

1 **Exercise Activates Compensatory Thermoregulatory Reaction in Rats: A Modeling Study**

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19 **Running head:** Body Temperature Dynamics During Exercise

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21

22 **Abstract**

23 The importance of exercise is increasingly emphasized for maintaining health. However,
24 exercise itself can pose threats to health such as the development of exertional heat shock in
25 warm environments. Therefore, it is important to understand how thermoregulation system
26 adjusts during exercise and how alterations of this can contribute to heat stroke. To explore this
27 we measured the core body temperature of rats (T_c) running for 15 min on a treadmill at various
28 speeds in two ambient temperatures ($T_a=25^\circ\text{C}$ and 32°C). We assimilated the experimental data
29 into a mathematical model that describes temperature changes in two compartments of the body,
30 representing the muscles and the core. In our model the core body generates heat to maintain
31 normal body temperature, and dissipates it into the environment. The muscles produce additional
32 heat during exercise.

33 According to the estimation of model parameters, at $T_a=25^\circ\text{C}$, the heat generation in the core
34 was progressively reduced with the increase of the treadmill speed to compensate for a
35 progressive increase in heat production by the muscles. This compensation was ineffective at
36 $T_a=32^\circ\text{C}$, which resulted in an increased rate of heat accumulation with increasing speed, as
37 opposed to the $T_a=25^\circ\text{C}$ case. Interestingly, placing an animal on a treadmill increased heat
38 production in the muscles even when the treadmill speed was zero. Quantitatively, this “ready-
39 to-run” phenomenon accounted for over half of the heat generation in the muscles observed at
40 maximal treadmill speed. We speculate that this anticipatory response utilizes stress-related
41 circuitry.

42 **Glossary**

43

$\frac{dT_c}{dt}$	The rate of change in the core temperature over time ($^{\circ} C/min$)
$\frac{dT_m}{dt}$	The rate of change in the temperature of the muscles over time ($^{\circ} C/min$)
P_0	Difference between overall heat production in the core and dissipation to the environment ($^{\circ} C/min$)
P_m	Additional heat produced by the muscles due to exertion ($^{\circ} C/min$)
T	Time (min)
T_a	Ambient temperature ($^{\circ} C$)
T_c	Core body temperature from the mathematical model ($^{\circ} C$)
T_m	Temperature in muscles from the mathematical model ($^{\circ} C$)
T_t^j	Averaged core body temperature at time t min of rats running on the treadmill with speed j m/min ($^{\circ} C$)
$\{T_t^j\}_{t=0}^{15}$	The series of averaged core body temperature at every minute for 15 minutes of rats running on the treadmill with speed j m/min ($^{\circ} C$)
$T(P_0, P_m, T_0, t)$	Core body temperature as a function of four parameters $P_0, P_m, T_0,$ and t ($^{\circ} C$)
$s_t^2(s_t)$	The sample variance (standard deviation) of the experimental measurement of the core body temperature at time t min of rats running on the treadmill with speed j m/min . ($^{\circ} C$)
η	heat transfer coefficient between muscles and core (min) ⁻¹
$\sigma^2(\sigma)$	Averaged value of the sample variance s_t^2 (square root of averaged value of the sample variance s_t^2) ($^{\circ} C$)

44

45

46 **Introduction**

47 To compensate for overall life style-induced decrease of exercise (26), millions of people
48 around the world participate in various sports. Many of these people choose to exercise and
49 compete outdoors. Since outdoor running occurs most frequently in the warmer months of the
50 year, the participants are at risk for developing heat-related illnesses. The most devastating heat-
51 related illness is exertional heat stroke. Occurring in otherwise healthy people, heat stroke affects
52 people at the most productive times of their lives. Between 1990 and 2010 an average of 2 high
53 school and college football players died each year due to heat stroke (3). The US Centers for
54 Disease Control estimate that each year 660 heat related deaths occur (1). Along with the risk for
55 death, those suffering heat stroke lose the ability to work or perform normal activities and may
56 suffer life-long medical complications even after recovery. As the number of marathoners in
57 USA exceeds a half a million (35), the ability to predict who is prone to heat stroke could benefit
58 thousands of people. The dynamics of the body temperature is a major determinant of a heat
59 stroke (14), so the ability to predict a heat stroke is largely an ability to predict changes in body
60 temperature.

