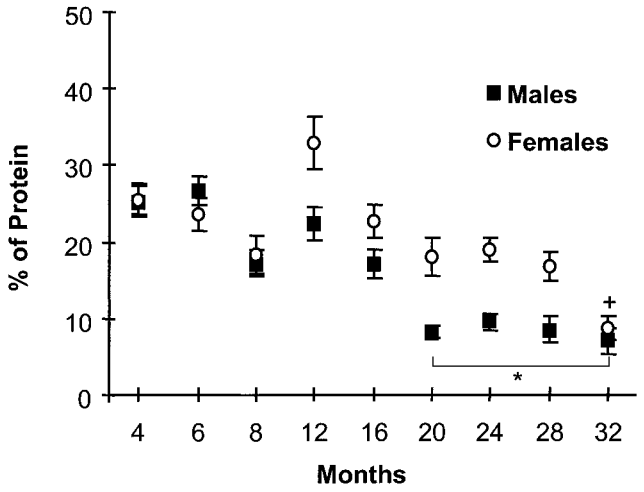

Figure 5. Age-related changes in protein intake. Each point represents the average of protein percentage of total intake (in grams) recorded in longitudinal and cross-sectional studies in all rats. The drop of protein already observed with age was confirmed. Protein percentage in diet decreased between 16 and 20 months of age in males, whereas it decreased only in the oldest females (after 28 months). $^{*}p < .05$ versus 4, 8, and 12 months; $^{+}p < .05$ versus 28 months.

months. In contrast, males decreased their caloric intake from the age of 16 to 18 months onward (44 ± 1.7 kcal in younger groups vs 39 ± 1.5 kcal in 28-month-old senescent animals). Generally, 28-month-old females and males showed no significant differences in daily energy intake. It is noteworthy that 33-month-old male survivors, as well as females, showed an increased daily caloric consumption.

Macronutrient preferences.—When rats were allowed to choose between the three macronutrients (Figure 4), an age-related modification in diet composition (percentage of each macronutrient in the daily caloric intake) was demonstrated. In young mature rats, carbohydrate was the main source of calories for both males and females. Later, after 8 months in the experiment presented here, males chose a high fat regimen (up to 89.5% of the total energy intake). Females selected up to 78% of their total energy intake as fat, and they selected a larger part of their energy as protein and carbohydrate than males.

An important difference between males and females was observed for protein intake. Males strongly reduced their protein intake from 16 months onward, whereas females maintained it up until the age of 28 months. The drop in protein with age was confirmed by analysis of data obtained from the overall group of 350 males and females (Figure 5). Protein percentage in diet decreased between 16 and 20 months in males but only after 28 months in females. The age-related sexually dimorphic decrease in protein intake was some of the strongest and most reproducible data resulting from our experiments (6–8).

Biological and Physiological Changes During Aging (Cross-Sectional Studies)

Biochemical parameters.—Biochemical parameters in blood plasma (Table 2) were well maintained during aging. Ion composition (Na, K, Cl, Mg, Ca, and P) and glucose concentration were not significantly modified until 26 months of age, but K was increased and glucose and Cl were decreased in the 35-month-old senescent group. Creatinine concentration was also well maintained but dropped dramatically in the 35-month-old animals. Total protein and urea decreased from 26 months of age.

Body composition.—No obesity was seen in LOU/c/jall rats (Figure 6). A strong correlation between BW and lean mass was found ($r^2 = 1$; $p < .0007$). Whatever the sex, the percentage of fat mass remained rather stable from 8 to 23 months. The same stability was observed when we considered the bone mineral density (results not shown). The BW loss in the oldest animals is due to a loss of fat-free mass as well as to a loss of fat mass.

Whereas no significant difference in percentage of fat was observed between 3-month-old Wistar and LOU/c/jall rats, obesity (increased BW correlated with increased percentage of fat) appeared from 12 months in male and female Wistar rats (Table 3). Such a precocious obesity was also reported by McCarter and Palmer (9) in Fisher 344 rats.

