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RESEARCH ARTICLE

Rats with higher intrinsic exercise capacities exhibit greater preoptic dopamine levels and greater mechanical and thermoregulatory efficiencies while running

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Rabelo PC, Cordeiro LM, Aquino NS, Fonseca BB, Coimbra CC, Wanner SP, Szawka RE, Soares DD. Rats with higher intrinsic exercise capacities exhibit greater preoptic dopamine levels and greater mechanical and thermoregulatory efficiencies while running. *J Appl Physiol* 126: 393–402, 2019. First published June 21, 2018; doi:10.1152/japplphysiol.00092.2018.—The present study investigated whether intrinsic exercise capacity affects the changes in thermoregulation, metabolism and central dopamine (DA) induced by treadmill running. Male Wistar rats were subjected to three incremental exercises and ranked as low-performance (LP), standard-performance (SP), and high-performance (HP) rats. In the first experiment, abdominal (T_{ABD}) and tail (T_{TAIL}) temperatures were registered in these rats during submaximal exercise (SE) at 60% of maximal speed. Immediately after SE, rats were decapitated and concentrations of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) were determined in the preoptic area (POA). In the second experiment, oxygen consumption was measured and mechanical efficiency (ME) was calculated in these rats during an incremental exercise. HP rats ran for longer periods and were fatigued with higher T_{ABD} values, with no difference in T_{TAIL}. Nevertheless, thermoregulatory efficiency was higher in HP rats, compared with other groups. DA and DOPAC concentrations in the POA were increased by SE, with higher levels in HP compared with LP and SP rats. $\dot{V}O_2$ also differed between groups, with HP rats displaying a lower consumption throughout the incremental exercise but a higher $\dot{V}O_2$ at fatigue. ME, in turn, was consistently higher in HP than in LP and SP rats. Thus, our results show that HP rats have greater T_{ABD} values at fatigue, which seem to be related to a higher dopaminergic activity in the POA. Moreover, HP rats exhibited a greater thermoregulatory efficiency during exercise, which can be attributed to a lower $\dot{V}O_2$, but not to changes in tail heat loss mechanisms.

NEW & NOTEWORTHY Our findings reveal that rats with higher intrinsic exercise capacities have greater thermoregulatory efficiencies and increased dopaminergic activity in the preoptic area, a key brain area in thermoregulatory control, while exercising. Moreover, higher intrinsic exercise capacities are associated with decreased oxygen consumption for a given exercise intensity, which indicates greater mechanical efficiencies. Collectively, these findings help to advance our knowledge of why some rats of a given strain can exercise for longer periods than others.

fatigue; monoamines; oxygen consumption; performance; temperature

INTRODUCTION

Fatigue is defined as a reduction in maximal voluntary force produced by contracting muscles that may result from changes in the periphery or central nervous system (10, 11, 48). This phenomenon has been accepted as a protective mechanism that prevents the organism from achieving a physiological condition that would result in tissue damage (30). Depending on the environmental conditions (14, 40) and some physical exercise features, e.g., intensity and duration (39, 49), fatigue can be differentially influenced by alterations in multiple physiological responses that signal early exercise cessation, including hyperthermia (4, 31–33), degradation of energy substrates (17), and changes in the levels of neurotransmitters (7, 8, 13, 53). Regarding the thermoregulatory influence on exercise fatigue, the increased heat production by the contracting muscles requires activation of heat loss mechanisms to maintain the core body temperature in a range compatible with physical performance and even life (51).

In rats, the primary mechanism that allows dry heat loss from the body to the environment depends on increased tail blood flow, which is caused by a withdrawal of sympathetic vasoconstrictor activity (4). During prolonged and intense exercises, heat production may surpass heat loss, thereby increasing core temperature, which, in turn, reduces voluntary activation of muscles and performance (4, 32). Marked increases in core temperature also place a great burden on the cardiovascular system, which may impair blood flow distribution between different beds, causing accumulation of metabolites and insufficient blood supply to the active muscles and skin vessels (6). Previous studies have demonstrated that pharmacological manipulations of neurotransmission systems override afferent signals arising from increased core temperature values that would cause fatigue (1, 40, 53, 54). For instance,

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the intracerebroventricular injection of dopamine (DA) allows exercising rats to achieve higher core temperatures and improves their aerobic performance (1).

Fatigue is a complex phenomenon that, alongside its thermoregulatory triggers, is also influenced by genetic variations that determine the intrinsic exercise capacity (22, 25). Previous experiments have identified that rats exhibit a large intrinsic variation in exercise performance (23–25) with this heterogeneity being associated with differences in multiple physiological responses (3, 19, 21, 38). In this context, we have previously identified that rats with different intrinsic capacities to exercise show altered monoaminergic responses in the caudate-putamen (CPu), also known as the dorsal striatum (36, 37). Such neurotransmitter changes might influence mechanical efficiency (ME), metabolic heat production, and the increase in core temperature during exercise (1).

Thus, considering the body of evidence linking thermoregulation and brain DA levels in the modulation of exercise fatigue, this study aimed to investigate whether rats with different intrinsic exercise capacities exhibit changes in thermoregulatory adjustments and dopaminergic neurotransmission in the preoptic area of the hypothalamus (POA) when subjected to exercise. We investigated the dopaminergic neurotransmission in the POA because of its modulatory effects on thermoregulation and physical performance in rats subjected to treadmill running (18, 53, 54). In addition, we also determined the effects of the intrinsic capacities on oxygen consumption ($\dot{V}O_2$) and ME during exercise.

MATERIALS AND METHODS

Animals

Adult male Wistar rats (240–280 g body wt), 2 months old, were used in all experiments. Initially, the rats were housed in collective cages (4 animals/cage) under a controlled light/dark cycle (14/10 h), at room temperature (24 ± 2°C) with free access to water and rat chow. In the first experiment, rats were housed in individual cages after the surgery for implantation of an abdominal temperature probe. In the second experiment, rats were kept in collective cages throughout the study. Experiments were approved by the Ethics Commission for the Use of Animals at the Universidade Federal de Minas Gerais (protocols 061/2011 and 367/2014), and the rats were obtained from the Animal Care Facility at the same university. During all experimental procedures, the ambient temperature was maintained at 24 ± 1°C. To control the influence of circadian oscillations in the physiological variables measured, experiments were always performed between 08:00 and 12:00 h.

