

Physical exercise-induced changes in the core body temperature of mice depend more on ambient temperature than on exercise protocol or intensity

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Abstract The mechanisms underlying physical exercise-induced hyperthermia may be species specific. Therefore, the present study aimed to investigate the effects of exercise intensity and ambient temperature on the core body temperature (T_{core}) of running mice, which provide an important experimental model for advancing the understanding of thermal physiology. We evaluated the influence of different protocols (constant- or incremental-speed exercises), treadmill speeds and ambient temperatures (T_{a}) on the magnitude of exercise-induced hyperthermia. To measure T_{core} , a telemetric sensor was implanted in the abdominal cavity of male adult Swiss mice under anesthesia. After recovering from the surgery, the animals were familiarized to running on a treadmill and then subjected to the different running protocols and speeds at two T_{a} : 24 °C or 34 °C. All of the experimental

trials resulted in marked increases in T_{core} . As expected, the higher-temperature environment increased the magnitude of running-induced hyperthermia. For example, during incremental exercise at 34 °C, the maximal T_{core} achieved was increased by 1.2 °C relative to the value reached at 24 °C. However, at the same T_{a} , neither treadmill speed nor exercise protocol altered the magnitude of exercise-induced hyperthermia. We conclude that T_{core} of running mice is influenced greatly by T_{a} , but not by the exercise protocols or intensities examined in the present report. These findings suggest that the magnitude of hyperthermia in running mice may be regulated centrally, independently of exercise intensity.

Keywords Animal experimentation · Fatigue · Heat · Mouse · Thermoregulation · Treadmill

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Introduction

The muscle contractions required to produce movement and locomotor activity increase muscular metabolic rate, resulting in heat production (Gonzalez-Alonso et al. 2000). This locally produced heat is absorbed via the blood that flows through the muscles and is then distributed to other body tissues. Because blood flows at higher rates during physical exercise (Rowell 1974), the body core is warmed rapidly and core body temperature (T_{core}) increases (Lind 1963; Saltin and Hermansen 1966).

This increase in T_{core} is a physiological response observed in virtually all exercising mammals, particularly when exercise is performed in warm environments (Marino 2004). The magnitude of exercise-induced hyperthermia depends, among

other factors, on the intensity of effort (Saltin and Hermansen 1966), ambient temperature (Galloway and Maughan 1997) and hydration status (Sawka et al. 1985). Although the exercise-induced increase in T_{core} is clear and reproducible, whether this response is regulated by the central nervous system or occurs passively as a result of an undesirable accumulation of heat remains unclear (Briese 1998). Webb (1995) proposed that the increase in T_{core} observed with the initiation of exercise is not a regulated response but occurs due to an imbalance between the rate of heat production (independent variable) and the rate of heat loss (dependent variable). According to Webb's hypothesis, the absolute exercise intensity (and, consequently, the rate of metabolic heat production) should determine the magnitude of hyperthermia. However, evidence obtained from human studies indicates that the exercise-induced increase in T_{core} does not differ across a wide range of ambient temperatures (Lind 1963) and is more closely associated with the relative, rather than the absolute, intensity of effort (Saltin and Hermansen 1966; Greenhaff 1989; Gant et al. 2004), suggesting that the degree of exercise hyperthermia is regulated by central mechanisms.

Another relevant factor related to this phenomenon is that exertional hyperthermia may be brought about by distinct patterns of thermoeffector activity in different mammalian species, as animals have developed different thermoregulatory strategies to address environmental challenges (Marino 2004). Compared to humans and rats, mice exhibit a higher body surface area-to-mass ratio (Pinkel 1958; Davies and Morris 1993), which facilitates the passive loss of body heat to the environment under temperate conditions (Gordon et al. 1986). In mice, facilitated passive heat loss is compensated by a higher resting metabolic rate (Schefer and Talan 1996). Moreover, small-sized rodents display a limited ability to dissipate heat through evaporative means while exercising and are more active during the dark phase of the day, when ambient temperatures are usually lower (Shellock and Rubin 1984). In fact, increased locomotor activity is suggested to be a thermoeffector in small rodents such as mice (Mount and Willmott 1967; Weinert and Waterhouse 1999; Kanizsai et al. 2009).