Organ and Muscle Weight

Organ weight is given in Table 4. A constant organ weight–BW ratio was observed from 4 until 24 months of age. As described for other strains, there was an increase in brain and heart mass, and a decrease in liver mass in senescent animals (29- and 34-month-olds). Pituitary gland weight increased significantly in both aged males and females, but the change occurred early in females (from 18 months vs 29 months in males). This well-known fact is probably linked to the hyperplasia of the lactotroph cells in middle age, depending on the age-related dysfunction of the female reproductive system.

Table 2. Age-Related Changes in Biochemical Parameters in LOU/c/jall Rats

Age (mo)	Sex	Urea (mmol/l)	Creatinine (μmol/l)	Protein (g/l)	Glucose (mmol/l)	Na (meq/l)	K (meq/l)	Cl (meq/l)	Ca (mmol/l)	P (mmol/l)	Mg (mmol/l)
4	male	7.3 ± 0.3	67 ± 3	78 ± 0.8	8.1 ± 2	149 ± 1.3	7.2 ± 0.3	116 ± 1.2	2.7 ± 0.02	2.1 ± 0.06	0.99 ± 0.02
	female	9.5 ± 0.4	65 ± 2	77 ± 0.9	8.0 ± 0.2	148 ± 0.7	6.2 ± 0.03	114 ± 0.8	2.7 ± 0.06	1.8 ± 0.06	0.95 ± 0.015
16	male	7.0 ± 0.3	66 ± 3	78 ± 0.6	7.7 ± 0.1	148 ± 0.1	6.2 ± 0.03	113 ± 0.7	2.7 ± 0.02	1.8 ± 0.06	0.95 ± 0.02
	female	9.5 ± 0.4	74 ± 4	80 ± 0.8	8.1 ± 0.3	124 ± 1.3	6.1 ± 0.02	120 ± 0.1	2.8 ± 0.01	1.5 ± 0.06	0.94 ± 0.02
20	male	6.5 ± 0.15	65 ± 2	79 ± 0.8	8.0 ± 0.2	152 ± 1.3	6.4 ± 0.01	116 ± 0.8	2.8 ± 0.04	1.9 ± 0.03	0.94 ± 0.02
	female	8.1 ± 0.2	66 ± 0.8	81 ± 1.1	7.7 ± 0.1	151 ± 0.9	6.1 ± 0.04	116 ± 0.7	2.8 ± 0.02	1.5 ± 0.07	0.97 ± 0.02
26	male	6.0 ± 0.4*	66 ± 8	72 ± 1.2*	7.4 ± 0.3	152 ± 3	6.4 ± 0.07	110 ± 0.3	2.8 ± 0.07	1.9 ± 0.08	1.00 ± 0.05
	female	6.9 ± 0.3*	67 ± 2	74 ± 2.1*	7.4 ± 0.2	153 ± 1.5	6.2 ± 0.03	114 ± 0.1	2.7 ± 0.05	1.7 ± 0.06	1.04 ± 0.03
35	male	4.9	48	73	5.0	144	6.9	106	2.7	1.9	9.00
	female	6.8 ± 0.2*	49 ± 1*	71 ± 1.6*	6.0 ± 0.5*	149 ± 1.7	8.6 ± 0.05*	109 ± 0.8*	2.7 ± 0.01	2.0 ± 0.14	1.05 ± 0.03

Notes: Biochemical parameters were studied in male and female groups ($n = 8$). Few samples (females $n = 4$, males $n = 1$) were obtained from 35-month-old rats. Animals were fed ad libitum with a standard chow. Data are presented as mean ± standard error. An analysis of variance showed no significant effect of age on sodium (Na), calcium (Ca), phosphate (P), or magnesium (Mg) concentration. Creatinine, glucose, potassium (K), and chloride (Cl) plasma levels were not significantly modified until 26 months of age, but they were significantly decreased (or increased for K) in senescent 35-month-old animals. Urea and total protein decreased from 26 months of age.

*$p < .05$ vs all other groups.

Age-related changes in skeletal muscles are reported in Table 5. An increase of muscle mass was observed from 2 to 4 months, followed by a proportional gain in BW until 18 months. A heterogeneous decline in muscle mass and in the ratio of muscle to body mass appeared after 18 months of age, depending on the sex of the animals and the type of muscle. In males, the decline was significant at 24 months for gastrocnemius and at 29 months for the two other muscles. Decline was delayed in females.

