Experimental Gerontology 98 (2017) 70–79

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Age-related changes of physiological performance and survivorship of bank voles selected for high aerobic capacity

Agata Marta Rudolf[a,*], Maciej Jan Dańko[b], Edyta Teresa Sadowska[a], Geoffrey Dheyongera[a,1], Paweł Koteja[a]

[a] Institute of Environmental Sciences, Jagiellonian University, Gronostajowa 7, 30-387 Krakow, Poland
[b] Max Planck Institute for Demographic Research, Konrad-Zuse-Strasse 1, 18057 Rostock, Germany

ARTICLE INFO

Section Editor: Christiaan Leeuwenburgh

Keywords:
Selection experiment
Maximum metabolic rate
Forced running
Senescence

ABSTRACT

Variation in lifespans is an intriguing phenomenon, but how metabolic rate influence this variation remains unclear. High aerobic capacity can result in health benefits, but also in increased oxidative damage and accelerated ageing. We tested these contradictory predictions using bank voles (*Myodes* = *Clethrionomys glareolus*) from lines selected for high swim-induced aerobic metabolism (A), which had about 50% higher maximum metabolic rate and a higher basal and routine metabolic rates, than those from unselected control lines (C). We measured sprint speed (VSmax), forced-running maximum metabolic rate (VO_2run), maximum long-distance running speed (VLmax), running speed at VO_2run (VVO_2), and respiratory quotient at VO_2run (RQ) at three age classes (I: 3–5, II: 12–14, III: 17–19 months), and analysed survivorship. We asked if ageing, understood as the age-related decline of the performance traits, differs between the A and C lines. At age class I, voles from A lines had 19% higher VO_2run, and 12% higher VLmax, but tended to have 19% lower VSmax, than those from C lines. RQ was nearly 1.0 for both A and C lines. The pattern of age-related changes differed between the lines mainly between age classes I and II, but not in older animals. VSmax increased by 27% in A lines and by 10% in C lines between age class I and II, but between classes II and III, it increased by 16% in both selection directions. VO_2run decreased by 7% between age class I and II in A lines only, but in C lines it remained constant across all age classes. VLmax decreased by 8% and VVO_2 by 12% between age classes II and III, but similarly in both selection directions. Mortality was higher in A than in C lines only between the age of 1 and 4 months. The only trait for which the changes in old animals differed between the lines was RQ. In A lines, RQ increased between age classes II and III, whereas in C lines such an increase occurred between age classes I and II. Thus, we did not find obvious effects of selection on the pattern of ageing. However, the physiological performance and mortality of bank voles remained surprisingly robust to ageing, at least until the age of 17–19 months, similar to the maximum lifespan under natural conditions. Therefore, it is possible that the selection could affect the pattern of ageing in even older individuals when symptoms of senility might be more profound.

1. Introduction

One of the most intriguing phenomena of evolutionary biology is the variation in lifespans and underlying mortality patterns across the tree of life. Even closely related, or individuals of the same species, can have very different average lifespans (Barja, 2013; Jones et al., 2014), which cannot be explained solely by differences in environmental factors. Therefore, the presence of such variation still requires to be explained in the context of evolutionary theory and also by the underlining physiological mechanisms. Ageing is a complex process, and detrimental changes occur simultaneously at various levels of biological organization. Senescence is typically defined as an increase of mortality and decline in fertility with age after maturity (Schaible et al., 2015), but it is also manifested as decreased quality of life caused by a declined physiological performance (Margolick and Ferrucci, 2015). The pattern of this decline appears similar in various species and taxa, therefore, a decrease of physiological performance is commonly used in medical and evolutionary-ecology studies as a proxy of the ageing process (Marck et al., 2016).

An increased physiological aerobic performance, associated with regular physical activity, is well documented to be associated with a wide range of health benefits and prevents or even reduces symptoms of

* Corresponding author at: Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, West Beichen Road, Beijing, China.
E-mail addresses: agata.rudolf@gmail.com, agata.rudolf@genetics.ac.cn (A.M. Rudolf).
[1] Current address: Directorate of Fisheries Resources, Ministry of Agriculture, Animal Industry and Fisheries, Lugard Avenue Entebbe, Uganda.

http://dx.doi.org/10.1016/j.exger.2017.08.007
Received 28 November 2016; Received in revised form 20 July 2017; Accepted 6 August 2017
Available online 10 August 2017
0531-5565/

age-related disorders, such as obesity, diabetes, inflammation (Kokkinos and Myers, 2010) and cardiovascular diseases (Thompson, 2003). Therefore, increased physiological performance contributes to healthy ageing and prolonged lifespan. On the other hand, it has been shown that high aerobic capacity is correlated with increased basal and routine metabolic rates (Sadowska et al., 2015; Wone et al., 2015), which, according to the classical 'rate of living' (ROL) theory, is negatively correlated with lifespan (Rubner, 1908; Pearl, 1928).

One of the leading mechanistic explanations behind the hypothetical metabolism - rate of living nexus used to be the 'free radical theory' of ageing (FRT) (Harman, 1956). It was based on the assumption that high overall rates of aerobic metabolism can be associated with the excessive production of reactive oxygen species (ROS). Surplus ROS can damage mitochondria, which in turn produce more ROS. Therefore, this can lead to oxidative stress, manifested by increased oxidative damage to molecules, such as protein carbonylation or epigenetic changes to DNA, and hence accelerated ageing (Bratic and Trifunovic, 2010). However, although it is known that oxidative stress is associated with age-related diseases such as cancer, atherosclerosis, diabetes, heart and neurodegenerative diseases (Ortuño-Sahagún et al., 2014), the role of ROS as a factor underlining ageing remains unclear (Selman et al., 2012). This is not only because animals can increase the efficiency of anti-oxidative defence systems (Vaanholt et al., 2008), but also because ROS help to maintain homeostasis at a cellular level by playing important roles in intracellular signalling of various physiological processes (Zarse et al., 2012). Moreover, exercise-induced ROS can even prolong lifespan (Ristow and Schmeisser, 2014). The other plausible and widely discussed mechanistic explanations of ageing include shortening of telomeres, a limited number of cell divisions, disrupted protein turnover equilibrium, or accumulation of somatic mutations to nucleic and mitochondrial DNA with progressive age (Balcombe and Sinclair, 2001), i.e. processes that can also depend on the rate of metabolism. Thus, the actual mechanistic explanation behind the ROL theory remains a subject of debate.

Irrespective of the ambiguities concerning a hypothetical mechanism underlying the association between metabolic rate and ageing, extensive studies on a wide range of species cast serious doubts on the association itself. Comparative analyses showed no clear correlation between lifespan and the rate of metabolism (De Magalhães et al., 2007), or even a reversed pattern to that predicted by the ROL theory (Barja, 2004; Brunet-Rossinni and Austad, 2004; Ricklefs, 2010; Montgomery et al., 2011; Mcnab, 2005). Analyses of several intraspecific correlations showed confusing results (Speakman et al., 2003, 2004; Oklejewicz and Daan, 2002; Niitepõld and Hanski, 2013; Hulbert et al., 2004; Melvin et al., 2007). Results of studies based on experimental manipulation of energy expenditure were also contradictory: average lifespan increased in response to elevated exercise in mice and rats (Chigurupati et al., 2008; Holloszy, 1997; Navarro et al., 2004), but mice selectively bred for high wheel-running activity had higher metabolic rate and shorter lifespan than non-selected control ones (Vaanholt et al., 2010). However, the selected mice without access to wheels had a similar rate of metabolism to controls, yet their lifespan also decreased, indicating no clear link between energy expenditure and lifespan.

Locomotor performance is a complex trait, limited by various anatomical, morphological and physiological features, as well as the current health state (Swallow et al., 2009). Although all types of locomotor performance are susceptible to age-related disorders, the relevance of particular limiting features to the process of ageing varies among locomotor performance types (Koch and Britton, 2001; Koch et al., 2011; Dlugosz et al., 2009; Bundle and Weyand, 2012). For example, aerobic capacity, associated with long-distance running, can be compromised by multiple dysfunctions to the cardiovascular system and decreased mitochondria efficiency, which alters energy supply and muscle strength (Speakman et al., 2003; Vaanholt et al., 2010; Navarro et al., 2004). Some disorders can also be associated with changes in body mass, possibly due to the degeneration of internal organs (Lessard-Beaudoin et al., 2015). Moreover, age-related muscle-tendon stiffness tends to increase limb load and cause posture or coordination disorders, which lead to gait abnormalities in both long- and short-distance running (Horner et al., 2011). Therefore, to obtain a relevant evaluation of the ageing process, various parameters of organismal performance must be assessed.

Experimental approaches used in many previous ageing-related studies primarily involved intra-specific correlations or phenotypic manipulations of metabolic rate, and such data are unable to be used to draw conclusions about genetic correlations between traits. A promising tool is offered by an experimental evolution approach, which enables to investigate genetically-based relations under a controlled experimental regime (Swallow et al., 2009). However, only a few selection experiments have focused on physiological performance traits (Swallow et al., 1998; Koch and Britton, 2001; Hayes and Garland, 1995; Konarzewski et al., 1997). Moreover, these studies have been based on model species, for which the observed effects can be strongly affected by domestication: laboratory mice and rats underwent a long-term selection for increased parameters of reproduction, rapid growth (Ricklefs, 2010; Selman et al., 2012) and sedentary phenotype (Garland et al., 2011). In this study, we took advantage of a unique experimental-evolution model system, based on a non-laboratory species, the bank vole (*Myodes* = *Clethrionomys glareolus*; Sadowska et al., 2008). The voles from lines selected for increased swim-induced aerobic metabolism achieved about 50% higher maximum rate of oxygen consumption compared to those from unselected control lines (Sadowska et al., 2015; Konczal et al., 2015). Furthermore, the selected voles have increased basal metabolic rate (Sadowska et al., 2015), daily activity and food consumption (Koteja et al., 2009; Dheyongera et al., 2016), and different expression of some genes involved in metabolism in the liver (Konczal et al., 2015).