Experimental Design

This study consisted of two sets of experiments. In the first set (*experiment 1*), we investigated whether rats with different intrinsic exercise capacities would exhibit distinct thermoregulatory adjustments and changes in dopaminergic transmission in the POA in response to constant and submaximal exercise (SE) at 60% of their maximal speed. In the second set (*experiment 2*), we investigated whether rats with different intrinsic exercise capacities also have distinct $\dot{V}O_2$ kinetics and ME during an incremental speed exercise.

Experiment 1: Effect of intrinsic exercise capacity on thermoregulation during SE. Rats were familiarized with running on a treadmill over five consecutive days. In the second week, all rats were subjected to three incremental speed exercises until they were fatigued, with a 48-h interval between tests for recovery. Based on the maximal exercise time (TE_{max}) obtained in one of the three tests, rats were ranked by using a previously described criterion (36, 37) into different categories of intrinsic exercise capacity [low-performance (LP), standard-performance (SP) and high-performance (HP)]. Briefly, to be included in the LP group, rats needed to have a TE_{max} lower than 24.9 min, whereas the rats with a TE_{max} between 24.9 and 57.1 min were ranked in the SP group. HP rats had a TE_{max} higher than 57.1 min. After this categorization, 16 rats from each group were randomly assigned to *experiment 1*. One day after the last of the three incremental exercise tests, half of the rats ($n = 8$ in each group) were subjected to a surgery for implantation of the temperature sensor in the abdominal cavity. After one week of recovery, these rats were subjected to an experiment under resting conditions for analysis of thermoregulatory variables. Two days after this experiment, the rats underwent an SE for analysis of running-induced changes in thermoregulatory variables. Immediately after the SE, the animals were decapitated. The other rats (again $n = 8$ for each group) were subjected to a sham implant of the temperature sensor and later decapitated under basal conditions (sedentary controls), i.e., without being subjected to the SE.

Experiment 2: Effect of intrinsic exercise capacity on $\dot{V}O_2$ and ME during incremental exercise. Similar to *experiment 1*, rats were ranked as LP, SP, and HP ($n = 8$ per group). Forty-eight hours after the last of the three incremental speed exercises that determined the intrinsic capacities $\dot{V}O_2$ was measured in rats of the three groups while resting on the treadmill. Twenty-four hours after this experiment, the rats were subjected to a fourth incremental speed exercise while their $\dot{V}O_2$ was continuously measured; these $\dot{V}O_2$ values were later used to calculate the ME. Rats were decapitated 48 h after the last exercise session.

Familiarization with Treadmill Running

All rats were familiarized with running on a treadmill designed for small animals (Columbus Instruments, Columbus, OH) over five consecutive days. Each day, the animals rested for 5 min and then ran for 5 min at incremental speeds (10, 10, 11, 13, and 15 m/min). In all familiarization sessions and experimental trials, the treadmill inclination was kept at 5 degrees, and an electrical stimulus was set to 0.28 mA. This light electrical stimulation was applied to encourage animals to run (36, 37).

Implantation of the Abdominal Sensor

For the surgery, the rats were anesthetized with intraperitoneal ketamine and xylazine (80.0 and 10.5 mg/kg body wt, respectively). After anesthesia, the animals were treated with a prophylactic dose of intramuscular antibiotic (0.1 ml applied to each hind paw; 48,000 IU/kg benzyl penicillin, Fort Dodge Animal Health, Fort Dodge, IA). The temperature sensor (G2 E-Mitter, ER-4000 model; Mini-Mitter Respironics, Bend, OR) was implanted in the abdominal cavity through an incision in the linea alba of the rectus abdominis. The abdominal sensor was implanted according to a previously described technique (26, 35, 51). After the surgery, the rats were treated with a subcutaneous analgesic (flunixin meglumine, 2 mg/kg body wt). The rats of the sedentary group were subjected to a sham surgery that was identical to the surgery described above, except that the sensor was not implanted.

Resting Experiments

In *experiment 1*, one week after the surgical procedures, all rats had recovered their presurgical body weight. Thermoregulatory variables, including abdominal (T_{ABD}) and tail-skin (T_{TAIL}) temperatures, were registered for 1 h while the rats were resting on the treadmill; these one-hour recordings were preceded by another hour for stabilization of temperatures. T_{ABD} was considered as an index of core body temperature and the increase in T_{TAIL} represented the activation of tail-skin heat loss, the major mechanism for heat dissipation in

exercising rodents (51). In *experiment 2*, rats were subjected to a recording of resting $\dot{V}O_2$. Initially, rats rested for one hour on the treadmill until $\dot{V}O_2$ stabilization, and in the subsequent hour, $\dot{V}O_2$ was continuously registered, as previously described (49).

Incremental Speed Exercise

The tests began at 10 m/min and the speed was increased by 1 m/min every 3 min until fatigue, which was determined when rats could no longer keep the running pace and stayed on the electric grid for 10 s (34).

Submaximal Exercise

Two days after the recording of thermoregulatory variables at rest, in *experiment 1*, the rats were subjected to a fatiguing exercise at 60% of their maximal speed (S_{max}). The S_{max} corresponded to the speed attained during one of the three initial incremental exercises when each rat had its best performance. Thermoregulatory variables were recorded during the SE.

Euthanasia

Rats of *experiment 1* were decapitated immediately after SE or after being taken from the vivarium (sedentary group). The brain was quickly removed and the POA was dissected, according to a previously described technique (43, 44). All dissections were performed bilaterally and by the same researcher. The POA was immediately stored at −80°C until assay for determination of DA and 3,4-dihydroxyphenylacetic acid (DOPAC) via high-performance liquid chromatography with electrochemical detection.

The rats of *experiment 2* also were decapitated and their brains were stored for future analyses and, therefore, not included in this manuscript.

Measured Variables

T_{ABD}. T_{ABD} was recorded every 15 s by telemetry. The radio waves emitted by the abdominal sensor were captured by a receiving plate (ER-4000 energizer/receiver; Mini-Mitter Respironics) positioned next to the treadmill. The radio wave frequencies were converted into temperature values by software (Vital View), and the data were stored online (26).