Behavioral Parameters

In LOU/c/jall rats, the well-known higher activity of females compared with that of male counterparts was found in each experiment (Figure 7). In the first experiment, no dramatic change during most of their life span was seen in total activity or specific exploration. Only older males (29 months) showed a decrease of activity compared with the other groups.

The second experiment in which exploration was specifically studied confirmed these data. The oldest groups showed a weaker initial level of exploration, within the first minute in males and until the fifth minute in females. Consequently, a lower habituation expressed by the decline of exploration with time was observed in senescent males and females compared with younger groups.

The difference between senescent males and females could be explained by the fact that the oldest males are survivors ($n = 3$).

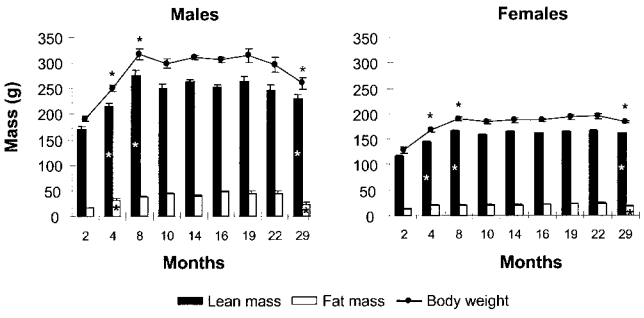

Figure 6. Whole body weight, and lean mass and fat mass in LOU/c/jall rats from 2 to 29 months of age. Lean mass was correlated with body weight, whereas fat mass did not change between 8 and 22 months of age. Both lean and fat masses decreased in senescent 29-month-old rats. *$p < .05$ versus adjacent age group.

Mechanical Sensitivity

A decrease in the response threshold was seen (Kruskal–Wallis test, $H = 11.6$; $p < .02$), showing that mechanical sensitivity (Table 6) was increasing with age, specifically in males and in senescent groups.

Discussion

No dramatic changes appeared in biochemical parameters. The ionic status does not show any deterioration with age, and the fact that glucose does not increase with age rules out any latent diabetes. The decrease of creatinine value in the very old animals is probably linked to the dramatic loss of lean body mass observed in senescence. The lack of urea increase with age excludes any renal insufficiency in this strain and confirms the data of Dodane and colleagues (10), which showed, in a Wag Lou M strain close to the LOU/c strain, the absence of any severe chronic nephropathy that is frequently found in old rats of more commonly used strains. Moreover, macroscopic pituitary tumors in aged LOU/c/jall rats were never observed, whereas an age-dependent occurrence of spontaneous pituitary tumors that reaches 60–85% has been described in rats over the age of 24 months (11,12). Animals may live up to the age of 28 months without obvious severe pathologies. The first results reported here suggest that LOU/c/jall rats present a healthy aging process. Nevertheless, before such a conclusion could be taken into consideration, further data on pathology and histopathology are needed.

The analysis of physiological parameters shows that the basic phenomena of aging, also described in humans (13) and in other strains of rodents (14–16), appeared after 16–18 months in LOU/c/jall rats. A loss in BW took place concurrently with a decline in caloric intake; a loss of skeletal

Table 3. BW and Fat Percentage in Wistar Versus LOU/c/jall Rats

		Wistar			LOU/c/jall		
Sex	Age (mo)	BW	% Fat	r^2	BW	% Fat	r^2
Male	3	381 ± 18*	11 ± 0.6		205 ± 7.5	7 ± 0.5	
	12	621 ± 30*.**	22 ± 2*.**	.84	306 ± 6**	9 ± 0.5	.29
	19	675 ± 21*.**.***	27 ± 2*.**.***		299 ± 4**	11 ± 0.9	
Female	3	242 ± 5*	7.7 ± 2		140 ± 2.5	6.8 ± 0.5	
	12	340 ± 15*.**	21 ± 4*.**	.69	186 ± 5**	6.5 ± 0.5	.002
	19	373 ± 10*.**	23 ± 8*.**		198 ± 3**	7 ± 0.7	

Notes: An analysis of variance was performed on the fat rate on animals fed with a standard diet given ad libitum. BW = body weight. Data showed an interaction of Age × Strain; $F(2,109) = 17$; $p < .0001$.

