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Wheel-running activity and energy metabolism in relation to ambient temperature in mice selected for high wheel-running activity

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Abstract Interrelationships between ambient temperature, activity, and energy metabolism were explored in mice that had been selectively bred for high spontaneous wheel-running activity and their random-bred controls. Animals were exposed to three different ambient temperatures (10, 20 and 30°C) and wheel-running activity and metabolic rate were measured simultaneously. Wheel-running activity was decreased at low ambient temperatures in all animals and was increased in selected animals compared to controls at 20 and 30°C. Resting metabolic rate (RMR) and daily energy expenditure (DEE) decreased with increasing ambient temperature. RMR did not differ between control and selected mice, but mass-specific DEE was increased in selected mice. The cost of activity (measured as the slope of the relationship between metabolic rate and running speed) was similar at all ambient temperatures and in control and selected mice. Heat generated by running apparently did not substitute for heat necessary for thermoregulation. The overall estimate of running costs was 1.2 kJ/km for control mice and selected mice.

Abbreviations

COT	Costs of transport
CTRL	Control mice
DEE	Daily energy expenditure
ERS	Event recording system
HP	Heat production
MR	Metabolic rate
RMR	Resting metabolic rate
RWA	Wheel-running activity
SD	Standard deviation
SEL	Selected mice
SEM	Standard error of the mean
V	Running speed

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Introduction

Homeothermic animals maintain a rather constant body temperature over a wide ambient temperature range. At low ambient temperature resting homeotherms elevate metabolic levels to compensate for elevated heat loss, while at high ambient temperatures metabolic rates should be low to avoid hyperthermia (Mount 1966; Tieleman et al. 2002). This temperature dependence of metabolic rate becomes more complicated when animals exhibit high locomotor activity,

which is known to be energetically expensive (Taylor et al. 1970). In the cold, high levels of activity may be favourable if activity-related metabolic costs can be used for temperature regulation. The excess heat produced by activity might theoretically substitute for shivering thermogenesis during rest. In principle, if substitution takes place, then the cost of locomotion, formally measured as the energy turnover during activity minus the energy turnover during inactivity, will be reduced at low temperature. If no substitution takes place, then the costs for activity will be added to those for thermoregulation (addition).

The empirical literature is ambiguous on this issue. Several studies demonstrate substitution [in White crowned sparrows, *Zonotrichia leucophrys gambelii* (Paladino and King 1984), potoroo, *Potorous tridactylus* (Baudinette et al. 1993) deer mice, *Peromyscus maniculatus* (Chappell et al. 2004), rat, *Rattus novegicus* (Arnold et al. 1986; Makinen et al. 1996)], but there are also results consistent with addition [Kowari, *Dasyroides byrnei* (MacMillen and Dawson 1986), Chipmunk, *Eutamias merriami* (Wunder 1970) patas monkey, *Erythrocebus patas* (Mahoney 1980)]. This discrepancy among studies may well be related to different conditions. Activity may; for instance, simultaneously lead to reduced insulation and increased heat loss in situations where animals huddle or use bedding material while resting and could therefore mask substitutive effects of activity. If substitution occurs, this would lead to low net costs of activity at low temperatures and thereby should lead us to expect increased activity in the cold.

We decided to exploit mice specifically selected for high activity to test the hypothesis of substitutive metabolic rate in this species. Swallow et al. (1998) have selected mice for high spontaneous wheel-running activity during many generations [for selection procedure see (Swallow et al. 1998)], which make these animals profitable to further explore interrelationships between ambient temperature, activity, and energy metabolism. Animals were bred under ambient temperatures of approximately 22°C. The intensity of spontaneous wheel-running activity has increased over generations and reached an apparent plateau around generation 16 (Bronikowski et al. 2001). In addition, selected animals have become smaller and leaner (I. Girard et al., submitted; Swallow et al. 2001), thereby diminishing whole-animal costs of running in these mice (Rezende et al. 2006). Smaller animals also have larger surface-to-volume ratios, which could make them more susceptible to heat loss at low ambient temperatures. During the selection process for high spontaneous

wheel-running activity, animals seemed to exhibit annual cycles regarding their spontaneous wheel-running activity (Bronikowski et al. 2001) which might be attributed to variations in ambient temperature. In order to evaluate the effects of genetically increased activity in the selected mice, we studied animals from control and selected lines at various ambient temperatures and recorded their wheel-running activity, body temperature, resting metabolic rate and daily energy expenditure.

