

# Exercising for Life? Energy Metabolism, Body Composition, and Longevity in Mice Exercising at Different Intensities

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## ABSTRACT

Studies that have found a positive influence of moderate, non-exhaustive exercise on life expectancy contradict the rate-of-living theory, which predicts that high energy expenditure in exercising animals should shorten life. We investigated effects of exercise on energy metabolism and life span in male mice from lines that had been selectively bred for high voluntary wheel-running activity and from the nonselected control lines. Mice were divided into the following three groups ( $n = 100$  per group): active high-runner mice (housed with wheels; HR+), sedentary high-runner mice (no wheels provided; HR-), and active control mice (C+). Sixty animals from each group were left undisturbed throughout their lives to create survival curves. In the remaining 40 animals in each group, energy metabolism and body composition was measured at 2, 10, 18, or 26 mo of age. Wheel-running activity was increased by ~50% throughout life in HR+ mice compared with C+ mice, and mass-specific daily energy expenditure was increased by ~30% in HR+ mice compared with both C+ mice and HR- mice. Median life span was similar in HR+ mice and HR- mice (740 and 733 d, respectively), and it was significantly shorter in these mice than it was in C+ mice (828 d). Thus, increasing the amount of voluntary aerobic exercise (as a result of selective breeding or housing with wheels) did not result in extended life span in mice, and we found no evidence for a direct link between energy expenditure and life span.

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## Introduction

Moderate, nonexhaustive physical activity is thought to have a positive influence on health and life expectancy in both laboratory rodents (Holloszy 1988; Navarro et al. 2004) and human beings (Lee and Skerrett 2001; Warburton et al. 2006). Beneficial effects of exercise include lowered body adiposity due, in part, to increased energy expenditure, and a reduced risk of developing the metabolic syndrome and associated diseases (e.g., diabetes mellitus, cardiovascular disease, hypertension, etc.; for review, see Carroll and Dudfield 2004; Warburton et al. 2006). Beneficial effects of exercise have been shown experimentally in mice and rats by providing them with access to running wheels (Goodrick 1980; Holloszy et al. 1985; Holloszy 1993) or by exercising them on treadmills (Navarro et al. 2004). Rats with wheel access show an increase in median (but not maximum) life span of approximately 10% compared with their sedentary counterparts (Goodrick 1980; Holloszy et al. 1985).

Beneficial effects of exercise contrast with expectations from the rate-of-living theory that proposes an inverse relationship between energy expenditure and life span (Rubner 1908; Pearl 1928). In principle, this relationship might come about by the production of free radicals (or reactive oxygen species [ROS]), which occurs during aerobic metabolism in mitochondria and that can cause oxidative damage to macromolecules in cells, thereby contributing to senescence (the free-radical theory of aging (Harman 1956; Beckman and Ames 1998)). In agreement with these ideas, several intraspecific studies have shown a reduction in life span in exercising animals; for instance, honeybees that were made to carry extra loads while foraging had reduced life spans (Wolf and Schmid-Hempel 1989), as did kestrels that had increased workloads when caring for enlarged families (Daan et al. 1996). In humans, professional athletes have shorter life expectancies than the general population (Samaras et al. 2002), and a recent study found a negative relationship between basal metabolic rate and life span (Ruggiero et al. 2008). In contrast, other intraspecific studies of rodents, such as those where animals were exposed to cold or exercise (Holloszy and Smith 1986; Holloszy 1988; Navarro et al. 2004; Selman et al. 2008b; Vaanholt et al. 2009) or where the relationship of individual variation in metabolic rate to life span was examined (Speakman et al. 2004), have failed to find a negative relationship between energy expenditure and life span. In most of these studies, however, no effort was made to measure the energy expenditure of the experimental groups

over their life spans in order to obtain accurate estimates of lifetime energy potential (LEP; kJ life<sup>-1</sup>; Vaanholt et al. 2009), nor have changes in body composition during life been taken into account (Greenberg 1999).

The lack of a consistent relationship between energy expenditure and life span in previous studies may be explained by the fact that ROS are not produced as a fixed proportion of total oxygen consumed, as was previously thought (Chance et al. 1979). Rather, the number of ROS produced per oxygen molecule consumed depends on many factors, including the state of respiration (state 3 vs. state 4) and the amount of mitochondrial uncoupling that occurs (Brand 2000; Barja 2007). In addition, other important factors including the levels of endogenously produced antioxidants (e.g., superoxide dismutase, catalase), exogenous antioxidants (e.g., vitamin C, beta-carotenoids), and repair mechanisms can help to protect the body against oxidative stress (Beckman and Ames 1998).

It is well known that the amount of ROS increases in muscles during exercise, and although these radicals can have deleterious effects on cells, growing evidence indicates that ROS produced during exercise have a physiological role in the adaptation to exercise (Navarro et al. 2004; Boveris and Navarro 2008; Gomez-Cabrera et al. 2008). In this respect, low levels of ROS that occur in muscles during moderate exercise will stimulate the expression of defense systems, including antioxidant enzymes (Selman et al. 2002; Lambertucci et al. 2007; Gomez-Cabrera et al. 2008). The majority of evidence in human beings suggests that mortality risk decreases with increasing levels of moderate physical activity in both men and woman (Lee and Skerrett 2001; Warburton et al. 2006), although to our knowledge this has never been experimentally tested (i.e., all existing data are strictly observational).

To investigate the effects of different intensities of lifelong voluntary aerobic exercise on energy expenditure and life span, we made use of strains of mice that had been selectively bred for high early-age voluntary wheel-running activity (HR mice; see Swallow et al. 1998; 2009). From generation 16 onward, at the age of 6–8 wk mice from the four replicate HR lines ran, on average, 2.5–3.0 times as much as did mice from the four nonselected control lines (Li et al. 2004; Rhodes et al. 2005). We have previously reported on measurements of protein synthesis rates and antioxidant enzyme activities at various ages throughout life in these lines of mice (Vaanholt et al. 2008*b*). Here we report on metabolic rate, body composition, and longevity. We studied the relationships between energy expenditure, metabolism, exercise status, and life span in high-runner mice housed with running wheels (HR+), and we compared these mice with their counterparts that were housed without a running wheel (sedentary; HR-) and with mice from the nonselected control lines that were housed with wheels (C+).

## Material and Methods

### *Animals and Housing*

Male mice (*Mus domesticus*) that had been selectively bred for high voluntary wheel-running activity over 30 generations and

nonselected controls were used in these experiments. For a detailed description of the selection procedure, see Swallow et al. (1998). In short, outbred Hsd:ICR mice (Harlan Sprague Dawley, Indianapolis, IN) were randomly divided into eight separate populations with 10 pairs per line. Four lines were then selectively bred for increased wheel-running activity at 6–8 wk of age and four were bred without regard to how much they ran, thus serving as control lines. At generation 30, mice from the four selected lines ran approximately 2.7 times more revolutions per day than did control mice at 6–8 wk of age (Li et al. 2004; Rhodes et al. 2005). At generation 30, 80 breeding pairs (10 per line) were shipped from the lab of T.G. to the Netherlands and were bred at the Zoological Laboratory in Haren. Male mice from the first generation of offspring, which were born between July 31, 2002, and January 27, 2003, were used in the experiments described below. After weaning at 21 d of age, mice were housed with their littermates (two to four mice per cage) until the age of 5 mo; then, animals were individually housed with or without a running wheel for the rest of their lives (Macrolon Type II cage adapted to fit a plastic running wheel with a diameter of 14.5 cm). The following three experimental groups of 100 mice each were created: (1) control mice housed with a running wheel (C+), (2) high-runner mice housed with running wheels (HR+), and (3) high-runner mice housed without wheels (HR-). Animals had ad lib. food (Standard Rodent Chow RMB-H [2181], Hope Farms, Woerden, the Netherlands) and water access, and they were housed at 22° ± 1°C on a 12L:12D cycle (lights turned on at 8:00 GMT+1). Body mass of all animals was measured once a month and cages were cleaned every 2 wk. All procedures concerning animal care and experimental treatment were performed in accordance with the protocol approved by the University of Groningen's ethical committee for the use of experimental animals (license DEC 2777).