*$p < .05$ vs the same age group of LOU rats.

.*$p < .05$ vs, respectively, 3- and 12-month-old groups of the same strain (a posteriori test).

muscle mass occurred earlier in fast-contracting locomotor muscles than in slow-contracting ones (17). A biochemical study performed on the muscles of these rats (18) has shown a decrease of glycolytic metabolism and a slight increase of oxidative metabolism without modification of adenosine triphosphatase activity. After 29 months, a markedly greater rate of body and muscle change was observed concomitantly with changes in the weight of the organs (decrease of liver mass and adrenals, and increase of heart and brain mass).

As the primary purpose of aging studies is to determine changes occurring as a function of time, lifelong studies are the most appropriate design. However, many experiments do not allow longitudinal studies, and therefore cross-sectional designs are the most common model. As reported by Florez-Duquet and McDonald (19), efficient cross-sectional design of animal experimentation requires a fairly precise determination of the age at which a species becomes senescent, and for the sake of comparison, the age at which the animal reaches maturity. The data presented here allowed us to specify, in the life of LOU rats, a "hinge" period (16–18 months) at which they enter into old age. The loss of BW and the decline in caloric intake can easily be used to follow this physiological aging (20), specifically in males.

Table 4. Age-Related Changes in Organ Weight in LOU/c Rats

Age (mo)	BW (g)	a or b	Brain (g)	Liver (g)	Heart (g)	Kidneys (g)	Pituitary (mg)	Adrenals (mg)
					Males			
2	196 ± 9*	a	1.6 ± 0.02*	7.5 ± 0.4	0.71 ± 0.02*	—	6.2 ± 0.2*	—
		b	0.8 ± 0.04*	3.8 ± 0.1*	0.4 ± 0.02*	—	3.2 ± 0.3	—
4	281 ± 7*.**	a	1.8 ± 0.02**	8.8 ± 0.02**	0.91 ± 0.02**	1.5 ± 0.05	8.9 ± 0.3**	33 ± 1
		b	0.59 ± 0.01**	3 ± 0.1**	0.3 ± 0.005**	0.5 ± 0.013	3 ± 0.09	11 ± 0.9
10	316 ± 8**	a	1.9 ± 0.04	9 ± 0.4	0.93 ± 0.03	—	8.4 ± 0.4	35 ± 3
		b	0.63 ± 0.02	2.9 ± 0.1	0.3 ± 0.009	—	2.7 ± 0.18	10.6 ± 1
18	309 ± 7	a	1.8 ± 0.02	8 ± 0.5	1 ± 0.03	1.6 ± 0.04	8.7 ± 0.3	31 ± 1.8
		b	0.61 ± 0.02	2.6 ± 0.1	0.3 ± 0.01	0.5 ± 0.007	2.9 ± 0.06	10.4 ± 0.4
24	296 ± 8	a	1.8 ± 0.02	8.5 ± 0.4	0.93 ± 0.02	1.5 ± 0.04	8.8 ± 0.4	35 ± 6.4
		b	0.61 ± 0.01	2.9 ± 0.1	0.3 ± 0.01	0.6 ± 0.01	2.95 ± 0.14	13.5 ± 2.3
29	254 ± 13*.**	a	1.8 ± 0.02	7.8 ± 0.5	1 ± 0.04	1.7 ± 0.01	9.6 ± 0.8*.**	43 ± 5**