A careful analysis of the literature reveals that the potential existence of an interspecies difference in the control of thermoregulation has been overlooked in previous investigations in which mice were subjected to physical exercises protocols. Therefore, considering that the use of genetic tools (including experiments with knockout mice) may represent an important strategy for understanding the mechanisms underlying exercise-induced hyperthermia, the present study aimed to examine the changes in T_{core} of running mice. To better understand the effects of running intensity on thermoregulation in mice, we subjected these animals to different exercise protocols, including constant-speed running at two treadmill speeds and incremental-speed running. These protocols were performed using a treadmill setup that enabled precise control

of exercise intensity. We also investigated the impact of a warm environment on the physical performance and exercise-induced hyperthermia of running mice.

Materials and methods

Animals

Sixteen male adult Swiss mice weighting 20–22 g (4 weeks of age), provided by the animal care center at the Faculty of Pharmacy of the Universidade Federal de Minas Gerais, were used in all experiments. The mice were initially transferred to the animal facilities of the Institute of Biological Science, where the experiments were conducted. The mice were housed in collective cages under controlled light (5:00 a.m. to 7:00 p.m.) and temperature (24.0 ± 2.0 °C) conditions, with water and chow provided ad libitum. Prior to the implantation of an abdominal probe, the animals were habituated to the new facilities for at least 2 weeks.

All experimental procedures were approved by the local Ethics Committee for the Care and Use of Laboratory Animals (protocol 006/2011) and were conducted in accordance with the policies described in the Committee's Guiding Principles Manual.

Implantation of a telemetry transmitter

The mice were anesthetized with ketamine (84 mg/kg body wt, i.p.) and xylazine (8 mg/kg body wt, i.p.), and a telemetry transmitter (G2 E-Mitter series, Mini Mitter, Bend, OR) was then implanted in the peritoneal cavity. A small incision was made in the linea alba of the abdominal muscle, and the peritoneal cavity was exposed. The sensor was inserted and affixed to the left lateral abdominal wall using sutures. The abdominal muscle and skin were then sutured in layers (Steiner et al. 2007).

The mice were given 7 days to recover from surgery prior to being familiarized with the treadmill exercise. This recovery period was sufficiently long for the mice to recover and to regain their presurgical body mass (27.9 ± 1.9 g presurgical vs 31.8 ± 1.3 g postsurgical; the telemetric probes had an average mass of 1.1 g).

Familiarization to treadmill exercise

The familiarization protocol consisted of running on a treadmill designed for small rodents (Modular Treadmill, Columbus Instruments, OH) over 5 consecutive days; the daily exercise sessions are described in Table 1. The mice were encouraged to run by light electrical stimulation (0.5 mA), which was provided by a grid located at the rear end of the treadmill belt. Following the familiarization protocol, the mice could run for

Table 1 Five-day familiarization protocol designed to teach the mice to run on the treadmill^a

Day	Stage 1	Stage 2	Stage 3	Distance traveled (m)
1	3 min of resting	5 min at 5 m/min	3 min at 6 m/min	43
2	3 min of resting	5 min at 6 m/min	3 min at 8 m/min	54
3 to 5	3 min of resting	5 min at 6 m/min	5 min at 8 m/min	70

^a Treadmill inclination was always set at 5 %

5 min at a constant speed of 8 m/min and 5 % inclination with minimal exposure to the electrical stimulation.

During the familiarization sessions and all of the experimental trials, an electrical fan, which generated an airflow rate of 2.0–2.5 m/min, was positioned in front of the treadmill belt. The ambient temperature (T_a) was measured using a thermocouple (YSI-400A, Yellow Springs Instruments, Yellow Springs, OH) placed inside an acrylic chamber that contained the treadmill. The thermocouple was positioned on the ceiling halfway between the fan and the electrical grid. T_a was controlled at 24 ± 1 °C during the familiarization sessions.

Incremental-speed exercise until voluntary interruption of effort

After completing the familiarization sessions, eight mice were subjected to incremental-speed exercises until they showed voluntary interruption of effort. On the day of the experimental trials, the animals were weighed and allowed to rest for at least 2 h in the experimental procedure room. The animals were then transferred from their home cages to the motor-driven treadmill. The incremental workload tests were initiated at a speed of 6 m/min, with an increase of 1 m/min every 3 min (the inclination was always set at 5 %). The experimenter interrupted the exercise when the animals were unable to keep pace with the treadmill for at least 10 s (Wanner et al. 2007, 2011).

Each mouse in this group was subjected to two experimental trials: incremental-speed exercises at a T_a of 24 °C or 34 °C. To heat the environment inside the acrylic chamber that contained the treadmill, an electrical heater (Britânia model AB 1100, Curitiba, Brazil) was positioned at the same level \approx 40–45 cm distant from the fan and turned on at 1,200 W (Lima et al. 2013).