We asked whether the selection for high aerobic metabolism affected the pattern of ageing at the organismal level. We investigated survivorship, body mass, and five aspects of locomotor performance: the maximum speed achieved during sprint and long-distance running (with gradually increasing speed), the maximum run-induced aerobic metabolic rate (VO_2run), respiratory quotient and the speed achieved at VO_2run. We expected that the physiological performance traits are improved in the selected compared to the control lines and that they decline with age, and asked whether and how survivorship and the age-related changes of performance are altered by the selection. Our previous studies showed that the selection resulted in both an elevated metabolic rate and improved locomotor performance (Sadowska et al., 2015; Jaromin et al., 2016), but not in an elevation of oxidative stress markers (in breeding young-adult females; Ołdakowski et al., 2015). However, the increased aerobic metabolism could lead to a ROS production rate that exceeds the antioxidant and repair systems capacity at senile age, and consequently, to oxidative damage and faster ageing. On the other hand, increased physical activity can be associated with health benefits, such as an augmented capacity of the cardiovascular and antioxidant systems, not only at a young age but also, or even particularly, at senescence (Koch et al., 2011; Vaanholt et al., 2008). If this is true, voles from the selected lines should be characterized by increased survivorship and a less profound age-related decline of organismal performance. Thus, contradictory predictions concerning the effect of the selection on the ageing pattern can be legitimately proposed.

2. Materials and methods

2.1. The experimental model

The work was based on an experimental evolution model system, in which four replicate lines of bank voles (*Myodes* = *Clethrionomys glareolus* Schreber 1780) were selected for high aerobic metabolism

Table 1
Body mass and physiological performance traits of bank voles from lines selected for high swim-induced aerobic metabolism (A) and unselected control lines (C) in three age classes: descriptive statistics (N and simple means with SD).

Trait	Selection direction	Age class					
		I (3–5 months)		II (12–14 months)		III (17–19 months)	
		N	Mean (SD)	N	Mean (SD)	N	Mean (SD)
BM	A	95	23.9 (3.7)	72	29.0 (4.1)	65	28.8 (3.7)
	C	95	21.9 (4.3)	77	27.7 (5.2)	69	27.9 (4.9)
VSmax	A	95	73.2 (18.6)	72	90.6 (21.0)	65	105.0 (18.6)
	C	95	88.2 (22.8)	77	93.0 (25.2)	69	106.8 (21.6)
VO_2run	A	95	5.5 (0.8)	66	5.8 (0.6)	60	5.8 (0.6)
	C	94	4.3 (0.6)	68	5.0 (0.7)	64	4.9 (0.7)
VLmax	A	95	50.1 (14.2)	66	48.9 (14.7)	60	44.9 (14.0)
	C	94	44.0 (10.7)	68	44.2 (10.3)	64	41.3 (10.8)
VVO_2	A	95	35.0 (10.0)	66	35.1 (10.6)	60	30.4 (9.4)
	C	94	31.0 (8.8)	68	33.5 (8.3)	64	30.3 (8.5)
RQ	A	95	0.98 (0.07)	66	1.00 (0.6)	60	1.03 (0.6)
	C	94	0.98 (0.07)	68	1.01 (0.6)	64	1.02 (0.6)

BM = body mass [g]; VSmax = maximum sprinting speed [m/min]; VO_2run = maximum metabolic rate during running [ml O_2/min]; VLmax = maximum long-running speed [m/min]; VVO_2 = running speed achieved at VO_2run [m/min]; RQ = respiratory quotient at VO_2run.

achieved during swimming (VO_2swim; A = aerobic lines), and four lines were maintained as unselected control (C = control lines) (Sadowska et al., 2008; Sadowska et al., 2015; Supplement I). In generations 11–14, voles from the A lines achieved about 50% higher VO_2swim than those from the C lines (Supplement I, Fig. S1.a; Chrząścik et al., 2014). The selection resulted also in about 9% increase of the basal metabolic rate (BMR) (Fig. S1.b; Sadowska et al., 2015), and about 20% increase of daily locomotor activity inside home-cages (measured at generation 7) and food consumption rate (measured at generations 11 and 18; Koteja et al., 2009; Dheyongera et al., 2016) in the young-adult voles from the A lines. Moreover, analyses of the complete transcriptome from heart and liver indicated differences between the A and C lines in the expression levels of SNP (single nucleotide polymorphism) allele frequencies in several genes involved in carbohydrate and lipid metabolism, including the rate-limiting step of glucose metabolic pathway in liver (Konczal et al., 2015). Thus, the A line voles differ from the C line ones not only in locomotor performance, but also in the overall rate of metabolism, and therefore provide a suitable model to investigate the relationship between metabolic rate and ageing.

2.2. Animals used

The main part of this study was conducted on voles sampled from generation 13 of the selection experiment. The animals were randomly chosen from the first and second litters of 15–20 families from each of the four A and four C lines, with the provision that not more than one male and one female represented a full-sib family (A: 51 males and 57 females; C: 53 males and 52 females). Following the standard colony protocols, the animals were weaned from mothers at the age of 17 days, maintained in full-sib family groups until the age of 30–32 days, and then separated into same-sex groups of 2–3 individuals per cage. The voles were maintained in standard plastic rodent cages provided with sawdust bedding, at a temperature of 20 °C (± 1 °C), photoperiod 16 h light: 8 h dark with light phase starting at 2:00 am. Water and food (standard rodent chow: 24% protein, 3% fat, 4% fibre; Labofeed H, Kcynia, Poland) were provided ad libitum.

The average life expectancy of young-adult bank voles in the wild is about 3 months, and the maximal life expectancy is about 10–15 months, but occasionally individuals can achieve even 18–21 months (Bernshtein et al., 1999; Petrusewicz, 1983). Therefore, the measurements described below were performed in three age classes: (I) 3–5 months: young adult animals, (II) 12–14 months: middle aged animals (maximum lifespan achieved under natural conditions), and (III) 17–19 months: elderly animals (advanced senility).

The original plan of the experiment assumed maintaining the voles until the age of about 22 months. However, we discovered that the colony had been infected with *Puumala* hantavirus, which can cause severe illness in people (e.g., Krüger et al., 2011), and the experiment had to be terminated prematurely. The virus, commonly present in many populations of voles (Ali et al., 2014), has not been detected immediately because, under standard housing conditions, such as applied in this experiment, the infection does not inflict any pathological effects in the bank voles (Bernshtein et al., 1999). Even though some data suggest that the infection may decrease vole's survival under harsh winter conditions (Kallio et al., 2007), it remains irrelevant in the case of our study. Moreover, we confirmed that the parameters of reproduction (litter mass and litter size during weaning), mortality, and adult body mass in the 'infected' generations did not differ from the preceding 'uninfected' generations. Therefore, it is highly unlikely that the infection influenced results of this experiment.

In most cases, the measurements of physiological performance traits and body mass were performed on the same individuals throughout the three age classes. However, the number of individuals tested in subsequent age classes decreased because of mortality and planned euthanasia (animals used at age class I: $N = 190$, II: $N = 149$ and III: $N = 134$; Table 1).

Additionally, we measured food consumption rate in a subsample of 64 individuals from generation 13 at the age of 13.5–14.5 months (similar to age class II used in the analysis of physiological performance) and additional 64 individuals from generation 14 at the age of 6.5–7.5 months (detailed description is provided in Supplement II).

To conduct survival analysis, we used mortality data of animals from two entire selection generations 13 and 14 combined together (gen. 13: A: $N = 1259$, C: $N = 1182$; gen. 14: A: $N = 1330$, C: $N = 1263$). A subsample of these animals (gen. 13: A: $N = 97$, C: $N = 98$; gen. 14: A: $N = 101$, C: $N = 97$) was used to diagnose the age-related diseases.

2.3. Measurements of performance traits, mortality and age-related ailments

Within each experimental session (age class) performance traits were measured in randomised groups of 16–32 individuals, which represented both selection directions, all replicate lines within the selection directions and both sexes. A blinded experimental procedure was applied, i.e. during the experimental and animal care procedures neither the researchers nor technicians knew from which selection

direction a given animal originated.

The maximum sprint speed (VSmax) was measured using a photocell-lined racetrack, following standard procedures for rodents (Dlugosz et al., 2009). The racetrack was made of an opaque plastic. It consisted of 4.5 m long, 7.5 cm wide tunnel, with 30 cm high walls, which prevented the animal from escaping, and a textured rubber ribbon bottom. The track was divided into 0.5 m long sections with 8 infrared photocell sensors attached to the walls at height of 1.5 cm. Binary data from the sensors were streamed through a U3-HV interface (LabJack Co, Lakewood, CO, USA) and recorded using a custom-made program working under DAQFactory Express 5.84 software (Azeotech, Aschland, OR, USA). Each animal was weighed and placed inside the track. Next, the voles were chased about 12 times along the track (6 times in right, and 6 times in left direction). The test was repeated on 2 consecutive days. Sprint speed was calculated from the time elapsed between passing subsequent photocell sensors. The maximum sprint speed was calculated from the minimum time achieved at a 1 m distance during any of the trials on a given day (similarly as in Dlugosz et al., 2009) and expressed as m/min (for compatibility with the long-distance running described below).