Materials and methods

Animals and housing

House mice (*Mus domesticus*) that had been selected for high wheel-running activity and their random bred controls were obtained from the lab of Prof. Dr. T. Garland Jr, Riverside, CA, USA. Originally, eight lines of mice consisting of ten pairs each had been created, four in which mice were randomly bred and four in which mice were selected for high wheel-running activity. Selection took place at 6–8 weeks of age during a 6-day trial on wheel running (1.12 m circumference). The most actively running female and male within each family were chosen as breeders for the next generation, without allowing sibling mating.

Eighty breeding pairs (ten per line) from generation 31 of selection were sent to the Zoological Laboratory in Haren (NL) to start a breeding colony without further selection. In the present study, 16 male mice (eight control and eight selected) at the age of 6–8 weeks were used from one of the control (lab designation is line 2) and one of the selection lines (line 7). The mice were individually housed in cages equipped with wheel runnings (Macrolon type I cages ($15 \times 30 \times 15$ cm); UNO Roestvast staal, Zevenaar, The Netherlands; adapted to fit in a wheel running with a diameter of 14 cm) and wood shavings as bedding two weeks prior to the experiments. The mice were on a 12:12 light–dark cycle (lights on at 0800 hours CET) and food (Standard lab chow RMB-H (2181), HopeFarms B.V., Zevenaar, The Netherlands) and water were provided ad libitum.

Experimental protocol

At the start of the experiment animals were randomly divided into two groups (each consisting of four controls and four selected animals) and housed in two separate temperature-controlled rooms. The mice

stayed in these rooms throughout the experiment. All animals were exposed to three ambient temperatures (10, 20, and 30°C) over a time course of three weeks. Each week ambient temperature was increased or decreased by ten degrees, starting at 10°C in room 1 and at 30°C in room 2. Wheel-running activity was recorded on a PC-based event recording system (ERS) with 2 min resolution. Body weight was measured every day at 12 pm.

At noon on day 6 of each stay at a set ambient temperature animals were put with their home cage in a respirometry chamber ($25 \times 35 \times 25$ cm), in the same room as they were housed. Oxygen consumption $\left(\dot{V}O_2, l/h \right)$ and carbon dioxide $\left(\dot{V}CO_2, l/h \right)$ production was then recorded for each individual for 24 h by indirect calorimetry. Our eight-channel open circuit system has been described earlier by Oklejewicz et al. (1997). In brief, oxygen and carbon dioxide concentration of dried inlet and outlet air (drier: molecular sieve 3 Å, Merck) from each chamber was measured with a paramagnetic oxygen analyzer (Servomex Xentra 4100) and carbon dioxide by an infrared gas analyzer (Servomex 1440). The system recorded the differentials in oxygen and carbon dioxide between dried reference air and dried air from the metabolic chambers. Oxygen and carbon dioxide analyzers were calibrated with two gas mixtures with known amount of O_2 and CO_2 prior to each measurement. Flow rate of inlet air was measured with a mass-flow controller (type 5850 Brooks) and set at 30 l/h. Of the respiration air a subsample was passed at a rate of 6 l/h through the drying system and subsequently through the gas analyzers. Ambient temperature in the chamber and cage were measured simultaneously. Data were collected every 10 min for each animal and automatically stored on a computer. Oxygen consumption was calculated according the equation two of Hill (1972) to correct for volume changes with respiratory quotient below one and expressed in standard temperature and pressure. The respirometric chambers fitted the complete home cage of the animals. Animals therefore did not need to be handled and had access to their own running wheel throughout the measurements. Water and food were provided ad libitum. Wheel-running activity was also measured throughout the respirometry measurement using the ERS.

Body temperature was measured with a rectal probe (NTC type C, Ahlborn, Holzkirchen, Germany) immediately after the respirometry measurement. Body weight was also measured at this time. After these measurements the ambient temperature in the rooms was changed.

Data analysis

Continuous recordings of wheel-running activity were available for day 3–5 in each condition, just prior to the respirometry. These data were used for further analysis, excluding days 1 and 2 after the temperature transition. Average wheel-running activity per day (distance run), time spent running and average running speed were calculated for each temperature. In addition, maximum wheel-running activity per temperature was calculated over the same days by determining the maximum amount run in a 2 min interval. The same variables of wheel-running activity were determined for the 24 h interval in the respirometer. Wheel-running recordings during this time were not available for all animals and sample size for controls and selected mice were, respectively, 5 and 3 at 10°C, 6 and 6 at 20°C and 5 and 6 at 30°C.