The experimental trials were separated by at least 48 h and were performed between 10:00 a.m. and 4:00 p.m. Each mouse was always tested at similar times on different days.

Constant-speed exercise

After completing the familiarization sessions, another group of eight mice was subjected to the constant-speed exercises. On the day of the experimental trials, the mice were prepared as described above. The constant-speed exercises consisted of running at constant speeds of 8 m/min or 10 m/min (with 5 % inclination) at a T_a of 24 °C or 34 °C. The exercise duration was set at 60 min.

A Latin square experimental design was used to prevent familiarization with the protocol and to prevent heat acclimation from interfering with the results. Every mouse in this group was subjected to four experimental trials that were separated by at least 48 h. The trials were performed between 10:00 a.m. and 4:00 p.m.

Measurements

The abdominal temperatures were measured every 30 s and were used as the T_{core} value. The time until the voluntary interruption of effort was determined as the interval between the beginning of exercise and the moment at which the mice were unable to keep pace with the treadmill.

The time until the interruption of effort, the maximal speed (S_{max}) attained and the distance traveled were considered to be indices of physical performance during the incremental-speed exercises.

Calculations

The rate of increase in T_{core} was calculated as the change in abdominal temperature within a specific time interval. The S_{max} values that were attained during the incremental-speed exercise tests were calculated by adapting the following equation that was previously proposed by Kuipers et al. (1985) for the calculation of the maximal power output: $S_{max} = S1 + (S2 \times t/180)$, where S1 is the speed reached in the last completed stage, S2 is the increase in treadmill speed in each stage, and t is the time spent (in seconds) in the uncompleted stage. The S_{max} achieved in the warm environment was also expressed as a percentage relative to the speed achieved at temperate conditions and was calculated in the following manner: $(S_{max} \text{ at } 34 \text{ °C} / S_{max} \text{ at } 24 \text{ °C}) \times 100$. The same method was used to express the rate of the increase in T_{core} at 34 °C as a percentage relative to the increase observed at 24 °C.

Statistical analysis

The data are reported as mean \pm SEM, unless otherwise stated. For the incremental-speed exercise protocol, physical performance indices were compared between the two T_a levels using paired t -tests. The effect size was calculated by subtracting the mean of the performance index at 34 °C from the mean of the same index at 24 °C and then dividing the result by a standard

deviation for the data. The curves for the S_{\max} data were analyzed using the logrank test (Bland and Altman 2004)—a statistical analysis generally used in survival experiments (Wanner et al. 2012). Differences in T_{core} between T_a and over time were evaluated using a two-way analysis of variance (ANOVA) with repeated measures followed by post-hoc Tukey's test. The correlation between the rate of increase in T_{core} and S_{\max} or the time to the voluntary interruption of effort were assessed using Pearson's correlation coefficient.

For the constant-speed exercise protocol, differences in T_{core} over time and between the different treadmill speeds and T_a were evaluated using a three-way ANOVA followed by post-hoc Tukey's test. The curves depicting the maximal exercise duration tolerated were analyzed using the logrank test.

We also compared the increase in T_{core} among running protocols (i.e., incremental- or constant-speed exercises with different treadmill speeds) at a given T_a . In this case, differences in T_{core} over time and among running protocols were evaluated using a two-way ANOVA with repeated measures followed by post-hoc Tukey's test. The significance criterion was set at $P < 0.05$.

Results

Incremental-speed exercise

At 24 °C (i.e., the temperate environment), the mice ran for an average of 28.6 ± 2.3 min and achieved a maximal speed of 14.5 ± 0.8 m/min. Running performance was greatly impaired during the incremental-speed exercise in the heat ($P < 0.05$; logrank test; Fig. 1a). At 34 °C, a shorter exercise

time until voluntary interruption of effort was observed ($P < 0.01$), and the S_{\max} achieved was decreased by 16 % ($P < 0.01$, Table 2). The mice also traveled a significantly shorter distance at 34 °C than at 24 °C ($P < 0.01$, Table 2).

In the temperate environment, all of the mice completed the sixth stage of the incremental exercise, which corresponded to a treadmill speed of 11 m/min. However, in the warmer environment, the S_{\max} achieved for all of the animals was only 9 m/min (Fig. 1a). Moreover, 37.5 % of the mice were still running at 24 °C when all of the animals had fatigued at 34 °C.