Next, the maximum exercise metabolic rate was measured as the maximum rate of oxygen consumption achieved during forced running (VO_2run; ml O_2/min) on a standard physiological treadmill for small rodents with electric stimulation (BTU-100-1U-M, BIO-SYS-TECH, Bialystok, Poland). Each animal was weighed, its belly was wetted to increase electrical conductivity (otherwise the voles ignored the mild shocks generated by stimulator), and it was placed on the treadmill inside the respirometric chamber. After about 1 min, the treadmill was turned on and the running speed was incrementally increased (by 6 m/min each minute) until the animal was not able to run any faster. At that moment the trial was stopped and the animal was placed back in its home cage. Measurements for each individual were performed on two subsequent days. The measurements were preceded by two days of mild training to accustom the animal to using the treadmill. Both training and the measurement trials were performed at a constant 22 °C temperature. During the measurement, fresh air dried with Silica gel was passed through the chamber at about 2000 ml/min regulated by a mass flow controller to ± 1% (two MFS-1 units; Sable Systems Int., USA). Oxygen and CO_2 concentrations were measured in a sample of ~200 ml/min of excurrent air (dried first with DG-1 or ND2; Sable Systems Int., USA, and finally with magnesium perchlorate; Anhydrone, J.T. Baker, USA) with FOX or FC10 oxygen analysers (depending on the experimental session; Supplement III.1) and CA2A CO_2 analyser (Sable Systems Int., USA). The data were recorded through an analog-to-digital UI2 interface with ExpeData 1.4.3 v PRO data acquisition program (Sable Systems Int., USA). The rates of oxygen consumption and CO_2 production were calculated using the respirometric equations with 'instantaneous correction' (Lighton, 2008). The highest 1-minute rate of oxygen consumption was treated as the estimate of VO_2run for each measurement day. The respiratory quotient (RQ) was calculated as the ratio of carbon dioxide production to oxygen consumption at the VO_2run.

The maximum speed of long-distance forced running (VLmax) was determined as the maximum speed that an animal was able to achieve during the VO_2run trial, i.e. after several minutes of running at an increasing speed. Another interesting performance trait measured in the context of this work was the maximum aerobic speed (MAS), i.e. the lowest speed at which an individual achieves VO_2max (Dlugosz et al., 2009). However, unlike in the case of measurements in humans, we could not ensure that the actual exercise effort increased as dictated by the treadmill speed, and consequently, MAS could not be reliably determined. Instead, we report the speed at the middle of the 1-min period when VO_2run is achieved (VVO_2), which sets the upper limit on MAS. VLmax and VVO_2 were expressed as m/min.

The mortality rate was determined daily. However, data were only collected from the age of 17 days (age at the weaning), therefore mortality before this age was not included in the survival analysis (left truncation). Among all 5034 individuals used in the analysis, 353 died of causes not related to experimental protocols, and these cases were included as natural, physiological deaths. Cases of death due to accidents (Ristow and Schmeisser, 2014) and planned euthanasia due to tissue sampling or selection protocols (4627), were included as non-natural deaths (right censoring).

To diagnose the presence of age-related diseases, observations from 349 individuals dissected in planned euthanasia were combined with data on 44 animals that died of natural causes. The animals were than divided into three age groups according to age at death (2–8 months: $N = 141$; 8–14 months; $N = 113$; 14–20 months: $N = 139$) and the observed ailments were assigned into 17 categories, according to affected organ system or observed age-related disease (Supplement V: Table S6). The results were expressed as a percentage of affected animals.

2.4. Statistical analyses

The statistical analyses of VSmax, VO_2run, VLmax, VVO_2, RQ, and body mass (BM) were performed with SAS 9.3 (SAS Institute Inc., Cary, NC, USA). Preliminary analyses showed that results obtained on subsequent measurement days within each of the three age classes were moderately repeatable (Supplement III.2: Table S1). Therefore, an average from the repeated trials was used in the main analyses. For VO_2run, VVO_2, and RQ, among the total of 894 repeated trials performed in all individuals at the three age classes, 50 trials were excluded from the analysis due to errors or because the animals refused to exercise, and then only one of the two trials repeated for an individual at a particular age class was used. Generalized Linear Mixed Model (SAS MIXED procedure) with REML method of estimation, variance constrained to positive values (default option) and with the Satterthwaite option to calculate degrees of freedom was applied. The model included three fixed categorical factors: Selection direction (A-Aerobic, C-Control), Sex (Males, Females) and Age class (I: 3–5, II: 12–14, and III: 17–19 months), and their all-way interactions. BM was included as a fixed covariate in analyses of the three running speeds, VO_2run and RQ. Replicate Line (A1-A4, C1-C4) nested within Selection direction, as well as its interactions with the main categorical factors, were included as random effects. Identification Number of an animal was included in the analysis as a random effect nested in Replicate Line. To test the assumption of homogeneity of slopes, initial models included interactions of BM with categorical factors. The interactions were not significant for VSmax, VLmax, VVO_2, and RQ, so they were removed from the final models. Factor effects were considered statistically significant when $P < 0.05$. A Tukey-Kramer correction was applied for post hoc comparisons among Age classes. The significance of random effects was assessed with chi square Likelihood Ratio test (using models with variances not constrained to be positive). To achieve a normal distribution of residuals, the analyses for VLmax and VVO_2 were performed on $\log_{10}$-transformed data. Results of the mixed model analyses are expressed as adjusted least square means (LSM) ± confidence intervals (CI), back-transformed to the original scale (when appropriate).

The survival analysis was performed in *R* language (survMisc and survival *R*-packages). We used Kaplan-Maier (product-limit) estimator with Greenwood confidence intervals (e.g. Klein and Moeschberger, 2006) to plot and compare survivorship curves (the fraction of subjects living). Additionally, to test equality of survival distributions we used two tests: log-rank test and Gehan-Breslow test (modified Wilcoxon test), which can be considered partially compensatory to each other: the log-rank test is sensitive mainly to late occurring events, whereas the Gehan-Breslow test gives more weight to early events. Also, the log-rank test is more powerful when proportional hazard assumption is fulfilled, whereas for the Gehan-Breslow test this assumption is less important (Martinez and Naranjo, 2010). The empirical mortality rates were smoothed by fitting one-dimensional Poisson penalized splines

Table 2
Results of Mixed Model analyses of body mass, maximum sprinting speed, maximum metabolic rate achieved during forced running, maximum running speed, running speed achieved at the maximum metabolic rate, and respiratory quotient at the maximum metabolic rate, of bank voles from lines selected for high swim-induced aerobic metabolism and unselected control lines at three age classes: test statistics (F with df) and significance levels (*P* values; marked with bold if < 0.05) of fixed factors in the Mixed Models.

Trait		Fixed effects							
		Body mass	Selection	Age	Sex	Selection × age	Selection × sex	Age × sex	Selection × age × sex
BM	F		3.07	124.33	64.86	0.29	2.71	1.1	1.28
	df		1, 5.96	2, 12.4	1, 6.41	2, 12.4	1, 6.41	2, 275	2, 275
	P		0.131	**< 0.001**	**< 0.001**	0.752	0.148	0.336	0.280
VSmax	F	1.75	1.81	100.56	0.68	7.58	1.65	0.87	1.71
	df	1, 459	1, 6.25	2, 349	1, 226	2, 284	1, 197	2, 284	2, 286
	P	0.187	0.225	**< 0.001**	0.412	**0.001**	0.200	0.422	0.183
VO_2run	F	179.95	133.95	7.67	6.56	8.84	0.87	0.67	0.38
	df	1, 12.7	1, 8.01	2, 319	1, 213	2, 319	1, 213	2, 267	2, 267
	P	**< 0.001**	**< 0.001**	**0.001**	**0.001**	**< 0.001**	0.353	0.511	0.682
VLmax	F	1.99	4.92	5.62	0.04	0.48	2.00	1.16	0.65
	df	1, 379	1, 6.11	2, 344	1, 224	2, 275	1, 195	2, 275	2, 276
	P	0.159	0.068	**0.004**	0.838	0.619	0.159	0.314	0.521
VVO_2	F	0.00	1.27	5.96	1.70	1.47	1.79	0.24	1.19
	df	1, 304	1, 6.15	2, 17.7	1, 209	2, 12	1, 185	2, 290	2, 290
	P	0.974	0.302	**0.011**	0.193	0.268	0.182	0.784	0.306
RQ	F	2.67	0.03	12.72	0.01	2.94	0.29	1.67	0.34
	df	1, 371	1, 6.04	2, 342	1, 185	2, 262	1, 160	2, 262	2, 262
	P	0.103	0.858	**< 0.001**	0.908	0.054	0.590	0.190	0.715

BM = body mass [g]; VS = maximum sprinting speed [m/min]; VO_2run = maximum metabolic rate during running [ml O_2/min]; VLmax = maximum long-running speed [m/min]; VVO_2 = running speed achieved at VO_2run [m/min]; RQ = respiratory quotient at VO_2run.

(MortalitySmooth *R*-package). Standard errors were calculated from smoothed log-mortality rates. Confidence intervals were calculated from the standard errors following the normality assumption.

3. Results

Body mass (BM) did not differ significantly between voles from A and C lines even though it tended to be 6.5% higher in the A lines (overall effect of selection: $P = 0.131$; Table 2, Fig. 1.a; only at age class I: $P = 0.425$, II: $P = 0.693$, III: $P = 0.740$; Supplement IV: Table S4). Body mass increased by 24% between the I and II age classes ($P < 0.001$), and remained constant later on (age classes II-III: $P = 0.956$; I-III: $P < 0.001$; Table S4; overall effect of age: $P < 0.001$; Table 2). The increase in BM with age was similar in A and C lines, i.e. no significant selection × age interaction was found ($P = 0.752$; Table 2, Fig. 1.a). Males had a 16% higher mass than females, but interactions of sex with selection or age were not significant (Table 2).