Heat production (HP, kJ/h) was calculated using the equation $HP = \left(16.18 \times \dot{V}O_2 \right) + \left(5.02 \times \dot{V}CO_2 \right)$ (Romijn and Lokhorst 1961). Instead of using a fixed gas exchange conversion factor this versatile equation enabled the calculation of heat production of different nutritional states [see also (Gessaman and Nagy 1988)]. Resting metabolic rate (RMR, kJ/h) was defined as the lowest (running) mean metabolic rate recorded over half an hour anywhere during the 24 h measurement. The average metabolic rate over 24 h was used to calculate daily energy expenditure (DEE, kJ/day). The body weight measured before and after the respirometry measurement was averaged and used to calculated mass-specific RMR and DEE (in kJ/g/day).

Independent t-tests were used to screen for differences between animals housed in the two separate rooms. No significant differences were found and data from both rooms were pooled for further analysis. For all traits, two-way repeated measures ANOVA were performed with a factor group (control vs. selection), temperature (10 vs. 20 vs. 30°C) and group × temperature using SAS 9.1 (PROC MIXED). Body mass is known to have a strong influence on metabolic rate and analysis of resting metabolic rate (RMR) and daily energy expenditure (DEE) were done using models with or without body mass as a covariate. In addition, we were interested in the relationship between parameters of wheel-running activity (distance run, time run, average running speed and maximal running speed) and DEE, and these parameters were added as an additional covariate to body mass in the model one at a time to explore these relationships. Data was normally distributed and thus not transformed before analysis. When the ANOVA showed significant

effects post hoc *t*-tests were performed. Significance was assumed at $P \leq 0.05$. All tests were two-tailed.

To determine the relationship between running speed (*V*, km/h) and heat production (HP, kJ/h) at the different ambient temperatures, average wheel-running activity (while in the respirometer) and HP of most mice (for sample size see above) were calculated in 30 min bins during the dark phase. We only used data from the dark phase (12 h) because mice are nocturnal and wheel-running activity is mainly limited to the dark phase (24 time points per mouse). We accounted for a 30 min. lag in the HP measurements caused by the low airflow rate through the respirometry system (see Fig. 1). At each temperature and for both groups we calculated the average running speed and heat production per 30 min bin. The relationship between running speed and heat production for all groups was plotted in Fig. 3. Using ANCOVA models we explored effects of group and temperature on the relationship between running speed and metabolic rate.

Results

Body mass, food intake and wheel-running activity

Table 1 shows the effects of ambient temperature on body mass, food intake and several measures of wheel-running activity in control and selected mice. We found no differences between control and selected mice in average body mass or food intake. Ambient temperature had no effect on body mass, but food intake was significantly higher at low ambient temperatures (10 and 20°C).

As expected, selected mice had significantly higher wheel-running activity (expressed as time spent running or distance run) than control mice (see Table 1). Ambient temperature significantly affected the distance run per day, running time per day and running speed. In both groups, wheel-running activity was significantly decreased at 10°C compared with 20°C. Maximum running speed was also significantly higher in selected mice than in control mice, but ambient temperature did not influence maximal running speed. Body mass was never a significant covariate in the models, indicating that body mass had no statistically detectable effects on food intake or any measure of wheel-running activity. This is most likely due to small variance in the body mass of the mice used for the experiments.

Metabolism and body temperature

Animals were put in respirometry chambers for 24 h at different ambient temperatures to measure resting metabolic rate and daily energy expenditure in control and selected mice at these temperatures (see Fig. 2). RMR was similar in control and selected mice and significantly decreased with increasing ambient temperature (see Table 2 for statistical analyses). Body mass was a significant predictor of RMR in the model, but did not influence the effects of group and temperature on RMR. Daily energy expenditure also did not significantly differ between control and selected mice and decreased with increasing ambient temperature. When body mass was included in the model as a covariate it significantly contributed to the explained variance in DEE and the group effect became significant, with a higher DEE in selected mice compared to
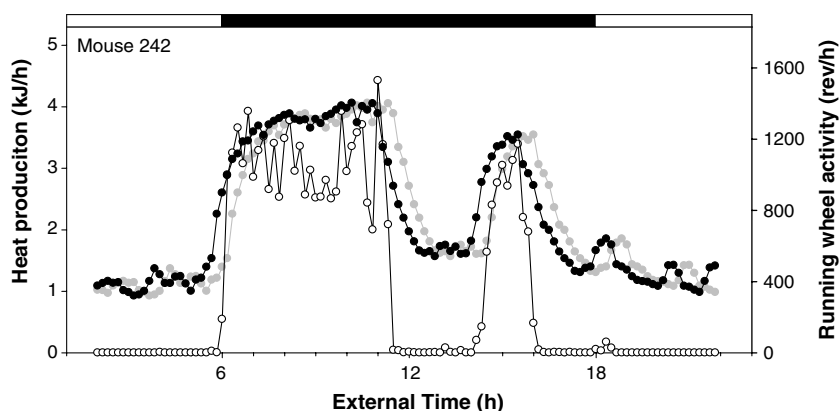