T_{TAIL}. T_{TAIL} was registered every minute via a thermocouple (series 409-B; Yellow Springs Instruments, Dayton, OH) fixed with impermeable adhesive tape (2-cm width) to the lateral surface of the tail, 1 cm from its base (12, 50). The thermocouple used in the experiments records temperature on only one side of its surface; the measuring surface was placed in direct contact with the tail. Both the use of adhesive tape and the placement of measuring surface help to buffer the temperature measured by the thermocouple from any influence of the ambient temperature.

DOPAC and DA. Concentrations of DOPAC and DA were measured in the POA with high-performance liquid chromatography with electrochemical detection, as previously described in Rabelo et al. (36, 37).

Calculated Variables

Heat loss index. The heat loss index (HLI) was used as a measure of tail-skin vasomotor tone. This index eliminates passive changes in T_{TAIL} caused by modifications in core body and ambient temperatures. It was calculated according to the following equation: HLI = (T_{TAIL} – ambient temperature)/(T_{ABD} – ambient temperature) (41).

Thermoregulatory efficiency. The ratio between the increase in T_{ABD} (°C) and the distance traveled by the rats (km) was calculated. The increase in T_{ABD} was calculated by subtracting final from initial T_{ABD}. This ratio was calculated at two different time points: at 24 min, when all rats from the three groups were still running, and at fatigue. This ratio is inversely related to the thermoregulatory efficiency; therefore, the lower the ratio, the higher the efficiency.

Heat loss threshold and heat loss sensitivity. The heat loss threshold (HL_{THR},°C) and heat loss sensitivity (HL_{SEN}, arbitrary units) were calculated using a method based on that described by Cheuvront et al. (5), which has been used to investigate thermoregulatory responses in humans (28) and rats (12). Briefly, the T_{TAIL} was plotted against T_{ABD}, the threshold for activation of tail heat loss was identified visually by two independent investigators, and a consensus-derived value was used. Next, the data before and after this threshold were separated. To describe the relationship between T_{TAIL} and T_{ABD}, linear regression analyses were performed for data before and after the threshold. The intersection of the regression lines was used to determine the HL_{THR}. At this moment, we could objectively verify whether visual identification of the threshold was correct. If not, the analysis was redone from the beginning. The HL_{SEN} was defined as the regression slope of the five points that followed the threshold and corresponded to the steepest part of the rising curve.

$\dot{V}O_2$ and ME. $\dot{V}O_2$ was continuously measured via open-flow indirect calorimetry (LE8700; Panlab, Harvard Apparatus, Cornella, Spain). For the $\dot{V}O_2$ experiments, a different treadmill setup was used (LE400; Panlab). The $\dot{V}O_2$ data were analyzed using the metabolism software (version 2.2.01; Panlab) and transformed into milliliters per minute and then relativized by body weight (ml $O_2 \cdot kg^{-1} \cdot min^{-1}$). During the incremental exercise, the highest $\dot{V}O_2$ value was considered $\dot{V}O_{2peak}$. The equipment was calibrated weekly with a known gas mixture (high O_2 = 49.99%, high CO_2 = 1.50%, low O_2 = 20.00%, low CO_2 = 0.00%) (49). The workload was calculated as follows: workload (Joule) = body wt·g·s·sinθ·t, where: body wt (kg), g = gravity force (9.8 m/s^2), s = speed (m/min), θ = inclination of the treadmill (5°), t = time spent in each stage in minute (2). The total workload was the sum of the workload in each stage of the incremental exercise (50). The $\dot{V}O_2$ and workload data were converted to kcal/min, and the ME was calculated as follows: ME (%) = (workload/$\dot{V}O_2$) ·100 (1, 44).

Statistical Analyses

All data are reported as means ± SE. Shapiro-Wilk and Levene's tests were used to assess the data normality and homoscedasticity, respectively. One-way ANOVA was used to compare initial body weight, TE_{max}, S_{max}, thermoregulatory efficiency, $\dot{V}O_{2peak}$, and the following parameters: T_{ABD}, T_{TAIL}, HLI, and ME, at fatigue between groups. Differences in the T_{ABD}, T_{TAIL}, HLI, $\dot{V}O_2$, and ME during exercise or resting conditions were compared between groups and across time points using two-way ANOVA with repeated-measures for only the factor consisting of time points. Dopaminergic variables in the POA were compared between groups and treatments using two-way ANOVA. The Student-Newman-Keuls post hoc test was used for multiple comparisons, whenever applicable. To identify the existence of outliers, the Grubbs' test was applied to all data (15). A statistical significance level of 5% was adopted.

RESULTS

Effect of Intrinsic Exercise Capacity on Thermoregulation during Exercise

Body weight and physical performance during the incremental exercise test. In the beginning of the experiment, there was no intergroup difference in body weight (LP, 283 ± 4 g; SP, 276 ± 4 g; HP, 280 ± 5 g; P = 0.527). The TE_{max} during the incremental speed exercise was 21 ± 2 min, 39 ± 1 min, and 66 ± 1 min for the LP, SP, and HP rats, respectively [$P <$ 0.001; data published (36)]. Additionally, the S_{max} attained by the rats was 16 ± 0.7 m/min, 21 ± 0.6 m/min, and 31 ± 0.7 m/min for LP, SP, and HP rats, respectively ($P <$ 0.001).

Therefore, both the TE_{max} and S_{max} were higher in HP rats compared with the SP, and LP rats and were lower in LP than in SP rats. In the subsequent experiments (i.e., SE), the rats were submitted to exercise at 60% of their S_{max}, which corresponded to 10 m/min, 13 m/min, and 19 m/min for the LP, SP, and HP rats, respectively ($n = 16$ per group).

T_{ABD} and T_{TAIL} loss under resting conditions. When rats were allowed to rest on the treadmill no significant differences were observed in the T_{ABD}, T_{TAIL}, and HLI between groups (T_{ABD}, $P = 0.658$; T_{TAIL}, $P = 0.302$; HLI, $P = 0.465$) or over time (T_{ABD}, $P = 0.614$; T_{TAIL}, $P = 0.793$; HLI, $P = 0.998$). In addition, no interactions were observed between the two factors for these three temperature parameters. In all experimental groups the average T_{ABD} values fluctuated in a range of 36.5 to 37.0°C, whereas average T_{TAIL} values fluctuated in a wider range of 26 to 28°C (Fig. 1). HLI fluctuated between 0.13 and 0.24 (data not shown), which suggests that the rats from the three groups were within the lower end of the thermoneutral zone (41).