The maximum sprint speed (VSmax) did not change with body mass ($P = 0.187$; Table 2, Supplement IV: Fig. S3). The overall effect of selection was not significant ($P = 0.225$; Fig. 1.b). However, the VSmax increased with age by 18% between age class I and II ($P < 0.001$), and by 16% between II and III ($P < 0.001$; I-III: $P < 0.001$; Table S4; overall effect of age: $P < 0.001$; Table 2), with a highly significant selection × age interaction ($P = 0.001$; Table 2). At age class I, VSmax tended to be 19% lower in A than in C lines ($P = 0.126$), but the difference diminished at age classes II and III ($P = 0.991$ and $P = 0.972$; Table S4). Thus, the interaction was apparently due to a different pattern of changes in A and C lines only between age classes I and II (Table 2, Fig. 1.b): VSmax increased with age by 27% in the A lines ($P < 0.001$), and by 10% in the C lines ($P = 0.009$; Table S4). However, between age classes II and III the increase of VSmax was similar in A and C lines (by about 16%; both $P < 0.001$; Table S4). To further investigate the selection × age interaction in the older age class, crucial for testing the hypotheses concerning the ageing process, an additional analysis was performed for age classes II and III only. This analysis confirmed that the selection × age interaction was clearly non-significant for the age classes II-III ($P = 0.751$; Supplement IV: Table S5). In both models, the VSmax did not differ between sexes (Table 2, Table S5), but the model for age classes II-III showed a significant sex × selection interaction: VSmax was 12% higher in C-line males than females (Table S5).

The maximum forced exercise-induced metabolic rate (VO_2run) increased with body mass (the common slope ± CI: 0.082 ± 0.022 ml O_2/(min g); $P < 0.001$; Table 2, Fig. 2.a). However, preliminary analysis showed significant interactions of body mass with selection ($F_{1,362} = 5.44$, $P = 0.020$) and body mass with age ($F_{2,228} = 8.63$, $P < 0.001$), which apparently were caused by a steeper regression slope in A-line voles at age class I compared to that in all other age classes (Fig. 2.a). Therefore, to investigate if VO_2run differed

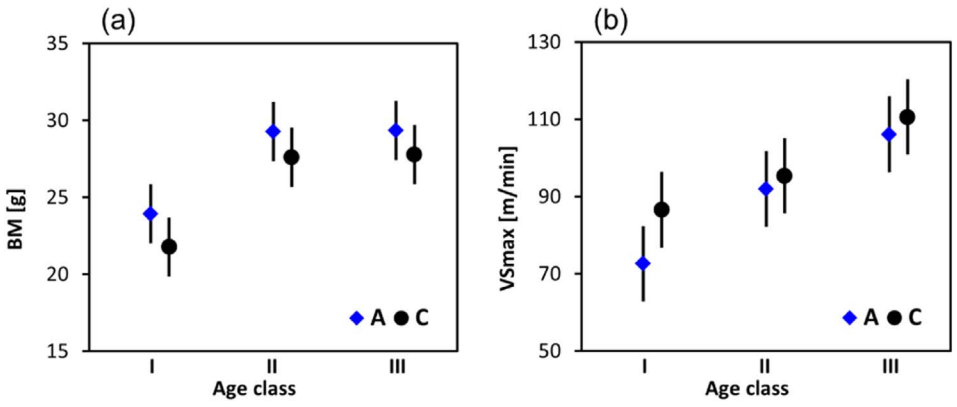

Fig. 1. (a) Body mass [BM; g] and (b) maximum sprint speed [VSmax; m/min] of bank voles from lines selected for high aerobic capacity (A) and from unselected control lines (C) in three age classes (I: 3–5, II: 12–14, III: 17–19 months); adjusted LSM ± 95% CI.

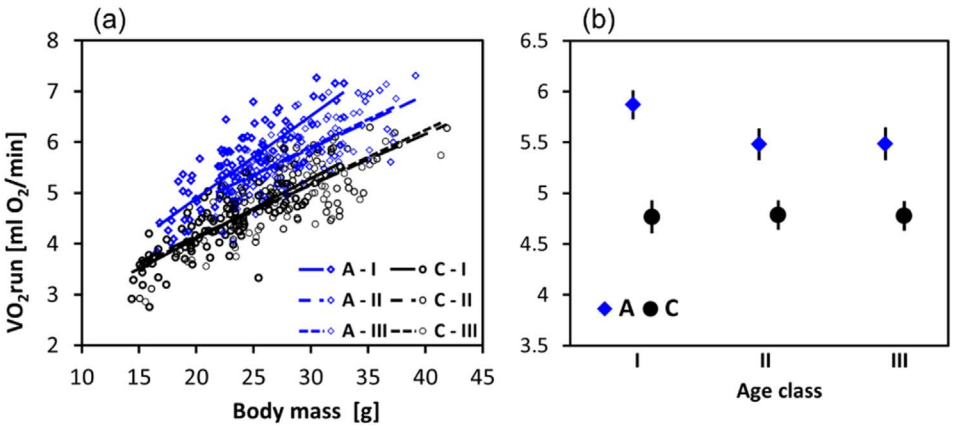

Fig. 2. (a) Individual values of the maximum forced exercise-induced metabolic rate [VO_2run; ml O_2/min] of bank voles from lines selected for high aerobic capacity (A) and from unselected control lines (C) in three age classes (I: 3–5, II: 12–14, III: 17–19 months) in relation to body mass [g] and (b) adjusted LSM ± 95% CI of VO_2run in the selection and age classes, computed for the overall mean body mass (26.14 g).

between the selection directions and the age classes at observed range of body mass, we tested the differences between these groups with a model including the interactions, at carefully chosen set of body masses: 15 g = minimum value for A and C lines at age class II, 26.14 g = the overall mean value, and 33 g = maximum value for A and C lines at age class I (Supplement IV: Table S2).

The VO_2run was 13% higher in A than in C lines at the lower end of body mass range (BM = 15 g; Table S2). The difference increased with increasing body mass: to 15% at mean body mass (BM = 26.14 g; $P < 0.001$; Table S2, Table 2) and to 16% at the upper end of the mass range (BM = 33 g; Table S2). Thus, irrespective of the differences in regression slopes, VO_2run was higher in A than in C lines for the entire observed range of body mass. At the minimum body mass (15 g), VO_2run did not differ between age classes I and II or III (Table S2). However, the regression slope was steeper in age class I than in II or III (slope ± CI: age class I: 0.111 ± 0.025; II: 0.093 ± 0.025; III: 0.096 ± 0.024 ml O_2/(min g). Therefore, at the mean observed body mass (26.14 g) the VO_2run was about 3.5% higher in age class I than in II ($P = 0.001$) or III ($P = 0.002$; Table S2; overall effect of age: $P = 0.001$; Table 2), and the difference increased further to about 8% at 33 g (Table S2). The VO_2run did not differ between age classes II and III throughout the entire range of observed body mass (BM = 26.14 g, $P = 1.000$; Table S2). Therefore, irrespective of the different regression slopes between age classes, in the entire range of body mass, the VO_2run was higher in age class I than in II or III, and did not differ between age classes II and III.

The above comparisons of VO_2run across age classes are, however, complicated by a highly significant selection × age interaction ($P < 0.001$; Table 2, Fig. 2.b). At mean body mass (26.14 g), the VO_2run was 19% higher in A than in the C lines at age class I ($P < 0.001$), whereas at age classes II and III the difference decreased to 13% (both $P < 0.001$; Table S4). Therefore, the selection × age interaction apparently resulted from different pattern of changes only between age classes I and II (Fig. 2.b): VO_2run decreased in the A lines by about 7% ($P < 0.001$), but did not change between age class II and III ($P = 1.000$), whereas in the C lines, it remained constant across all three age classes (both $P = 1.000$). To further investigate VO_2run changes in older age, crucial for the inferences concerning ageing, we performed an additional analysis, restricted to age classes II and III only. For these two age classes the regression slopes were homogeneous (body mass × selection: $F_{1,138} = 0.16$, $P = 0.689$; body mass × age; $F_{1,73.2} = 0.35$, $P = 0.557$; Fig. 2.a), and therefore all interactions with body mass were excluded from the final model. The results concerning simple effects of selection and age were qualitatively the same as in the model for age classes I-III (Table S5). However, the selection × age interaction was clearly not significant ($P = 0.665$; Table S5). In both models, the VO_2run was about 3% higher in males than females, with no significant interactions of sex with selection or age (Table 2, Table S5).

The maximum long-distance running speed (VLmax) and the speed achieved at VO_2run (VVO_2) did not change significantly with body mass (VLmax: $P = 0.159$; VVO_2: $P = 0.974$; Table 2, Supplement IV: Fig. S4, Fig. S5). The mass-adjusted VLmax tended to be about 9% higher in A than in C lines ($P = 0.068$; Table 2, Fig. 3.a; only at age class I: $P = 0.119$, II: $P = 0.506$; III: $P = 0.715$; Table S4). It did not change between age class I and II ($P = 0.832$) and decreased by 8% between age class II and III ($P = 0.003$, I-III: $P = 0.118$; Table S4; overall effect of age: $P = 0.004$; Table 2). However, the pattern of changes with age was similar in A and C lines (selection × age interaction: $P = 0.619$; Fig. 3.a). Mass-adjusted VVO_2 did not differ between A and C lines ($P = 0.302$; Table 2, Fig. 3.b; at age class I: $P = 0.356$, II: $P = 0.995$, III: $P = 0.100$; Table S4). It did not change between age classes I and II ($P = 0.273$) and decreased by 12% between age classes II and III ($P = 0.008$; I-III: $P = 0.067$; Table S4; overall effect of age: $P = 0.011$; Table 2), similarly in A and C lines (selection × age interaction: $P = 0.268$; Fig. 3.b). The effect of sex and its interactions with selection or age were not significant for either VLmax or VVO_2 (Table 2).