Fig. 1 Simultaneous measurements of wheel-running activity (*white dots*) and HP (*grey dots*) for a mouse representative of the group (10 min averages) from 4 h prior to the dark phase to 4 h after the dark phase (*black bar*) at 30°C. At the flow rate employed a 30 min time lag is detectable in our respirometry system and therefore data on HP were corrected with 30 min to determine the relationship between heat production and running speed (*black dots*)

Table 1 Body mass, food intake and wheel-running activity of control and selected mice at various ambient temperatures

Group	Ambient temperatures (°C)			P-values for repeated measures ANOVA	
	10	20	30	Group	Temp
Body mass (g)					
Control	28.7 ± 1.5	28.2 ± 1.6	28.0 ± 2.2	0.151	0.085
Selected	27.2 ± 2.2	26.8 ± 1.9	26.6 ± 2.3		
Food intake (g/day)					
Control	8.4 ± 2.3	8.0 ± 2.8	4.4 ± 0.5	0.190	**0.001**
Selected	9.3 ± 2.4	9.1 ± 1.4	5.6 ± 0.6		
Distance run (km/day)					
Control	7.1 ± 4.1	10.7 ± 3.7	8.8 ± 3.2	**0.024**	**0.003**
Selected	9.6 ± 3.0	14.1 ± 5.1	13.1 ± 3.4		
Time spent running (h/day)					
Control	6.1 ± 2.4	7.7 ± 2.1	7.8 ± 1.8	**0.048**	**0.001**
Selected	7.5 ± 1.1	9.1 ± 1.0	9.5 ± 2.3		
Average speed (km/h)					
Control	1.1 ± 0.3	1.4 ± 0.2	1.1 ± 0.2	0.092	**0.005**
Selected	1.3 ± 0.2	1.6 ± 0.5	1.4 ± 0.3		
Maximum speed (km/h)					
Control	2.3 ± 0.4	2.5 ± 0.4	2.3 ± 0.3	**0.048**	0.092
Selected	2.5 ± 0.4	2.7 ± 0.6	2.9 ± 0.2		

Mean ± SD are given for several variables, for control and selected mice at three different ambient temperatures seperately. Two-way repeated measures ANOVA were performed on all variables with group as a between subjects factor and temperature (temp) and group × temp as within subjects factors. P values for effects of age and group are given in the table and are bold when the effect was statistically significant ($P < 0.05$). No significant interaction effects between group and age were found ($P > 0.1$), and P values are therefore not shown in the table. Sample size was eight in both groups, except for the measures of wheel-running activity where data of one mouse in the selected group were missing

controls. Post hoc comparison showed that DEE was significantly different between lines at 30 and 20°C, but not 10°C. We were interested in how wheel-running activity as measured during the respirometry measurement contributes to the explained variance in

Fig. 2 Mass-specific resting metabolic rate (*RMR*) and daily energy expenditure (*DEE*) in mice selected for high wheel-running activity (*SEL*) and their random bred controls (*CTRL*) at various ambient temperatures. Values represent mean ± SD. Asterisks show at which temperatures *DEE* significantly differed between control and selected mice ($P < 0.05$)

DEE, and included activity variables into the model with body mass, one at a time. All variables were positively related to DEE. Only distance run and time spent running significantly contributed to the variance in DEE in these models. They fully accounted for the group effect, but not for temperature. The effect of ambient temperature remained significant in these models.