Thermoregulation and physical performance during SE. Time of exercise during SE was higher in HP rats compared with the SP and LP rats and was lower in LP than in SP rats [LP, 49.0 ± 8.4 min; SP, 109.4 ± 8.3 min; HP, 224.6 ± 26.7 min; $P < 0.001$, (36)]. Because of technical problems with recording the T_{ABD} data during treadmill running, an $n = 7$ was used in each of the three groups. During this exercise, the T_{ABD} exhibited the expected increases that were similar in the three groups for most of the exercise period. However, the T_{ABD} at fatigue was ~1.0°C higher in HP than in LP and SP rats ($P < 0.05$; Fig. 2, *A* and *C*). We then calculated the ratio between the increase in T_{ABD} and distance traveled, which is inversely associated with thermoregulatory efficiency. At 24 min (when all rats in the three groups were still running) or at fatigue, the ratio was lower in HP than in SP and LP rats ($P < 0.01$; Fig. 2*E*) indicating that HP rats had the greatest thermoregulatory efficiency. Besides that, the efficiency was also higher in SP than in LP rats at fatigue ($P < 0.01$; Fig. 2*F*).

T_{TAIL} and HLI also exhibited the expected increases during exercise, implying augmented cutaneous heat loss. No intergroup differences and no interactions between groups and time points were observed in T_{TAIL} ($P = 0.213$, Fig. 2*B*) and HLI (data not shown, $P = 0.218$) during exercise. In addition, HL_{THR} and HL_{SEN} did not differ between groups (Table 1), indicating that these parameters related to cutaneous heat loss were not influenced by the intrinsic exercise capacity. At fatigue, T_{TAIL} did not differ between groups ($P = 0.063$; Fig. 2*D*), although HLI was higher in SP rats than in the two other groups (LP, 0.42 ± 0.04; SP, 0.53 ± 0.01; HP, 0.44 ± 0.02; $P = 0.029$).

Collectively, our results indicated that HP rats have a greater thermoregulatory efficiency that does not result from an improved tail heat loss. We thus hypothesized that differences in ME and the consequent lower metabolic heat production might explain the greater thermoregulatory efficiency in HP rats. *Experiment 2* was therefore conducted to test this hypothesis.

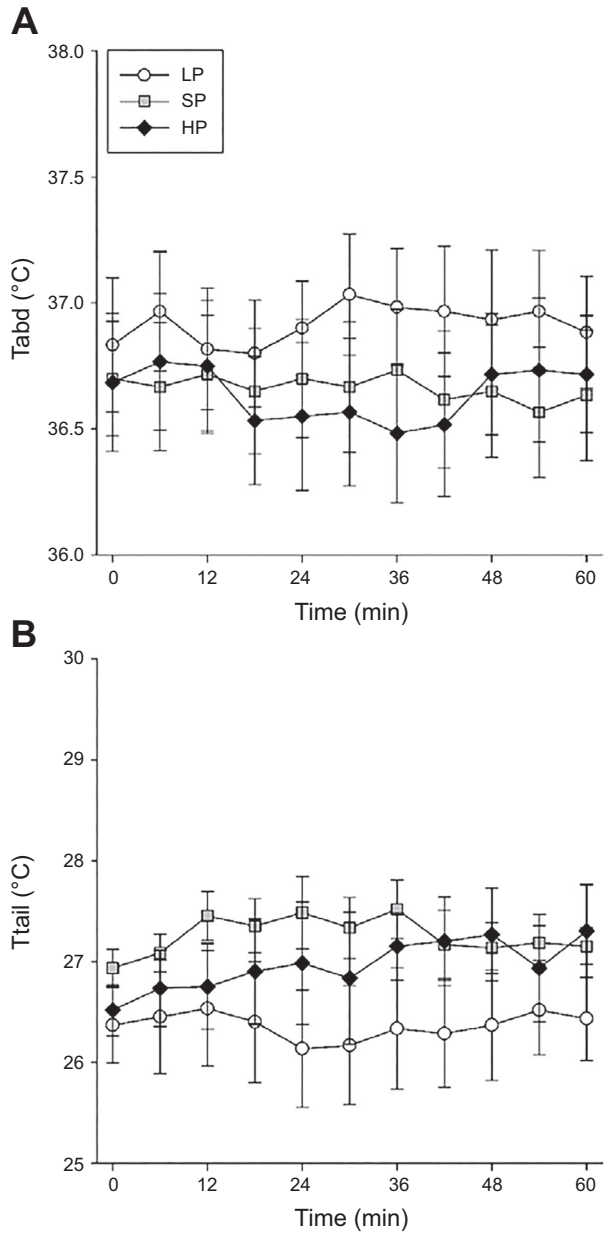

Fig. 1. Thermoregulatory parameters under resting conditions in rats with different intrinsic exercise capacities. Low performance (LP), standard performance (SP), and high performance (HP) rats ($n = 8$ per group) had their abdominal temperature ($T_{ABD;}$ *A*) and tail skin temperature (T_{TAIL}; *B*) measured for 60 min while resting. Values are expressed as means ± SE. Statistical analysis: two-way ANOVA with repeated-measures (one factor repetition).

Dopaminergic responses in the POA. In sham rats, which consisted of animals that were decapitated under baseline

Fig. 2. Thermoregulatory parameters in rats with different intrinsic exercise capacities subjected to a submaximal exercise (SE). Low performance (LP; $n = 7$), standard performance (SP; $n = 6$), and high performance (HP; $n = 7$) rats underwent SE at 60% of maximal speed until they were fatigued. The following parameters were measured: abdominal temperature (T_{ABD}, *A*), tail-skin temperature (T_{TAIL}, *B*), T_{ABD} at fatigue (*C*), T_{TAIL} at fatigue (*D*), the ratio between the change in T_{ABD}, and distance traveled at 24 min of exercise (*E*) and at fatigue (*F*). Values are expressed as means ± SE. #Difference compared with LP ($P < 0.05$); and +difference compared with SP ($P < 0.05$). Statistical analyses: two-way ANOVA with repeated-measures (one factor repetition) in *A* and *B*, and one-way ANOVA in *C*, *D*, *E*, and *F*.