Respiratory quotient at VO_2run (RQ) did not change with body mass ($P = 0.103$; Table 2, Supplement IV: Fig. S6). The simple effect of selection was not significant ($P = 0.858$; Table 2; Fig. 3.c). At age class I the RQ was 0.98, but it increased to 1.00 between age classes I and II ($P = 0.036$), and additionally to 1.02 between age classes II and III

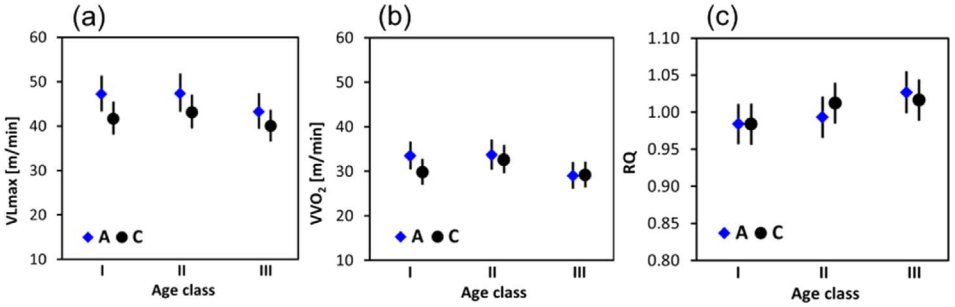

Fig. 3. (a) Maximum long-distance running speed [VLmax; m/min], (b) running speed achieved at maximum metabolic rate [VVO_2; m/min] and (c) respiratory quotient at maximum metabolic rate [RQ], in bank voles from lines selected for high aerobic capacity (A) and from unselected control (C), in three age classes (I: 3–5, II: 12–14, III: 17–19 months); adjusted back-transformed LSM ± 95% CI.

($P = 0.008$; I-III: $P < 0.001$; Table S4; overall effect of age: $P < 0.001$; Table 2). However, a marginally significant selection × age interaction was found ($P = 0.054$; Table 2, Fig. 3.c). The RQ did not differ between voles from A and C lines at any of the three age classes (I: $P = 1.000$, II: $P = 0.855$, III: $P = 0.989$; Table S4). However, in the A lines RQ remained nearly constant between age classes I and II ($P = 0.928$), and increased to 1.03 between age classes II and III ($P = 0.003$), whereas in the C lines it increased to 1.01 between age class I and II ($P = 0.40$), and remained nearly constant between II and III ($P = 0.997$; Table S4). Next, an additional analysis was performed for age classes II and III only. In this model, the selection × age interaction was clearly significant ($P = 0.017$; Table S5), and the pattern observed in the main model was confirmed: RQ increased with age in the A lines, but not in the C lines (Fig. 3.c). In both models, RQ did not differ between sexes, and the interactions of sex with selection or age were not significant (Table 2, Supplement IV: Table S5).

Likelihood ratio test showed significant random effect of differences among replicate lines within selection direction in the case of BM ($\chi^2 = 19.1$, $P < 0.001$), VSmax ($\chi^2 = 14.8$, $P < 0.001$), VO_2run ($\chi^2 = 5.8$, $P = 0.016$), VLmax ($\chi^2 = 5.4$, $P = 0.020$) and RQ ($\chi^2 = 11.1$, $P = 0.001$), but not VVO_2 ($\chi^2 = 3.1$, $P = 0.078$). The random line × age interaction was significant only in BM ($\chi^2 = 8.1$, $P = 0.004$), whereas line × sex interaction only in VLmax ($\chi^2 = 4.4$, $P = 0.036$).

The food consumption rate was about 8% higher in A than in C lines at the age of 7 and 14 months (mean with confidence limits: A: 6.12 (5.87–6.38), C: 5.62 (5.39–5.86) g/d; $P = 0.006$), but it was not significantly affected by age ($P = 0.173$), sex, or interactions of these factors (Supplement II: results and Fig. S2).

The survival distributions did not differ between selection generations 13 and 14 in either A or C lines (log-rank test: A: $\chi^2 = 3.21$, $P = 0.073$; C: $\chi^2 = 0.083$, $P = 0.774$; Gehan-Breslow-Wilcoxon test: A: $\chi^2 = 3.22$, $P = 0.073$, C: $\chi^2 = 1.43$, $P = 0.232$) or between males and females (log-rank test: A: $\chi^2 = 0.00$, $P = 0.970$, C: $\chi^2 = 0.42$, $P = 0.422$; Gehan-Breslow-Wilcoxon test: A: $\chi^2 = 0.06$, $P = 0.814$, C: $\chi^2 = 0.44$, $P = 0.506$). Therefore, the main analyses of survivorship and mortality were performed on combined data for both generations and sexes. Survivorship was lower in the young-adult bank voles from A than from C lines (Gehan-Breslow-Wilcoxon test: $\chi^2 = 10.56$, $P = 0.0012$; Fig. 4.a). Moreover, detailed analysis by Kaplan-Meier estimators revealed that the differences between survivorship curves concerned only at the age range between 32 and 135 days (Fig. 4.a). However, although in middle-aged and senile age ranges the mortality rates tended to be lower in animals from A than in those form C lines, the difference was not significant (log-rank test: $\chi^2 = 3.11$, $P = 0.079$; Fig. 4.b).

Post mortem diagnoses showed that animals suffered a wide range of age-related diseases at old age, which included cases of urinary system disorders (degeneration of kidney tissue, nephrolithiasis, and urolithiasis), obesity, tumours, male reproductive system disorders, fur loss, and problems with eyes. The age-related ailments and diseases, however, were similar in A and C animals (Supplement V: Table S6).

4. Discussion

The selection for high swim-induced maximum aerobic metabolism (VO_2swim) resulted also in changes of a few physiological performance traits. As young adults, voles from selected (A) lines tended to have lower sprint speeds (VSmax, Fig. 1.b) than ones from control (C) lines (age class I: 3–5 months). However, selection for a partly voluntary exercise led to an increase in the maximum metabolic rate achieved during forced running (VO_2run, Fig. 2). The difference in VO_2run between voles from A and from C lines were highest at age class I, but it was also profound later in life (age classes II: 12–14, III: 17–19 months). The voles from A lines also tended to have higher maximum long-distance running speed (VLmax, Fig. 3.a), but not speed at VO_2run (VVO_2, Fig. 3.b), however, the difference between A and C lines tended to be higher in young-adults, than later on. Therefore, in the case of these performance traits, the selection seemed to act strongest at age class I, which is similar to the age when it is applied. Apparently, the selection tended to act inversely on the VSmax and the VLmax. A reversed association between sprint and aerobic running was also found in mice selected for high daily wheel running (Dlugosz et al., 2009), where selected animals ran faster on wheels than control ones, but had lower sprinting speeds. Voles from the A and C lines did not have a different overall respiratory quotient at VO_2run (RQ, Fig. 3.c), which in young-adults was nearly equal at 1.0, indicating that the main source of their metabolism was carbohydrate oxidation (Anderson and Rhodes, 1989). All of the investigated features are known to be susceptible to age-related ailments (Koch et al., 2011; Horner et al., 2011). However, surprisingly for such a short lived species, the voles from either A or C lines seemed to suffer only some changes to physiological fitness and mortality.

Body mass (BM, Fig. 1.a) increased with age between age classes I and II, after which it remained constant. This pattern of BM increase is in agreement with previous studies on mice, and it presumably could be caused by changes in adiposity, bone mass, or hypertrophy of the internal organs (Lessard-Beaudoin et al., 2015), which is however unknown in this study. The BM of the voles from A and C lines followed similar patterns of changes across all three age classes (Fig. 1.a). Regardless, all the results concerning the investigated physiological performance traits were corrected for body mass.

Unexpectedly, the maximum sprint speed (VSmax, Fig. 1.b) of voles from A and C lines were not lower, but progressively higher at consecutive age classes. It was shown that quadrupeds are able to vary their

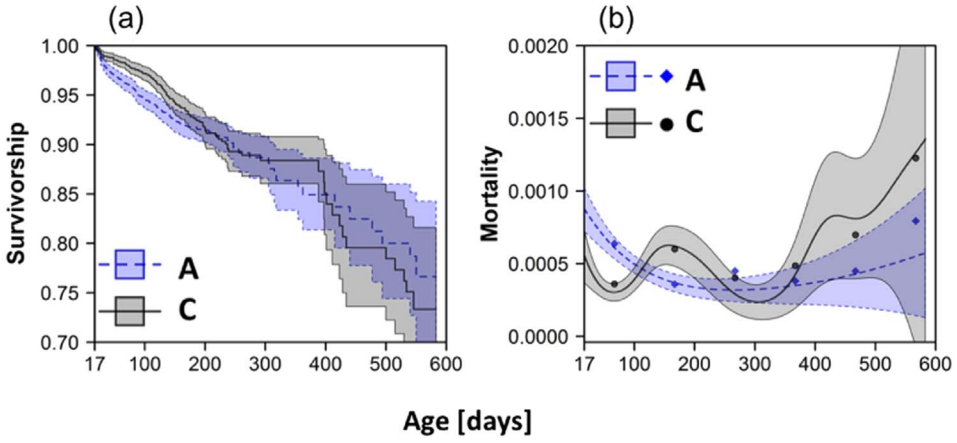

Fig. 4. (a) Survivorship based on Kaplan-Meier estimator and (b) mortality rates fitted with P-splines, in bank voles from lines selected for high aerobic capacity (A) and unselected control lines (C). The diamonds (A) and dots (C) represent empirical mortality rates calculated for consecutive 30-day intervals from aggregated daily mortality. The shaded areas represent 95% CI.

footfall pattern to try to compensate for effects of ageing by modifying the speed, metabolic efficiency and individual limb load (Horner et al., 2011). The biological age of a peak in sprint performance is a subject of debate, however, in some species, it can be achieved rather late after sexual maturity (Marck et al., 2016). However, the large and progressive increase of sprint speed with age is unexplained. Sprint performance is determined primarily by musculoskeletal function and limb mechanics (Bundle and Weyand, 2012). In an ecological framework, sprint speed is mainly vital to short activities, like escaping predators (Andrew et al., 2016). It is possible, that natural selection favors some changes in physiology or mechanics, which cause a shift into high sprint capacity in old age. However, the more likely explanation could be behavioural. During the measurements of VSmax, the animals were chased from one side of a racetrack to another. It is possible that young-adult animals are bolder and try to resist the procedure, whereas older ones are easier to urge to run because they do not intend to struggle. Learning the test procedure is an unlikely explanation of the increase of VSmax with age because the test did not include any reward earned depending on how fast the vole would run. However, the increased performance could be due to an attuned reaction to stress. It is known that rodents often react to stress by immobility (freezing) rather than escaping. However, throughout their life, the voles are getting accustomed to being handled by people. Therefore, the adverse effects of stress associated with the procedure can decrease with age, which can result in an increased sprint speed. At any rate, because between age classes I and II the VSmax in A lines increased more than in the C lines (Fig. 1.b), the initial difference between selection directions vanished already at age class II. However, at senescence, selection clearly did not cause changes in the rate of increase in VSmax with age.

