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Housing conditions modulate spontaneous physical activity, feeding behavior, aerobic running capacity and adiposity in C57BL/6J mice

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ABSTRACT

There is evidence of reduced adiposity in rodents living in a large cages (LC) as compared to animals housed in small cages (SC). Because spontaneous physical activity (SPA) provides an important portion of the total daily energy expenditure, an increase of SPA in rodents kept in LC could explain their reduced body fat accumulation. The relationship between SPA and components of physical fitness (i.e. aerobic and anaerobic fitness and body leanness) has not been previously determined. We examined the effects of eight weeks of LC exposure on SPA, body composition, feeding behavior, as well as aerobic and anaerobic running capacity in adult C57BL/6J mice. Male mice were housed in cages of two different sizes for 8 weeks: a small (SC, $n = 10$) and large (LC n = 10) cages with 1320 cm^2 and 4800 cm^2 floor space, respectively. SPA was measured gravimetrically, and food and water intake were recorded daily. Mice had critical velocity (CV) and anaerobic running capacity (ARC) evaluated at the beginning, middle course (4th week) and at the end of study (8th week). Despite non-significant differences in each week LC-mice were more active than SC-mice by considering all SPA values obtained in the entire period of 8 weeks. The difference in SPA over the whole day was mainly due to light phase activity, but also due to activity at dark period (from 6 pm to 9 pm and from 5 am to 6 am). LC-mice also exhibited higher food and water intake over the entire 8-wk period. LC-mice had lower content of fat mass (% of the eviscerated carcass) than SC-mice (SC: 8.4 ± 0.4 vs LC: 6.3 ± 0.3, $p < 0.05$). LC-mice also exhibited reduced epididymal fat pads (% of body mass) compared to SC-mice (SC: 1.3 ± 0.1 vs LC: 0.9 ± 0.1, $p < 0.05$) and retroperitoneal fat pads (SC: 0.4 ± 0.05 vs LC: 0.2 ± 0.02, $p < 0.05$). The LC-group showed significantly higher critical velocity than SC-group at the fourth week (SC: 14.9 ± 0.6 $m \cdot min^{-1}$ vs LC: 18.0 ± 0.3 $m \cdot min^{-1}$, $p < 0.05$) and eighth week (SC: 17.1 ± 0.5 $m \cdot min^{-1}$ vs LC: 18.8 ± 0.6 $m \cdot min^{-1}$, $p < 0.05$). Our findings demonstrate that eight weeks of LC housing increases SPA of C57BL/6J mice, and this may lead to reduced fat accumulation as well as higher aerobic fitness. Importantly, our study implies that SC limits SPA, possibly generating experimental artifacts in long-term rodent studies.

1. Introduction

Laboratory rodents are commonly kept in cages with small dimensions with continuous food availability, and minimal locomotion. The adverse effects of these housing conditions have been reported (Martin et al., 2010; Spangenberg et al., 2005). We have built a cage with large dimensions that allows animals to move freely. This “large cage” (LC) is also kept under a controlled temperature, luminosity and humidity environment. Rodents who lived in LC had a significantly lower visceral fat compared with their counterparts kept in regular, small cage (SC) housing conditions (Scariot et al., 2015). Although the physiological reasons for this finding are under investigation, a major factor is likely to be that the rodents kept in LC expended more energy through the higher daily physical activity. More specifically, it is conceivable that LC may stimulate rodents to perform more natural movements, commonly defined as spontaneous physical activity (SPA).

SPA refers to daily living activities such as fidgeting, spontaneous muscle contractions, posture maintenance and ambulation (Kotz et al., 2017). The energy spent while engaging in SPA accounts for an important portion (~30%) of the total energy expenditure (Garland et al.,

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2011). There is evidence that selectively-bred obesity-resistant rats (with a greater resistance to fat accumulation) had an increased SPA (Teske et al., 2012). Independent from bioenergetics, SPA may have an influence on aerobic and anaerobic fitness as well as muscular endurance, traditionally being regarded as components of physical fitness related to health and performance. However, it is unknown, whether an increase in SPA is sufficient to elicit improvements in aerobic fitness and body fatness.

This study aimed to investigate the influence of keeping isogenic mice over eight weeks in LC as compared to SC on SPA and feeding behavior, and on aerobic and anaerobic running capacities. We also aimed to compare the body composition of SC and LC-mice following eight weeks of housing, and to analyze whether energy-storage mobilization is increased in mice kept in LC, as determined by glycogen alterations induced by fasting. Our main hypothesis was that LC can induce SPA, especially during the dark period, given the nocturnal habits of rodents (Ikeda et al., 2000). Based on the role of SPA in increasing energy expenditure, we also hypothesized that food intake would be higher and body adiposity would be reduced in mice kept in a LC. We also postulated that aerobic and anaerobic running capacities would be elevated in mice living in LC. Because of greater energy demand, we expected to find a pronounced depletion of glycogen stores in different tissues of mice kept in LC when compared to mice kept in SC.

2. Material and methods

2.1. Animal care

All animal procedures and protocols were approved by the Committee for Ethical Use of Animals (CEUA). Twenty 5-month old mice (C57BL/6J) were utilized, having an initial body weight of approximately 28 g. During the experimental period, the mice were kept in a room with a controlled environment, including the temperature (23 ± 1 °C), relative humidity (45–55%), noise (< 80 dB) and a photoperiod with a 12:00 h light/dark cycle (illumination from 6.00 am to 6.00 pm).

2.2. Study design

After weaning, twenty male mice were kept in small cages until reaching 150 days-old. At that age animals were randomly allocated into two types of housing space conditions: small cage (SC, $n = 10$) and large cage (LC, n = 10). More details on housing conditions are provided at the following Material and Methods sections. SPA as well as food and water intake were recorded daily. Mice had critical velocity (CV) and anaerobic running capacity (ARC) evaluated at the beginning, in the middle (4th week) and at the end of experiment (8th week). The CV and ARC were regarded respectively as aerobic and anaerobic running capacities. At the end of experiment, animals were euthanized for the determination of body composition (visceral fat and organs, as well as the water, fat mass and fat-free mass of carcass) and tissue glycogen stores.

2.3. Housing conditions

In SC, mice were kept (ten animals per cage, floor area of 1320 cm^2) in polyethylene cages (SC dimensions: length: 40 cm, width: 33 cm and height: 16 cm). For this study, a special cage was built, called by us as large cage (LC), in which animals were kept in groups of 10 (LC dimensions: length: 80 cm, width: 60 cm, height: 33.3 cm, floor area of 4800 cm^2). All cages were lined with autoclave-sterilized sawdust that was replaced four times a week. New mice ("intruders") were not placed in cages with already-established social groups.

2.4. Measurement of SPA

SPA was measured gravimetrically according to Biesiadecki et al. (1999). The gravimetric method was previously validated in terms of linearity, precision and repeatability (Biesiadecki et al., 1999). Moreover, this method has been used successfully on many studies involving animal models (Beck et al., 2016; Chausse et al., 2014; Moes and Holden, 2014; Scariot et al., 2016). The principle of method is that activities produced by mice induce a force on the platform that can be registered (by load cells) as a change in weight. We build two apparatus, which operates as a top balance, to measure SPA of animals in their respective habitat. In each housing condition (SC and LC), we used three load cells of similar design (MKPW®, MK Controle e Instrumentação, BR). The full scale of the load cells is 2 kg and the nominal sensibility is 2 mV/V. The three load cells were coupled in a triangular configuration to detect forces variations on the metallic platform, as drawn in supplementary figure. The following instruments were used for amplifying (MKTC5-10®, MK Controle e Instrumentação, BR) and conditioning the signals (NI-USB 6008®, National Instruments, USA; SC-2345-SCC®, National Instruments, USA). The signals were captured at a frequency of 200 Hz using an acquisition software (Labview Signal Express®, National Instruments, USA). SPA recordings were performed for 19 continuous hours (12 h of dark period and 7 h of light period). The SPA recording was started at 11:00 am and continued until 6:00 pm. Mice were treated (cage cleaning) between 6:00 and 10:00 am; this period was also reserved for recording food/water intake as well as body mass. During SPA recordings, the mice were collectively housed (10 mice *per* cage). Our choice to preserve social housing instead of providing a cage for each animal was because chronic social isolation may induce anxiety and aggressivity in social rodents (Goldsmith et al., 1978; Parker and Morinan, 1986; Weiss et al., 2004). Moreover, there are reports demonstrating that chronic social isolation alters metabolic parameters (Moore, 1968; Sharp et al., 2002; Sun et al., 2014). We obtained three regression equations since our apparatus was supported by three load cells. The SPA was obtained from signals of the three load cells, individually calibrated. The gravimetric system was calibrated by positioning known weights on top of each load cell. Regression equations ($R^2 \geq 0.99$) were then computed enabling conversions of volts signals to grams (g) units. After acquisition, the signals were treated using Matlab ® software. The raw signals were filtered using a 4-order butterworth filter with a cutoff frequency of 5 Hz. The cutoff value adopted for the low-pass filter application was used for eliminating electrical noise. The SPA was calculated according to the mathematical method proposed originally by Biesiadecki et al. (1999). First, signal variations were obtained by the difference values between consecutive samples (CS). Signals variations values were squared, and the square root was taken for transforming all variations to absolute values (module value). Absolute values need to be calculated because downward movements register positive values whereas upward movement register negative values (Biesiadecki et al., 1999). Converting all changes to absolute values allow summation of all weight changes throughout time, as is described in the equation below.

$$SPA = \sum_{i=1}^{n} \sqrt{(Cs_{i+1} - Cs_i)^2}$$

Finally, the SPA value was divided by the sum of the body mass (grams) of all animals in the cage. For example, the SPA values were divided by the mass of all mice of cage 300 g (taken ten mice weighting 30 g, for example). We calculated SPA for each hour of the day (Fig. 2). SPA values from 11 am to 6 pm (lamps on) were used to calculate the average for the light period while SPA values from 6 pm to 6 am (lamps off) for the dark period.