### *Experimental Procedures*

The experimental protocol applied in this study is similar to the procedures described in Vaanholt et al. (2009), a study on the effects of cold exposure on energy metabolism and life span. Each experimental group of 100 mice was randomly split into two subgroups of 60 and 40 individuals. The subset of 60 animals (life span cohort) was left undisturbed throughout life (except for during cage cleaning and monthly weighing); time of spontaneous death was noted to construct survival curves. Of the other 40 animals (test cohort; see below), we used eight mice at each of four ages (2, 10, 18, and 26 mo) to measure food intake and metabolic rate and then sacrificing them to collect tissues and determine body composition. At the later ages, after some mortality had occurred, sample sizes were smaller than eight in some of the groups (see samples sizes in Table 3). Data on hormone levels, antioxidant enzyme activities, and protein synthesis rates for various tissues have previously been published for mice from this test cohort (Vaanholt et al. 2007*b*, 2008*b*).

### *Life Span Cohort: Body Mass, Wheel-Running Activity, and Life Span*

Body mass of all animals was measured once a month on the same day. Because of variation in birth date, not all animals were the same age at time of measurement; therefore, for statistical analyses, body mass data were sorted into 30-d blocks, starting at 15 d of age (e.g., body mass measured in mice aged 15–44 d old was averaged to get the average age at 1 mo, 45–74-d-old mice constituted the 2-mo-old group, etc.).

Individual wheel-running activity was not measured continuously. Rather, it was sampled across the life spans of mice from the C+ and the HR+ groups by using an event-recording system (ERS). The ERS logged the number of wheel revolutions in 2-min bins. Because of limitations of the experimental set up, not all mice could be measured simultaneously (maximum of 64 measurement channels for 120 animals housed with wheels). Mice were thus rotated on the channels and were measured for ~30 consecutive days every other month. Nearer the end of the experiment, when mortality had occurred, the remaining animals could be measured continuously. Mean wheel-running activity (distance run;  $\text{km d}^{-1}$ ) was determined for all animals and averaged over 2-mo blocks for statistical analysis. In addition, time spent running, average running speed, and maximum running speed were determined over a period of 2 wk in a random sample of 6-mo-old mice ( $n = 32$ : 16 C+, 16 HR+). Time spent running was obtained by deducting the number of 2-min intervals in which no running occurred from 24 h. Average running speed was determined by dividing the total distance run ( $\text{km d}^{-1}$ ) by the time spent running ( $\text{h d}^{-1}$ ), and maximum wheel-running activity ( $\text{km h}^{-1}$ ) was calculated by determining the maximal amount run in a 2-min interval. At one point during the protocol, food intake was measured for all the animals in the life span cohort that were alive at that time (mean age  $\pm$  SD =  $668 \pm 70$  d) by determining the amount of food remaining in the hopper after 7 d (food orts in the bedding were not measured; see Koteja et al. 2003).

Mice were checked every day, and date of spontaneous death was noted. Most mice were found dead in their cage. A few ( $n = 6$ ) animals that showed clear symptoms of disease (e.g., tumors, were irresponsive) were euthanized, and the day of termination was used as their death date in survival analysis. One HR+ mouse died at 65 d of age, which is unusually young (see Bronikowski et al. 2006). However, we found no indication of a problem such as a malfunctioning water bottle, and visual inspection of the body did not show any obvious cause of death. Therefore, this individual was not excluded from analyses.

### *Test Cohort: Food Intake, Metabolic Rate, and Body Composition*

Food intake and metabolic rate were measured in animals from the test cohort at ages 2 (mean  $\pm$  SD =  $70 \pm 6$  d), 10 ( $313 \pm 7$  d), 18 ( $558 \pm 8$  d), and 26 ( $783 \pm 13$  d) mo ( $n = 6$ –8 per group at each age; see also Vaanholt et al. 2009). Food

intake ( $\text{g d}^{-1}$ ; corrected for changes in humidity) was measured over a period of 3 d by determining the amount of food missing from the hopper. Daily energy expenditure (DEE;  $\text{kJ d}^{-1}$ ) was determined in each mouse by using the doubly labeled water (DLW) technique (Speakman 1997): Mice were weighed to the nearest 0.1 g and injected with about 0.1 g DLW ( $^2\text{H}$  and  $^{18}\text{O}$  concentrations of the mixture were 37.6% and 58.7%, respectively). The exact dose was quantified by weighing the syringe to the nearest 0.0001 g before and after administration. An initial blood sample was collected after 1 h, using the tail nick procedure, and it was stored in three glass capillary tubes each filled with about 15  $\mu\text{L}$  of blood. These capillaries were immediately flame-sealed with a propane torch. Thereafter, the mouse was returned to its home cage. After 48 h, the animal was weighed again and a final blood sample was collected as described above.

Determinations of  $^2\text{H} : ^1\text{H}$  and  $^{18}\text{O} : ^{16}\text{O}$  isotope ratios of the blood samples were performed at the Groningen Centre for Isotope Research, following Visser and Schekkerman (1999), using a SIRA 10 isotope ratio mass spectrometer. For a detailed description of the procedure, see Vaanholt et al. (2009).

Three days after we performed the DLW measurements, mice were moved to a respirometry room to determine their resting metabolic rate (RMR;  $\text{kJ d}^{-1}$ ). Mice were put in flow-through cages ( $15 \times 10 \times 10$  cm), where oxygen consumption ( $\dot{V}\text{O}_2$ ;  $\text{L h}^{-1}$ ) and carbon dioxide production ( $\dot{V}\text{CO}_2$ ;  $\text{L h}^{-1}$ ) were measured simultaneously along with ambient temperature and activity (with a passive infrared [PIR] detector [Optex Wonderex FX-35] on the lid of the cage), as described previously by Vaanholt et al. (2007a). In short, eight animals were measured simultaneously with each channel sampled for 1 min every 10 min, and a reference channel was sampled every 5 min. At the end of each minute, before switching channels,  $\text{O}_2$  and  $\text{CO}_2$  concentrations of dried inlet and outlet air (dryer: molecular sieve 3 Å, Merck) were measured with a paramagnetic oxygen analyzer (Servomex Xentra 4100) and an infrared carbon dioxide gas analyzer (Servomex 1440), respectively. The system recorded the differentials in oxygen and carbon dioxide between dried reference air and dried air from the metabolic cages. Flow rate of inlet air was measured with a mass-flow controller (type 5850, Brooks). All mice were measured for a total of 24 h at an ambient temperature of 22°C while provided with ad lib. food (standard chow) and water. Body mass remained fairly stable during the respirometry measurements, with a mean change of  $-0.14 \pm 0.99$  g (mean  $\pm$  SD). Metabolic rate (MR;  $\text{kJ h}^{-1}$ ) was calculated using the following equation:  $\text{MR} = 16.18 \times \dot{V}\text{O}_2 + 5.02 \times \dot{V}\text{CO}_2$  (Hill 1972). RMR ( $\text{kJ h}^{-1}$ ) was defined as the lowest metabolic rate value calculated in half-hour running means when the animals were apparently post-absorptive (respiratory quotient,  $\sim 0.7$ ) and inactive (on the basis of PIR measurements).