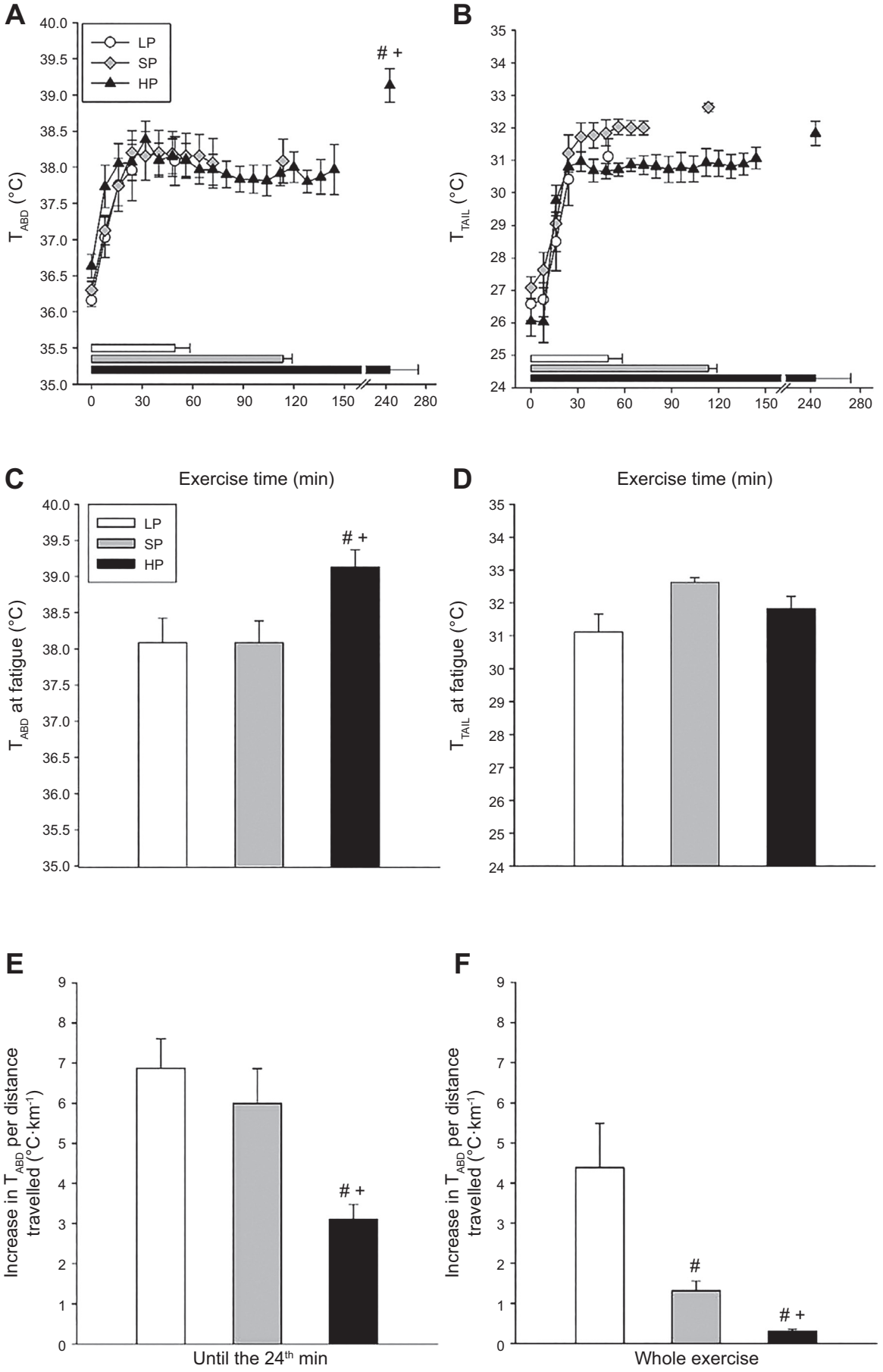

Table 1. *Heat loss threshold and sensitivity in rats with different intrinsic exercise capacities subjected to submaximal exercise*

	LP	SP	HP	*P*
HL_{SEN}, AU	6.8 ± 1.3	4.4 ± 1.4	7.2 ± 1.7	0.400
HL_{THR}, °C	37.4 ± 0.3	36.8 ± 0.5	37.7 ± 0.2	0.279

Values are expressed as means ± SE. Low performance (LP, $n = 7$), standard performance (SP, $n = 6$), and high performance (HP, $n = 7$) rats were subjected to submaximal exercise until they were fatigued. AU, arbitrary units; HL_{THR}, heat loss threshold; HL_{SEN}, heat lost sensitivity. Statistical analysis: one-way ANOVA.

conditions, no intergroup differences were observed in the concentrations of DA and DOPAC in the POA (Fig. 3). On the contrary, in exercised rats that were decapitated immediately after fatigue, DOPAC ($P < 0.01$) and DA ($P < 0.01$) concentrations increased in SP and HP rats, but not in LP rats, relative to baseline concentrations. Furthermore, both parameters were higher in HP than in LP and SP rats [(DOPAC, $P < 0.05$; DA, $P < 0.01$), Fig. 3, *A* and *B*]. No intergroup differences were observed in the DOPAC/DA ratio at baseline conditions or at fatigue.

Effect of intrinsic exercise capacity on $\dot{V}O_2$ and ME during exercise

Body weight and physical performance during incremental exercise. As in *experiment 1*, the initial body weight did not differ between groups (LP, 285 ± 6 g; SP, 281 ± 6 g; HP, 275 ± 4 g; $P = 0.492$). In addition, TE_{max} was higher in HP than in SP and LP rats and was also higher in SP than in LP rats (LP, 12 ± 2 min; SP, 45 ± 2 min; HP, 63 ± 3 min; $P < 0.001$).