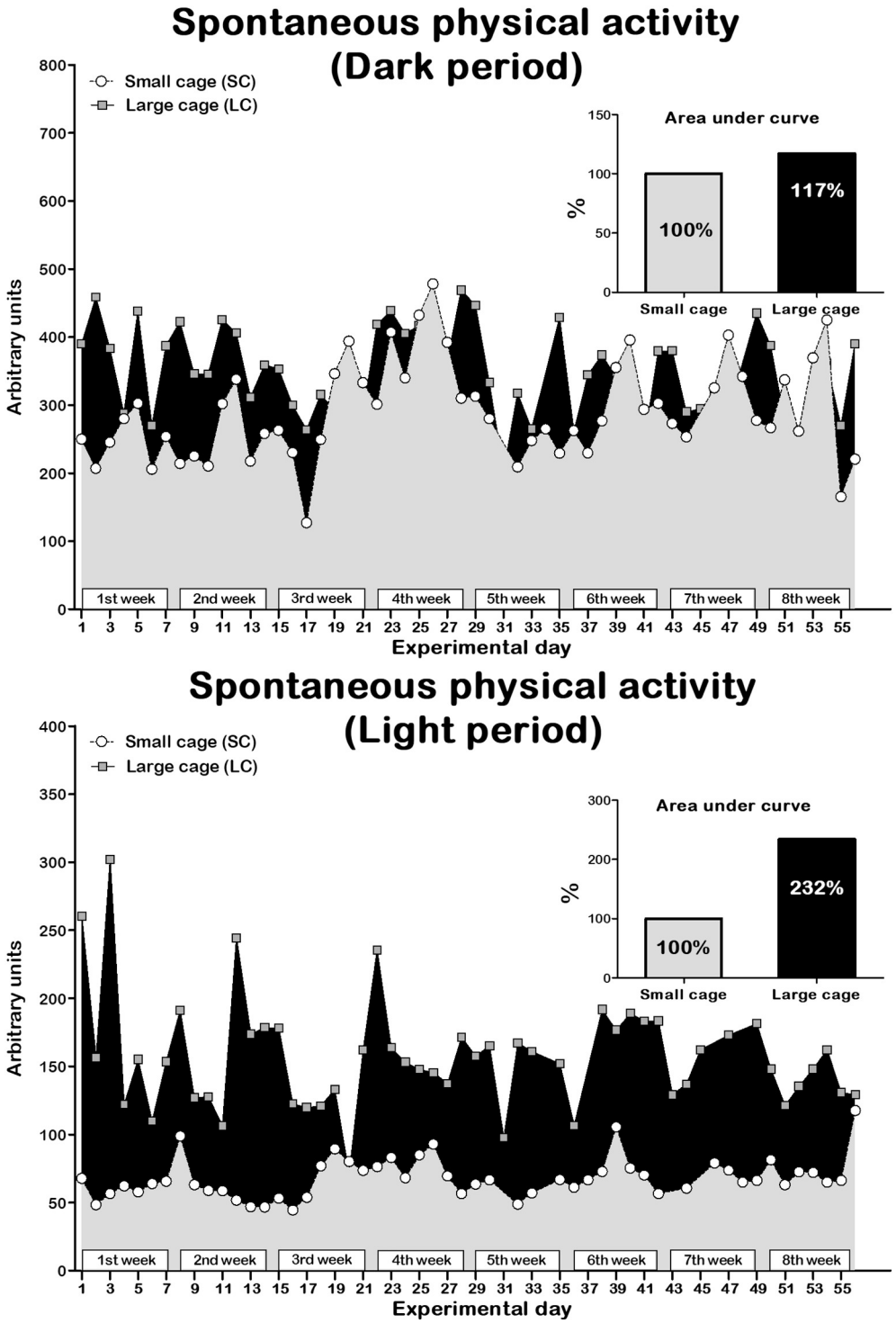

Fig. 1. SPA in arbitrary units at dark and light periods for the groups housed in SC or LC over 8 weeks. The lateral graphs represent the area under the curve (over the entire 8-wk period) where the SC group has been set at 100%.

2.5. Determination of food and water intake

Food intake was measured by weighing the amount of daily food left after 24 h and subtracting it from the initial amount of food. In a similar way, the water intake was obtained by subtracting the water remaining at the end of 24 h from the initial amount in the water bottle. Food and water intake were measured on a per cage basis. Food (g) and water (ml) intake were normalized by the body mass of all mice of the cage (i.e. food consumed in grams was divided by 300 g, if considering ten mice weighting 30 g). Thus, we calculated the amount of food consumed *per* gram of body mass. For instance, 274.3 g in body mass of mice ate 38.7 g of food, therefore 1 g of mice would have eaten 0.141 g of food. Mice were weighed five times per week. The mice received commercial standard chow (Nuvilab® CR-1; Nuvital, BR). The food and water were accessible ad libitum to prevent competition and disturbances of social relationships. To better understand whether food intake was adjusted in response to changes in SPA, we calculated a ratio between the food intake and daily SPA. To understand whether water

Table 1
SPA recorded over 8 weeks for SC and LC mice housed at the density of ten animals per cage.

	SPA (Dark)			SPA (Light)		
	Small cage	Large cage	Stat. analysis	Small cage	Large cage	Stat. analysis
1st week (n = 7)	249.3 ± 13.4	373.4 ± 26.7	$P = 0.586$ Cohen's d = 2.34	60.4 ± 2.5	179.9 ± 27.4*	$P < 0.001$ Cohen's d = 3.02
2nd week ($n = 7$)	252.3 ± 18.9	373.6 ± 16.7	$P = 0.630$ Cohen's d = 2.57	60.7 ± 6.8	164.1 ± 17.9*	P = 0.001 Cohen's d = 3.16
3rd week (n = 7)	277.6 ± 33.5	277.1 ± 26.8	P = 1.000 Cohen's d = 0.01	67.4 ± 6.4	129.6 ± 13.0	$P = 0.464$ Cohen's d = 2.43
4th week (n = 7)	380.1 ± 24.8	407.0 ± 16.8	$P = 1.000$ Cohen's d = 0.49	76.0 ± 4.6	164.9 ± 12.5*	$P = 0.020$ Cohen's d = 3.91
5th week ($n = 6$)	257.5 ± 15.1	332.0 ± 38.3	$P = 0.996$ Cohen's d = 1.14	60.6 ± 3.4	150.0 ± 10.7	$P = 0.087$ Cohen's d = 5.28
6th week (n = 7)	302.3 ± 21.3	329.5 ± 20.6	$P = 1.000$ Cohen's d = 0.49	72.7 ± 6.0	171.8 ± 13.2*	$P = 0.007$ Cohen's d = 4.11
7th week (n = 6)	312.4 ± 21.0	333.6 ± 24.6	P = 1.000 Cohen's d = 0.37	68.9 ± 3.3	156.5 ± 10.1	$P = 0.158$ Cohen's d = 5.84
8th week (n = 7)	292.3 ± 34.0	311.0 ± 25.0	P = 1.000 Cohen's d = 0.24	76.9 ± 7.2	139.3 ± 5.3	$P = 0.458$ Cohen's d = 3.77

Data for SPA at dark and light period are in the mean and standard error of the mean. SPA data are in arbitrary units (AU). Statistical analysis: Scheffe post hoc and effect size (Cohen's d) for pair-wise comparisons reveals the differences between groups within the same week.

* Significant difference ($P < 0.05$) in relation to the SC group.

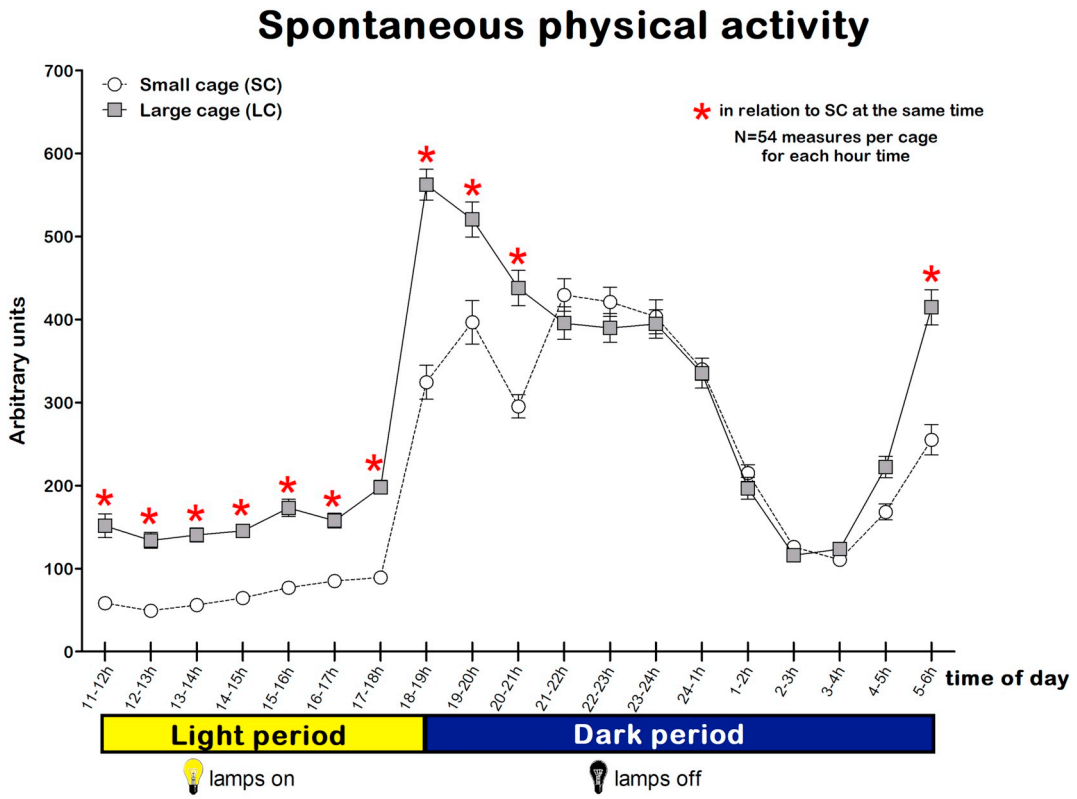

Fig. 2. SPA at each hour from 11 am to 6 am (12 h of dark period and 7 h of light period). This analysis considered all data obtained over the entire 8-wk period ($N = 54$ for each hour time). *Significant difference ($P < 0.05$) in relation to the SC within the same hour-time interval.

intake was adjusted in response to changes in daily physical activity, the ratio between daily water intake and daily SPA was calculated.