61 Mammals have developed multiple thermoregulatory mechanisms to maintain their core
62 body temperature within a relatively narrow range. In cold environments, the body produces heat
63 through non-shivering thermogenesis and decreases heat dissipation through cutaneous
64 vasoconstriction (34). Correspondingly, in warm environments, non-shivering thermogenesis is
65 shut off and excess heat is dissipated by increasing blood flow near the skin and, in humans,
66 through evaporative heat loss through sweat.

67 During exercise an additional heat is produced by contractions of the skeletal muscles.
68 Unlike “classical” heat stroke where the primary cause of heat stroke is ineffective dissipation of

69 heat in hot and/or humid environment, exertional heat stroke develops due to excessive
70 production of heat from work/exercise. In these cases, heat stroke is produced by heat production
71 outstripping maximal heat dissipation. It is logical to hypothesize that during exercise heat
72 production unrelated to exercise would be suppressed (for review see (21)). However, activity-
73 thermoregulatory heat substitution had not been analyzed in terms of resulting body temperature
74 dynamics. Thermoregulatory processes affecting heat production during exercise are difficult to
75 study, as the corresponding experimental paradigms require measuring the metabolism in, and
76 heat exchange between, different body compartments in conscious running animals. In this
77 study, we use mathematical modeling to characterize thermoregulatory processes from easy to
78 measure body temperature dynamics observed at different ambient temperatures and exercise
79 intensities.

80

81

82 **Methods**

83 *Animals and experimental design*

84 Male Sprague-Dawley rats (280-350 g) were individually housed with a 12 h light cycle at a
85 room temperature of 23-25°C with free access to food and water. All animals for which data are
86 reported remained in good health throughout the course of surgical procedures and experimental
87 protocols as assessed by appearance, behavior, and maintenance of body weight. All procedures
88 described here were approved by the Indiana University Institutional Animal Care and Use
89 Committee and followed NIH guidelines.

90 Rats were anesthetized with 1.5-2% isoflurane in oxygen. The concentration of isoflurane
91 was adjusted as needed, and we have monitored heart rate and oxygen saturation during surgery

92 using a Pulse Oximeter monitor (model LS1P-10R, Nonin, Plymouth, MN). To measure core
93 body temperature TA-F40 telemetric transmitters (DSI, St. Paul, MN) were implanted
94 intraperitoneally. Briefly, animals were placed in a dorsal recumbent position with abdominal
95 skin shaved along midline. A longitudinal medial skin incision (appr. 2 cm) was made, followed
96 by a longitudinal incision of the muscular wall along the white line. The body of the transmitter
97 was placed into the abdominal cavity, and the muscle was sutured followed by the skin. Animals
98 were returned to their cage for at least 1 week before being familiarized to the treadmill.

99 All rats were familiarized to running on the treadmill. Mild electric stimulus at the back of
100 the treadmill chamber promoted learned running behavior. On the first day of familiarization the
101 rats were placed on the treadmill at standby and were given 10 min to explore the surroundings.
102 After 10 min the treadmill was activated at 6 m/min for 5 min. In two consecutive days rats were
103 familiarized with running in 5 min sessions at increasing speeds.

104 Two series of experiments were performed in two separate groups of rats – running at room
105 temperature ($T_a = 25^\circ\text{C}$, N=6) and running at high ambient temperature ($T_a = 32^\circ\text{C}$, N=4). Each
106 rat was subjected to four experiments: stayed on the idle treadmill belt (0 m/min with zero
107 incline) or ran on the treadmill at one of three speeds (6, 12 and 18 m/min with zero incline). The
108 experiments were performed every other day with a speed which was selected using a random
109 number generator. We did not use higher speeds (e.g. 24 m/min), because not all rats were able
110 to keep such speeds for 15 min without getting excessive prodding electric stimuli.

111 Baseline temperatures were recorded for at least 20 min in the home cage at room
112 temperature. Then the animal was transferred to the treadmill, equilibrated at ambient
113 temperature, and the belt was activated. Animals ran for 15 min at set speed, while body
114 temperature was recorded, then the treadmill was inactivated. During the run no rat received

115 more than 3 consecutive prodding shocks. After the run the rats were immediately transferred
116 back to the home cage for recovery.