After the respirometry measurements, animals were weighed and sacrificed with  $\text{CO}_2$  gas and then decapitated. Trunk blood was collected in tubes containing anticoagulant (EDTA or heparin) for later hormone analysis (results are published elsewhere [Vaanholt et al. 2007b]). Heart, liver, kidneys, brown adipose

tissue (BAT; interscapular), white adipose tissue (WAT; epididymal), intestines, stomach, lung, brain, testes, and skin (from the entire body but excluding that of the head and tail) were dissected out and weighed to the nearest 0.1 mg. Samples of heart, liver, one kidney, skeletal muscle from the hind leg (thigh), BAT, and WAT were immediately frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$  for later measurement of protein synthesis and antioxidant enzyme activities (data published elsewhere: Vaanholt et al. 2008b). The gut fill of the stomach and the intestines was removed before obtaining the fresh mass of these organs. Tissues were stored at  $-20^{\circ}\text{C}$  until the water and fat contents were determined. Water content was determined by drying for 4 h to constant mass in an oven at  $103^{\circ}\text{C}$  following International Standards Organization (ISO) protocol (ISO 6496-1983(E)). Fat was extracted with a Soxhlet apparatus and petroleum ether. Following fat extraction, samples were dried to constant mass at  $103^{\circ}\text{C}$  again. Dry lean masses of the organ samples that had been frozen (i.e., heart, liver, and kidney) were calculated using the remaining, unfrozen part of the organ, with the assumption that fat and water content of this part were representative of the whole tissue. In six healthy male mice (Hsd:ICR(CD-1) mice; Harlan, France), the water and fat contents of hind limb muscle, WAT, and BAT were determined. Water content (mean  $\pm$  SD) was  $70\% \pm 7\%$ ,  $31\% \pm 6\%$ , and  $9\% \pm 3\%$  in muscle, WAT, and BAT, respectively; fat content was  $12\% \pm 8\%$ ,  $90\% \pm 4\%$ , and  $59\% \pm 7\%$  in muscle, WAT, and BAT, respectively. For estimation of the dry lean mass of the remainder of each carcass, we calculated the dry lean mass of leg muscle, BAT, and WAT by assuming these water and fat contents.

### LEP

LEP ( $\text{kJ life}^{-1}$ ) is the product of the rate of energy expenditure and life span (Rubner 1908), and it was calculated for each group by combining data collected from animals in the life span and the test cohorts. Traditionally, LEP has been estimated on the basis of measurements of RMR and maximum life span (Colder 1985). However, as life is not spent solely in the resting state, LEP is better measured as the product of DEE and life span (Speakman et al. 2002; Speakman 2005; Vaanholt et al. 2009). RMR might be used as an estimator of DEE if the two occur in a fixed ratio, but in most animals that is obviously not the case. For example, in this study, RMR was on average 83%, 68%, or 87% of DEE in C+, HR+, and HR- mice, respectively (see "Results"). These differences call for the use of DEE to calculate LEP as a more accurate estimate of the LEP. Maximum life span represents only a single event in each group and, therefore, its estimation is subject to large variance. Using the median or mean survival of the oldest 10% yields a more reliable measure of life span (Speakman et al. 2002; Selman et al. 2008a).

Taking these considerations into account, we estimated LEP on the basis of measurements of DEE (with DLW) and the mean age at which the oldest 10% of the animals had died. LEP was estimated for each individual in the test group by

multiplying its (mass-specific) value of DEE by the life span (mean age of oldest 10%) of its group (i.e., 1,019, 1,062, and 1,060 d for C+, HR+, or HR- mice, respectively). Because different parts of the body contribute differently to energy metabolism (Greenberg 1999), LEP was then corrected for various measures of body composition by dividing by body mass, dry lean mass, or metabolically active organ mass (sum of dry lean heart, liver, kidney, and brain mass) values that we had for the test animals. Differences between groups were tested using one-way ANOVAs (see also Vaanholt et al. 2009).

### Statistical Analysis

Group means  $\pm$  SEM are presented unless stated otherwise. To test for effects of group and/or age, we applied nested ANCOVA (ANCOVA) models to the mixed procedure in SAS for Windows (ver. 9.1). In the life span cohort, body mass and running-wheel activity were measured repeatedly on the same individuals; however, repeated-measures analysis is complicated by the fact that not all mice have measurements at all ages (because of limitations of the wheel-measuring system as well as mortality). Therefore, we also applied one-way AN(C)OVA with group as a fixed factor, to different age categories. Our life span mice represented all four replicate controls and four replicate selected lines in sufficient numbers, and therefore we applied a nested design to the model, where replicate lines nested within group was added as a random effect. Contrasts were added to the model and we tested a priori for differences between C+ groups versus HR+ groups and HR+ groups versus HR- groups.

In the test animals, we applied two-way AN(C)OVA with group, age, and group  $\times$  age as fixed factors to data from mice 10, 18, and 26 mo of age. Data at 2 mo of age were analyzed separately using a one-way AN(C)OVA because at this age, only two groups were present (i.e., no mice had yet been given wheel access). Because sample sizes were relatively small, the different lines were not distributed evenly and/or not all the lines were represented in each cohort, and line was not added as a random effect in the analysis of data for the test animals. Covariates were added to the models where appropriate (e.g., body mass for measures of food intake, body composition, and metabolic rate). Data were  $\log_{10}$ -transformed or squared (see text) when necessary to attain a normal distribution of residuals.

Survival data were analyzed with Kaplan-Meier survival analysis in SPSS for Windows (ver. 14.0) by using the Breslow (Generalized Wilcoxon) test to compare survival distributions among groups (in these analyses, line effects could not be included as a random effect; see also "Results"). In addition, instantaneous mortality rates ( $\mu$ ) over intervals of 100 d were calculated; instantaneous mortality at age  $x$  (in days):  $\mu_x = \ln(1 - N_c/N_b)$ , where  $N_c$  is the number of animals left over at the end of the interval ( $x$  days), and  $N_b$  is the number of animals at the start of the interval ( $x - 100$  d; plotted in Fig. 3B). Several models of increasing mortality with age (Gompertz and Logistic) were then tested for goodness-of-fit to these data with the Winmodest program developed by Pletcher (1999): the