$\dot{V}O_2$ under resting conditions. While rats were resting on the treadmill, the $\dot{V}O_2$ was not different between groups ($P = 0.596$, Fig. 4) or over time ($P = 0.133$, Fig. 4), and no interactions were observed between these two factors. In all groups, the average $\dot{V}O_2$ values fluctuated in the range of 20 to 26 ml O_2·kg·min.

$\dot{V}O_2$ and ME during incremental exercise. In response to the incremental exercise, $\dot{V}O_2$ markedly increased in all groups ($P < 0.001$, Fig. 5*A*). However, $\dot{V}O_2$ was lower in HP compared with LP rats from the 6th to the 12th min and with SP rats from the 6th to the 12th min and from the 24th to the 33rd min of exercise ($P < 0.05$, Fig. 5*A*). Nevertheless, the $\dot{V}O_{2peak}$ was higher in HP than in LP and SP rats and was also higher in SP than in LP rats ($P < 0.001$, Fig. 5*C*). We then calculated ME to determine the relationship between the workload produced by the rats and their energy expenditure. ME also increased in response to the incremental exercise in all three groups ($P < 0.001$; Fig. 5*B*). Moreover, ME was higher in HP rats compared with LP rats from the 3rd to the 12th min and compared with SP rats from the 3rd to the 12th min and from the 24th to the 33rd min of exercise ($P < 0.001$, Fig. 5*B*). Like the $\dot{V}O_{2peak}$, ME at fatigue depended on the intrinsic exercise capacity of the rats, such that the higher the capacity the higher the ME ($P < 0.001$, Fig. 5*D*).

DISCUSSION

The current findings show that rats with different intrinsic exercise capacities exhibit distinct thermoregulatory responses, $\dot{V}O_2$, ME, and DA neurotransmission in the POA during treadmill running. More specifically, HP rats exhibited lower $\dot{V}O_2$ at submaximal intensities and greater thermoregulatory efficiency, ME, DOPAC, and DA levels while exercising than SP and LP rats. Moreover, HP rats also exhibited greater $\dot{V}O_{2peak}$, a physiological parameter commonly used to assess cardiorespiratory fitness (20) and a determinant of endurance performance. Collectively, these findings evidence some physiological features that may influence the intrinsic running capacity and enhance our knowledge regarding the mechanisms underlying the innate variability of exercise performance.

As expected, the core temperature data during the SE in temperate conditions allowed for the identification of two distinct phases, i.e., the dynamic and steady-state phases of T_{ABD} (51). At the beginning of the exercise, abrupt increases in T_{ABD} (dynamic phase) resulted from augmented metabolic rate in working muscles and cutaneous vasoconstriction. This abrupt increase was independent of the intrinsic exercise capacity. Indeed, this initial increase in core temperature induced by treadmill running seems to be a stereotypic response, which, for instance, occurs regardless of exercise intensity (51). However, after 15–20 min of exercise, the sympathetic activity for the tail is withdrawn, resulting in skin vasodilation, heat dissipation to the environment, and stabilization of T_{ABD} (steady-state phase). Of note, close to fatigue, the T_{ABD} increased again in HP rats. This second increase in T_{ABD} was not observed in SP and LP rats, suggesting that in these rats, fatigue was caused predominantly by nonthermoregulatory factors.

To determine the thermoeffector activity associated with cutaneous heat loss, the T_{TAIL} was plotted as a function of T_{ABD}, and then HL_{THR} and HL_{SEN} were calculated. The HL_{THR} and HL_{SEN} have been traditionally accepted as indicators of central and peripheral modulation of cutaneous heat loss, respectively (50). The differential intrinsic capacities to exercise influenced neither the HL_{THR} nor the HL_{SEN}. In addition, when the HLI was calculated, no intergroup differences were observed during most of the exercise period; however, at fatigue, SP rats presented a greater HLI than the other groups. This result was unexpected and difficult to explain due to the marked differences in the time-to-fatigue between groups (~2 h higher in HP than in SP rats), and because the greatest HLI value was not observed in rats with extreme performances (i.e., HP and LP rats). In addition, reinforcing the notion that tail heat loss is not dependent on changes in preoptic DA, a previous study reported that rats treated with buproprion had greater DA concentrations in the POA but unchanged T_{TAIL} during treadmill running (18).

A recent study highlighted that tail skin vasodilation does not fully explain heat loss in a running rat; Malheiros-Lima et al. (29) reported that rats subjected to tail artery denervation presented greatly impaired tail heat loss, but a normal exercise induced increase in T_{ABD}. In the present study, differences in alternative pathways for dissipating heat other than the tail-skin vasodilation could explain the distinct thermoregulatory efficiencies between the three groups. The paw is probably not a major pathway for dry heat loss in running rats due to the friction between the paws and treadmill belt, which generates heat. In contrast, ear vasodilation may be an alternative functional pathway for dissipating heat during exercise, although its