2.6. Critical velocity protocol

The critical velocity (CV) and anaerobic running capacity (ARC) were determined by a CV protocol which has been previously used in animal models of exercise (Billat et al., 2005; Manchado-Gobatto et al., 2010). This protocol is based on mathematical analysis from the relationship between exercise intensity and time to achieve the exhaustion, as previously reported in several studies (Gaesser et al., 1995; Hill et al., 2011; Jones et al., 2010; Monod and Scherrer, 1965). Based on the mathematical principle of the model, CV may be interpreted as the intensity of exercise which could be maintained for "a very long time without fatigue", or even "maintainable almost indefinitely" (Jones et al., 2010). It has been proposed that CV demarcates the lower bound of the severe intensity exercise domain, standing slightly above the anaerobic threshold intensity (Gaesser and Poole, 1996; Jones et al., 2010). Animals were submitted to four exhaustive efforts of running in a calibrated treadmill. It is important to

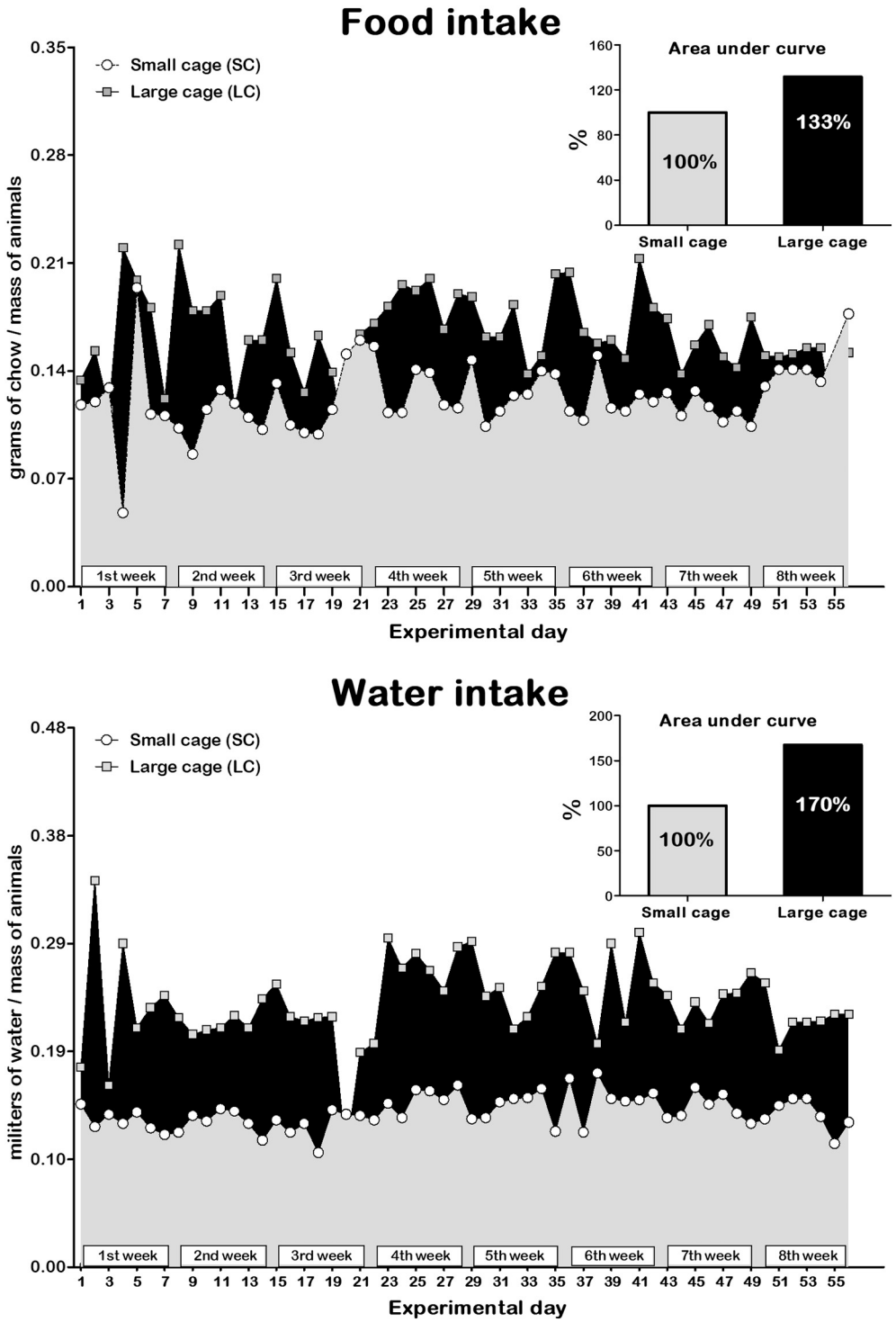

Fig. 3. Food intake ($g \cdot g^{-1}$) and water intake ($ml \cdot g^{-1}$) for the groups confined in SC or LC. The lateral graphs represent the area under the curve (over the entire 8-wk period) where the SC group has been set at 100%.

note that before the beginning of the actual CV protocol, mice were adapted to the treadmill exercise for three days. This adaptation consisted of runs performed at velocity ranging 5–10 $m \cdot min^{-1}$ and with duration of 1–5 min. All tests were conducted at the same time of day (between 6 am to 10 am). Tests were performed within a week in random order. In each test, mice ran on a treadmill at constant speed. Before the test, each mouse performed a warm-up exercise at 8 $m \cdot min^{-1}$ for 2 min. Exercise intensities ranging 10 to 30 $m \cdot min^{-1}$ were individually selected so that the time to was not more than 15 min and not less than 1 min, as previously recommended (Manchado-Gobatto et al., 2010). The time to exhaustion (TE) was recorded in seconds and determined when the mice were unable to run properly on the treadmill, despite encouragement given by the researcher. Electrical stimulus was not used in this experiment. The mice were encouraged to run by gentle tapping using a soft brush. Each test ended with a cool-down walk (1 min). Distance traveled (D) was obtained from the velocity and time to exhaustion data. Distance and time to exhaustion data obtained from the four tests were plotted in a linear regression graph. The CV

Table 2
Food intake recorded over the course of 8 weeks for SC and LC mice housed at the density of ten animals per cage. Raw (g) and normalized ($g \cdot g^{-1}$) amount of food consumed by mice in the cage.

		Small cage	Large cage	Stat. analysis
1st week ($n = 7$)	g	34.2 ± 4.7	46.9 ± 4.2	$P = 0.742$, Cohen's d = 1.09
	$g \cdot g^{-1}$	0.119 ± 0.016	0.161 ± 0.015	$P = 0.797$, Cohen's d = 1.01
2nd week ($n = 7$)	g	31.5 ± 1.5	51.6 ± 3.7*	$P = 0.039$, Cohen's d = 2.90
	$g \cdot g^{-1}$	0.109 ± 0.005	0.172 ± 0.012	$P = 0.100$, Cohen's d = 2.74
3rd week ($n = 7$)	g	35.5 ± 2.6	44.7 ± 4.0	$P = 0.980$, Cohen's d = 1.06
	$g \cdot g^{-1}$	0.123 ± 0.009	0.147 ± 0.013	$P = 0.998$, Cohen's d = 0.80
4th week ($n = 7$)	g	35.9 ± 1.8	55.7 ± 1.5*	$P = 0.047$, Cohen's d = 4.54
	$g \cdot g^{-1}$	0.128 ± 0.006	0.185 ± 0.005	$P = 0.223$, Cohen's d = 3.86
5th week ($n = 7$)	g	36.2 ± 1.6	51.2 ± 2.6	$P = 0.446$, Cohen's d = 2.65
	$g \cdot g^{-1}$	0.127 ± 0.006	0.169 ± 0.009	$P = 0.788$, Cohen's d = 2.20
6th week ($n = 7$)	g	34.8 ± 1.5	53.8 ± 2.8	$P = 0.075$, Cohen's d = 3.42
	$g \cdot g^{-1}$	0.121 ± 0.005	0.176 ± 0.009	$P = 0.312$, Cohen's d = 2.83
7th week ($n = 7$)	g	33.9 ± 0.9	50.7 ± 1.9	$P = 0.223$, Cohen's d = 4.56
	$g \cdot g^{-1}$	0.115 ± 0.003	0.158 ± 0.006	$P = 0.765$, Cohen's d = 3.52
8th week ($n = 7$)	g	41.2 ± 2.1	47.0 ± 2.2	$P = 0.999$, Cohen's d = 1.02
	$g \cdot g^{-1}$	0.144 ± 0.006	0.145 ± 0.007	$P = 1.000$, Cohen's d = 0.07

Data are in the mean and standard error of the mean. Food intake was normalized by the mass of animals in the cage. Statistical analysis: Scheffe post hoc and effect size (Cohen's d) for pair-wise comparisons reveals the differences between groups within the same week.
* Significant difference ($P < 0.05$) in relation to the SC group.

Table 3
Water intake recorded over the course of 8 weeks for the SC and LC housed at the density of ten animals per cage. Raw (ml) and normalized ($ml \cdot g^{-1}$) amount of water ingested by mice in the cage.