		b	0.69 ± 0.03	3 ± 0.1	0.4 ± 0.01*	0.75 ± 0.1	3.7 ± 0.2*.**	17 ± 2.8
34	231 ± 12*	a	1.8 ± 0.1	7 ± 0.5*	1 ± 0.04	1.75 ± 0.1	10 ± 1.3*	14 ± 1.2*.**
		b	0.74 ± 0.02*	2.9 ± 0.1*	0.4 ± 0.01*	0.75 ± 0.05	4.1 ± 0.5*	5.3 ± 1.3*.**
					Females			
2	132 ± 3*	a	1.5 ± 0.02*	4.9 ± 0.2	0.54 ± 0.02*	—	6.4 ± 0.3*	—
		b	0.8 ± 0.04*	3.8 ± 0.1	0.4 ± 0.02	—	4.9 ± 0.2*	—
4	166 ± 4*.**	a	1.6 ± 0.01**	4.8 ± 0.03	0.59 ± 0.03*	1 ± 0.03	8.1 ± 0.6*.**	38 ± 1.6
		b	0.59 ± 0.01**	2.9 ± 0.1	0.4 ± 0.01	0.6 ± 0.004	4.8 ± 0.3*	22 ± 1.2
10	182 ± 5**	a	1.6 ± 0.02	5.4 ± 0.2	0.69 ± 0.01	1 ± 0.03	9 ± 0.4*	—
		b	0.63 ± 0.02	3 ± 0.1	0.4 ± 0.06	0.6 ± 0.006	4.9 ± 0.2*	—
18	194 ± 8	a	1.7 ± 0.02	5.2 ± 0.2	0.76 ± 0.08	1.3 ± 0.03	13 ± 0.7**	42 ± 3.1
		b	0.61 ± 0.02	2.7 ± 0.1	0.4 ± 0.01	0.7 ± 0.02	6.8 ± 0.3**	21 ± 1.7
24	177 ± 8	a	1.6 ± 0.03	5.4 ± 0.3	0.68 ± 0.02	1.2 ± 0.03	11.8 ± 0.6	38 ± 3.1
		b	0.61 ± 0.01	3 ± 0.1	0.4 ± 0.01	0.65 ± 0.1	6.6 ± 0.5	21 ± 4.5
29	180 ± 5	a	1.7 ± 0.02	5.3 ± 0.2	0.76 ± 0.02	1.25 ± 0.04	11.3 ± 0.9	42 ± 0.4
		b	0.69 ± 0.03	3 ± 0.1	0.5 ± 0.01	0.7 ± 0.02	6.4 ± 0.4	23 ± 2.4
34	173 ±10*	a	1.7 ± 0.2	5 ± 0.3	0.78 ± 0.03	1.35 ± 0.04	10.4 ± 0.5	21 ± 8.2*.**
		b	0.74 ± 0.02*	3 ± 0.2	0.5 ± 0.015*	0.8 ± 0.03*.**	6.2 ± 0.3	13.5 ± 4*.**

Note: BW = body weight; a = weight in grams or milligrams; b = grams or milligrams/100 grams of BW.

*$p < .05$ vs 18 months of age, **$p < .05$ vs adjacent age group, a posteriori tests.

Table 5. Age-Related Changes in Muscle Mass in LOU/c/jall Rats

		Soleus		EDL		GM	
Age (mo)	a or b	Male	Female	Male	Female	Male	Female
2	a	89.5 ± 5.3*	63 ± 2.9*	97 ± 4.5*	67 ± 1.5*	495 ± 25*	331 ± 11*
	b	45.5 ± 1.4*	48 ± 1.5*	49.2 ± 0.7*	51 ± 0.7	252 ± 3	251 ± 5
4	a	131 ± 7.5*.**	89 ± 3.8**	148 ± 3.8**	88 ± 3.7*.**	717 ± 23**	456 ± 25
	b	47 ± 1.8*	54 ± 4.1**	53.2 ± 2.1**	53 ± 1.1	256 ± 10	275 ± 12
10	a	153 ± 20	95 ± 4.8	158 ± 9	97 ± 2.7	745 ± 21	486 ± 22
	b	48 ± 5.7	52 ± 4	50.2 ± 1.8	53 ± 1.6	237 ± 5	266 ± 9
18	a	153 ± 8	105 ± 8	156 ± 12	100 ± 2.6	735 ± 16	458 ± 16
	b	49.5 ± 3.2	54 ± 3	51 ± 5	51.7 ± 1	238 ± 9	237 ± 5
24	a	155 ± 1.2	108 ± 8.5	145 ± 6	102 ± 5.3	576 ± 20*	388 ± 28
	b	52 ± 2.6	61 ± 6.9	49 ± 2.5	56.4 ± 1.9	194 ± 6*	212 ± 6
29	a	109 ± 5.6*.**	82 ± 3*.**	97 ± 5*.**	79 ± 2.1*.**	371 ± 24*.**	288 ± 8*.**
	b	43 ± 1*.**	46 ± 2*.**	38 ± 1.7*.**	46 ± 2.5*.**	146 ± 8*.**	159 ± 5*.**
34	a	91 ± 4.4*.**	77 ± 4*	112 ± 4.3*	73 ± 3.7*	337 ± 21*	259 ± 16*
	b	39 ± 2*.**	45 ± 1*	48.6 ± 2*.**	42.5 ± 1*	145 ± 4*	150 ± 8*