117

118

119 *Data Processing*

120 The running on the treadmill created momentary artifacts (<5 msec) on the temperature
121 signal from the probe recorded by the telemetric system. When animals were not running, there
122 were no artifacts. Artifacts were also absent when the electrical motor was working at a speed
123 needed for the highest treadmill speed used in described experiments (18 m/min) with the clamp
124 connecting the shaft of the motor to the treadmill belt removed, so the rat was resting. When an
125 animal was running at the highest speed, the mean value could be significantly affected by
126 artefacts. Therefore, we could not rely on the mean value collected by hardware every data
127 acquisition cycle. Instead, every minute we have recorded a 10 sec “waveform” signal at intrinsic
128 250 Hz, and used median of collected 2500 points as a value for this minute. The artifacts
129 constituted less than 10% of all samples, so the median was a robust statistic and reliable
130 measure. To calculate the median, we have exported “waveform” data to ASCII file, which was
131 then filtered by the program developed by one of authors (YIM). Further processing of
132 experimental data was performed using Microsoft Excel. Results were compared using a one
133 way ANOVA with repeated measures followed by a Fisher’s LSD post hoc test, where
134 appropriate. A value of $p < 0.05$ was considered to indicate a significant difference in all
135 comparisons.

136

137

138 *Model design*

139 The time constant of telemetric probe measured by us was relatively small (0.96 ± 0.01 min),
140 which allowed us to neglect the inertia of measurements in the model.

141 In a model containing only one compartment any additional heat production due to running
142 will result in immediate increase of body temperature. This contradicts the dynamics of body
143 temperature in first minutes after an initiation of running, when body temperature does not
144 change or can even slightly decrease. Therefore, we excluded one compartment models from
145 further consideration.

146 Our working mathematical model contained two compartments which generate heat aptly
147 named core body and muscles. We considered that muscles perform the physical work, and are
148 producing heat during exercise. Muscles are insulated from environment, and as such can
149 dissipate heat only to the core. Biologically we expect the heat generated by the muscles to be
150 transferred to the core proportionately to the difference of temperature between the two
151 compartments. In turn changes of heat production in the core are not a direct result of performing
152 the physical work, and are of regulatory origin. The core can also dissipate heat to the
153 environment with heat loss proportionate to the difference between core body and ambient
154 temperatures.

155 In rats, the bones and muscles together constitute 45-50% of body weight (11), while
156 skeleton comprises only approximately 3% (9). Therefore, we explicitly assumed that two
157 compartments of the model (core body and muscles) have equal heat capacities. We introduced
158 net heat accumulation in the core P_0 as the difference between overall heat production in the core
159 and dissipation to the environment. We assumed that at rest all heat to be generated in the core.
160 Based on the experimental data (13) the temperature of the muscles was considered equal to the

161 temperature of the core without exercise as an initial condition. P_m was defined as the additional
162 heat produced by the muscles due to exertion. Both P_0 and P_m were considered constant
163 throughout exercise. Initial model fitting was performed by varying the parameters P_0 and P_m in
164 order to check the basic validity and the ability to reproduce experimental data.

165 Differential equations describing the change in temperature for each compartment were
166 written as follows. The rate of change in the temperature of the core over time (dT_c/dt , $^{\circ}\text{C}/$
167 min) is affected by the net heat accumulation in the body and the heat transfer from muscles.

$$\frac{dT_c}{dt} = P_0 + \eta(T_m - T_c) \quad (1),$$

168 where η is a heat transfer coefficient, which indicates the percent of temperature differential
169 transferred from the muscles to the core per minute and measured in min^{-1} . We found that
170 $\eta = 0.125 \text{ min}^{-1}$ provided an average value of the rate of heat transfer, which best reproduced
171 the data.

172 Correspondingly, the rate of change in temperature of the muscles (dT_m/dt , $^{\circ}\text{C}/\text{min}$) was
173 affected by heat produced due to running and heat exchange with the core.

$$\frac{dT_m}{dt} = P_m - \eta(T_m - T_c) \quad (1)$$

174 At equilibrium we know that the temperature is not changing indicating that

$$dT_c/dt = dT_m/dt = 0 \quad [3]$$

175 At initial resting conditions, there is no additional heat generated by exercise, $P_m = 0$, and
176 the net heat accumulation in the core $P_0 = 0$. To satisfy condition [3] and considering resting
177 conditions we set the initial temperature of the muscles equal to the initial temperature of the
178 core $T_m(0) = T_c(0) = T_0$. This temperature is higher than ambient temperature due to baseline

179 heat production by the organism. Any baseline heat production in muscles before the exercise is
 180 considered as a part of the P_0 , while the P_m reflects only the heat produced from exercise.