Irrespective of T_a , mice that were subjected to physical exercise presented marked increases in T_{core} (Fig. 1b). In the cool environment, treadmill running induced a sharp increase in T_{core} from the 4th to the 13th min of exercise; thereafter, T_{core} remained stable (≈ 38.6 to 38.9 °C) until voluntary interruption of effort. In the warm environment, treadmill running not only induced a sharp increase in T_{core} but also exaggerated the observed exercise-induced hyperthermia; the mice exhibited higher T_{core} values from the 3rd min until the end of exercise when compared with their values during running at 24 °C (40.06 ± 0.10 °C vs 38.85 ± 0.10 °C; at the interruption of effort; $P < 0.001$).

To assess whether the increase in hyperthermia was associated with the decreased performance that was observed at 34 °C, the achieved S_{\max} was evaluated with respect to the rate of increase in T_{core} . Both parameters exhibited a significant negative correlation regardless of whether the rate of increase in T_{core} was calculated from the beginning until the 13th min of exercise ($r = -0.56$; $P < 0.05$; data not shown) or until voluntary interruption of effort ($r = -0.84$; $P < 0.001$; Fig. 2a). Moreover, the higher percentage rate of increase in T_{core} in the warmer environment was correlated negatively with the percentage decrease in S_{\max} ($r = -0.71$; $P < 0.05$; Fig. 2b).

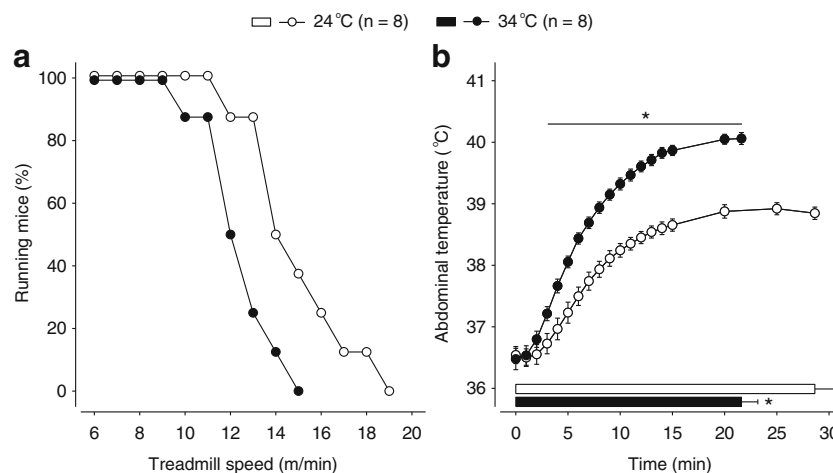


Fig. 1 **a** Maximal speeds achieved by mice subjected to incremental-speed treadmill exercises at two different ambient temperatures: white circles 24 °C, black circles 34 °C. Data are expressed as proportions of mice that were still running at specific speeds. **b** Abdominal

temperature of mice subjected to incremental-speed exercises. The bars at the bottom represent the time until the voluntary interruption of effort. Data are expressed as mean \pm SEM. * Significant differences ($P < 0.05$) compared with the 24 °C trial

Table 2 Physical performance of mice subjected to incremental-speed exercises at 24 °C (temperate ambient) and 34 °C (warm ambient)

Performance index	Temperate (24 °C)	Warm (34 °C)	Reduction (%)	<i>t</i> statistics	Effect size
Running time (min)	28.6±2.3	21.6±1.6	24.5	0.002	1.26
Maximal speed (m/min)	14.5±0.8	12.2±0.5	16.1	0.002	1.22
Distance traveled (m)	301±36	200±19	33.5	0.005	1.23

Constant-speed exercise

After determining the achieved S_{\max} during the incremental-speed exercises, we subjected another group of mice to constant-speed exercises. Two treadmill speeds were chosen: 8 m/min and 10 m/min. Each speed corresponded to different exercise intensities according to the environment examined: at 24 °C, 8 m/min and 10 m/min represented 55 % and 69 % of S_{\max} , respectively. At 34 °C, 8 m/min and 10 m/min represented 66 % and 82 % of S_{\max} , respectively.