The maximum forced exercise-induced metabolic rate (VO_2run, Fig. 2) decreased with age between age classes I and II, but not between age classes II and III. Therefore, in the case of VO_2run the actual effect of ageing, understood as age-related detrimental changes at senescence, in this study, was not observed. Aerobic capacity reflects cardiovascular efficiency and adaptations of skeletal muscles to use oxygen, according to energy needs (Koch et al., 2011). During ageing, aerobic capacity can be altered by a range of age-related critical effects (Vaanholt et al., 2010; Navarro et al., 2004). However, it is possible that alterations of VO_2run in the bank vole species appear even later in life than it was shown in this study. Moreover, the VO_2run decreased between the I and II age classes only in the A lines and it remained constant later on, while in the C lines it remained constant throughout all age classes (Fig. 2.b). This observation is consistent with other studies, in which selected mice had distinctly higher voluntary wheel-running activity than unselected control ones until the age of a few months, before the difference diminished (Vaanholt et al., 2008, 2010), and with studies on fruit flies, where selection for high reproductive output led to a fast decrease of reproductive capacity (Rose et al., 2008). However, even though VO_2run in the voles from A lines decreased, it was still elevated compared to those from C lines until senility.

The maximum long-distance running speed (VLmax, Fig. 3.a) and speed at VO_2run (VVO_2, Fig. 3.b) of the voles did not change between age classes I and II, and decreased later on, between II and III. A similar pattern was observed in various species, where a peak of physiological performance was followed by a decline with ageing (Marck et al., 2016). Long-distance running depends primarily on traits associated with aerobic capacity, including efficiency of the cardiovascular system or mitochondria number and function (Koch and Britton, 2001; Koch et al., 2011; Dlugosz et al., 2009). Age-related decline in running speed is a consequence of decreased muscle aerobic capacity (McGregor et al., 2014), and defects of locomotor mechanics, including the weakening of muscle-tendon units, postural changes, and loss of balance control (Horner et al., 2011). The age at which peak performance is achieved can vary among species from different taxa, but it is typically achieved around sexual maturity (Marck et al., 2016). Unexpectedly, it seems, that in bank voles peak performance was achieved rather late in life, at the age of about one year, before decreasing in senile individuals. Aerobic locomotion is largely essential to long-term activities in small rodents, such as foraging for food or patrolling territories (Andrew et al., 2016). Therefore it is likely, that in the short lived bank vole species, most energy is invested into traits associated with reproduction and feeding the offspring until the age of a few months. For individuals born in early spring this could maximize chances of rearing more than one litter during summer, when resources are in abundance, whereas for individuals born later in the season, it could increase breeding chances in the months preceding winter when the resources become more scarce. However, even though in this study a peak of running performance was observed later in life than could be expected, it is clear that at senility, the voles from A lines suffered similar detrimental changes than ones from the C lines (Fig. 3.a–b), which is consistent with another study conducted on mice selected for high wheel-running activity (Marck et al., 2016).

The RQ at VO_2run in bank voles increased with age from below 1.0 to about 1.02 (RQ, Fig. 3.c), which indicates changes in the proportion of metabolic processes. Apparently, at a young age animals used mainly oxidation of carbohydrates in aerobic metabolism, whereas RQ exceeding 1.0 at senility indicate, that the lactate threshold lowered and a higher proportion of anaerobic metabolism was used, so the contribution of anaerobic glycolysis became higher (Anderson and Rhodes, 1989). Between the age classes of I and II, the RQ increased only in the C lines, whereas between the age classes of II and III, only in the A lines (Fig. 3.c). So, voles from A lines were able to sustain high performance until quite advanced age, but ageing, as age-related detrimental changes observed at senility, was clearly more profound than in voles from C lines. On the other hand, in voles from C lines performance decreased earlier, but at senility, it was better sustained than in voles from A lines. Therefore, this result can be understood as consistent with the classical ROL theory of ageing.

The mortality rates were significantly higher in voles from A than in those from C lines, but only within the age range of 32–135 days (Fig. 4.b). Importantly, in our colony, at the age of 30–32 days, individuals are placed in a new cage, together with two to three unfamiliar same-sex cage-mates. It is therefore likely, that these individuals tend to arrange a hierarchy and engage in fighting (Łopucki, 2007). Our observations suggest that animals from A lines tend to fight more than those from C lines. The explanation could also be physiological, as a consequence of a trade-off between sustaining high aerobic capacity and effective immune system, among which the other is especially costly during the critical developmental period (Lee, 2006). On the other hand, in older voles (above 135 days), mortality tended to be lower in A than in C lines, but the difference was not significant (Fig. 4). It can be speculated that the difference could be due to beneficial health effects of increased locomotor exercise (Navarro et al., 2004), observed in voles from A lines. It should be noted, however, that even though mortality was evaluated using a sample of over 5000 individuals, only a small number of them died from natural causes (353), or were kept until the age of advanced senility (213). Consequently, the estimates of survivorship bear a large error (Fig. 4), and therefore results do not warrant a strong conclusion. Interestingly, post mortem diagnoses revealed that already at the age ranges used in this study, animals suffered severe disorders of multiple organ systems (Table S5). Additionally, no clear differences between the disorders found in voles from A and C lines were detected.

Results of our work are similar to a study conducted on mice artificially selected for high wheel-running activity (Vaanholt et al., 2010), in which the link between physiological performance and lifespan was not clear. The results are, however, inconsistent with a study on rats selectively bred for high and low endurance treadmill-running capacity (Koch et al., 2011), where high-endurance rats had better survival than the low capacity ones. However, because the low-endurance rats had inherently compromised parameters of the cardiovascular system (Koch et al., 2011), this selection experiment is more suited as a model of cardiovascular

diseases in geriatric biology, rather than to test the basic theories of ageing. Both of these studies were performed on laboratory species, which underwent domestication (Ricklefs, 2010; Selman et al., 2012; Garland et al., 2011). In contrast, the selection experiment used here, incorporates the benefits of using non-laboratory species and experimental evolution directed towards a physiological performance trait. At senescence, the voles from A lines manifested only some health benefits, as they retain increased VO_2run and tended to have lower mortality, than voles from C lines. The lack of clearly visible health benefits during ageing is unexpected, given that high physiological performance is linked to decreased risk of atherosclerotic cardiovascular disease (Thompson, 2003), obesity, or inflammation (Kokkinos and Myers, 2010). However, the comparison between voles forms A and C lines is similar to one between healthy individuals and high capacity athletes. It is not certain how a largely increased respiration can affect ROS production (Barja, 2007), but the level of antioxidant defence can remain unchanged with age (Barreiro et al., 2006). In our earlier work, breeding bank voles from A and C lines that were only a few months-old did not differ in levels of oxidative damage or antioxidants, but the level of antioxidants was inversely correlated with the oxidative damage (Ołdakowski et al., 2015). Here, voles from A lines were more affected by ageing than those from C lines only in the case of RQ. Apparently, voles from A lines sustained effective antioxidant defence, not only as young-adults, but also later in life. Another likely explanation can be related to the protein turnover rate. Proteins are targets of post-translational damage, but the accumulation of damaged proteins depends not only on increased damage occurrence, but also on the efficiency of cellular systems responsible for the elimination of such damaged proteins, which, however, can be disrupted with ageing (Ortuño-Sahagún et al., 2014). Possibly, the rate of protein turnover is higher in voles from the A lines, and it remains higher throughout the entire life. It is also possible that differences in the rate of ageing between A and C lines would be more profound only in presence of additional physiological burden.

Although in our study age-related changes were observed in investigated physiological performance traits, the detrimental changes at senility were found only in the case of VLmax, VVO_2, and RQ. However, we did not observe remarkable and consistent differences in the pattern of age-related changes between the A and C lines. Also, differences in mortality rates between the A and C lines were not present in the old age ranges. Therefore, we conclude that selection for increased aerobic exercise metabolism does not inflict either obvious positive or negative effects in the ageing process. However, perhaps the most surprising finding of our work is that the physiological performance and mortality of the voles remained quite robust to ageing within the investigated age range. Therefore, as the symptoms of ageing were only moderately visible, it is possible that the selection would inflict changes in the pattern of ageing at a still older age when symptoms of senility could be more profound. Note, however, that the animals were maintained until the age of about the maximum lifespan of bank voles under natural conditions, achieved in nature by only a small percentage of individuals (Bernshtein et al., 1999). According to the evolutionary theory of ageing, natural selection acts in favour of repairing biological machinery at a young age, but does not support mechanisms preventing deterioration at old age, because most individuals die of reasons other than ageing, long before they achieve the age of senility (Jones et al., 2014). Apparently, the biochemical mechanisms supporting high performance in young voles help also to sustain health in the older age range, even though natural selection acting at this age is weak. Alternatively, it is possible that elderly individuals, although small in number, do have a significant contribution to whole-population reproduction (e.g., as a result of their experience and enhanced competitive abilities). Both of these possibilities should be subjected to further research. Therefore, even though the premature termination of the experiment made it less interesting from the point of view of geriatric medicine, it remains adequate in the context of evolutionary processes, and the basic aspects of ageing research.