		Small cage	Large cage	Stat. Analysis
1st week (n = 7)	ml	37.5 ± 1.0	69.2 ± 6.8*	$P < 0.001$, Cohen's d = 3.09
	$ml \cdot g^{-1}$	0.131 ± 0.004	0.237 ± 0.024*	$P < 0.001$, Cohen's d = 2.94
2nd week ($n = 7$)	ml	37.4 ± 1.1	65.3 ± 1.1*	$P < 0.001$, Cohen's d = 9.45
	$ml \cdot g^{-1}$	0.129 ± 0.004	0.218 ± 0.004*	$P = 0.003$, Cohen's d = 8.46
3rd week (n = 7)	ml	36.7 ± 1.3	61.9 ± 5.6*	$P < 0.001$, Cohen's d = 2.77
	$ml \cdot g^{-1}$	0.127 ± 0.005	0.204 ± 0.019*	$P = 0.031$, Cohen's d = 2.47
4th week (n = 7)	ml	41.5 ± 1.2	78.7 ± 3.6*	$P < 0.001$, Cohen's d = 5.76
	$ml \cdot g^{-1}$	0.148 ± 0.005	0.262 ± 0.012*	$P < 0.001$, Cohen's d = 5.18
5th week (n = 7)	ml	40.3 ± 1.4	75.2 ± 3.0*	$P < 0.001$, Cohen's d = 5.92
	$ml \cdot g^{-1}$	0.142 ± 0.005	0.249 ± 0.011*	$P < 0.001$, Cohen's d = 5.17
6th week (n = 7)	ml	43.6 ± 1.8	77.9 ± 3.9*	$P < 0.001$, Cohen's d = 4.55
	$ml \cdot g^{-1}$	0.152 ± 0.006	0.255 ± 0.014*	$P < 0.001$, Cohen's d = 3.81
7th week (n = 7)	ml	41.7 ± 1.3	76.0 ± 2.2*	$P < 0.001$, Cohen's d = 7.40
	$ml \cdot g^{-1}$	0.142 ± 0.004	0.237 ± 0.006*	$P < 0.001$, Cohen's d = 6.57
8th week (n = 7)	ml	38.8 ± 1.1	71.7 ± 2.2*	$P < 0.001$, Cohen's d = 7.65
	$ml \cdot g^{-1}$	0.136 ± 0.005	0.222 ± 0.007*	$P = 0.005$, Cohen's d = 5.42

Data are in the mean and standard error of the mean. Water intake was normalized by the mass of animals in the cage. Statistical analysis: Scheffe post hoc and effect size (Cohen's d) for pair-wise comparisons reveals the differences between groups within the same week.
* Significant difference ($P < 0.05$) in relation to the small cage group.

and ARC were calculated, respectively, from the slope (angular coefficient) and the intercept (linear coefficient) of the regression line, according to the equation $D = CV * TE + ARC$. CV and ARC were regarded respectively as aerobic and anaerobic running capacities. Coefficient of determination (R^2) was calculated as indices of the goodness of fit of the linear regression.

2.7. Collection of biological material

Animals were euthanized at rest to verify chronic adaptations. All mice (SC and LC groups) were fasted overnight (12 h of fasting), and euthanasia was performed by cervical dislocation. Euthanasia was performed 48 h after the CV protocol to avoid any acute effect of exercise on experimental variables. Blood was collected and centrifuged to obtain serum for the determination of glucose and triglycerides. Euthanasia began at 6:30 am (30 min after lights on). Tissues collected include liver, heart and kidney, as well as different muscles such as vastus lateralis, triceps brachii, gluteus and tibialis anterior. The epididymal and retroperitoneal visceral fat were removed and weighted. All visceral organs were removed. The carcass of animals was processed as described in the following section.

2.8. Characterization of body composition

Visceral organs mass was calculated as the difference between body mass and eviscerated carcass. The eviscerated carcass was weighed before and after dehydration at a temperature of 100 degrees centigrade. The dehydrated carcass was crushed in the presence of organic solvent (benzene), and again dehydrated at a temperature of 100 degrees centigrade. Water and fat mass were obtained from the weight differences after dehydration and fat removal. The fat-free mass was defined as the remaining amount of carcass. The results were expressed as a percentage of the eviscerated carcass.

2.9. Biochemical measurements

Immediately following dissection, portions of tissues weighing around 150 mg were digested in potassium hydroxide (KOH) at a concentration of 30%. Sodium sulfate (Na_2SO_4) and ethanol [70%] were added for the precipitation of glycogen. After centrifugation, the

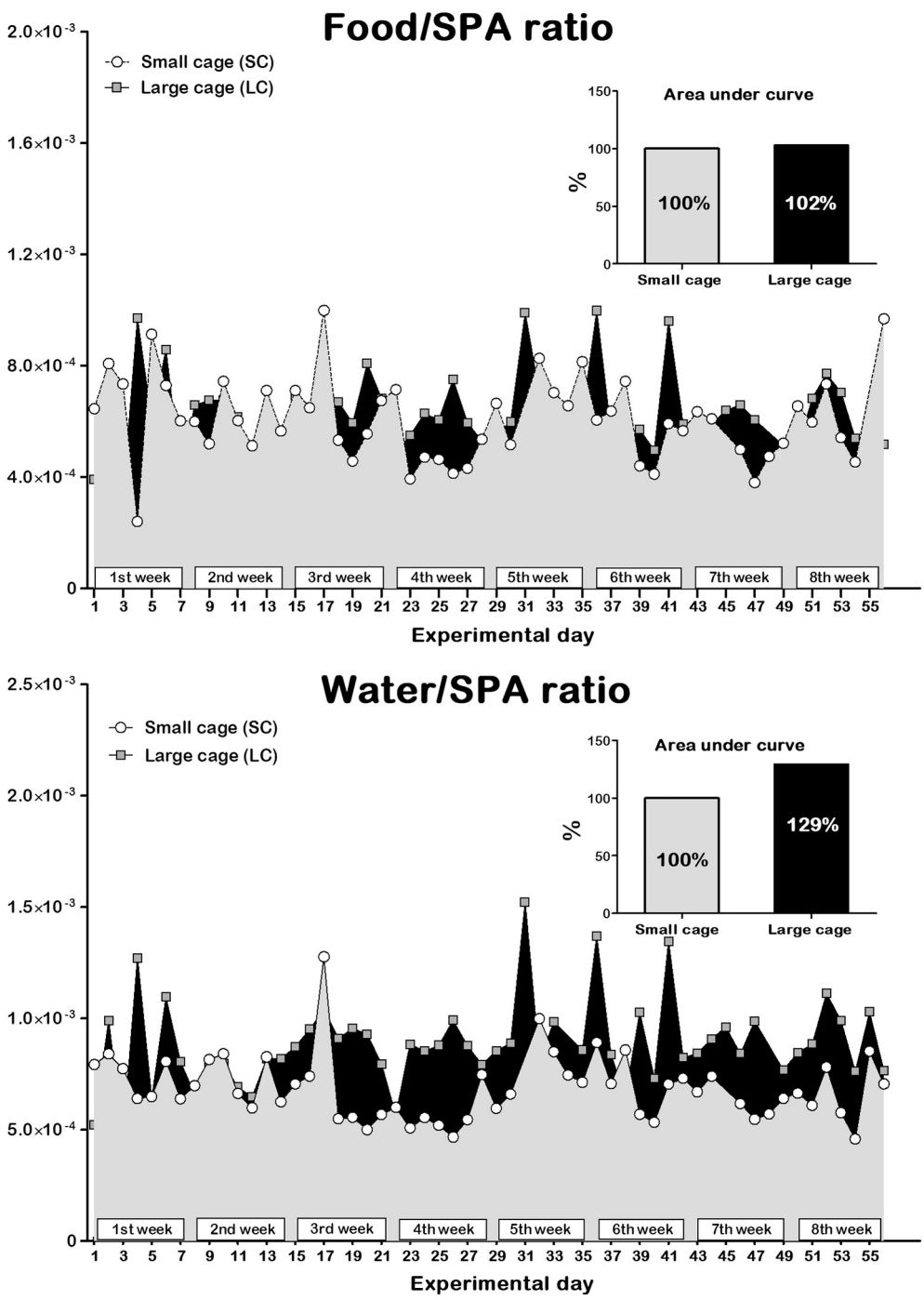

Fig. 4. Ratio between the daily food intake and daily SPA (Food/SPA ratio). This figure also shows the ratio between daily water intake and daily SPA (Water/SPA ratio). Ratios were obtained from measurements carried out on samples collected over the entire 8-wk period for the groups confined in a small cage or a large cage. The lateral graphs represent the area under the curve where the SC group has been set at 100%.

precipitate was dissolved in distilled water. The glycogen concentration was measured by a colorimetric reaction using phenol (C_6H_5OH) and sulfuric acid (H_2SO_4) (Dubois et al., 1956). The colored reaction product was quantified spectrophotometrically at 492 nm. A standard curve was constructed from glucose solutions of known concentration. The concentration of triglycerides in serum was determined using commercial kits for enzymatic colorimetric methods (Laborlab ®, BR). Serum glucose was determined by the glucose oxidase method using a commercial kit (Laborlab ®, BR).

2.10. Statistical procedures

All data are presented in means and standard error of the mean (SEM). Data normality was checked using the Shapiro-Wilk test. Measurements taken multiple times over eight weeks, such as SPA, body mass, food and water intake were analyzed by two-way ANOVA to determine the housing (SC vs LC) and time (eight weeks) effects. Scheffe post-hoc test was chosen. Another two-way ANOVA was run to determine the differences in SPA at each hour between different housing conditions (SC vs. LC) and time of day (from 11 am to 6 am). This analysis (showed in the Fig. 2) is statistically very powerful

Table 4
Weekly average of the ratio between the daily food intake and SPA (Food/SPA ratio). The table also shows the daily water intake-SPA ratio (Water/SPA ratio).

	Food/SPA ratio			Water/SPA ratio		
	Small cage	Large cage	Stat. analysis	Small cage	Large cage	Stat. analysis
1st week	0.67 ± 0.08	0.57 ± 0.10	P = 0.999 Cohen's d = 0.42	0.73 ± 0.03	0.83 ± 0.12	P = 1.000 Cohen's d = 0.46
2nd week	0.61 ± 0.03	0.59 ± 0.05	P = 1.000 Cohen's d = 0.18	0.72 ± 0.04	0.74 ± 0.03	*P* = 1.000 Cohen's d = 0.22
3rd week	0.65 ± 0.07	0.67 ± 0.03	P = 1.000 Cohen's d = 0.13	0.70 ± 0.10	0.92 ± 0.03	*P* = 0.977 Cohen's d = 1.29
4th week	0.49 ± 0.04	0.59 ± 0.03	P = 0.999 Cohen's d = 1.06	0.56 ± 0.03	0.83 ± 0.05	*P* = 0.874 Cohen's d = 2.43
5th week	0.70 ± 0.05	0.68 ± 0.07	*P* = 1.000 Cohen's d = 0.13	0.76 ± 0.06	0.99 ± 0.11	*P* = 0.987 Cohen's d = 1.09
6th week	0.57 ± 0.04	0.67 ± 0.08	*P* = 0.999 Cohen's d = 0.61	0.71 ± 0.05	0.97 ± 0.11	*P* = 0.922 Cohen's d = 1.21
7th week	0.52 ± 0.04	0.60 ± 0.02	P = 1.000 Cohen's d = 1.15	0.63 ± 0.03	0.88 ± 0.03	*P* = 0.962 Cohen's d = 3.31
8th week	0.66 ± 0.07	0.60 ± 0.04	P = 1.000 Cohen's d = 0.40	0.66 ± 0.05	0.91 ± 0.05	*P* = 0.937 Cohen's d = 1.86

Data are in the mean and standard error of the mean (n = 6–7 per week) over the course of 8 weeks for the groups confined in a small cage or a large cage. The ratio values were multiplied by 1000 for better visualization of the values. Daily SPA was correspondent to the values taken during the light and dark period. Statistical analysis: Scheffe post hoc and Cohen's d analysis reveals the differences between groups within the same week.*Significant difference ($P < 0.05$) in relation to the SC group.