The duration of the constant-speed exercise was predetermined as 60 min; however, not every animal was able to complete the task, with the rate of completion depending on the trial (Fig. 3a). For the 8 m/min–24 °C trial, all of the mice were able to run the established duration. In the 10 m/min–24 °C and 8 m/min–34 °C trials, the rate of completion was 88 % and 63 %, respectively; these rates were not significantly different from that of the 8 m/min–24 °C trial. However, in the 10 m/min–34 °C trial, the rate of completion was only 38 % and was significantly lower than that of the 8 m/min–24 °C ($P<0.01$; logrank test) and 10 m/min–24 °C ($P<0.05$; logrank test) trials.

We next pooled the data to assess the specific effects of T_a and treadmill speed. T_a , but not treadmill speed, affected the rate of completion; the percentage of mice that were able to run for 60 min was significantly lower at 34 °C than at 24 °C (50 % vs 94 %; $P<0.01$; Fig. 3b).

All four of the constant-speed exercise trials induced marked increases in T_{core} (Fig. 4a). After exhibiting a high rate of increase during the initial minutes of exercise, T_{core} peaked and then remained stable until the voluntary interruption of effort. At the same T_a , the treadmill speed (8 or 10 m/min) did not influence the magnitude of exercise hyperthermia. However, at a given treadmill speed, T_a greatly influenced exercise hyperthermia. During the trials that were performed in the warm environment, T_{core} was higher from the 5th min until the end of the exercise period relative to the trials that were performed at 24 °C (39.97 ± 0.11 °C vs 38.59 ± 0.07 °C; $P<0.001$; data pooled from the 8 and 10 m/min trials at the same T_a).

Following the interruption of exercise, T_{core} decreased slightly in mice that were subjected to exercise at 24 °C (Fig. 4b); at the end of the 60-min post-exercise period, T_{core} was lower than that observed in the 10th min of the 24 °C–8 m/min trial (38.18 ± 0.24 °C vs 38.77 ± 0.13 °C; $P<0.05$) and was lower than that observed upon interruption of effort during the 24 °C–10 m/min trial (37.86 ± 0.21 °C vs 38.61 ± 0.11 °C; $P<0.01$). After exercise in warm conditions, T_{core} decreased by 2.0 °C until reaching equilibrium at ≈ 38.0 °C, which is similar to the post-exercise values observed in mice that were subjected to exercise at 24 °C. At the end of the post-exercise period, T_{core} values were higher than the resting values in all of the experimental trials (37.97 ± 0.10 °C vs 36.30 ± 0.09 °C; pooled data from all of the trials; $P<0.001$).

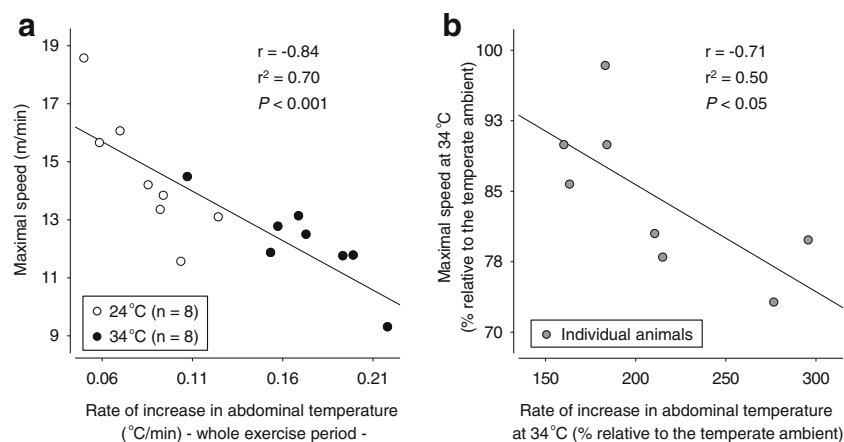


Fig. 2 **a** Correlation between the rate of increase in abdominal temperature (calculated from the beginning until voluntary interruption of the exercise) and the maximum achieved speed during the two incremental exercises. *White circles* Individual data from the 24 °C trial, *black*

circles data from the 34 °C trial. **b** Correlation between the higher rate of increase in abdominal temperature in the warmer environment and the heat-induced decrease in the maximum achieved speed. *Gray circles* Data from animals subjected to both the 24 °C and 34 °C trials