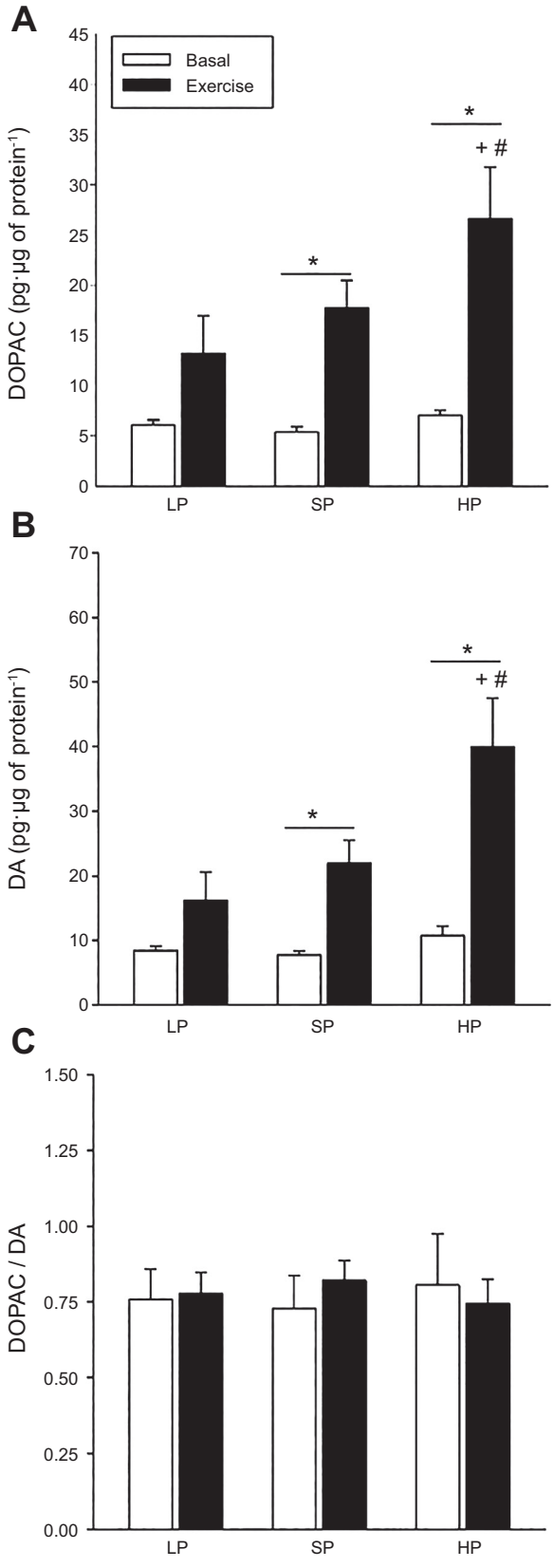

Fig. 3. Concentrations of dopaminergic variables in the preoptic area of the hypothalamus (POA) in rats with different intrinsic exercise capacities. Low performance (LP), standard performance (SP), and high performance (HP) rats were decapitated under basal conditions or after submaximal exercise (SE) until fatigue ($n = 8$ per group, except that 2 outliers subjected to SE were excluded from analysis: 1 LP rat and 1 SP rat). The following dopaminergic variables were measured: dopamine (DA; *A*) and 3,4-dihydroxyphenylacetic acid (DOPAC; *B*) concentrations and the ratio between DOPAC/DA in the POA (*C*). Values are expressed as means ± SE. *Difference compared with basal conditions ($P < 0.05$); #difference compared with LP ($P < 0.05$); and +difference compared with SP ($P < 0.05$). Statistical analysis: two-way ANOVA.

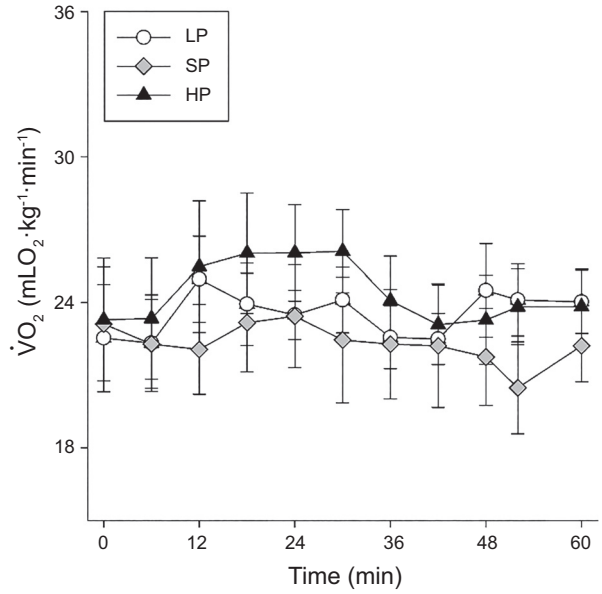

Fig. 4. Oxygen consumption ($\dot{V}O_2$) under resting conditions in rats with different intrinsic exercise capacities. $\dot{V}O_2$ was measured for 60 min in low performance (LP), standard performance (SP), and high performance (HP) rats ($n = 8$ in each group) at rest. Values are expressed as means ± SE. Statistical analysis: two-way ANOVA with repeated-measures (one factor repetition).

effectiveness has never been investigated under such conditions. Regarding evaporative heat loss, the evaporation of water from the respiratory tract is dependent upon exercise intensity (47) and, therefore, may also contribute to thermoregulation in exercising rats. Saliva-spreading behavior is an important adjunct thermolytic mechanism, particularly in hot environments (16). However, it is noteworthy that the importance of this thermolytic pathway is minor during treadmill running because rats are unable to spread saliva over their fur (42). Taken together, the above-mentioned information indicates that we cannot rule out that HP rats have greater cutaneous heat loss relative to the SP and LP rats in surface areas other than the tail skin (i.e., dry heat loss through the ears and evaporative loss from the respiratory tract).

DA and DOPAC levels in the POA increased in SP and HP rats in response to exercise, and both parameters were markedly higher in HP rats than in the other groups. These results suggest that treadmill running at 60% of S_{max} for ~2 h or more leads to an increase in preoptic DA synthesis in the POA possibly through the activation of tyrosine hydroxylase, the rate-limiting enzyme in DA synthesis, as previously indicated (45, 46). As DOPAC is considered to be a reliable index of DA