Body temperature at the different ambient temperatures was measured at the moment when animals came out of the respirometry chambers. In control mice body temperature was on average 36.7 ± 0.5, 37.3 ± 0.8 and 37.3 ± 0.4 (mean ± SD) at 10, 20 and 30°C, respectively, and in selected mice it was 36.7 ± 0.4, 37.8 ± 0.5 and 37.3 ± 0.4. Body temperature did not differ significantly between control and selected mice and decreased with ambient temperature in both groups (see Table 2).

Cost of transport

Estimates of the incremental cost of transport (COT, kJ/km) are generally derived from the slope of the regression of heat production and running speed. The relationship between HP and running speed (*V*) for control and selected mice in the present study is shown in Fig. 3 (see also Table 3). This figure plots the
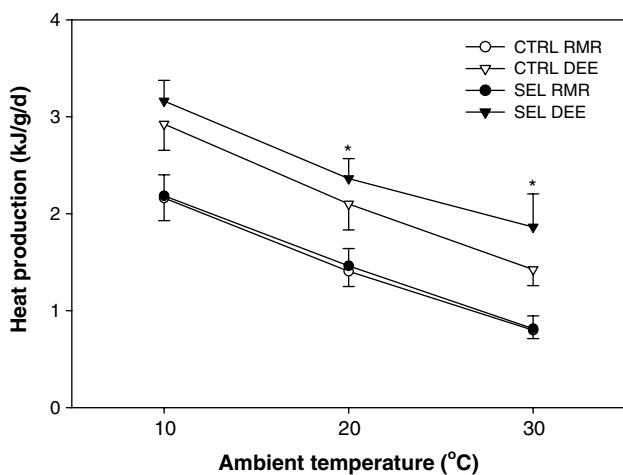
interindividual average metabolic rate for each 30 min bin of running speed at each of the three temperatures. The figure clearly shows that at each temperature, the metabolic rates of both lines were distributed around the same positive regression with speed. The highest speeds were more often observed in the selected line. There was a thermal gradient, with higher metabolism at lower temperature, but at each temperature the slope appeared to be similar. We tested for effects of temperature and group in an ANCOVA model with HP as the dependent variable and running speed as a covariate, where we looked at effects of group (selection vs. control), temperature, and their interactions with running speed. Temperature strongly affected the relationship between HP and running speed ($F_{2,51} = 676.5$, $P < 0.001$). This supports the visual inspection of Fig. 3, with obviously different intercepts (HP at zero running) at the ambient temperatures measured. There was no interaction effect between temperature and running speed, supporting similar slopes of all relationships (slope = 1.19, 95% CI 1.09–1.29). Hence the incremental costs of running were equal at all temperatures measured. Group did not significantly affect the regression ($F_{1,44} = 361$, $P = 0.064$). The slope of the regression between running speed and HP was slightly lower in selected mice (see Table 3), but not significantly so. Costs of running were thus similar in both groups. Even though body mass is known to affect COT, body mass did not contribute significantly to the explained variance in HP.

Again this is probably caused by small variance in mass. The relationship between HP and body mass was positive in the models used, though. The only factor that significantly influenced the relationship between HP and running speed was thus ambient temperature. The solid lines in Fig. 3 show the regressions for the three ambient temperatures measured with both groups combined and without taken body mass taken into account. The equations for these regression lines are: at 10°C: HP = 1.16 V + 3.63, at 20°C: HP = 1.20 V + 2.55 and at 30°C: HP=1.20 V + 1.82 ($P < 0.001$ for all regressions).

Discussion

We explored effects of ambient temperature on wheel-running activity, body temperature and metabolic rate in mice that had been selected for wheel-running activity for 31 generations and their random bred controls.

We expected that at low ambient temperatures the heat generated by activity might (partially) substitute thermostatic metabolic rate and therefore mice might run more in the cold. At high ambient temperatures animals were expected to reduce their activity to prevent hyperthermia, as has been shown in humans (Cheuvront and Haymes 2001) and birds (Davies 1982; Spinu et al. 2003). Ambient temperature did indeed significantly affect wheel-running activity, but opposite

Table 2 Results for repeated measures ANOVA on metabolic measurements and body temperature