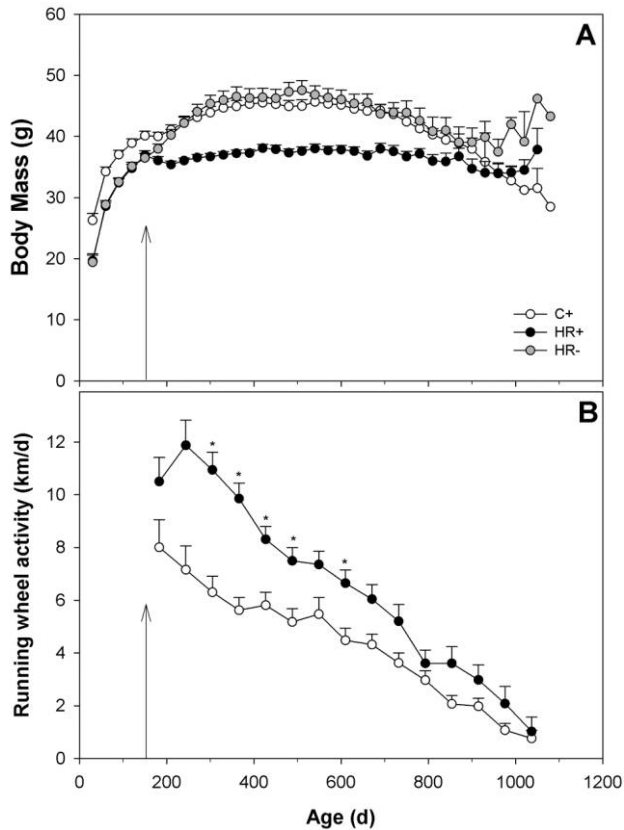


Figure 1. Development of body mass (A; 1-mo means + SE) and wheel-running activity (B; binned into 2-mo means + SE) in mice selectively bred for high wheel-running activity (HR+, black filled circles) and nonselected controls (C+, open circles) housed with wheels. Gray filled circles represent high-runner mice housed without a running wheel (HR-). An asterisk in Figure 1B denotes a significant difference between the groups at this age ( $P < 0.05$ ) on the basis of the nested (including line effects) one-way ANOVA for this age. Arrows indicate when animals first had access to running wheels.

Gompertz model ( $\mu_x = Ae^{bx}$ ) is determined by two parameters, one estimating the initial mortality rate ( $A$ ) and one that represents the rate of exponential increase with age ( $b$ ); the Logistic model has an additional parameter ( $s$ ) that represents the deceleration in the increase in mortality at the end of life ( $\mu_x = Ae^{bx}[1 - (As/b)(e^{bx} - 1)]^{-1}$ ; if  $s = 0$ , Logistic = Gompertz); and both the Gompertz and the Logistic models were combined with a constant additive term for early adulthood mortality (Makeham term,  $c$ ). Maximum log likelihoods were used to determine the model that best described our data, and the WinModest program calculated the associated probabilities. When models were fitted, a likelihood ratio test was used to test for significant variation in parameters among the groups. In the types of survival analysis described above, possible variation among the replicate lines within the HR or C line types is ignored. To investigate line effects, we therefore analyzed data on age of death using a nested ANOVA design in SAS Procedure Mixed, where line was a random effect nested within group. The significance level was set at  $P < 0.05$ , and all tests were two tailed.

## Results

### Life Span Cohort

**Development of Body Mass and Wheel-Running Activity.** We tested for group differences in body mass around the ages of 70, 300, 560, and 780 d (i.e., same ages as when measurements were taken in the test group) using one-way ANCOVA with line nested within group as a random effect and age at time of measurement (in days) as covariate, testing a priori for differences of C+ versus HR+ and HR+ versus HR-. Data on body mass (in monthly categories) were  $\log_{10}$ -transformed to attain a normal distribution. Body mass (fig. 1A) was already significantly lower, around 70 d of age in HR+ mice, compared with C+ mice ( $F_{1,8} = 9.4$ ,  $P = 0.016$ ); this difference was maintained throughout life, but it was no longer statistically significant after approximately 780 d of age ( $F_{1,8} = 1.6$ ,  $P = 0.23$ ). When HR mice were housed without a running wheel, their body masses were greater, and from approximately 300 d of age, HR- mice had significantly higher body masses when compared with HR+ mice ( $F_{1,8} = 7.4$ ,  $P = 0.024$ ), although this difference also disappeared nearer to the end of life ( $F_{1,8} = 2.9$ ,  $P = 0.12$ ).

Mean wheel-running activity (2-mo averages per individual; total distance run;  $\text{km d}^{-1}$ ) was increased by approximately 50% in HR+ mice compared with C+ mice throughout life (see Fig. 1B). On the basis of one-way nested ANOVAs performed for each 2-mo block separately, wheel-running activity was no longer significantly higher in HR+ mice compared with C+ mice from approximately 600 d (20 mo) onward ( $F_{1,8} = 4.4$ ,  $P = 0.069$ ). This may be attributed to the lower sample sizes present at these ages; although wheel-running activity strongly decreased later in life, mean wheel-running activity remained increased in HR+ mice compared with C+ mice by approximately 50% from 600 d onward. Detailed analysis of wheel running in the random sample of mice at 6 mo of age (Table 1) showed that the increased total distance run by HR mice was related to both increased time spent running per day and higher maximum running speeds (i.e., running at greater intensities), although these differences did not reach statistical significance in the tests that included line effects (see Table 1).

**Food Intake.** Food intake measured in the life span subgroup

Table 1: Wheel-running activity at 6 mo of age

Parameter	C+	HR+	Two-Tailed $P$
Total distance ( $\text{km d}^{-1}$ )	7.9 (1.3)	14.1 (1.2)	.016
Time (h)	7.2 (1.1)	10.5 (1.0)	.073
Mean speed ( $\text{km h}^{-1}$ )	1.1 (.1)	1.4 (.1)	.132
Maximum speed	2.0 (.1)	2.4 (.1)	.096

Note. Total distance run, time spent running, and mean and maximum running speeds in mice selectively bred for high wheel-running activity and their controls was determined over 2 wk in a random sample of 6-mo-old life span mice. C+ = control mice housed with wheels; HR+ = high-runner mice housed with wheels. For each group,  $n = 16$ . Values shown are least squares means and associated SE obtained from SAS Procedure Mixed. This particular sample included no individuals from control line 1 (lab designation).

at  $668 \pm 70$  d was  $76.1 \text{ kJ d}^{-1}$  (SD = 17.8;  $n = 44$ ),  $85.9 \text{ kJ d}^{-1}$  (SD = 13.0;  $n = 32$ ), and  $68.0 \text{ kJ d}^{-1}$  (SD = 16.2;  $n = 30$ ) in C+, HR+, and HR- mice, respectively. The increase in food intake in HR+ mice was significant compared with both C+ and HR- mice (one-way nested ANCOVA with age and body mass as covariates;  $F_{1,9} = 7.6$ ,  $P = 0.022$  and  $F_{1,9} = 6.3$ ,  $P = 0.034$ , respectively). Mean body masses ( $\pm$ SD) for this subsample of mice were  $43.7 \pm 5.8$ ,  $36.4 \pm 3.8$ , and  $45.0 \pm 9.2$  g for C+, HR+, and HR- mice, respectively.

**Survival.** Median survival was approximately 90 d shorter in both groups of high-runner mice (HR+, HR-) compared with C+ mice (Fig. 2A; Table 2). Kaplan-Meier survival analysis showed that these effects on survival were significant for both pairwise comparisons, that is, HR+ versus C+ ( $P = 0.045$ ) and HR- versus C+ ( $P = 0.032$ ). The HR mice housed with wheels did not statistically differ from those housed without wheels ( $P > 0.1$ ). In this type of survival analysis, possible variation among the replicate lines within the selected or control line types is ignored. To investigate line effects, we therefore analyzed data on age of death using a nested ANOVA design (SAS Procedure Mixed). No significant difference was found among groups ( $F_{2,9} = 0.29$ ,  $P = 0.75$ ), but the analysis did show significant variation in mean age of death among the eight lines (natural log likelihood ratio test,  $\chi^2_1 = 23.5$ ;  $P < 0.0001$ ). Subsequent analyses of the separate groups showed that line effects were present in all three groups (C+, HR+, and HR-; all  $P < 0.01$ ).