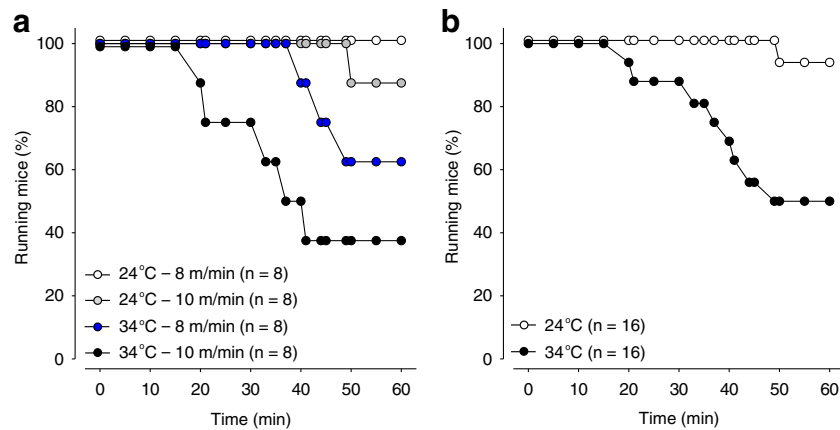


Fig. 3 **a** Maximal exercise duration tolerated by the mice subjected to constant-speed exercises on the treadmill at two ambient temperatures and two treadmill speeds: *white circles* 24 °C, 8 m/min; *gray circles* 24 °C, 10 m/min; *blue circles* 34 °C, 8 m/min; *black circles* 34 °C, 10 m/min

min. b The same analysis as in panel A, but the two treadmill speeds are pooled for each ambient temperature: 24 °C (*white circles*) or 34 °C (*black circles*). In both panels, the data are expressed as proportions of mice that were still running at given time points

When the data from all of the exercise trials (i.e., incremental- or constant-speed exercises) performed at the same T_a were plotted together, no changes in the profile of running-induced hyperthermia were observed (Fig. 5a, b). At the time when effort was interrupted, the average T_{core} values ranged from 38.57 to 38.85 °C at 24 °C and from 39.82 to 40.12 °C at 34 °C.

Discussion

The present study investigated the T_{core} of mice subjected to different running exercise protocols (i.e., incremental- or constant-speed exercises) at two T_a . Our results demonstrate that T_{core} increased in response to exercise performed in a temperate

environment (24 °C) and that this hyperthermia was enhanced in a warmer environment (34 °C). Consequently, the physical performance of mice subjected to prolonged treadmill running at the higher T_a was markedly impaired. Additionally, within the range of treadmill speeds examined, the magnitude of exercise hyperthermia was not dependent on treadmill speed.

During experiments performed at the temperate ambient, treadmill running induced a sharp increase in T_{core} that was observed as soon as 4 min following the initiation of exercise (Fig. 5a). T_{core} increased rapidly until the 9th to 13th min of exercise (at a rate of increase of 0.18–0.25 °C/min) prior to plateauing. Thereafter, T_{core} remained elevated until the mice were unable to keep pace with the treadmill. This pattern of T_{core} response during submaximal treadmill running at temperate environments is similar to previous findings in humans

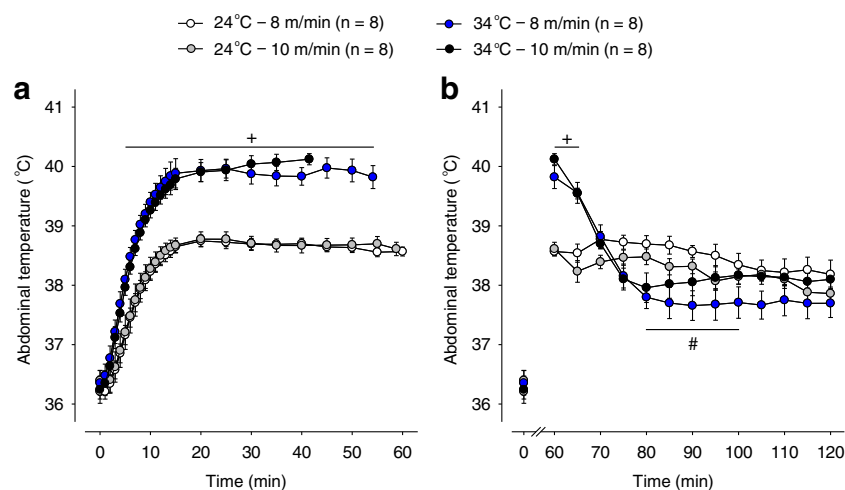
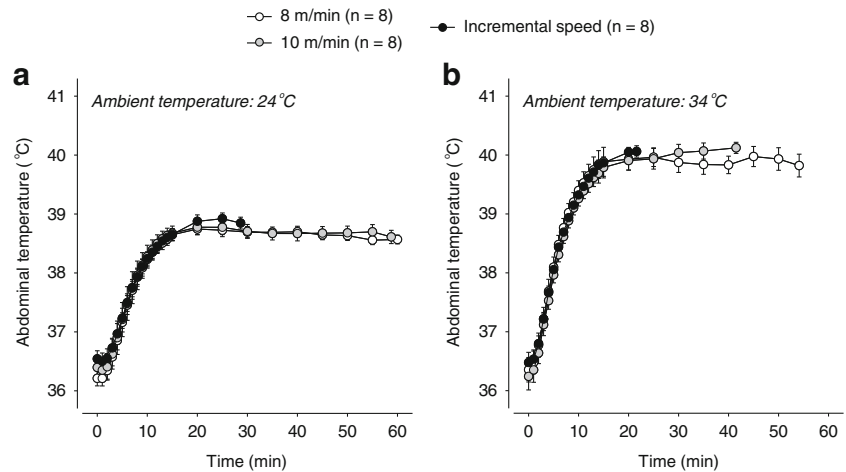