Variable	n	Group			Temperature			Covariate	
		df	F	P	df	F	P		P
RMR	16	1, 14	0.6	0.441	2, 28	408.3	**<0.001**	None	
RMR	16	1, 14	0.1	0.851	2, 27	457.3	**<0.001**	Body mass	**0.009**
DEE	16	1, 14	3.0	0.103	2, 28	208.7	**<0.001**	None	
DEE	16	1, 14	7.9	**0.014**	2, 27	203.7	**<0.001**	Body mass	**0.017**
DEE	15	1, 13	0.1	0.935	2, 10	167.8	**<0.001**	Body mass	0.179
								Distance	**0.005**
DEE	15	1, 13	0.1	0.764	2, 10	115.7	**<0.001**	Body mass	0.103
								Time	**0.042**
DEE	15	1, 13	0.2	0.679	2, 10	107.5	**<0.001**	Body mass	0.556
								Speed	0.059
DEE	15	1, 13	0.1	0.971	2, 10	117.2	**<0.001**	Body mass	0.968
								Max speed	0.074
Body temp	16	1, 14	0.9	0.356	2, 28	11.9	**<0.001**	None	

Repeated measures ANOVA were performed on all variables with group as a between subjects factor and temperature and group × temperature as within subjects factors. In addition, where appropriate body mass and wheel-running activity variables were added into the model as covariates. Degrees of freedom (df), F and P values for each factor are given in the table. P values are bold when the effect was statistically significant ($P \leq 0.05$). No significant interaction effects between group and age were shown and P values are therefore not shown in the table. Sample size was eight in both groups, except for the measures of wheel-running activity where data of one mouse in the selected group were missing
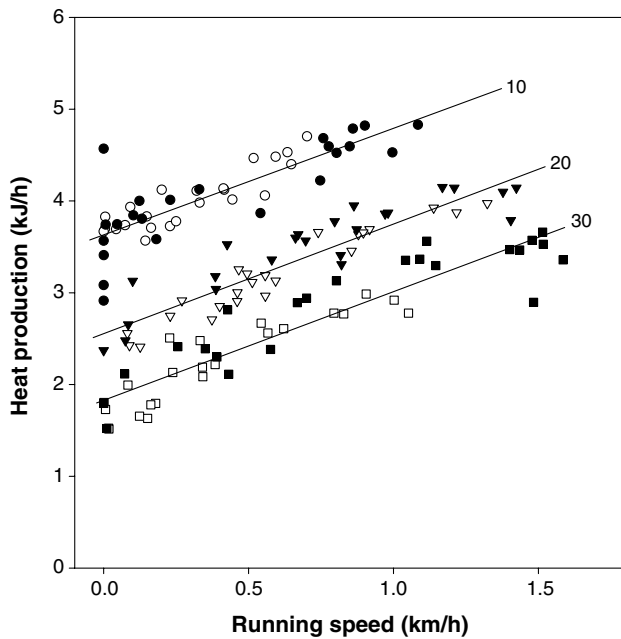
Fig. 3 Heat production (HP, kJ/h) of control and selected mice during voluntary running as a function of running speed (V, km/h). Each symbol represents average running speeds and metabolic rates of mice at that temperature for each half hour of the dark phase. Control mice are in white and selected mice in black. *Circles* represent 10°C, *triangles* represent 20°C and *squares* represent 30°C. The solid lines are the regression lines for the different ambient temperatures (see text)

Table 3 Effect of temperature and group on linear regressions between running speed (km/h) and metabolic rate (kJ/h)

Temperature (°C)	Slope	Intercept	R^2
Control			
10	1.25 ± 0 .14	3.64 ± 0 .05	0.79
20	1.30 ± 0 .08	2.41 ± 0 .06	0.92
30	1.31 ± 0 .13	1.71 ± 0 .07	0.83
Selected			
10	1.16 ± 0 .18	3.61 ± 0 .10	0.68
20	1.08 ± 0 .11	2.72 ± 0 .10	0.82
30	1.06 ± 0 .11	1.99 ± 0 .11	0.82

Using linear regression, slopes and intercepts of the relationship between running speed and metabolic rate (Fig. 2) were determined (without body mass as a covariate). Slopes and intercepts for all separate groups are shown as mean ± SEM. All regressions were highly significantly different from zero ($P < 0.001$)

was no longer significant at low ambient temperature. The mice have been selected at ambient temperatures of approximately 22°C and at 10°C thermoregulatory costs might be too high for mice to maintain high levels of activity. Indeed, mice at 10°C had body temperatures decreased by approximately 0.6°C which could reflect difficulties to maintain constant body temperature. Lowering of body temperature may also be a strategy to lower costs for thermoregulation while resting. Body temperature was measured once in the middle of the light phase (rest phase) and the variation assessed between ambient temperatures may just reflect ambient temperatures at rest and may not have persisted while running.