Conflict of interest statement

The authors declare no conflicts of interest.

Acknowledgments

We would like to thank Katarzyna Baliga-Klimczyk, Katarzyna M. Chrząścik, Joanna Hajduk, Ilona Nowak, Krystyna Ścigajło and Monika Skrzypczak for their excellent assistance with animal care, and Clare Stawski for a language help. Approval for all procedures in this study was granted by the Local Ethical Committee for Ethics in Animal Research in Krakow, Poland, decisions No. 99/2006 and No. 62/2011.

Funding

This work was supported by the Polish National Science Centre (grant numbers 2011/01/N/NZ4/00124 to A.M.R. and N N303 816740 to P.K.); Jagiellonian University, Krakow, Poland (grant numbers DS/WBINOZ/INoŚ/757 to P.K. and DS/MND/WBiNoZ/INoŚ/22/2011 to A.M.R); and the European Union under the European Social Fund (POKL.04.01.01-053/09) to A.M.R.

Appendix A. Supplementary methods and results

Supplementary methods and results to this article can be found online at http://dx.doi.org/10.1016/j.exger.2017.08.007.

References

Ali, H.S., Drewes, S., Sadowska, E.T., Mikowska, M., Groschup, M.H., Heckel, G., Koteja, P., Ulrich, R.G., 2014. First molecular evidence for *Puumala* hantavirus in Poland. Virus 6 (1), 340–353. http://dx.doi.org/10.3390/v6010340.

Anderson, G.S., Rhodes, E.C., 1989. A review of blood lactate and ventilatory methods of detecting transition thresholds. Sports Med. 8 (1), 43–55 (doi: 0112-1642/89/0007-00043).

Andrew, J.R., Saltzman, W., Chappell, M.A., Garland Jr., T., 2016. Consequences of fatherhood in the biparental California mouse (*Peromyscus californicus*): locomotor performance, metabolic rate, and organ masses. Physiol. Biochem. Zool. 89 (2), 130–140. http://dx.doi.org/10.1086/685435.

Balcombe, N.R., Sinclair, A., 2001. Ageing: definitions, mechanisms and the magnitude of the problem. Best Pract. Res. Clin. Gastroenterol. 15 (6), 835–849. http://dx.doi.org/10.1053/bega.2001.0244.

Barja, G., 2004. Free radicals and aging. Trends Neurosci. 27 (10), 595–600. http://dx.doi.org/10.1016/j.tins.2004.07.005.

Barja, G., 2007. Mitochondrial oxygen consumption and reactive oxygen species production are independently modulated: implications for aging studies. Rejuvenation Res. 10 (2), 215–224. http://dx.doi.org/10.1089/rej.2006.0516.

Barja, G., 2013. Updating the mitochondrial free radical theory of aging: an integrated view, key aspects, and confounding concepts. Antioxid. Redox Signal. 19 (12), 1420–1445. http://dx.doi.org/10.1089/ars.2012.5148.

Barreiro, E., Coronell, C., Laviña, B., Ramírez-Sarmiento, A., Orozco-Levi, M., Gea, J., 2006. Aging, sex differences, and oxidative stress in human respiratory and limb muscles. Free Radic. Biol. Med. 41 (5), 797–809. http://dx.doi.org/10.1016/j.freeradbiomed.2006.05.027.

Bernshtein, A.D., Apekina, N.S., Mikhailova, T.V., Myasnikov, Y.A., Khlyap, L.A., Korotkov, Y.S., Gavrilovskaya, I.N., 1999. Dynamics of *Puumala* hantavirus infection in naturally infected bank voles (*Clethrinomys glareolus*). Arch. Virol. 144 (12), 2415–2428. http://dx.doi.org/10.1007/s007050050654.

Bratic, I., Trifunovic, A., 2010. Mitochondrial energy metabolism and ageing. Biochim. Biophys. Acta - Bioenergetics 1797 (6–7), 961–967. http://dx.doi.org/10.1016/j.bbabio.2010.01.004.

Brunet-Rossinni, A.K., Austad, S.N., 2004. Ageing studies on bats: A review. Biogerontology 5 (4), 211–222. http://dx.doi.org/10.1023/B:BGEN.0000038022.65024.d8.

Bundle, M.W., Weyand, P.G., 2012. Sprint exercise performance: does metabolic power matter? Exerc. Sport Sci. Rev. 40 (3), 174–182. http://dx.doi.org/10.1097/JES.0b013e318258e1c1.

Chigurupati, S., Son, T.G., Hyun, D.H., Lathia, J.D., Mughal, M.R., Savell, J., ... Mattson, M.P., 2008. Lifelong running reduces oxidative stress and degenerative changes in the testes of mice. J. Endocrinol. 199 (2), 333–341. http://dx.doi.org/10.1677/JOE-08-0306.

Chrząścik, K.M., Sadowska, E.T., Rudolf, A., Koteja, P., 2014. Learning ability in bank voles selected for high aerobic metabolism, predatory behavior and herbivorous capability. Physiol. Behav. 135, 143–151. http://dx.doi.org/10.1016/j.physbeh.2014.06.007.

De Magalhães, J.P., Costa, J., Church, G.M., 2007. An analysis of the relationship between metabolism, developmental schedules, and longevity using phylogenetic independent contrasts. J. Gerontol. A Biol. Sci. Med. Sci. 62 (2), 149–160. http://dx.doi.org/10.1093/gerona/62.2.149.

Dheyongera, G., Grzebyk, K., Rudolf, A., Sadowska, E.T., Koteja, P., 2016. The effect of chlorpyrifos on thermogenic capacity of bank voles selected for increased aerobic exercise metabolism. Chemosphere 149, 383–390. http://dx.doi.org/10.1016/j.chemosphere.2015.12.120.

Dlugosz, E.M., Chappell, M.A., McGillivray, D.G., Syme, D.A., Garland Jr., T., 2009. Locomotor trade-offs in mice selectively bred for high voluntary wheel running. J. Exp. Biol. 212 (16), 2612–2618. http://dx.doi.org/10.1242/jeb.029058.

Garland Jr., T., Schutz, H., Chappell, M.A., Keeney, B.K., Meek, T.H., Copes, L.E., ... Eisenmann, J.C., 2011. The biological control of voluntary exercise, spontaneous physical activity and daily energy expenditure in relation to obesity: human and rodent perspectives. J. Exp. Biol. 214 (2), 206–229. http://dx.doi.org/10.1242/jeb.048397.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11 (3), 298–300.

Hayes, J.P., Garland Jr., T., 1995. The evolution of endothermy: testing the aerobic capacity model. Evolution 49 (5), 836–847. http://dx.doi.org/10.1111/j.1558-5646.1995.tb02320.x.

Holloszy, J.O., 1997. Mortality rate and longevity of food-restricted exercising male rats: a reevaluation. J. Appl. Physiol. 82 (2), 399–403.

Horner, A.M., Russ, D.W., Biknevicius, A.R., 2011. Effects of early-stage aging on locomotor dynamics and hindlimb muscle force production in the rat. J. Exp. Biol. 214 (21), 3588–3595. http://dx.doi.org/10.1242/jeb.055087.

Hulbert, A.J., Clancy, D.J., Mair, W., Braeckman, B.P., Gems, D., Partridge, L., 2004. Metabolic rate is not reduced by dietary-restriction or by lowered insulin/IGF-1 signalling and is not correlated with individual lifespan in *Drosophila melanogaster*. Exp. Gerontol. 39 (8), 1137–1143. http://dx.doi.org/10.1016/j.exger.2004.04.006.

Jaromin, E., Sadowska, E.T., Koteja, P., 2016. A dopamine and noradrenaline reuptake inhibitor (bupropion) does not alter exercise performance of bank voles. Curr. Zool. 62 (3), 307–315. http://dx.doi.org/10.1093/cz/zow026.

Jones, O.R., Scheuerlein, A., Salguero-Gómez, R., Camarda, C.G., Schaible, R., Casper, B.B., ... Vaupel, J.W., 2014. Diversity of ageing across the tree of life. Nature 505 (7482), 169–173. http://dx.doi.org/10.1038/nature12789.

Kallio, E.R., Voutilainen, L., Vapalahti, O., Vaheri, A., Henttonen, H., Koskela, E., Mappes, T., 2007. Endemic hantavirus infection impairs the winter survival of its rodent host. Ecology 88 (8), 1911–1916. http://dx.doi.org/10.1890/06-1620.1.

Klein, J.P., Moeschberger, M.L., 2006. Survival Analysis: Techniques for Censored and Truncated Data. Springer Science & Business Media, New York.

Koch, L.G., Britton, S.L., 2001. Artificial selection for intrinsic aerobic endurance running capacity in rats. Physiol. Genomics 5 (1), 45–52.

Koch, L.G., Kemi, O.J., Qi, N., Leng, S.X., Bijma, P., Gilligan, L.J., ... Wisløff, U., 2011. Intrinsic aerobic capacity sets a divide for aging and longevity. Circ. Res. 109 (10), 1162–1172. http://dx.doi.org/10.1161/CIRCRESAHA.111.253807.

Kokkinos, P., Myers, J., 2010. Exercise and physical activity clinical outcomes and applications. Circulation 122 (16), 1637–1648. http://dx.doi.org/10.1161/CIRCULATIONAHA.110.948349.

Konarzewski, M., Sadowski, B., Jozwik, I., 1997. Metabolic correlates of selection for swim stress-induced analgesia in laboratory mice. Am. J. Phys. Regul. Integr. Comp. Phys. 273 (1), 337–343.

Konczal, M., Babik, W., Radwan, J., Sadowska, E.T., Koteja, P., 2015. Initial molecular-level response to artificial selection for increased aerobic metabolism occurs primarily through changes in gene expression. Mol. Biol. Evol. 32 (6), 1461–1473. http://dx.doi.org/10.1093/molbev/msv038.