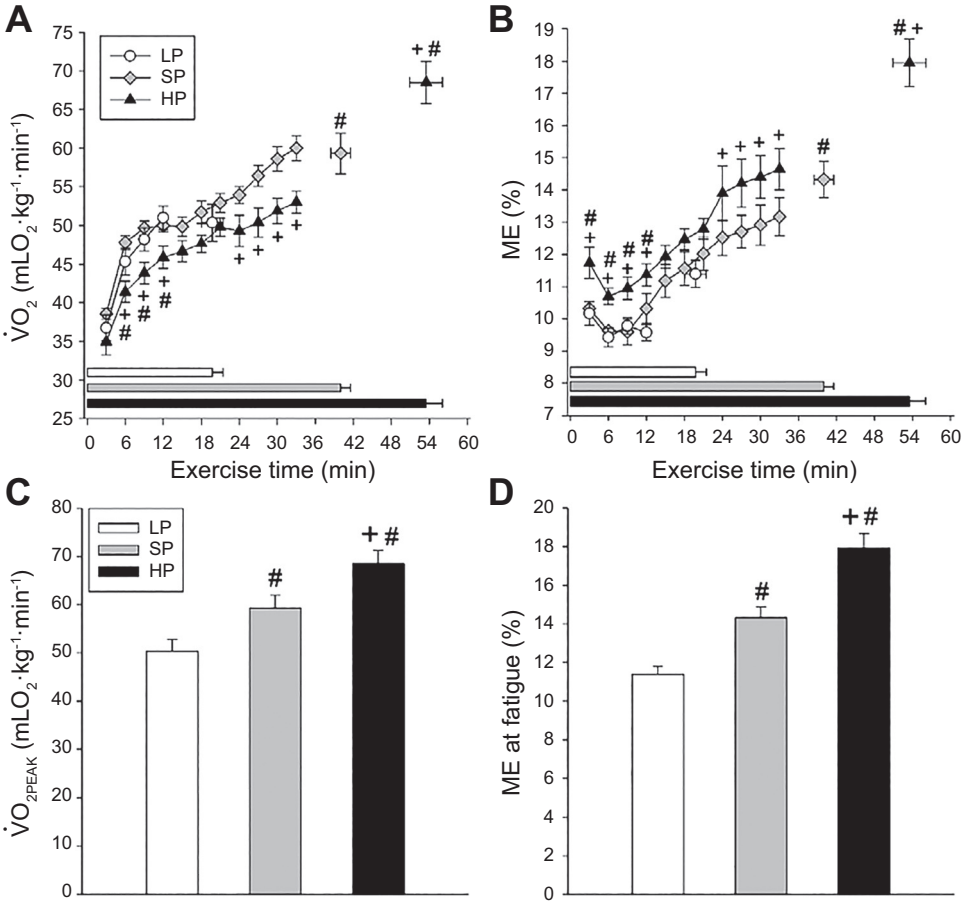

Fig. 5. Oxygen consumption ($\dot{V}O_2$) and mechanical efficiency (ME) in rats with different intrinsic exercise capacities subjected to an incremental exercise. Low-performance (LP), standard-performance (SP), and high-performance (HP) rats ($n = 8$ in each group) underwent an incremental exercise until they were fatigued. The following parameters were measured: $\dot{V}O_2$ (*A*), ME (*B*), peak oxygen consumption ($\dot{V}O_{2peak}$; *C*) and ME at fatigue (*D*). Values are expressed as means ± SE. #Difference compared with LP ($P < 0.05$) and +difference compared with SP ($P < 0.05$). Statistical analyses: two-way ANOVA with repeated-measures (one factor repetition; *A* and *B*) and one-way ANOVA (*C* and *D*).

release (27), it can be stated that both the synthesis and release of DA seem to be enhanced in the POA of HP rats. Interestingly, the present results also contradict the hypothesis of central fatigue, which predicts that dopaminergic activity decreases close to fatigue due to a serotonergic inhibition (11).

HP rats were fatigued with higher T_{ABD} levels, suggesting a greater tolerance to exercise-induced hyperthermia that would be associated with higher dopaminergic activity in the POA. Accordingly, DA has been considered to be a neurotransmitter that modulates the tolerance to exercise-induced hyperthermia (1, 9, 40, 53). In fact, Balthazar et al. (1) identified that intracerebroventricular administration of DA before exercise improves performance and allows for the achievement of higher core temperatures in running rats. Our data reinforce the idea that DA in the POA likely alters the inhibitory signals triggered by the increase in core temperature which would eventually stop exercise (52). Despite the body of evidence supporting that higher core temperatures at fatigue in HP rats are due to greater thermal tolerance, this conclusion should be considered with caution, because our experiments were not conducted in a hot environment that allows proper investigation of thermal tolerance.

At 24 min and at fatigue (both during SE), the thermoregulatory efficiency was higher in HP than in LP and SP rats. At fatigue, the thermoregulatory efficiency was also higher in SP rats compared with the LP group. As tail heat loss, the main pathway for dissipating body heat in exercising rodents, was not influenced by intrinsic exercise capacity, we hypothesized that ME would be greater, and, consequently, heat production would be lower in HP rats. The latter hypothesis was confirmed in the *experiment 2*, in which HP rats subjected to an incremental exercise exhibited greater ME than the LP and SP rats. Besides that, the $\dot{V}O_{2peak}$ and ME at fatigue were dependent on intrinsic capacity, gradually increasing in LP, to SP, to HP rats. Based on these results, we speculate that animals with differences in intrinsic exercise capacity exhibit distinct biomechanical features while running.

The greater ME in HP rats can also be explained by central factors, particularly by parameters associated with dopaminergic neurotransmission. In previous studies, we reported that HP rats exhibited higher dopaminergic activity in the CPu, a brain area related to motor control, in comparison with rats with lower performance (36, 37). Thus, this greater dopaminergic activity in the CPu could result in a higher ME during treadmill running, a hypothesis that is also supported by the findings of Balthazar et al. (1). In the latter study, intracerebroventricular injection of DA in rats attenuated the reduction of ME during incremental exercise, relative to the reduction observed after treatment with saline (1). Moreover, this treatment with DA increased the $\dot{V}O_{2peak}$ (1).

In summary, our results indicate that HP rats have a greater exercise-induced increase in core temperature, which likely results from higher dopaminergic activity in the POA. In addition, HP rats exhibited a greater ME, favoring a greater thermoregulatory efficiency. Thus, our findings contribute to enhance our knowledge about the intrinsic variability of exer-

cise capacities. Considering the thermoregulatory differences among rats with different intrinsic exercise capacities, future experiments should investigate the thermoregulatory responses of these rats during exercise under environmental heat stress, and in response to physical training or heat acclimation protocols.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

P.C.R. and R.E.S. conceived and designed research; P.C.R. and B.B.F. performed experiments; P.C.R., L.M.S.C., S.P.W., and R.E.S. analyzed data; P.C.R., L.M.S.C., B.B.F., S.P.W., and R.E.S. interpreted results of experiments; P.C.R., L.M.S.C., S.P.W., and R.E.S. prepared figures; P.C.R., L.M.S.C., N.S.S.A., B.B.F., C.C.C., S.P.W., R.E.S., and D.D.S. drafted manuscript; P.C.R., L.M.S.C., N.S.S.A., B.B.F., C.C.C., S.P.W., R.E.S., and D.D.S. edited and revised manuscript; P.C.R., L.M.S.C., N.S.S.A., B.B.F., C.C.C., S.P.W., R.E.S., and D.D.S. approved final version of manuscript.

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