One could speculate that there is a restraint on running at low ambient temperatures (due to slower muscle contraction). Maximal running speeds, however, did not significantly vary between temperatures in this study. A study in deer mice likewise provided no evidence for effects of temperature on wheel-running activity (Chappell et al. 2004).

No differences in body temperatures were observed between control and selected mice at any of the ambient temperatures measured, which is in agreement with previous measurements of body temperature in mice of the same strain at an ambient temperature of 22°C (Rhodes et al. 2000). Regulation of body temperature at rest thus appears unchanged in mice selected for high wheel-running activity and there does not appear to be a difference in thermoregulatory capacity, at least at the temperatures studied here. Nonetheless, we cannot exclude differences between the lines in shivering or non-shivering thermogenesis. Moreover, mice from selected lines show elevated heat shock protein 72 expression in the triceps surae muscle (Belter et al. 2004).

As expected for a small endotherm, energy expenditure decreased with increasing ambient temperature. RMR increased 1.6-fold from 20 to 10°C and 1.8-fold from 30 to 20°C. DEE was also affected by ambient temperature with a 1.4-fold increase from 20 to 10°C and a 1.5-fold increase from 30 to 20°C. These results are similar to values found in a study in deer mice (Chappell et al. 2004) housed at 3, 10 and 25°C. Wheel-running activity (distance run and running time) was positively correlated with the simultaneously measured DEE. In concurrence with an increase in wheel-running activity, mass-specific DEE was significantly increased in selected mice compared with controls. RMR did not differ between control and selected mice, even though there are differences in body composition between them (Swallow et al. 2005, 2001). Apparently, the costs for thermoregulation and maintenance of the

to the prediction on the basis of themogenetic substitution, wheel-running activity (distance run, time spent and average running speed) was decreased by approximately 60% in control as well as selected mice at low ambient temperature (10°C). As expected, selected mice ran a longer distance (+42%), more time (+22%) and at faster speeds (+19%) than control mice did. This difference between control and selected mice

Table 4 Overview of studies investigating whether cost of running were substitutive or additive to heat necessary for thermoregulation

Species	Latin name	Temperature range (°C)	Additive (A) or substitutive (S)	Temperature at which substitution occurred (°C)	Voluntary (V) or forced (F)	Reference
Mammals						
Patas monkey	*Erythrocebus patas*	0 to 55	A	–	F treadmill	Mahoney (1980)
Potoroo	*Potorous tridactylus*	5 to 25	S	5	F treadmill	Baudinette et al. (1993)
Wapiti	*Cervus elaphus*	−25 to 15	A	–	V activity in cage	Gates and Hudson (1979)
Deer mouse	*Peromyscus maniculatus*	3 to 25	S	10	V wheel running	Chappell et al. (2004)
Random-bred controls	*M. domesticus*	10 to 30	A	–	V wheel running	This study
Mice selected for high wheel-running activity	*Mus domesticus*	10 to 30	A	–	V wheel running	This study
Rat	*R. norvegicus*	−20 to 22	S	0	F treadmill	Makinen et al. (1996)
Rat	*Rattus norvegicus*	4 to24	S	4	F treadmill	Arnold et al. (1986)
Chipmunk	*Eutamias merriami*	5 to 40	S	5	F treadmill	Wunder (1970)
Antelope ground squirrel	*Ammospermophilus leucurus*	10 to 35	A	–	F treadmill	Yousef et al (1973)
Birds						
Common redpoll	*Acanthis flammea*	−50 to 30	S	−45	F hopping on rotating bars	Pohl and West (1973)
White crowned sparrow	*Zonotrichia leucophrys*	−10 to 25	S	−10	F treadmill	Paladino et al. (1984)

body are similar in control and selected mice. Given that RMR did not differ between the groups and the group difference in DEE disappeared when correcting for variables of wheel-running activity, the difference in DEE between groups can be fully attributed to energy spent on activity.