In comparing the likelihood of the four models for mortality (Gompertz, Logistic, and both Gompertz and Logistic with the Makeham term), the Gompertz model was never rejected (i.e., the likelihood for the Gompertz model did not differ significantly from any of the other models;  $\chi^2 < 1.0$ ;  $P > 0.1$ ). We therefore accepted the simpler Gompertz model for our data. The better fit of the Gompertz model over the Logistic model means that increasing age-specific mortality did not decelerate or plateau at the end of life, and the better fit of the Gompertz model alone without a constant early-adult Makeham term means that mortality rates began increasing in early adulthood in our mice. Gompertz models ( $\mu_x = Ae^{bx}$ ) were fitted to the instantaneous mortality data (see Fig. 2B). We used likelihood ratio tests to compare models that assume common  $A$ , common  $b$ , or common  $A$  and  $b$  values to models that independently estimated both  $A$  and  $b$ . Comparing C+ with HR+ mice showed the highest likelihood for the model with both parameters estimated, and this model fit the data significantly better than did the models with common  $A$  and  $b$  values ( $\chi^2 = 8.7$ ,  $P = 0.013$ ). Inspection of the parameter estimates (for C+:  $\mu_x = 1.6 \times 10^{-3}e^{0.0076x}$ ; for HR+,  $\mu_x = 13.0 \times 10^{-3}e^{0.0048x}$ ; for HR-:  $\mu_x = 11.5 \times 10^{-3}e^{0.0050x}$ ) indicates that initial mortality ( $A$ ) was higher and the rate of exponential increase ( $b$ ) was slightly lower in HR+ mice compared with C+ mice. The lower median age at death in the HR mice can thus be attributed to an increase in mortality early in life (Table 2; Fig. 2B). In addition, because of a slightly lower exponential increase in mortality in HR mice, estimated maximum life span did not

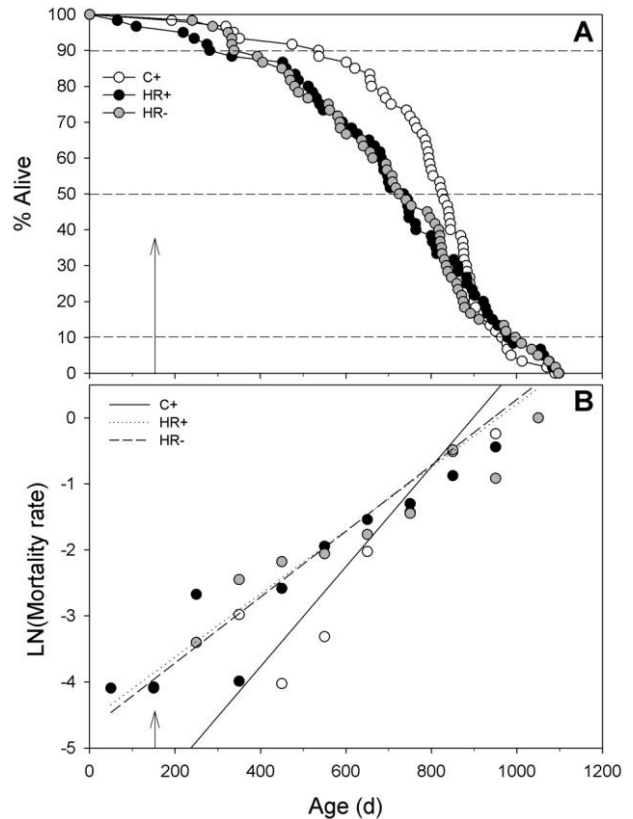


Figure 2. Survival (A; % alive) and natural log of mortality rates (B; see text for formula). Open circles represent nonselected controls housed with wheels (C+), black filled circles represent high-runner mice housed with wheels (HR+), and gray filled circles are high-runner mice housed without wheels (HR-). Arrows indicate when animals first had access to running wheels.

differ between the groups. No differences in mortality rates between HR+ and HR- mice were apparent ( $\chi^2 = 0.7$ ,  $P = 0.99$ ).

**Correlates of Life Span.** To evaluate relationships between body mass, wheel-running activity, food intake, and life span, we calculated mean body mass and wheel-running activity in early life (0–250 d old), middle age (251–500 d old), old age (501–750 d old), and over each individual's entire life span. At all ages (shown for middle age in Fig. 3) there was a significant negative association (Pearson correlation of all individuals, ignoring potential line effects) between body mass and wheel-running activity in C+ mice (Pearson correlation: young:  $r = -0.49$ ,  $P = 0.03$ ; middle aged:  $r = -0.53$ ,  $P < 0.001$ ; old:  $r = -0.50$ ; life:  $r = -0.46$ ,  $P < 0.001$ ) but not in HR+ mice (young:  $r = -0.02$ ,  $P = 0.92$ ; middle aged:  $r = -0.05$ ,  $P = 0.74$ ; old:  $r = 0.08$ ,  $P = 0.64$ ; life:  $r = 0.09$ ,  $P = 0.53$ ). Linear regression was applied to each group separately to evaluate whether body mass, food intake, or wheel-running activity could predict life span. Body mass and food intake did not predict future age at death. Mean wheel-running activity over life did significantly predict life span in HR+ mice ( $R^2 =$

0.26,  $P < 0.001$ ), and it almost reached significance in C+ mice ( $R^2 = 0.07$ ,  $P = 0.053$ ), with an increase in wheel-running activity leading to a reduction in life span. These results must be interpreted with care, however, because wheel-running activity significantly decreases with age, and thus animals that die young will have a higher mean wheel-running activity than animals that die at an older age. To circumvent this problem, we estimated the total distance run per life for each animal. Total distance run per life did not significantly predict life span in either group (HR+:  $R^2 = 0.07$ ,  $P = 0.062$ ; C+:  $R^2 = 0.01$ ,  $P = 0.58$ ).

### Test Cohort

**Metabolism.** In a subgroup of test mice, RMR (by respirometry) and DEE (by DLW technique) were measured at 2, 10, 18, and 26 mo of age (Fig. 4). RMR was similar in HR+, C+, and HR- mice around  $47.5 \text{ kJ d}^{-1}$  (SD = 7.0) throughout life, with no significant contribution to the explained variance by either age or group. DEE was significantly increased in HR+ mice compared with both HR- and C+ mice throughout life (ANCOVA:  $F_{2,56} = 7.6$ ,  $P = 0.001$ ). On average, DEE ( $\text{kJ d}^{-1}$ ;  $\pm$ SD) was  $59.0 (\pm 7.7)$ ,  $69.5 (\pm 11.9)$ , and  $61.2 (\pm 7.6)$  in C+, HR+, and HR- mice, respectively. DEE significantly decreased with age ( $F_{2,56} = 4.7$ ,  $P = 0.013$ ), and there was a significant interaction between group and age ( $F_{4,56} = 3.3$ ,  $P = 0.018$ ), indicating that DEE decreased slightly faster with age in HR+ mice than in the other groups (Fig. 4).