181 The explicit solution of the system of equations can be found for any given time t as:

$$T_c = T_0 + P_0 \left(\frac{t}{2} + \frac{1 - e^{-2\eta t}}{4\eta} \right) + P_m \left(\frac{t}{2} - \frac{1 - e^{-2\eta t}}{4\eta} \right) \quad [4]$$

$$T_m = T_0 + P_0 \left(\frac{t}{2} - \frac{1 - e^{-2\eta t}}{4\eta} \right) + P_m \left(\frac{t}{2} + \frac{1 - e^{-2\eta t}}{4\eta} \right) \quad [5]$$

182

183 *Model parameter estimation*

184 A temperature time-series for each running speed at each ambient temperature consisted of
 185 16 points for each rat (15 min running), which were calculated by obtaining medians for
 186 corresponding time intervals as described in Data Processing. For each ambient temperature T_a
 187 and speed j we calculated the mean temperature among rats at time t as $\{T_t^j\}_{t=0}^{15}$. Also, we
 188 denote the predicted value of T_c from our model using [4] at time t as $T(P_0, P_m, T_0, t)$.

189 For a fixed speed j , we considered, T_t^j , of the time series $\{T_t^j\}_{t=0}^{15}$ as independent identically
 190 distributed normal random variables with mean $T(P_0, P_m, T_0, t)$ and variance $\sigma^2 = \sum_{t=0}^{15} s_t^2 / 16$,
 191 where s_t^2 is the sample variance found for each time $t=0,1,\dots,15$, i.e. $T_t^j \sim N(T(P_0, P_m, T_0, t), \sigma^2)$.

192 Then, the likelihood function, the conditional joint probability density function, is given by
 193 the product of the conditional distributions as noted: $\prod_{t=0}^{15} f(\{T_t^j\}_{t=0}^{15} | T_0, P_0, P_m)$. The likelihood
 194 function can be calculated as recommended in (31-33):

$$L(\{T_t^j\}_{t=0}^{15} | T_0, P_0, P_m) = \frac{1}{(2\pi)^{\frac{16}{2}} \sigma^{16}} \exp \left\{ -\frac{1}{2\sigma^2} \sum_{t=0}^{15} (T_t^j - T(P_0, P_m, T_0, t))^2 \right\} \quad [6]$$

196 where $j = 0, 6, 12, 18$ (m/min).

198 By maximizing the likelihood [6] we obtained a best-fit set of parameter values (P_0, P_m, T_0)
199 for each speed at two different ambient temperatures, 25 °C and 32 °C. Then by assuming that the
200 likelihood [6] represents a Bayesian posterior probability distribution for the model parameters,
201 we calculated the 95% confidence regions of parameter estimates on the (P_0, P_m)-plane, which
202 demonstrated strong negative correlation between estimates of these two parameters. This
203 correlation results in large variability of estimate for individual parameters. This also indicated
204 that any independent prior limitations on the value of one of the parameters could significantly
205 improve the precision of the estimates for both of them.

206 To formulate the prior restrictions on the parameters, we considered two hypotheses. First,
207 we hypothesized that the heat production in the core at high ambient temperature is at its
208 minimum, and hence cannot be further lowered by exercise, while heat dissipation remains
209 constant. Mathematically this means that the net heat production in the core P_0 at $T_a = 32$ °C
210 remains equal to 0 for all treadmill speeds. The statistical analysis confirmed that the estimate for
211 P_0 is not statistically significantly different from 0 for any speed. Second, we hypothesized that
212 the heat production in the muscles P_m is independent of T_a and depends only on the intensity of
213 the exercise (speed of the treadmill). Following these hypotheses, at $T_a = 32$ °C, we fixed
214 $P_0 = 0$ for all speeds, and used the likelihood function to determine the best-fit P_m values for
215 each speed. Then, these P_m values were substituted into the model for $T_a = 25$ °C, which was
216 used to obtain the distribution of P_0 values for different speeds at 25 °C.