Note: EDL = extensor digitorum longus muscle; GM = gastrocnemius muscle; a = weight in milligrams; b = weight in milligrams/100 grams of BW.
*$p < .05$ vs 18 months of age, **$p < .05$ vs adjacent age group, a posteriori tests.

When they became older, rats selected more fat and less carbohydrate. A decline in spontaneous protein consumption, more important and more precocious in males than in females, was also observed. Two mechanisms are probably involved: lipid and carbohydrate intake could be modulated by changes in energetic metabolism, whereas dietary protein selection appeared to be correlated with the decline of the somatotrope function and with physiological aging (7,21).

In most animals, including humans, a female survival advantage has been documented (22,23). Females live longer than males in the LOU/c/jall strain as well. Basic phenomena of aging expressed by BW loss and muscle atrophy as well as by caloric and protein intake declines were delayed in female groups compared with male groups. Nevertheless, we previously reported that self-selecting LOU/c/jall male rats had a longer life expectancy than those eating a standard chow, and they showed delayed atrophy of glycolytic muscle (7). These data emphasize the influence of diet composition on aging processes. From a general point of view, we think that the sexual differences in aging could be used to elucidate the factors likely to contribute specifically to

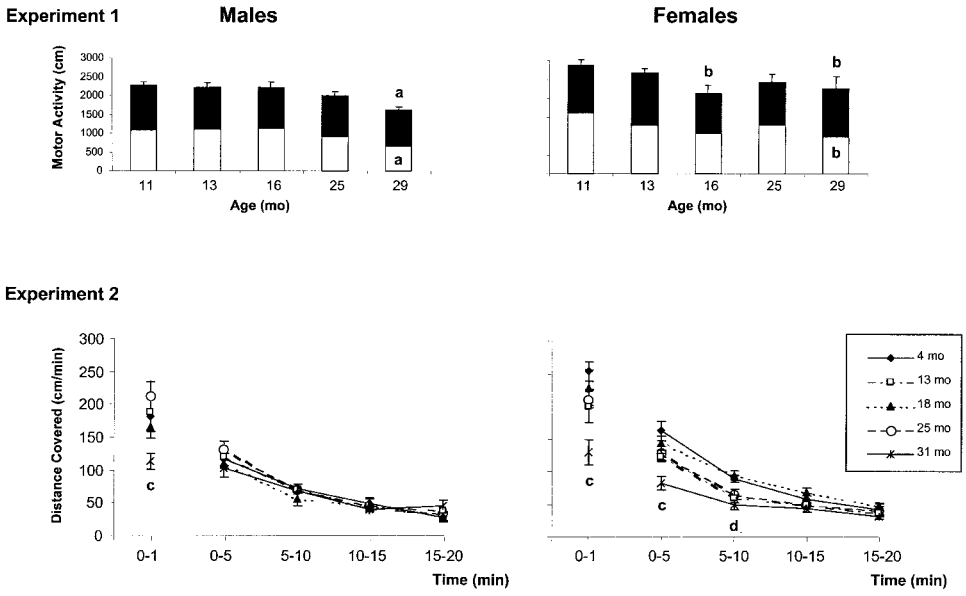

Figure 7. Age-related changes in general activity. For Experiment 1, total motor activity and locomotion (white part of bar chart) are represented. An analysis of variance showed an effect of age. Oldest animals showed a decrease of activity and locomotion compared with other groups. For Experiment 2, locomotion across time is represented. In females, a higher total locomotion than in males was observed during the first 10 minutes ($p < .05$). The covered distance was lower in the first minute and until the fifth minute in 31-month-old male and 31-month-old females, respectively. A decline of exploration with time (habituation) was observed in senescent males and females. Experiment 1: **a**, $p < .05$ versus 11-, 13-, and 16-month-old groups; **b**, $p < .05$ versus 11-month-old group (a posteriori mean least-squares test). Experiment 2: **c**, $p < .05$ versus all other groups; **d**, $p < .05$ versus 4- and 13-month-old groups (a posteriori mean least-squares test).

Table 6. Measure of Mechanical Sensitivity of LOU/c/jall/ Rats as a Function of Age

Sex	4 mo	9 mo	18 mo	22 mo	28 mo
Male	2.5 ± 0.9	1.1 ± 0.5	1.1 ± 0.5	0.9 ± 0.2	0.5 ± 0.1*
Female	1.7 ± 0.8	2.1 ± 0.7	1.7 ± 0.5	2.1 ± 0.9	0.6 ± 0.1*

Notes: A decrease in the response threshold (minimum pressure in grams required to elicit the paw withdrawal) was seen (Kruskal–Wallis test, $H = 11.6$; $p < .02$). Mechanical sensitivity increased with age, specifically in males.

*$p < .05$ vs all other age groups.

the onset of age-related disorders and ultimately death. More studies on aging that compare males and females would be very fruitful.