**Body Composition.** Using data from mice aged 300, 560, and 760 d, we tested for effects of group, age, and the age  $\times$  group interaction on body composition (ANCOVA; descriptive statistics are presented in Table 3; see Table 4 for statistical results). Body mass was always included as a covariate, as it has been shown to correlate strongly with organ mass in many studies of mice (Swallow et al. 2005; Rezende et al. 2006a). Statistically significant differences among groups were found for dry lean mass, fat mass, and heart, lung, and skin mass. Dry lean mass was increased in HR+ mice compared with HR- mice, but it did not differ significantly between HR+ and C+ mice. Fat mass was lowest in HR+ mice, but it differed significantly only from HR- mice. Heart and lung mass were increased and skin mass was decreased in the running mice (HR+, C+) compared with the sedentary mice (HR-). Age significantly affected lean mass, fat mass, and the masses of the heart, kidney, skin, and remainder of the carcass. Lean mass and heart and kidney mass

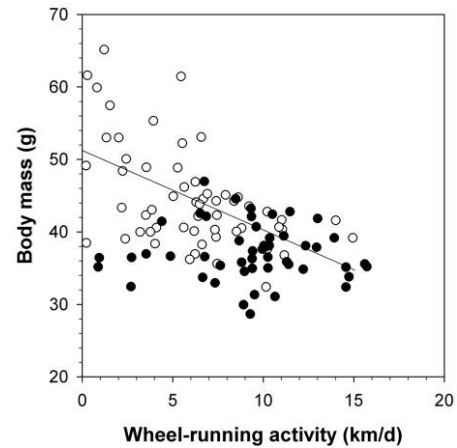


Figure 3. Statistically significant negative relationship between wheel-running activity and body mass in control mice housed with wheels (C+, open circles and solid regression line) but not in high-runner mice housed with wheels (HR+, black filled circles). Points represent mean body mass and wheel-running activity for individual mice between 250–500 d of age.

significantly increased between 10 and 26 mo, and skin and fat masses significantly decreased with age.

**LEP.** Traits measured in the life span cohort (i.e., life span) and in the test cohort (i.e., metabolic rate and body composition) were combined to calculate LEP (Table 5). The rate-of-living theory predicts that LEP per gram of body mass should be constant. LEP calculated based on maximum life span (i.e., mean survival of the oldest 10%) was considerably higher in HR+ mice compared with both C+ and HR- mice (ANOVA with post hoc Turkey,  $P < 0.001$ ), and this increase in LEP remained apparent when values were expressed relative to body mass, dry lean mass, or metabolically active organ mass ( $P < 0.001$  for all; see Table 5). No statistically significant differences were found for LEP between HR- and C+ mice.

### Discussion

Mice selectively bred for high wheel-running activity represent a valuable animal model for the study of physiological effects of increased voluntary exercise (Vaanholt et al. 2007b, 2008b; Nehrenberg et al. 2009; Swallow et al. 2009). Here we investigated the effect of varying intensities of exercise on metabolism and body composition throughout life, in addition to its effects on life span.

Table 2: Descriptive statistics for survival data

Group	<i>n</i>	Median	Mean Life Span (SE)	Range	Oldest 10% (SE)	Youngest 10% (SE)
C+	60	828	787 (23)	192–1,090	1,019 (20)	368 (50)
HR+	60	740	704 (32)	65–1,099	1,062 (15)	199 (37)
HR-	60	733	711 (29)	240–1,098	1,060 (14)	309 (16)

Note. Survival of mice from control (nonselected) lines housed in cages with wheels (C+), high-runner mice housed with wheels (HR+), and high-runner mice housed without wheels (HR-). All values except *n* are in days.

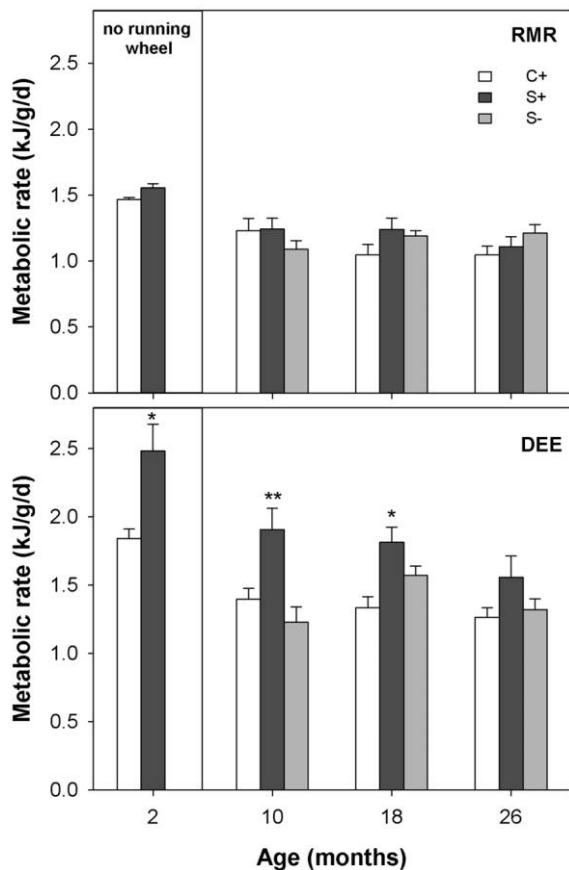


Figure 4. Mass-specific resting metabolic rate (RMR) and daily energy expenditure (DEE) in high-runner mice housed with wheels (HR+, black bars) or nonselected controls housed with wheels (C+, white bars) at different ages. At the age of 2 mo (separate panel on the left), all mice were still housed without running wheels. Gray bars represent high-runner mice that were housed without wheels throughout life (HR-). Values are simple means and SE. For the different ages, statistically significant ( $P < 0.05$ ) differences between C+ and HR+ mice are indicated by one asterisk and differences between HR+ and HR- mice are indicated by two asterisks.

As expected, mean spontaneous wheel-running activity was higher (+50%) throughout life in high-runner mice (HR+) from breeding generation 30 than it was in controls (C+), although this difference was no longer statistically significant after ~20 mo of age. These findings agree with measurements in an earlier generation (16), although the difference in wheel running was slightly lower in that study (+30% throughout life; Morgan et al. 2003). High-runner mice covered these greater distances by running at higher speeds and by increasing the time spent running, as compared with control lines (see Table 1; Vaanholt et al. 2007a). These results are in agreement with previous studies of these mice, despite the fact that mice were housed with metal versus plastic wheels that had larger diameters than did those used in our study (30 cm compared with 14.5 cm in this study). This is important, because some behavioral differences between HR and C mice (see Girard et al. 2001) might not be apparent in smaller, lighter wheels.

Wheel-running activity declined with age in both groups, but it did so more rapidly in HR+ mice, from ~12 km d<sup>-1</sup> at 150 d of age to 1 km d<sup>-1</sup> at the end of life (~1,100 d). These results agree with findings in previous studies on the same mouse lines from generation 16 (Morgan et al. 2003), and they correspond with the general pattern of decline in spontaneous activity with age that has been known in rodents for nearly a century (Richter 1922; Aschoff 1962; Ingram 2000).