Fig. 4 Abdominal temperatures of mice during the four different constant-speed experimental trials (**a**) and during the post-exercise period (**b**). The following legends apply to both panels: *white circles* 24 °C, 8 m/min; *gray circles* 24 °C, 10 m/min; *blue circles* 34 °C, 8 m/min; *black circles*

34 °C, 10 m/min. Data are expressed as mean \pm SEM. + denotes that both trials at 34 °C are significantly different ($P < 0.05$) from the 24 °C trials. # denotes that the data for the 34 °C–8 m/min trial are significantly different ($P < 0.05$) from the data from the 24 °C–8 m/min trial

Fig. 5 Increases in the abdominal temperature of mice induced by three different types of treadmill running: *white symbols* constant-speed at 8 m/min, *gray symbols* constant-speed at 10 m/min, *black symbols* incremental-speed. These exercise trials were performed at 24 °C (a) and 34 °C (b). The data are expressed as mean \pm SEM



(Webb 1995) and rats (Lacerda et al. 2005). The similarities in the profile of exercise-induced hyperthermia indicate that running mice may be an adequate experimental model to investigate certain aspects of human thermal and exercise physiology.

An unexpected finding of the present study was that, irrespective of T_a , running at a constant-speed of 10 m/min did not exaggerate exercise-induced hyperthermia relative to running at 8 m/min (Fig. 4a). Even more unexpected was that incremental-speed exercise produced hyperthermia similar to that observed in both constant-speed exercise conditions (i.e., 8 and 10 m/min; Fig. 5a,b). At 34 °C, for example, the mice exhibited an average T_{core} of approximately 39.8–40.1 °C when the three different exercises protocols were interrupted. Although we speculate that the treadmill speed and, consequently, effort intensity are not the major determinants of the increase in T_{core} induced by exercise, we cannot rule out that longer exercise durations (> 1 h) would reveal effects of intensity on the T_{core} of running mice.

The observation that the running-induced increase in T_{core} was not dependent on exercise intensity (either absolute or relative) suggests the existence of a regulatory component within the hyperthermic response of exercising mice. Considering that different exercise intensities induced different metabolic rates (Schefer and Talan 1996), heat defense thermoeffectors were likely activated in a way that allowed T_{core} to be regulated at a similar level during the performance of distinct protocols. This finding suggests that physical exercise induces regulated hyperthermia in running mice, characterized by upward shifts in the thresholds of both heat loss and heat production, as observed under conditions of fever (Romanovsky 2004). This assumption contradicts the imbalance hypothesis proposed by Webb (1995) and must be confirmed through experiments designed to characterize tail skin temperature, metabolic rates and the T_{core} threshold for the activation of thermoeffectors in mice subjected to physical exercise.

The incremental-speed running performed in warm conditions exaggerated exercise hyperthermia relative to running at a temperate environment. When compared with the findings for same exercise performed at 24 °C, the exercise-induced increase in T_{core} was already enhanced after 3 min of running in the heat. Moreover, hyperthermia was exaggerated by 1.2 °C upon the interruption of effort (Fig. 1b). This exaggerated hyperthermia may be explained by the impaired ability of exercising rodents to dissipate heat through evaporative means (Shellock and Rubin 1984) and by relatively low heat loss through their tail skin, since the passive heat exchange is limited by a narrow skin temperature/ T_a gradient in hot environments.