Table 5
Critical velocity (CV), anaerobic running capacity (ARC) and coefficients of determination (R^2) for SC and LC groups at different times.

	Baseline		4th week		8th week	
	SC	LC	SC	LC	SC	LC
CV ($m \cdot min^{-1}$)	14.0 ± 1.1	13.9 ± 0.5	14.9 ± 0.6	18.0 ± 0.3*	17.1 ± 0.5	18.8 ± 0.6*
ARC (m)	6.3 ± 1.7	6.9 ± 1.7	7.3 ± 1.8	5.4 ± 1.3	5.6 ± 1.3	6.0 ± 0.6
R^2	0.98 ± 0.004	0.99 ± 0.002	0.98 ± 0.01	0.99 ± 0.001	0.99 ± 0.005	0.99 ± 0.002

Data are in the mean and standard error of the mean (n = 9–10).
* $P < 0.05$ compared to SC group within the same experimental time.

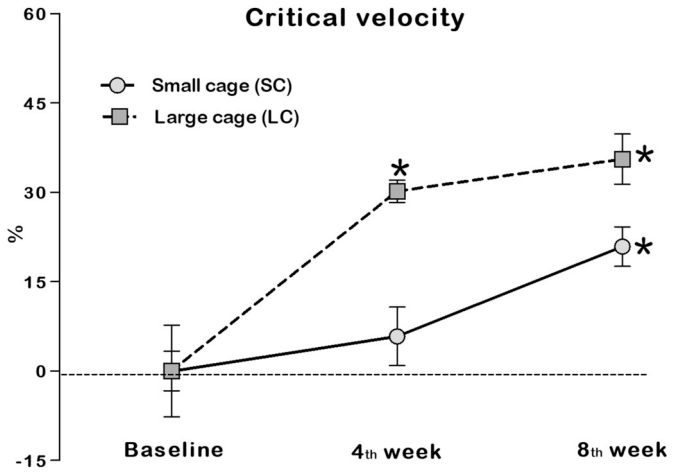

Fig. 5. Critical velocity for SC and LC changes during housing period. The data (mean ± SEM) are relative (%) to baseline evaluation, which has been set at 0%. ANOVA for repeated measures was carried out to determine intragroup differences over time. *Significant difference ($P < 0.05$) in relation to the baseline.

because SC and LC groups are compared by considering all SPA data obtained over the entire period of observation (a total of 54 measures per cage). Regarding CV, ARC and R^2 variables, an independent t-test was employed to compare the differences between groups within the same experimental time, and also, ANOVA for repeated measures was carried out to determine intragroup differences over time. Independent t-test was also used to compare the differences between groups for the variables measured at the end of experiment. Product-moment correlation (Pearson) was used to determine the association between variables food intake and daily SPA values per cage. This correlation was made using daily values of food intake and SPA (all mice housed in group) obtained over the entire period of 8 weeks. The significance level was set at $P < 0.05$ in all cases. The effect size (Cohen's d) for pair-wise comparisons was determined by the formula: (mean of SC group – mean of LC group)/pooled standard deviation. Cohen's d values of 0.2, 0.5, and 0.8 describe small, moderate, and large effects, respectively. Additionally, eta squared (η^2) was calculated for each ANOVA, providing an estimate of the magnitude of the effect of treatment (Cohen, 1973; Pierce et al., 2004). The following interpretation of eta squared was used: 0.01 = small effect, 0.06 = moderate effect and 0.14 = large effect. Eta squared was determined by dividing the sum of squares for each effect (SS_{effect}) by the total sum of squares (SS_{total}), as outlined in the equation below.

$$\eta^2 = \frac{SS_{effect}}{SS_{total}}$$

We applied the trapezoidal method to determine the total area under the curve (AUC) from time-course measurements obtained over the entire 8-wk period (i.e. SPA, food and water intake). AUC was obtained by calculating the area of small segments (rectangles and triangles) between two designated values on the on the x and y axis. The total sum of these individual areas thus represents the AUC. Calculations were made following the mathematical formulation proposed by Tai (1994):

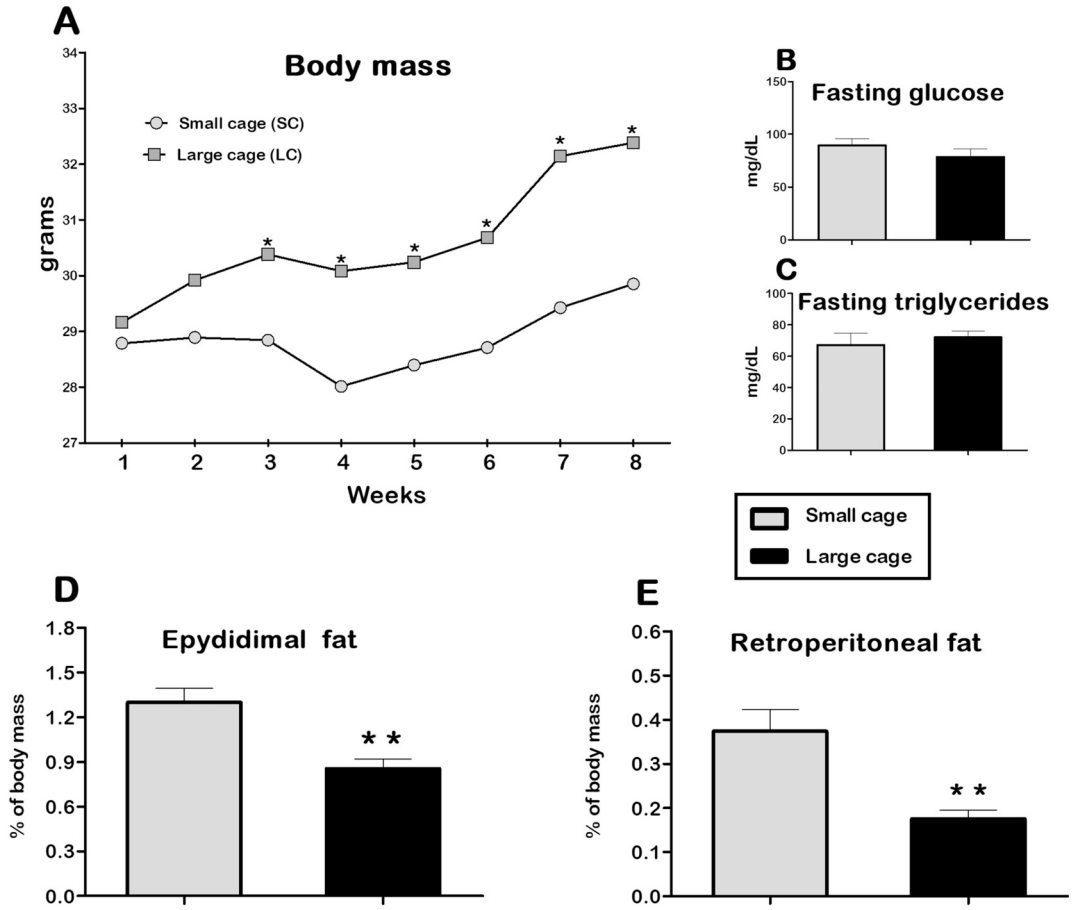

Fig. 6. Weekly average of body mass over the course of 8 wk. (Panel A). Fasting glucose (Panel B) and triglycerides (Panel C) at the end of experiment. The epididymal (Panel D) and retroperitoneal (Panel E) fat mass relative to body mass (%).The bars and lines in the graphs represent the mean and standard error of the mean for SC and LC groups. *P < 0.05 vs. small cage (SC) group, **P < 0.01 vs. SC group, ***P < 0.001 vs. SC group.

Table 6
Visceral organs mass, eviscerated carcass, water, fat mass and fat-free mass of carcass for SC and LC groups at the end of 8 week housing period.

		Small cage	Large cage	Stat. analysis
Visceral organs mass	g	8.3 ± 0.3	11.5 ± 0.3*	t = −7.2, P < 0.001, Cohen's d = 3.26
	%	29.4 ± 0.7	35.6 ± 0.5*	t = −7.2, P < 0.001, Cohen's d = 3.30
Eviscerated carcass	g	19.9 ± 0.4	20.7 ± 0.4	t = −1.3, P = 0.205, Cohen's d = 0.59
Water carcass	g	13.2 ± 0.3	14.2 ± 0.3*	t = −2.1, P = 0.048, Cohen's d = 0.95
	%	66.1 ± 0.5	68.5 ± 0.4*	t = −3.7, P = 0.001, Cohen's d = 1.70
Fat mass	g	1.66 ± 0.1	1.31 ± 0.1*	t = 3.5, P = 0.002, Cohen's d = 1.62
	%	8.4 ± 0.4	6.3 ± 0.3*	t = 4.2, P < 0.001, Cohen's d = 1.96
Free-fat mass	g	5.07 ± 0.1	5.22 ± 0.1	t = −1.0, P = 0.311, Cohen's d = 0.47
	%	25.5 ± 0.2	25.2 ± 0.2	t = 0.9, P = 0.367, Cohen's d = 0.42

Visceral organs mass is expressed as percentage of total body mass. Water carcass, fat mass and free-fat mass are express in absolute (g) or relative to eviscerated carcass (%). Data in mean and standard error of the mean (n = 10). Statistical analysis: t-test and effect size (Cohen's d) for pair-wise comparisons reveals the differences between groups.