217

218

219

220 **Results**

221 *Body temperature dynamics*

222 All rats were kept in home cages at room temperature before being placed on the treadmill.
223 The time during which the baseline temperature was recorded was sufficient to achieve uniform
224 values of core body temperature in all groups within $37.3 \pm 0.2^\circ\text{C}$. No statistical differences
225 between baseline temperatures in all experiments (for various ambient temperatures and speeds)
226 were found. The dynamics of the average temperature for each group is shown in Fig. 1. The
227 temperature in all groups eventually rose, including animals on the idle belt (Fig. 1, 0 m/min). In
228 a room temperature environment $T_a = 25^\circ\text{C}$ the core temperature decreased slightly before
229 starting to rise; this decrease was observed at all running speeds (Fig. 1A). In the warm
230 environment, $T_a = 32^\circ\text{C}$, the body temperature did not decrease initially, however the
231 temperature did not start to increase for at least 3 min (Fig. 1B). After 5 min body temperatures
232 were increasing in a linear fashion in all groups until the end of the run (15 min total, Fig. 1A,B).

233 The presence of a “hypothermic” phase is more obvious when experimental data for both
234 ambient temperatures are plotted together (Fig. 2A). We have used the estimate of the rate of
235 change in the core temperature from 6 to 11 minutes to determine the rate of heat accumulation.
236 In the control group which was placed on an idle treadmill, the rate of change of the core
237 temperature was almost identical for both ambient temperatures (Fig. 2B). However, at 25°C
238 running at the speed of 18 m/min subtly increased the rate of heat accumulation, whereas at 32°C
239 C running at 18 m/min led to almost two-fold faster increase in temperature (Fig. 2B).

240 After analysis of the experimental data we can summarize a few qualitative observations and
241 phenomena characterizing short-term running on a treadmill as follows. First, the running at the
242 treadmill results in increases in body temperatures which do not appear immediately, but rather

243 after a delay. Second, increases in temperature are preceded by slight decreases at room
244 temperature, while no initial change in temperature is observed in the warm environment.
245 Finally, the rate of heat accumulation is not significantly different between animals running with
246 different speeds at room temperatures. In a warm environment, however, the increase in the
247 running speed correlates with the rate of heat accumulation.

248

249 *The model with unrestricted parameter values*

250 At first, we utilized the likelihood function [6] to find the confidence regions for all model
251 parameters in all groups as described in Methods section. The analytically calculated (for the
252 high ambient temperature) 95% confidence regions on the (P_m, P_0) -parameter plane have an
253 elliptical shape and are shown on Fig. 3A. Figures 3B and 3C represent the best-fit estimates
254 and standard errors for P_0 and P_m respectively.

255 The P_0 and P_m estimates appear strongly correlated. This suggests that the model with
256 unrestricted parameter values is overdetermined. Practically, it means that the model with higher
257 heat production in the muscles and accordingly lower net heat production in the core can
258 describe the same temperature curve equally well. Consequently, while the ellipses in Fig. 3A
259 are largely separated, their projections onto P_m and P_0 axes (which represent the confidence
260 intervals for each parameter individually) heavily overlap. Accordingly, no conclusion can be
261 drawn about exercise intensity dependent changes in individual parameters, as those changes
262 appear statistically insignificant due to large standard errors.

263 The standard approach to overcome the problem of the overdetermined model is to introduce
264 appropriate prior restrictions on its parameter values. In this regard, we formulated two

265 biologically plausible hypotheses and verified that none of them contradicted our modeling
266 results with unrestricted parameter values.

267 First, Figure 3B shows that at room temperature P_0 drops to negative values for all speeds,
268 whereas this is not observed in warm environment. Such drop in P_0 represents a decrease in the
269 core metabolism. With an increase in ambient temperature, the core metabolism is decreasing to
270 maintain the appropriate body temperature until metabolism reaches a minimum value. Our first
271 hypothesis is that at $T_a = 32^\circ\text{C}$ this limit is reached, and accordingly the exercise cannot lead to
272 any further thermoregulatory decrease in the core heat production. Mathematically, this
273 hypothesis states that for all speeds at high ambient temperature P_0 remains equal to 0. Statistical
274 analysis indeed shows that at $T_a = 32^\circ\text{C}$, none of the P_0 estimates for different speeds are
275 statistically different from 0. This fact is reflected in Fig. 3A as confidence regions of P_0 for each
276 speed contained $P_0 = 0$.