Small-sized animals, such as mice, exhibit a high body surface area-to-mass ratio (Pinkel 1958; Davies and Morris 1993), which facilitates heat exchange with the environment. The consequences of different body sizes become clear when the effects of T_a on the hyperthermic response induced by exercise are compared between species. A number of investigations have found that a wide range of ambient temperatures does not change the extent of the rise in T_{core} during exercise in humans (Nielsen 1966; Lind 1963; Stolwijk et al. 1968). In contrast, small alterations in T_a (a reduction from 15 °C to 8 °C) have been found to alter the profile of the T_{core} response in rats exercising at the same absolute intensity, with the increase in T_{core} being replaced by a decrease (Guimaraes et al. 2013).

Recently, mice have been referred to as “average” homeotherms, as their T_{core} can increase or decrease as much as 2–4 °C from one hour to the next. Such a high variability is observed even in animals that are maintained under temperate environmental conditions (Gordon 2012). However, when averaged over several days, the mean T_{core} values of mice, rats and humans are quite similar despite large interspecies differences in body mass (Gordon 2012). Together with these data, our observations indicate that the T_{core} of

running mice is more sensitive to environmental heat stress than that of humans and rats. During exercise in the heat, the rate of increase in T_{core} was higher and T_{core} reached 40 °C earlier in mice (≈ 20 min, Fig. 5b) than in humans (≈ 50 min) exercising at a similar intensity and at a higher T_{a} (Nybo and Nielsen 2001). The greater dependence on T_{a} indicates the existence of a forced component (i.e., warming or cooling pressure from the environment) involved in the thermoregulation of small rodents during exercise.

The physical performance of the mice was greatly impaired in the warm environment: the distance traveled during incremental-speed exercise at 34 °C was decreased by 34 % relative to that observed for the temperate environment (Table 2). Given that exercising in heat imposes a great deal of stress on the cardiovascular system, restricting cutaneous blood flow and convective heat loss (Gonzalez-Alonso et al. 2008), we tested whether thermoregulation was associated with decreased performance in the present study. We observed that the higher rate of increase in T_{core} induced by exercise in the heat was associated with decreased maximal treadmill speed. These results are in agreement with previous observations in humans (Galloway and Maughan 1997; Gonzalez-Alonso et al. 1999) and rats (Rodrigues et al. 2003). Collectively, these results indicate that studies of running mice can, with certain limitations, help us to understand why the ability to exercise is impaired in hot environments. These limitations include interspecies differences in the body surface area-to-mass ratio and the higher capacity of exercising humans to dissipate heat through evaporative processes (i.e., evaporation of watery sweat; Lieberman and Bramble 2007; Jablonski 2010).

Despite not determining the magnitude of exercise-induced hyperthermia, the running protocol did determine the time elapsed until the voluntary interruption of effort, which was markedly shorter during the incremental-speed running exercise than during the constant-speed exercise at both 8 and 10 m/min (Fig. 5a and b). These results suggest that, in addition to high T_{core} and or high rates of increase for T_{core} (Nielsen et al. 1997; Fuller et al. 1998; Gonzalez-Alonso et al. 1999; Walters et al. 2000; Rodrigues et al. 2003; Tucker et al. 2006), other physiological responses that are associated with exercise intensity also contribute to the voluntary interruption of effort. That is, hyperthermia may be interpreted in different ways depending on the metabolic rate: 40 °C appears to be more dangerous for homeostasis when mice are running at 12 m/min (when the maximal incremental exercise duration was ≈ 22 min) than at 10 m/min (when 38 % of the mice were able to complete 60 min of exercise). Furthermore, the present data corroborate recent findings that demonstrated that high T_{core} is not the sole explanation for impaired aerobic performance in the heat (Cheung and Sleivert 2004). Physiological responses, such as increases in perceived exertion (Nybo and Nielsen 2001), changes in brain metabolism and blood flow

(Nybo et al. 2002; Rasmussen et al. 2010; Sakurada and Hales 1998) and endotoxemia-induced reductions in force generation by contractile proteins (Supinski et al. 2000), may also underlie the decrease in physical performance that is observed in conditions of environmental heat stress.

We conclude that the T_{core} of running mice was influenced greatly by T_{a} , but not by the exercise protocols or intensities examined in the present study. These findings suggest that the magnitude of hyperthermia, at least in running mice, is centrally regulated, independent of the rate of metabolic heat production. Our results provide the first systematic characterization of physical exercise-induced changes in the T_{core} of running mice and broaden the potential to use genetic tools, including knockout mice, for evaluating the association between exercise and hyperthermia.

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