* Significant difference (P < 0.05) in relation to the SC group.

$$AUC = \frac{1}{2} \sum_{i=1}^{n} \Delta x \, (y_{i-1} + y_i)$$

3. Results

3.1. LC-mice exhibit higher SPA over 8 weeks

Fig. 1 shows the daily records of SPA (at dark and light periods) of SC and LC-mice. Table 1 shows weekly average of SPA. Post hoc analysis did not detect significant differences in SPA (during the dark period) between groups within the same week (Table 1), however, SPA at dark period was higher for the LC-group than SC-group when considering the entire 8-wk period (based on ANOVA analysis). ANOVA showed a significant effect of housing on SPA at dark ($F_{1,\ 92}$ = 17.5, P < 0.001, η^2 = 0.11). A significant effect of housing on SPA between groups (SC and LC) at the light period ($F_{1,\ 88}$ = 224.2, P < 0.001, η^2 = 0.68) was found. In line with these statistical findings, the area under the curve of SPA (over the entire 8-wk period) was higher for the LC-group than SC-group (Fig. 1). These findings indicate that mice kept in LC exhibit higher SPA (at dark and light period) than mice kept in SC, when considering the entire 8-wk period. Interestingly, ANOVA detected an effect of time on SPA at the dark period ($F_{7,\ 92}$ = 4.0, P < 0.001, η^2 = 0.18), but post hoc did not detect any significant

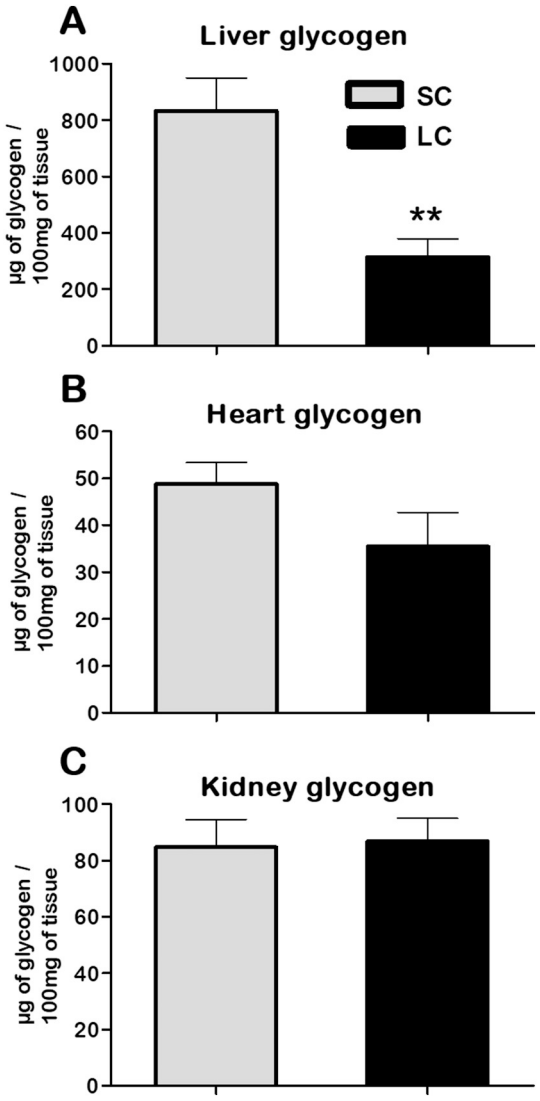

Fig. 7. Glycogen concentrations in the liver (Panel A), heart (Panel B) and kidney (Panel C) for SC and LC groups following an overnight fast. Mean and standard error of the mean (n = 8–10 per group) are shown. *P < 0.05 vs. small cage (SC) group, **P < 0.01 vs. SC group, ***P < 0.001 vs. SC group.

differences between weeks. No significant effect of time on SPA at the light period was found ($F_{7, 88} = 1.0$, $P = 0.419$, $\eta^2 = 0.01$). Finally, ANOVA did not show significant interaction between housing and time on SPA at dark period ($F_{7, 92} = 2.0$, $P = 0.062$, $\eta^2 = 0.09$) and light period ($F_{7, 88} = 1.4$, $P = 0.186$, $\eta^2 = 0.03$). The difference in SPA over the whole day was mainly due to light phase activity, but also due to activity at dark period, as demonstrated in Fig. 2.

3.2. Large cage increases food and water intake over 8 weeks

Food and water daily intake is shown in Fig. 3, whereas weekly average can be visualized in Tables 2 and 3. We found a significant effect of housing on normalized food intake ($F_{1, 95} = 77.7$, $P < 0.001$, $\eta^2 = 0.39$) and absolute food intake ($F_{1, 95} = 118.6$, $P < 0.001$, $\eta^2 = 0.51$). For normalized food intake, post hoc analysis did not detect any significant differences between groups, but moderate to large effect sizes were seen (Table 2). A significant effect of housing on normalized water intake was found ($F_{1, 96} = 364.5$, $P < 0.001$, $\eta^2 = 0.74$). In addition, an effect of housing on absolute water intake was observed ($F_{1, 96} = 479.7$, $P < 0.001$, $\eta^2 = 0.78$). The area under the curve of food and water intake (over the entire 8-wk period) was higher for group LC than group SC (Fig. 3). These findings show that mice kept in LC exhibit higher food and water consumption than mice kept in SC, when considering the entire 8-wk period. ANOVA did not detected an effect of time on normalized food intake ($F_{7, 95} = 1.2$, $P = 0.301$, $\eta^2 = 0.04$) and absolute food intake ($F_{7, 95} = 1.0$, $P = 0.380$, $\eta^2 = 0.03$), demonstrating that consumption of food was constant over the course of 8 wk. We found a significant effect of time on normalized water intake ($F_{7, 96} = 3.7$, $P < 0.001$, $\eta^2 = 0.05$) and absolute water intake ($F_{7, 96} = 4.0$, $P < 0.001$, $\eta^2 = 0.04$). ANOVA detected an interaction between housing and time on normalized food ingestion ($F_{7, 95} = 2.2$, $P = 0.036$, $\eta^2 = 0.08$). No interaction between housing and time on absolute food ingestion ($F_{7, 95} = 1.8$, $P = 0.095$, $\eta^2 = 0.05$). Finally, ANOVA did not show a significant interaction between housing and time on normalized water intake ($F_{7, 96} = 0.7$, $P = 0.621$, $\eta^2 = 0.01$) and on absolute water intake ($F_{7, 96} = 0.8$, $P = 0.510$, $\eta^2 = 0.01$). We found a significant relationship (tested by Pearson Correlation) between food intake and daily SPA values ($r = 0.50$, $P < 0.001$), suggesting that cages in which mice ingested a high amount of food also were those showing the highest SPA values.

Fig. 4 shows the ratio between the daily food intake and daily SPA. Fig. 4 also shows the ratio between daily water intake and daily SPA. Table 4 illustrates the weekly average of these ratios. ANOVA detected an effect of housing on water/SPA ratio ($F_{1, 92} = 36.2$, $P < 0.001$, $\eta^2 = 0.25$), but no effect on food/SPA ratio ($F_{1, 91} = 0.2$, $P = 0.642$, $\eta^2 = 0.002$). Post hoc did not detect any significant differences between weeks. No significant effect of time on food/SPA ratio ($F_{7, 91} = 1.4$, $P = 0.208$, $\eta^2 = 0.09$) and water/SPA ratio ($F_{7, 92} = 1.4$, $P = 0.192$, $\eta^2 = 0.07$) was detected by ANOVA. Finally, ANOVA did not show a significant interaction between housing and time on food/SPA ratio ($F_{7, 91} = 0.8$, $P = 0.513$, $\eta^2 = 0.06$) and water/SPA ratio ($F_{7, 92} = 1.0$, $P = 0.435$, $\eta^2 = 0.05$).

3.3. LC housing endows increased aerobic capacity

Table 5 expresses the critical velocity (CV) and anaerobic running capacity (ARC) as well as R^2 (obtained from the linear regression between distance and time to exhaustion data) for SC and LC groups at three different time points (baseline, 4 and 8 week). The LC-group showed significantly higher critical velocity than SC-group at the fourth week ($t = -4.6$, $P < 0.001$, Cohen's d = 2.25) and eighth week ($t = -2.1$, $P = 0.042$, Cohen's d = 0.98). No significant difference was found between groups at the baseline condition ($t = 0.0$, $P = 0.926$, Cohen's d = 0.04). Linear regressions resulted in R^2 around 0.99. This reveals a very high consistency in the determination of CV and ARC. We observed no significant differences in ARC values between groups (SC vs LC) at baseline ($t = -0.2$, $P = 0.805$, Cohen's d = 0.12), at fourth week ($t = 0.8$, $P = 0.399$, Cohen's d = 0.39) and at eighth week ($t = -0.2$, $P = 0.780$, Cohen's d = 0.14). No significant differences in R^2 values between groups (SC vs LC) at baseline ($t = -1.1$, $P = 0.246$, Cohen's d = 0.56), at fourth week ($t = -1.6$, $P = 0.109$, Cohen's d = 0.91) and at eighth week ($t = -0.1$, $P = 0.850$, Cohen's d = 0.09) were detected. With regard to intragroup differences over time (Fig. 5), we found CV increased from baseline to 4 weeks in the LC group, but not in the SC group. CV increased from baseline to 8 weeks for both groups (SC vs LC) (Fig. 5).