277 Our second hypothesis follows that the heat production in the muscles correlates with
278 exercise and its intensity, and does not depend on the ambient temperature. In support of this
279 idea no statistically significant differences were found between P_m values for different ambient
280 temperatures, at identical treadmill speeds (Fig. 3C).

281

282

283 *The restricted model*

284 The first condition which we applied to the model was fixing $P_0 = 0$ at the high ambient
285 temperature. As expected, this restriction allowed calculation of P_m with much better precision
286 (Fig. 3D). Increasing the treadmill speed resulted in progressive increases in heat production by
287 the muscles. Interestingly, just placing an animal on the treadmill resulted in heat production in

288 the muscles comparable to the non-zero speeds. Specifically, the heat production at zero speed is
289 approximately one half of the heat produced at the highest running speed of 18 m/min.

290 Following the second hypothesis that P_m depends on speed, but not on ambient temperature,
291 we used the P_m values shown in Fig. 3D to define changes of P_0 for the ambient temperature
292 $T_a = 25\text{ }^\circ\text{C}$ (Fig. 3E). Overall, the model reproduced experimental temperature dynamics:
293 calculated values were within one standard deviation (Fig.4). The dynamics of the predicted
294 temperature of the muscles is shown at the Fig.5.

295 Placing the animal on a treadmill (speed 0 m/min) resulted in a small but significant decrease
296 of core heat production. As the running speed increased the decrease in core heat production
297 became more significant. This decrease was not able to fully compensate for the production of
298 heat in muscles. At the highest running speed, the increase in muscle heat generation was
299 approximately $0.4\text{ }^\circ\text{C}/\text{min}$, while P_0 dropped by only $0.1\text{ }^\circ\text{C}/\text{min}$. The difference between P_m
300 values for 0 and 18 m/min was appr. $0.2\text{ }^\circ\text{C}/\text{min}$. This resulted in significant increases in overall
301 heat accumulation at the ambient temperature of $32\text{ }^\circ\text{C}$ (Fig. 2B). At room temperature the heat
302 produced by muscles was half-compensated by the reduced core heat generation. This led to an
303 increase in heat accumulation to be not significant (see Fig. 2B).

304

305

306 **Discussion**

307 Treadmill exercise results in the steady production of heat in animals (16). Our objective was
308 to mathematically model core body temperature changes as a result of exercise as a first step in
309 building a model to predict the expected core body temperature at any given point in time given
310 a specific running speed and ambient temperature. We also attempted to model physiologic and

311 metabolic responses, which are difficult to directly measure in exercising animals: muscle
312 temperature, and both muscle and core heat generation.

313

314 *Relationship between various parameters characterizing heat production*

315 Temperature changes in the body occur because metabolic processes generate heat. Increases
316 in temperature occur when heat production is greater than dissipation into the environment, and
317 decreases occur when it is less. In our model, heat production is separated into exercise-related,
318 which occurs in muscles (P_m), and exercise-independent, which we assume to occur in the core.
319 The heat production in the core was combined with the heat dissipation from the core indicating
320 overall net heat change in the core and it was quantified as P_0 .

321 Heat generation and dissipation is commonly expressed as power (P, energy divided by time)
322 and is measured in units of energy per unit of time (e.g. Watt=Joule/sec). Heat accumulation is
323 directly connected with changes in body temperature through heat capacity as $dT/dt=P/Heat$
324 Capacity (15). Both heat production during running and heat capacity are proportional to the
325 body weight, therefore changes of body temperature are independent of body weight and are
326 reflecting heat production per unit of weight. In this study the heat production/accumulation is
327 always expressed in °C/min which can be translated to the power by multiplying by the heat
328 capacity of the body.

329 As it will be discussed below, we accepted heat dissipation approximately constant
330 throughout these experiments, because the body temperature is not changed strongly enough to
331 activate thermoregulatory responses. Therefore, P_0 provides an estimate of the changes in heat
332 production mediated by the core, and a sum ($P_0 + P_m$) can be considered an estimate of a change
333 in total heat production in the organism.