Total energy spent on activity was thus higher in the selected mice. This does not imply that there were differences in the costs per unit distance between the groups (COT). At all ambient temperatures COT were approximately 1.2 kJ/km, (at an average body mass of 27.6 g) which is comparable to the COT 1.19 kJ/km obtained by forced locomotion on a treadmill by Taylor et al. (for a 21 g house mouse) (Taylor et al. 1970). COT is related to body mass, with higher costs of transport at higher body mass. In our study as well as previous work by Chappell et al. (2004) and Rezende et al. (2006), body mass was not a statistically significant predictor of COT. The incremental cost of terrestrial locomotion in relation to body mass can be estimated using the allometry given by Taylor et al. in 1982: COT (kJ/km) = $10.7 \times$ mass (kg)$^{0.684}$ (Taylor et al. 1982), and predicts a slope of 0.92 kJ/km for a 27.6 g animal. This is lower than the value we found for these mice. The mice measured by Taylor were forced to run on a treadmill. Animals on treadmills are forced to run at specific speeds, whereas voluntary running mice choose their preferred speed. This might render a different relationship between running speed and metabolic cost. A previous study on male selected mice at 22°C estimated a COT of 1.29 kJ/km (when using a conversion factor of 20.1 J/ml O_2) (Rezende et al. 2006), which is very similar to the value of 1.2 kJ/km we obtained. The slight difference may easily be attributed to the different wheels used (plastic wheels with a 7 cm radius in our study compared to metal wheels with a 18 cm radius in the study by Rezende et al. (2006). The study by Rezende et al. demonstrated that whole-body COT during voluntary wheel running was significantly lower in the selected lines, when combining analysis of males and females (Rezende et al. 2006). When body mass and/or maximal speed were added as a covariate the difference disappeared. These factors apparently caused the line difference. Similar to our study, analyzing males alone did not render a significant effect of selection on COT.

The novel result in our study is that COT was unaffected by ambient temperature. With decreasing ambient temperature the intercept of the relationship between metabolic rate of running speed did increase, indicating increased costs at rest at lower temperatures, as is also reflected in an increase in RMR. Heat generated by running apparently did not substitute for

thermoregulation costs at low ambient temperature in
our mice (Fig. 3). At all ambient temperatures the
slope of the relationship between metabolic rate and
running speed was statistically indistinguishable. Con-
tradictory evidence exists for other species of homeo-
therms, showing either addition or substitution of
activity-generated heat for thermoregulatory heat at
low ambient temperatures. Table 4 summarizes the
results for two studies on birds and several on various
mammals. We have listed whether heat generated by
activity was additive or substitutive and at which
temperature substitution first occurred. The two stud-
ies on birds indicate partial or complete substitution of
exercise-generated heat production for thermoregula-
tory costs usually at low ambient temperatures and
additive at moderately cold ambient temperature
(Paladino and King 1984; Pohl and West 1973). In
mammals the results are more scattered with cases of
total, partial and no substitution (see Table 4). The
ambient temperatures used vary widely amongst these
studies. In our study the ambient temperatures applied
might not have been extreme enough to show substi-
tution of activity-generated heat for thermoregulatory
heat. However, there is no theoretical basis to assume
that substitution should exclusively occur at very low
ambient temperatures. At all ambient temperatures
below the lower critical temperature substitution could
occur to a certain degree. Also, when partial substitu-
tion occurs, these effects may be masked by differences
in heat loss under resting or active conditions. For
example, when an animal leaves a well-insulated rest-
ing place to become active, thermoregulatory costs
may well simultaneously shoot up due to increased
surface area and reduced insulation, and thus coun-
teract substitutive effects of activity. In this case the net
effect on costs of transport may not be different and
partial substitution would not be noticed. In our ani-
mals housed in their home cage with bedding during
the measurements, these effects may have been more
pronounced than in other studies. At 10°C mice were
less active and may have chosen to use shivering
thermogenesis while well-insulated and curled up in
their nest instead of using heat generated by wheel-
running activity to offset increased heat loss (animals
did not have nesting material, but did built small nests
using wood shavings). Interestingly, in selected and
control mice the cost of running was found to be sim-
ilar and in both groups heat generated by activity could
not substitute for heat necessary for thermoregulation
at the lowest ambient temperature measured.

In summary, mice that have been selected for high
voluntary wheel-running activity had increased mass-
specific daily energy expenditure, but did not differ

from control mice with respect to resting metabolic
rate. Wheel-running activity decreased at low ambient
temperature (10°C) in both selected and non-selected
mice and was unchanged at high ambient temperature
(30°C) compared to control temperature (20°C). The
cost of transport was similar between the lines. It was
also indistinguishable between the ambient tempera-
tures measured, indicating that the energy spent on
activity was additive and did not substitute for heat
necessary for thermoregulation.

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