3.4. LC-mice have less visceral and carcass fat mass

Compared to SC-mice, LC mice exhibited reduced epydidimal fat ($t = -3.4$, $P = 0.002$, Cohen's d = 1.58) and retroperitoneal fat ($t = 3.5$, $P = 0.002$, Cohen's d = 1.74) (Fig. 6). No differences were found between SC and LC groups with regard the fasting serum glucose ($t = 1.0$, $P = 0.296$, Cohen's d = 0.53) and triglyceride ($t = -0.6$, $P = 0.534$, Cohen's d = 0.32). The LC-group showed increased body mass from the third week to the end of experiment (Fig. 6). Table 6 shows body composition parameters. No statistical difference was

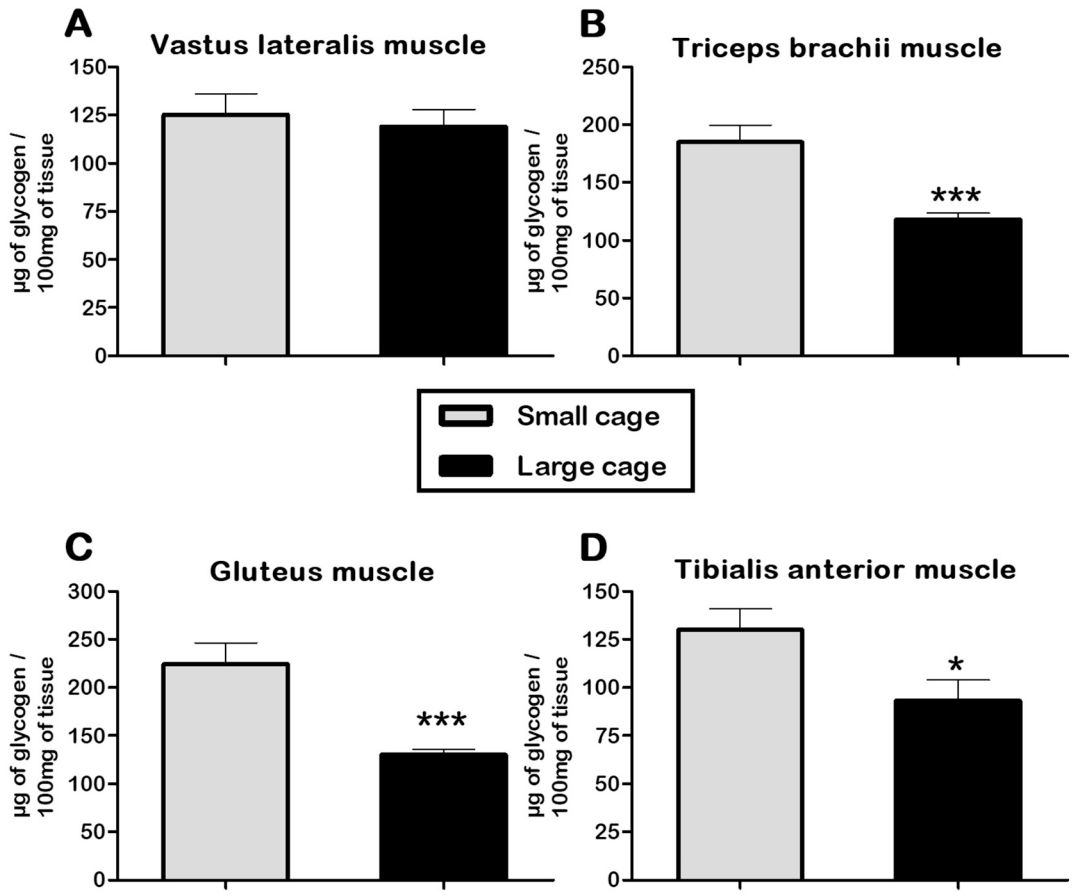

Fig. 8. Glycogen concentrations (µg/100 mg of tissue) in different skeletal muscle groups such as vastus lateralis (Panel A), triceps brachii (Panel B), gluteus (Panel C) and tibialis anterior (Panel D) for SC and LC groups. Mean and standard error of the mean (n = 8–10 per group) are shown. *P < 0.05 vs. small cage (SC) group, **P < 0.01 vs. SC group, ***P < 0.001 vs. SC group.

found in the eviscerated carcass mass between groups. Interestingly, LC-mice showed greater water carcass than SC-mice. In contrast to the water carcass, we found a lower amount of fat in the carcass of the LC group as compared to the SC group.

3.5. LC impacts tissue glycogen levels

Animals kept in LC exhibited lower overnight fasting liver glycogen than SC-mice ($t = 3.7$, P = 0.001, Cohen's d = 1.82) (Fig. 7). Regarding heart ($t = 1.6$, $P = 0.123$, Cohen's d = 0.77) and kidney fasting glycogen ($t = -0.1$, $P = 0.875$, Cohen's d = 0.07), we found no statistical differences between groups (Fig. 7). As shown in Fig. 8, we found significant differences between SC and LC groups for muscle glycogen concentrations in some of muscles such as triceps brachii ($t = 4.3$, P < 0.001, Cohen's d = 2.23), gluteus ($t = 4.1$, P < 0.001, Cohen's d = 2.16) and tibialis anterior ($t = 2.3$, $P = 0.028$, Cohen's d = 1.10). We found no statistical differences between groups regarding glycogen content in the vastus lateralis muscle ($t = 0.4$, $P = 0.667$, Cohen's d = 0.20).

4. Discussion

The most significant finding of the present investigation is that eight weeks of confinement in a LC increases SPA of inbred mice. In agreement with our hypothesis, eight weeks of confinement in a LC amplified feeding behavior and reduced fat depots of inbred mice. LC also modulated aerobic capacity, but not anaerobic capacity. In addition, fasting mice kept in LC exhibited a depletion of glycogen in the liver and in three out of four skeletal muscles studied.

The findings are very consistent in showing that mice kept in LC exhibit higher SPA than mice kept in SC, when considering the entire 8-wk period (Fig. 1). In addition, the difference in SPA over the whole day was mainly due to light phase activity, but also due to activity at dark period. Despite non-significant differences in each week (as tested by post hoc analysis), ANOVA showed a significant effect of housing on SPA at the dark period. This analysis is statistically very powerful because SC and LC groups are compared by considering all values obtained in the entire period of 8 weeks. LC-mice were more active than SC-mice only in some hours of the dark period (from 6 pm to 9 pm, and from 5 am to 6 am) and during all hours of light period (from 11 am to 6 pm). Fig. 2 also demonstrates a time-of-day variation in SPA. It is well-established that SPA exhibits circadian rhythmicity (Ikeda et al., 2000). Why did LC-mice become more active? We believe that LC elicits more locomotion opportunities to rodents than SC. A plausible explanation is that from a behavioral perspective, LC-mice were "re-wilded". As wildlife requires alertness, which is essential for mate and food searching, foraging, and for avoiding predation and competition, it is reasonable to consider that LC increased SPA by increasing time spent awake. Awake time may increase energy demand, explaining why the LC-mice ingested a higher amount of food than SC-mice (Fig. 3). An important question that remains is by which neurobiological mechanism LC can increase time spent moving. A plausible explanation is that orexigenic pathways may have been triggered in mice kept in LC. This may also explain why SPA of LC-mice was increased in the light period, which was the most intriguing finding of the present study. Supporting this hypothesis, it has been shown that orexin-A increases awake time of rodents, and this effect occurs irrespective of whether the orexin-A is administered at light or dark time points (Kotz et al., 2002;

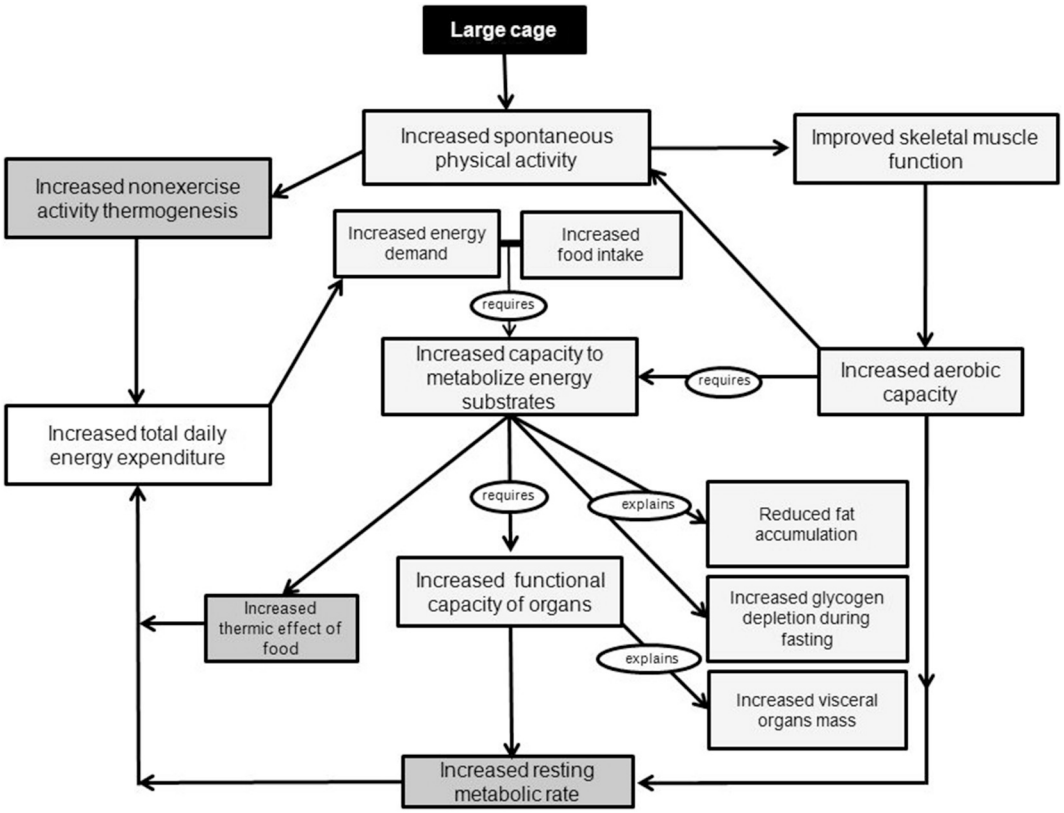

Fig. 9. Scheme of main causal links leading to an increased total daily energy expenditure and its physiological consequences. This scheme was developed based on findings from this study and based on theoretical considerations reported by Koteja (2000) and Hayes and Garland (1995).

Piper et al., 2000). Further, it has been consistently found that elevated orexin-A signaling is observed in rodents having high SPA (Novak et al., 2006; Perez-Leighton et al., 2013; Perez-Leighton et al., 2012).