Negative effects of increased energy expenditure on life span, which are expected on the basis of the rate-of-living theory, have been shown in several species (e.g., flies [Yan and Sohal 2000], honeybees [Wolf and Schmid-Hempel 1989], kestrels [Daan et al. 1996], and hamsters [Lyman et al. 1981]). In agreement with this, in comparing control and high-runner mice housed with running wheels, the HR mice spent more energy ( $LEP_{BW} = +43\%$ ) and had decreased mean and median life spans (~100 d shorter; see Fig. 2). However, no difference in life span was observed when comparing active and sedentary HR mice, although they also differed in LEP by 46%. We previously manipulated energy expenditure in lab mice by exposing them to cold (10°C; Vaanholt et al. 2009), which, for an endothermic animal, is one of the most powerful tools to increase energy expenditure (Hammond and Diamond 1997; Koteja et al. 2001). This resulted in an increase in energy expenditure of ~40% throughout life compared with that of control mice, and it did not affect life span (Vaanholt et al. 2009). In this study

Table 3: Body composition in control (C) and high-runner (HR) mice housed with (+) or without (-) a running wheel

Group, Measurement	Age (mo)			
	2	10	18	26
C+ mice	$n = 8$	$n = 8$	$n = 8$	$n = 8$
BM	32.9 (1.1)	42.5 (1.8)	44.4 (2.8)	39.3 (2.1)
FFM	29.5 (.8)	33.6 (1.0)	33.9 (1.2)	34.4 (1.3)
DLM	8.0 (.2)	9.4 (.2)	9.7 (.3)	8.9 (.3)
FM	3.4 (.4)	8.8 (1.2)	10.5 (2.3)	4.9 (1.2)
HR+ mice	$n = 8$	$n = 8$	$n = 7$	$n = 5$
BM	30.5 (1.2)	36.7 (2.1)	33.4 (1.4)	36.7 (1.8)
FFM	28.1 (1.1)	31.4 (.7)	28.7 (1.0)	33.3 (.9)
DLM	7.6 (.3)	8.3 (.2)	8.1 (.4)	8.6 (.3)
FM	2.4 (.2)	5.3 (1.9)	4.7 (.7)	3.4 (1.2)
HR- mice		$n = 8$	$n = 7$	$n = 7$
BM		39.6 (2.4)	39.5 (1.6)	39.0 (2.0)
FFM		28.9 (.9)	30.6 (.7)	31.0 (.5)
DLM		8.3 (.3)	8.8 (.2)	8.5 (.4)
FM		10.7 (1.7)	8.9 (1.7)	8.0 (1.7)

Note. Mean (SE) masses (in grams) of various components of the body are shown for C+, HR+, and HR- mice at various ages (data for separate organs not shown). At 2 mo of age, all animals were still housed without a running wheel and body composition measurements for HR+ and HR- mice were included in the same group ( $n = 8$ ); they are arbitrarily listed in the HR+ section of the table. Sample size ( $n$ ) for each group is shown. See Table 4 for statistical results. BM = body mass; FFM = fat-free mass; DLM = dry lean mass, FM = fat mass.



Table 4: Statistical analysis of body composition data

Mass (g)	N	df	Group		Age		Covariate P
			F	P	F	P	
Body	66	2, 57	6.8	<b>.002</b>	.3	.78	... <sup>a</sup>
Fat free	66	2, 56	13.8	<b>.001</b>	7.4	<b>.001</b>	<b>.001</b>
Dry lean	66	2, 56	6.2	<b>.004</b>	2.3	.11	<b>.001</b>
Fat	66	2, 56	13.8	<b>.001</b>	7.4	<b>.001</b>	<b>.001</b>
Heart	66	2, 56	2.5	<b>.04</b>	6.8	<b>.002</b>	.060
Liver	66	2, 56	1.4	.26	2.7	.079	<b>.001</b>
Kidney	66	2, 56	1.1	.35	3.2	<b>.05</b>	.089
Brain	66	2, 56	.4	.70	1.2	.30	.86
Stomach	66	2, 56	.3	.74	2.3	.11	<b>.005</b>
Intestine	66	2, 56	.8	.45	1.0	.37	<b>.001</b>
Lung	66	2, 56	3.4	<b>.04</b>	.9	.41	.70
Skin	66	2, 56	3.5	<b>.04</b>	7.5	<b>.001</b>	<b>.001</b>
Remainder of carcass	66	2, 56	.5	.64	14.2	<b>.001</b>	<b>.001</b>

Note. Two-way ANCOVA were performed with group, age, and group  $\times$  age as fixed factors. Body mass (BM) was included as a covariate. Data were analyzed at ages of 10, 18, and 26 mo for all groups. No significant interactions (group  $\times$  age) were found, except for in intestine mass ( $F_{4,56} = 6.1$ ,  $P = 0.001$ ), and interaction effects are therefore not shown in the table. When the ANOVA generated a significant group effect, post hoc *t*-tests were performed to establish which groups differed from each other (see text).

<sup>a</sup> No covariate *P* value available, as no covariate was included in the body mass analysis.

we used a similar protocol, but we manipulated energy expenditure by increasing exercise, and we again found no evidence of a direct relationship between energy expenditure and life span. Results from these two studies, wherein we manipulated energy expenditure by exposing mice to either cold (Vaanholt et al. 2009) or exercise (this study), thus indicate that life spans of house mice are not directly related to energy expenditure, and these results contradict the rate-of-living theory (see also Speakman et al. 2002; de Magalhaes et al. 2007). The results from these studies agree with other experimental (Holloszy and Smith 1986; Holloszy 1988; Navarro et al. 2004; Selman et al. 2008b) and correlative (Speakman et al. 2004) studies of laboratory rodents. Importantly, these two studies are the first in which accurate measurements of energy metabolism (both RMR and DEE) are made based on multiple measurements throughout life, and they include corrections for various measures of body composition (for related discussion, see Greenberg 1999; Speakman et al. 2002; Speakman 2005).

Our results do not undermine the fundamental relevance of aerobic metabolism to the aging process. ROS are inevitably produced during aerobic metabolism, and they can cause damage to macromolecules that may contribute to the aging process (Beckman and Ames 1998). The amount of ROS produced, however, depends on many factors, such as the state of respiration (3 vs. 4) and the amount of uncoupling that occurs (Barja 2007). During exercise, mitochondria are more coupled and in respiration state 3, which reduces the production of ROS (Brand 2000; Barja 2007). In addition, low levels of ROS that occur in muscles during moderate exercise have been shown to stimulate the expression of such defense systems as antioxidant enzymes (Lambertucci et al. 2007; Gomez-Cabrera et al. 2008). Differences in ROS production, antioxidant en-

zyme activity, and/or repair mechanisms may explain the differences we observed in life span between the HR mice and the C mice.

Enzyme activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) have been measured in heart, liver, and muscle tissue of the test mice in this study at 2, 10, 18, and 26 mo of age (Vaanholt et al. 2008b); however, as in the study by Bronikowski et al. (2002), no significant differences were found between HR mice and C mice. Differences in ROS production, repair mechanisms, and oxidative damage have not been studied, and the exact mechanism involved in the reduction of life span in HR mice remains to be elucidated. Moreover, the difference in life span between male HR mice and male C mice observed in the present study might, in principle, be related to any behavioral and/or physiological trait that differs between these sets of mouse lines. For instance, young HR females and males have increased circulating corticosterone levels (Girard and Garland 2002; Malisch et al. 2007, 2008), but old males do not (Vaanholt et al. 2007b), and one study of rats found that individuals with high corticosterone levels have significantly shorter life spans (Cavigelli and McClintock 2003), although increased levels of corticosterone are also found in calorically restricted mice with extended life spans (Masoro 2005).