334 At room temperature rats can run at the maximal speed used in this study, 18 m/min, for
335 extended periods of time. We have noticed that rats will run for more than an hour at room
336 temperature (unpublished observation). This means that at this speed, heat is generated by
337 oxidative processes without significant accumulation of metabolic byproducts such as lactic acid.
338 In this case the generated heat is proportional to oxygen consumption, because the differences
339 related to metabolic substrate comprise less than 7% per unit of oxygen: if the substrate is
340 carbohydrates, the energy generated from one liter of O₂ is 21.13 kJ, and it is 19.69 kJ if the
341 substrate is fat (28). Considering this, in the following discussion we will draw connections
342 between studies that used various end-points – body temperature, heat production or oxygen
343 consumption.

344 Via modeling, we have quantified several key phenomena. While some of those phenomena
345 may appear counterintuitive, we found multiple evidence supporting our observations.

346

347 *Delay in body temperature increase*

348 When animals began running, their body temperature did not increase immediately. Instead
349 there was a several minute long delay prior to the temperature elevation. Even more, there was a
350 decrease in the temperature observed at the beginning of the run at room temperature. A graph of
351 abdominal temperature versus running time revealed a delay in the temperature change at the
352 beginning of the run in the study by Fuller *et al.* (12). Hasegawa *et al* (19) also observed a
353 similar delay in the experiment in which rat was allowed to adapt to the idle treadmill first, and
354 treadmill belt was activated after body temperature reached new baseline. When a rat was
355 transferred from a home cage to an idle treadmill, there were at least two data points, shown in 5
356 min time increments, before the slope of the temperature curve became positive (see Fig. 2 in

357 (19)). Upon activation of a treadmill, there was an immediate temperature increase, however, the
358 maximum slope was reached only after a 10 min delay (19). In a separate study from the same
359 group a similar delay was shown (see Fig. 2 in (20)).

360 The mechanism of this delay at the beginning of run is explained by our model. As soon as
361 exercise begins, contracting muscles begin to produce heat, yet this heat is not transmitted to the
362 core immediately. Until muscles become physically warmer than the core, there is no net transfer
363 of the heat to the core, and no changes in the core body temperature occur. The rate of heat
364 transfer from the muscles to the core is proportional to the difference in temperatures between
365 the core and the muscles and the heat transfer coefficient η which defines the inertia of this
366 process. Therefore, the delay in the core temperature increase is concerned with the time needed
367 for sufficient heat accumulation in muscles to activate the heat transfer from muscles to the core.
368 When a larger time scale (1 h or longer) is considered, this delay would be hardly noticeable,
369 however, on a time scale of 15 min, this delay is an important part of the system's dynamics.

370

371 *A decrease in temperature in the beginning of the run at room temperature*

372 A slight decrease in body temperature in the beginning of each run was clearly present at
373 room temperature; this was absent at high ambient temperature. We speculate that the
374 mechanism of this "hypothermia" may be a compensatory adjustment of heat production in the
375 body core, which is not yet compensated by heat transfer from the muscles. It takes some time
376 for the produced heat to increase temperature of the muscles, and only after that additional heat
377 can be transferred to the core. During this time, the heat produced in the core by the decrease of
378 heat accumulation is less than the amount of heat dissipated to the environment. This results in a
379 transient decrease of the body temperature. That decrease is significantly smaller or nonexistent

380 in hot environments due to low baseline heat production and a smaller difference between the
381 temperatures of the environment and the core. An anticipatory decrease of heat accumulation in
382 the core occurs at all speeds including 0. So the very fact of the transfer of an animal to the
383 treadmill evokes preparation for the exercise.

384

385 *Relative independence of body temperature changes on exercise intensity at room temperature*

386 This phenomenon is best demonstrated by linear increase in body temperature in graded
387 exercise studies. An experimental protocol of Balthazar *et al.* (2) had initial speed of 10 m/min
388 with 5% incline and increased the speed every 3 min by 1 m/min. In this protocol of graded
389 exercise body temperature increases were virtually linear. Despite increasing speed (the maximal
390 speed was 16 m/min) the increase of body temperature was so close to linear that the authors
391 calculated the average slope over the whole run. The rate of change of the body temperature
392 measured by Hasegawa *et al.* (18) was also independent of the work load with speeds between
393 10 and 26 m/min (see Fig. 1 in (18)).