Since increased food intake could be due to increased energy demand required by SPA, we assessed the ratio between food intake and SPA, as described in Fig. 4 and Table 4. Interestingly, no difference was found between SC and LC groups with regard the food/SPA ratio. This result suggests that food intake is adjusted in response to changes in SPA, and that food intake and SPA are tightly balanced. Taken together, these observations clearly demonstrate the complexity of the hypothalamus in regulating daily physical activity and feeding behavior. In the future, additional experiments will be necessary to understand how LC modulates the activity of brain areas involved in the control of energy balance. Behavioral measures such as SPA and food intake are critical to increase the understanding of multiple physiological processes, and should be more widely appreciated by physiologists. With regard to the data on water intake, we hypothesize that a higher SPA in LC-mice could increase the metabolic heat production in the muscle tissue, leading to an increase in the rate of water loss, specially while foraging, like suggested by Levy et al. (2016). In line with this reasoning, Gavini et al. (2014) found elevated skeletal muscle energy (heat) dissipation in rodents selected as high-capacity runners (HCR), which are more physically active than their low-capacity runner (LCR) counterparts. We believe that the ratio values reported in Table 4 could be useful to better understand whether water intake was adjusted in response to changes in SPA. Contrary to our reasoning, however, the physical activity-adjusted values of water intake remain different between the groups (SC and LC). This result suggests that an increased SPA does not explain (at least not fully) the changes in water intake. This supports the possibility that other factors beyond SPA are responsible for the increased water intake in mice living in a LC. As LC-mice need to eat larger amounts of food, it is probable that they had a higher need for drinking water. Nonetheless, other pathways mediating the thermoregulatory responses (i.e. changes in brown adipose tissue) need be more thoroughly explored in future studies.

An important observation is that LC-mice exhibited a lean phenotype (i.e. low body adiposity) despite ingesting a higher amount of food. This is in line with work of Perez-Leighton et al. (2013), who showed that rats having intrinsically higher SPA remained lean despite high-fat feeding. Further, our data indicates that leanness cannot be explained just by reduced food intake. This is not entirely surprising, as it was already noted almost two decades ago by Levin (1991) who found that body mass gain resulted not only from hyperphagia but also from hypoactivity. Our findings are also in agreement with those of Novak et al. (2009).

Since leanness of LC-mice can't be attributed to a reduction in food consumption, it is likely that LC-mice experienced an overall negative energy status. It is obvious that LC-mice had an elevated non-exercise activity thermogenesis (NEAT), which is the energy expenditure attributable to SPA (Castaneda et al., 2005). A reduction of fat depots is a morphological change strongly associated with greater energy expenditure (Thompson et al., 2012). Given that the biochemical machinery necessary to sustain a high SPA is metabolically costly, it is very plausible that active animals have an improved ability for metabolizing energy substrates. This increased energy flux may play a role in preserving health by combating metabolic diseases associated with sedentary behavior. Our data also fits well with previous theories on the relationships between locomotor activity and energy expenditure (Hayes and Garland, 1995; Koteja, 2000). Some of these ideas were summarized in Fig. 9. Apart from NEAT, it is likely that resting metabolic rate (RMR, the largest component of daily energy expenditure) may have been altered in LC-mice. Our prediction is based on the fact that active animals need improved functional capacity of visceral organs, which are responsible for a greater proportion of the RMR (Koteja, 2000; Selman et al., 2001). As LC-mice need to eat more in order to satisfy their energy requirements, a higher energy expenditure in

visceral organs (e.g. intestine, liver, kidneys and heart) is also expected. This agrees with our results showing a higher weight of visceral organs in LC-mice (Table 6).

Interestingly, an increase of aerobic capacity (Table 5) also supports an enhancement in RMR (Almeras et al., 1991; Koteja, 2000; Poehlman et al., 1992). We found that LC in the absence of exercise training, endows increased aerobic running capacity. These observations are unprecedented and provide evidence that an increase of SPA is sufficient to elicit aerobic adaptations. We believe that an enhanced aerobic running capacity of the LC-mice can be explained by an improvement in skeletal muscle function. This is in agreement with other studies showing that rats selectively bred for high aerobic running capacity exhibit greater SPA than their low-endurance counterparts selectively bred as low-capacity runners (Gavini et al., 2014; Novak et al., 2010). Furthermore, it is important to note that some activities included within SPA may generate a high amount of muscle contractions, and for this reason, it is not surprising that an improvement of SPA may be able to enhance muscle performance. Based on our data, we suggest that LC may be useful for improving health status in mice. This suggestion is based on the observation low aerobic capacity and SPA are linked with various metabolic diseases (Gavini et al., 2014; Koch and Britton, 2018).

Another component of this study was the measurement of glycogen stores. As the harvest of tissues was performed in fasting mice in the evening that preceded euthanasia, it was possible to analyze whether energy-storage mobilization is dependent of the housing condition. Interestingly, we found lower fasting glycogen content in the liver of LC-mice than SC-mice (Fig. 7), but with no differences in fasting serum glucose (Fig. 6). The liver plays a very important role in glucose homeostasis. Given that blood glucose comes mainly from the breakdown of liver glycogen (Wilmore and Costill, 2001), we suggest that LC-mice exhibit higher hepatic glucose production than SC-mice. An increased locomotion for finding food sources can explain the higher energy needs of LC-mice. Our findings also suggest that glycogen in kidney cells was not required to maintain blood glucose concentration during fasting, at least for 12 h. Although the kidney does not have the largest amount of glycogen, there is evidence showing that content of glycogen in this tissue may contribute to the homeostasis of blood glucose (Froesch et al., 1958; Reinecke, 1943). Curiously, some studies have found an accumulation of glycogen in the kidney of diabetic animals (Froesch et al., 1958; Kang et al., 2005), suggesting that changes in kidney glycogen can be seen in severe metabolic perturbations.

From an evolutionary point of view, muscle glycogen serves as an energy fuel in fight or flight situations (Hargreaves, 2015; Jensen et al., 2011; Mul et al., 2015). In this regard, cardiac glycogen is a potential source of myocardial energy only in situations of oxygen deficiency, such as ischemia or hypoxia (Soraya et al., 2016). This may explain why we did not observe substantial changes in heart glycogen. Because it was hypothesized that anaerobic energy metabolism would increase in mice kept in LC, we were especially interested in analyzing whether glycogen-storage mobilization is increased in mice kept in LC. It is well know that glycogen stored in skeletal muscle provides energy during high-intensity physical activities (Hargreaves, 2015). It should be noted that LC-mice performed activities such as chasing, foraging, jumping, climbing and perching. Most of the force produced during anaerobic physical activities is due to the activity of fast glycolytic fibers, with a minimal contribution from slow oxidative fibers. Type IIx and IIb fibers have a high glycolytic activity, and exhibit a high contractile velocity and force generation (Chikani and Ho, 2014). Muscles dissected were the vastus lateralis, triceps brachii, gluteus and tibialis anterior muscle. In addition to these muscles being responsible for key movements of quadruped mammals, they were chosen because they are known to contain high percentage of type IIx and IIb fibers, classified as fast-twitch glycolytic fibers according to their content of myosin heavy chain (MyHC) (Eng et al., 2008). Thus, considerable utilization of glycogen reserves should be expected in muscles containing these fiber types. LC-mice exhibited a depletion of glycogen stores in three out of four skeletal muscles studied, in agreement with our prediction (Fig. 8). We found no statistical differences between groups regarding glycogen content in the vastus lateralis muscle, which coincidentally has a lower percentage of MyHC IIB fibers (64%) in comparison to gluteus (75%) and tibialis anterior (69%) (Eng et al., 2008). Contradicting our hypothesis, LC did not cause significant alterations in ARC, which have been assumed as the total amount of work that can be produced from anaerobic energy metabolism. This result is not surprising if we consider that this construct still is not well understood (Bosquet et al., 2007; Zagatto and Gobatto, 2012). It has been suggested that ARC is the total amount of energy which can be resulted from depletion of anaerobic stores (Billat et al., 2000). In practical terms, ARC is finite quantity of distance (meters) that can be covered above the CV by using energy derived from anaerobic glycolysis, phosphates, and stored oxygen within the muscle fibers (Jones and Whipp, 2002).

The current study provides evidence for the robustness of the gravimetric principle in evaluating SPA. The gravimetric principle can account for major movements associated with displacement, but also more sensitive movements such as grooming, rearing and fidgeting. Further, activities can be measured by this apparatus without the need for removing animals from their home cage. This enables researchers to measure SPA of rodents in their natural settings without disturbing their behavior. Thus, the gravimetric principle is very accurate in measuring natural behaviors (i.e. “pure” SPA). The gravimetric method could help researchers in drawing more accurate and consistent conclusions about SPA. Moreover, our experiment shows that the system can be used for recordings over long periods of time. In this regard, the SPA was recorded using six load cells with an acquisition frequency of 200 Hz during 19 continuous hours (per day). Over the entire experimental period, it becomes possible to record approximately 4.6×10^9 sample points. This large amount of SPA data is unusual in the field of physiology research.

Our findings suggest that SPA, which is “intrinsically” controlled, may be increased by modifying the environment (external stimuli). This agrees with human studies showing that altering environment at school or office can be associated with increased SPA (Ben-Ner et al., 2014; John et al., 2011; Koepp et al., 2017; Koepp et al., 2016). These findings have led to increased interest in strategies (modifying the obesogenic environment) to help sedentary individuals move more. There is an urgent need for finding natural approaches that can be applied to increase SPA in the prevention of metabolic diseases in humans. Understanding the role of SPA is becoming increasingly important in biological and biomedical research (Levine et al., 1999). In this regard, we believe that rodents may serve as a useful model in elucidating the mechanisms by which SPA is modulated by housing and environmental conditions. With the growing obesity epidemic, therapeutic approaches for increasing SPA have been proposed (Levine et al., 1999; Teske et al., 2008). In this regard, our data suggests that LC can be employed as an approach for stimulating SPA in rodents. This effect, to our knowledge, has never been achieved without genetic or pharmacological manipulations. The present results are obtained with an inbred strain of mice (C57BL6/J), and therefore other strains or species of rodents must be tested, before generalizing our findings. Our study supports other reports indicating that laboratory rodents kept in small cage are in a sedentary, obesogenic environment (Martin et al., 2010; Scariot et al., 2015) and provides an accessible solution to study and overcome this problem.

5. Conclusion

In conclusion, we have demonstrated that eight weeks of confinement in a large cage increases SPA in collectively housed mice. Moreover, inbred mice kept in a large cage exhibited high aerobic running capacity and were protected against fat accumulation despite increased food intake. In light of present study, the results are not

sufficient to demonstrate a causal effect of SPA on any morphological or physical characteristics. Rather, the data suggest that high SPA in mice housed in a large cage induces changes in adiposity, aerobic fitness and other metabolic traits.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.yhbeh.2019.07.004.

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