Koteja, P., Baliga-Klimczyk, K., Chrzascik, K.M., Damulewicz, M., Dragosz-Kluska, D., Morawska-Ploskonka, J., 2009. Laboratory model of adaptive radiation: activity and metabolic rates in bank voles from a multidirectional artificial selection experiment. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 153 (2), 146.

Krüger, D.H., Schönrich, G., Klempa, B., 2011. Human pathogenic hantaviruses and prevention of infection. Hum. Vaccin. 7 (6), 685–693. http://dx.doi.org/10.4161/hv.7.6.15197.

Lee, K.A., 2006. Linking immune defenses and life history at the levels of the individual and the species. Integr. Comp. Biol. 46 (6), 1000–1015. http://dx.doi.org/10.1093/icb/icl049.

Lessard-Beaudoin, M., Laroche, M., Demers, M.J., Grenier, G., Graham, R.K., 2015. Characterization of age-associated changes in peripheral organ and brain region weights in C57BL/6 mice. Exp. Gerontol. 63, 27–34. http://dx.doi.org/10.1016/j.exger.2015.01.003.

Lighton Jr., B., 2008. Measuring Metabolic Rates: A Manual for Scientists. Oxford University Press, New York.

Łopucki, R., 2007. Social relationships in a bank vole *Clethrionomys glareolus* (Schreber, 1780) population: video monitoring under field conditions. Pol. J. Ecol. 55 (3), 543.

Marck, A., Berthelot, G., Foulonneau, V., Marc, A., Antero-Jacquemin, J., Noirez, P., ... Hersen, P., 2016. Age-related changes in locomotor performance reveal a similar pattern for *Caenorhabditis elegans*, *Mus domesticus*, *Canis familiaris*, *Equus caballus*, and *Homo sapiens*. J. Gerontol. A Biol. Sci. Med. Sci. 72 (4), 455–463. http://dx.doi.org/10.1093/gerona/glw136.

Margolick, J.B., Ferrucci, L., 2015. Accelerating aging research: how can we measure the rate of biologic aging? Exp. Gerontol. 64, 78–80. http://dx.doi.org/10.1016/j.exger.2015.02.009.

Martinez, R.L.M.C., Naranjo, J.D., 2010. A pretest for choosing between logrank and Wilcoxon tests in the two-sample problem. Metron - Int. J. Stat. 68 (2), 111–125. http://dx.doi.org/10.1007/BF03263529.

McGregor, R.A., Cameron-Smith, D., Poppitt, S.D., 2014. It is not just muscle mass: a review of muscle quality, composition and metabolism during ageing as determinants of muscle function and mobility in later life. Longevity & Healthspan 3 (1), 9. http://dx.doi.org/10.1186/2046-2395-3-9.

Mcnab, B.K., 2005. Uniformity in the basal metabolic rate of marsupials: its causes and consequences. Rev. Chil. Hist. Nat. 78 (2), 183–198.

Melvin, R.G., Van Voorhies, W.A., Ballard, J.W.O., 2007. Working harder to stay alive: metabolic rate increases with age in *Drosophila simulans* but does not correlate with life span. J. Insect Physiol. 53 (12), 1300–1306. http://dx.doi.org/10.1016/j.jinsphys.2007.07.006.

Montgomery, M.K., Hulbert, A.J., Buttemer, W.A., 2011. The long life of birds: the rat-pigeon comparison revisited. PLoS One 6 (8), 24138. http://dx.doi.org/10.1371/journal.pone.0024138.

Navarro, A., Gomez, C., López-Cepero, J.M., Boveris, A., 2004. Beneficial effects of moderate exercise on mice aging: survival, behavior, oxidative stress, and mitochondrial electron transfer. Am. J. Phys. Regul. Integr. Comp. Phys. 286 (3), 505–511. http://dx.doi.org/10.1152/ajpregu.00208.2003.

Niitepõld, K., Hanski, I., 2013. A long life in the fast lane: positive association between peak metabolic rate and lifespan in a butterfly. J. Exp. Biol. 216 (8), 1388–1397. http://dx.doi.org/10.1242/jeb.080739.

Oklejewicz, M., Daan, S., 2002. Enhanced longevity in tau mutant Syrian hamsters, *Mesocricetus auratus*. J. Biol. Rhythm. 17 (3), 210–216.

Ołdakowski, Ł., Wasiluk, A., Sadowska, E.T., Koteja, P., Taylor, J.R., 2015. Reproduction is not costly in terms of oxidative stress. J. Exp. Biol. 218 (24), 3901–3910. http://dx.doi.org/10.1242/jeb.126557.

Ortuño-Sahagún, D., Pallàs, M., Rojas-Mayorquín, A.E., 2014. Oxidative stress in aging: advances in proteomic approaches. Oxidative Med. Cell. Longev. 2014. http://dx.doi.org/10.1155/2014/573208.

Pearl, R., 1928. The Rate of Living. University Press, London.

Petrusewicz, K., 1983. Ecology of the bank vole. In: Acta Theriol. Vol. 28 (1) Polish Scientific Publishers, Warsaw.

Ricklefs, R.E., 2010. Insights from comparative analyses of aging in birds and mammals. Aging Cell 9 (2), 273–284. http://dx.doi.org/10.1111/j.1474-9726.2009.00542.x.

Ristow, M., Schmeisser, K., 2014. Mitohormesis: promoting health and lifespan by increased levels of reactive oxygen species (ROS). Dose-Response 12 (2), 288. http://dx.doi.org/10.2203/dose-response.13-035.Ristow.

Rose, M.R., Burke, M.K., Shahrestani, P., Mueller, L.D., 2008. Evolution of ageing since Darwin. J. Genet. 87 (4), 363–371. http://dx.doi.org/10.1007/s12041-008-0059-6.

Rubner, M., 1908. Das Problem der Lebensdauer und seine Beziehungen zu Wachstum und Ernährung. Oldenbourg. 1908.

Sadowska, E.T., Baliga-Klimczyk, K., Chrząścik, K.M., Koteja, P., 2008. Laboratory model of adaptive radiation: a selection experiment in the bank vole. Physiol. Biochem. Zool. 81 (5), 627–640. http://dx.doi.org/10.1086/590164.

Sadowska, E.T., Stawski, C., Rudolf, A., Dheyongera, G., Chrząścik, K.M., Baliga-Klimczyk, K., Koteja, P., 2015. Evolution of basal metabolic rate in bank voles from a multidirectional selection experiment. Proc. R. Soc. Lond. B Biol. Sci. 282 (1806), 20150025. http://dx.doi.org/10.1098/rspb.2015.0025.

Schaible, R., Scheuerlein, A., Dańko, M.J., Gampe, J., Martínez, D.E., Vaupel, J.W., 2015. Constant mortality and fertility over age in Hydra. Proc. Natl. Acad. Sci. U. S. A. 112 (51), 15701–15706. http://dx.doi.org/10.1073/pnas.1521002112.

Selman, C., Blount, J.D., Nussey, D.H., Speakman, J.R., 2012. Oxidative damage, ageing, and life-history evolution: where now? Trends Ecol. Evol. 27 (10), 570–577. http://dx.doi.org/10.1016/j.tree.2012.06.006.

Speakman, J.R., van Acker, A., Harper, E.J., 2003. Age-related changes in the metabolism and body composition of three dog breeds and their relationship to life expectancy. Aging Cell 2 (5), 265–275. http://dx.doi.org/10.1046/j.1474-9728.2003.00061.x.

Speakman, J.R., Talbot, D.A., Selman, C., Snart, S., McLaren, J.S., Redman, P., ... Brand, M.D., 2004. Uncoupled and surviving: individual mice with high metabolism have greater mitochondrial uncoupling and live longer. Aging Cell 3 (3), 87–95. http://dx.doi.org/10.1111/j.1474-9728.2004.00097.x.

Swallow, J.G., Carter, P.A., Garland Jr., T., 1998. Artificial selection for increased wheel-running behavior in house mice. Behav. Genet. 28 (3), 227–237. http://dx.doi.org/10.1023/A:1021479331779.

Swallow, J.G., Hayes, J.P., Koteja, P., Garland Jr., T., 2009. Experimental evolution of mice in different thermal environments in mice endurance running in rats and voluntary wheel running in mice evolution of the rate of energy metabolism. In: Experimental Evolution: Concepts, Methods, and Applications of Selection Experiments. University of California Press 2009.

Thompson, P.D., 2003. Exercise and physical activity in the prevention and treatment of atherosclerotic cardiovascular disease. Circulation 107 (24), 3109–3116. http://dx.doi.org/10.1161/01.CIR.0000075572.40158.77.

Vaanholt, L.M., Speakman, J.R., Garland Jr., T., Lobley, G.E., Visser, G.H., 2008. Protein synthesis and antioxidant capacity in aging mice: effects of long-term voluntary exercise. Physiol. Biochem. Zool. 81 (2), 148–157. http://dx.doi.org/10.1086/525289.

Vaanholt, L.M., Daan, S., Garland Jr., T., Visser, G.H., 2010. Exercising for life? Energy metabolism, body composition, and longevity in mice exercising at different intensities. Physiol. Biochem. Zool. 83 (2), 239–251. http://dx.doi.org/10.1086/648434.

Wone, B.W.M., Madsen, P., Donovan, E.R., Labocha, M.K., Sears, M.W., Downs, C.J., ... Hayes, J.P., 2015. A strong response to selection on mass-independent maximal metabolic rate without a correlated response in basal metabolic rate. Heredity 114 (4), 419–427. http://dx.doi.org/10.1038/hdy.2014.122.

Zarse, K., Schmeisser, S., Groth, M., Priebe, S., Beuster, G., Kuhlow, D., ... Ristow, M., 2012. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. Cell Metab. 15 (4), 451–465. http://dx.doi.org/10.1016/j.cmet.2012.02.013.