The most important difference between LOU/c/jall and more common strains is the absence of obesity. In the present study, as in that of Newby and colleagues (24), the percentage of fat in Wistar rats was around 11% in young rats and reached 22% and 27% in 12-month-old and 19-month-old animals weighing more than 600 g. Surprisingly, females showed a similar precocious obesity despite lesser weight. In our LOU strain, the fat mass rate remained stable from 8 to 23 months of age in males and females. Thus, BW is a good criterion to evaluate the fat-free mass in LOU rats in contrast to more common strains.

The accumulation of total lipids in rats with increasing age is probably due to continuing lipid deposition brought about by a caloric intake exceeding caloric expenditure. Undoubtedly, food consumption of most laboratory rodents was enhanced by food and water given ad libitum, and by inactivity, leading to overfeeding and to obesity. We can hypothesize that stability of the percentage of fat mass in LOU/c/jall rats could be attributed to the ability of the animal to adjust caloric consumption to its caloric needs throughout life. In a recent study (25), it was shown that serum leptin concentration remained stable in old LOU/c/jall rats, whereas it increased considerably in old Wistar rats. Among numerous other factors regulating BW, leptin has been reported to be an endocrine signal involved in the regulation of fat storage. Consequently, further studies presently under investigation are required, aimed at determining the neuroendocrine status of LOU/c/jall rats.

We think that the use of the LOU/c/jall strain makes metabolic, pharmacological, and behavioral studies more valid. Indeed, the metabolic machinery, and the study of drug distribution, would not be influenced as much by differences in body composition as it is when fat body mass increases with age. Moreover, learning and memory tests commonly used in animals are based on the analysis of performance. Because LOU rats do not show obesity, differences in body mass and differences of buoyancy in swimming tests, which could induce differences in effort and then in performance, would not bias the results.

In old animals, it is difficult to separate the influence of age on sensory capacities and motor responses from the effect on learning and memory per se. The first data presented here showed that differences in motor and/or sensory capacities would have a minor influence on results obtained in numerous learning and memory tests performed in old, not senescent, animals. However, a decrease of activity and an increase of paw tactile sensitivity have to be taken into account when senescent, but not survivor animals, are studied. Before a definitive conclusion can be reached, further studies are needed, particularly to evaluate other sensory modalities.

In conclusion, the LOU/c/jall rat could be an appropriate model to study aging mechanisms, especially those concerned with differences that are due to sex and without age-related obesity. However, we are aware that further information has to be provided before this strain can be widely used as a model for aging research.

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