It is worth considering whether the levels of running on wheels that HR mice exhibit were moderate or possibly exhaustive and therefore stressful. Relative to mice from the C lines, both sexes of mice from the HR lines exhibit elevated corticosterone levels under basal conditions (Malisch et al. 2007, 2008, 2009) and during conditions of nightly wheel running (only studied in females; Girard and Garland 2002), at least at relatively young ages. By some definitions, the wheel

Table 5: Lifetime energy potential and its components

Parameter	C+	HR+	HR-
Mass (g):			
Body	39.8 (1.3)	34.1 (1.0)	39.4 (1.1)
Total dry lean	9.0 (.2)	8.1 (.2)	8.5 (.2)
Organ (dry lean)	.89 (.03)	.76 (.02)	.80 (.03)
DEE	59.0 (1.5)	69.5 (2.5)	55.6 (1.9)
DEE <sub>BM</sub>	1.5 (.1)	2.1 (.1)	1.4 (.1)
DEE <sub>DL</sub>	6.6 (.2)	8.7 (.4)	6.5 (.2)
DEE <sub>OM</sub>	68.1 (2.4)	92.8 (4.0)	70.6 (2.3)
Maximum life span (oldest 10%; d)	1,019 (20)	1,062 (15)	1,060 (14)
LEP	60,093 (1,509)	73,841 (2,623)	58,962 (2,016)
LEP <sub>BM</sub>	1,550 (58)	2,223 (109)	1,522 (68)
LEP <sub>DL</sub>	6,736 (191)	9,245 (404)	6,940 (243)
LEP <sub>OM</sub>	69,370 (2,401)	98,604 (4,295)	80,826 (2,443)

Note. Lifetime energy potential (LEP;  $\text{kJ life}^{-1}$ ) is the product of rate of energy expenditure and life span, and it was calculated for each group using individual daily energy expenditure (DEE,  $\text{kJ d}^{-1}$ ) of the test animals, and maximum life span (mean of the age of the oldest 10%) measured in the life span animals. In addition, as indicated by subscripts, DEE ( $\text{kJ d}^{-1} \text{g}^{-1}$ ) and LEP ( $\text{kJ g}^{-1} \text{life}^{-1}$ ) was corrected for various measures of body composition measured in test mice by dividing by body mass (BM; g), dry lean mass (DL; g), or metabolically active organ mass (OM; sum of dry lean heart, liver, kidney, and brain masses; g). All values shown are mean (SE). C+ = control mice housed with wheels; HR+ = high-runner mice housed with wheels; HR- = high-runner mice housed without wheels.

running of HR mice might thus be considered stressful. However, HR+ mice run for many hours per day, and the exercise can therefore not be exhaustive in a conventional sense. Also, although HR mice run voluntarily on wheels at speeds that are closer to their maximal aerobic speeds than do C mice, they generally do not reach  $\dot{V}O_{2\max}$  (i.e.,  $\dot{V}O_2$  at maximum running speeds in wheels was 82% and 73% of the  $\dot{V}O_{2\max}$  in HR+ and C+ mice, respectively, that were measured on a motorized treadmill; Rezende et al. 2005; 2006b). Finally, we have not found differences between HR and C mice in the respiratory exchange ratio at voluntary maximum oxygen consumption on the running wheels (Rezende et al. 2009).

When comparing the life spans of high-runner mice housed with (HR+) and without (HR-) access to a running wheel, we found that differences in activity were unrelated to longevity. This contrasts with other studies in rats and mice that have shown beneficial effects of exercise (i.e., absence or presence of a running wheel) on median (but not maximum) life span (Holloosy 1988; Navarro et al. 2004). Holloszy and Smith (1987) have suggested that exercise may bring about this “rectangularization” of the survival curves by counteracting deleterious effects of a sedentary life combined with overeating, making it possible for more of the animals to attain old age but without slowing primary aging. The fact that we did not find deleterious effects of a sedentary life in our HR mice may be related to the fact that they are more active than the controls even in the absence of a running wheel (infrared measurements in respirometer; data not shown; see also Rhodes et al. 2001; Malisch et al. 2008, 2009). As a consequence, body adiposity of HR- mice, although increased compared with HR+ mice (fat content was 13% of body mass in HR+ mice vs. 23% in HR- mice), was similar to that of active control mice (19%;

see also Swallow et al. 2001). Therefore, the sedentary high-runner mice may not have experienced deleterious effects of sedentary life due to a relatively high activity and a lean phenotype. Note that HR mice in general have very low body fat when compared with other strains (Nehrenberg et al. 2009).

A previous study by Bronikowski et al. (2006) investigated survival in these mouse lines at generation 16 of selective breeding and found similar results for male HR- and C- mice; that is, median life span of C- mice in this study was 863 d versus 828 d in Bronikowski’s (2006) study, and in HR- mice it was 760 d in this study versus 740 d in Bronikowski’s study. However, median survival differed by 153 d between studies for the HR- mice; that is, it was 880 d in Bronikowski et al. (2006) versus 733 d in this study. Looking at the mortality rates, this difference seems to be related to a higher mortality early in life and a slower exponential increase in mortality rate in HR- mice in the previous study. In the previous study, the initial survival was calculated on the basis of a sample size of 40 mice per group, but this sample size was reduced by half at ~590 d of age, which is before most of the mortality occurs; this may have caused the discrepancy in the results. In Bronikowski et al.’s (2006) study, an additional group of sedentary control mice was present. For males, this group had a shorter life span (i.e., 762 d) than the active control group (C+) but one that was similar to that of the active high-runner mice (HR+). Control mice housed with wheels thus had increased life spans compared with sedentary mice. This is in agreement with previous studies showing increased median life span in rats housed with wheels compared with rats housed without wheels (Holloosy 1988), and it strengthens the argument that the lack of a difference in life span between HR mice housed with or without wheels is related to the leaner and more active

phenotype of these mice in general. Thus, it may be that the sedentary high-runner mice did benefit from their more active lifestyle and lived longer than would be expected given the results of previous studies comparing sedentary and active rodents (Goodrick 1980; Holloszy et al. 1985; Navarro et al. 2004). This may be due in part to improvements to the metabolic profile of these mice that make them less prone to develop the metabolic syndrome and associated diseases. For instance, HR mice have lower circulating levels of leptin (corrected for body fat; Girard et al. 2007) and increased levels of adiponectin (Vanholt et al. 2007b, 2008a) than do C mice. In addition, an increase in AMP-activated protein kinase activity has been shown in the aorta of male HR mice (Zhang et al. 2006). All of these factors have been shown to be protective against developing the metabolic syndrome (Carroll and Dudfield 2004; Misra 2008). Exercise thus seems to have a positive effect on health status. This relationship does not appear to be linear, however, as has been suggested in human beings (Lee and Skerrett 2001; Warburton et al. 2006); HR mice housed with or without wheels had different levels of exercise but similar life spans, and HR+ mice that exercised the most had a shorter life span than did C+ mice. We could speculate that HR+ mice experienced detrimental effects of high levels of exercise that were unrelated to energy metabolism.

In summary, mice from lines that were selectively bred for high wheel-running activity have increased levels of wheel running at ages up to ~20 mo of age (see also Bronikowski et al. 2006), which results in an increase in whole-animal DEE of 18% and 25% over a life span compared with that in C+ mice and HR– mice, respectively, in spite of their smaller body size (Table 5). On a mass-specific basis, lifetime energy potential ( $LEP_{BM}$ ) in high-runner mice housed with wheels was increased by 43% and 46%, respectively, compared with high-runner mice housed without wheels or control mice with wheels. The median life span of the selectively bred lines was reduced by ~90 d when compared with that of control (nonselected) mice housed with a running wheel, but they did not differ from high-runner mice housed without a running wheel (Table 2). These results are not consistent with the rate-of-living theory, and they suggest that physiological and/or behavioral traits other than differences in metabolic rate underlie the observed differences in life span between control mice and high-runner mice.

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