394 In experiments presented in this manuscript, during the linear part of the temperature increase
395 the slope of heat accumulation in rats running at 18 m/min is significantly higher than in rats on
396 the idle belt of the treadmill in hot environment but not at room temperature (Fig. 2B). This slope
397 is dependent on both P_0 and P_m with $(P_0 + P_m/2)$ providing a good estimate. At high ambient
398 temperatures $P_0 = 0$ and cannot be decreased with increasing speed. Accordingly, the slope
399 increases as P_m increases (Fig. 3D). As a result, the slope is higher in running rats compared with
400 idle rats (Fig.2B). In contrast, at room temperature, P_0 decreases with the increasing speed (Fig.
401 3E), while P_m has the same dependence on the speed as in the hot environment. This means that
402 at room temperature an increase of the temperature slope with an increase in treadmill speed is

403 partially offset by decreasing heat accumulation in the core (Fig. 2). As a result, due to
404 variability present in all biological systems, the increase in the slope does not reach statistical
405 significance (Fig. 2B).

406 At the first glance, this independence contradicts not only common sense, but also
407 experimental data demonstrating that at higher speeds body temperatures of rats are higher (see,
408 for example, (38)). To interpret this contradiction, it is important to consider a time frame of the
409 experiments: Tanaka *et al.* (38) explicitly stated that they measured the rectal temperature at the
410 steady state (after 30 min of running). In the referenced study, the cutaneous dissipation of heat
411 is not effective in the beginning of the run, and body temperature rises due to the increased heat
412 production. As soon as body temperature reaches cutaneous vasodilation threshold, the steady
413 state is established. In other words, the steady state is defined by a thermoregulatory threshold of
414 cutaneous vasodilation, which is increasing with the running speed. Correlation between
415 thermoregulatory threshold and running speed is the reason of higher body temperature at higher
416 speeds. We argue below that in the time frame of our experiments the thermoregulatory heat
417 dissipation is not activated.

418 Alternatively, in studies showing initial phases of temperature increases in rats running at
419 various speeds, the slopes of temperature are impressively similar (Figs. 2 and 6 in (25)). The
420 temperature dynamics in different groups separates dramatically only after the (brain)
421 temperature exceeds heat dissipation threshold, and when thermoregulatory heat loss responses
422 are activated (25) .

423

424 *Transfer to a treadmill accounts for a large part of the temperature change during exercise*

425 When animals were placed on a treadmill and stayed at least 30 min, Hasegawa et al. (19)
426 found that their body temperature rose by 1 °C and remained elevated without any trend to return
427 to baseline. After stabilization of the increased temperature, running at 10 m/min raised the
428 temperature by an additional 0.5 °C in 15 min when it again reached a plateau. So placing the
429 animal on the idle treadmill accounted for approximately 2/3 of total increase of the body
430 temperature. From equations [2, 3] it follows that the additional overall heat accumulation
431 ($P_0 + P_m$ in our study) is proportional to the change in equilibrium temperature after sufficient
432 time. The sum ($P_0 + P_m$) when staying on the idle belt was about 0.17 °C/min (see Fig. 3D,E),
433 while at a treadmill speed of 12 m/min (closest to the speed used in (19)) it is appr. 0.25 °C/min,
434 so a transfer to the belt accounts for approximately the same 2/3 of the increase in the rate of heat
435 accumulation in the whole body during running at 12 m/min.

436 Further increases in speed do not dramatically increase heat production. Brooks & White (4)
437 reported that oxygen consumption was 50 ml/kg/min when running at 15 m/min speed on a
438 treadmill with 1% incline, and increased only to approximately 65 ml/kg/min at 45 m/min with
439 the same incline.

440

441 *Suppression of thermoregulatory metabolism by exercise*

442 In our study we showed that rats exposed to exercise exhibited a decrease in their core heat
443 production. Similar effects of thermoregulation suppression were described in the study by
444 Guimaraes et al. (17). When rats were exposed to 12°C, their core body temperature went up (a
445 well-known phenomenon from other studies (22)). However, when the animals were forced to
446 run at the same temperature, their body temperature was lower than at rest. Running at
447 progressively colder temperatures resulted in lower body temperatures at equilibrium, which

448 were achieved after 15-30 min of exercise. Similar observations were presented in (10).
449 Assuming that running produces the same amount of heat regardless of the ambient temperature,
450 one can conclude that in colder environment the exercise suppresses the thermoregulatory heat
451 production more in absolute values. In fact, Makinen et al. (29) observed that exercise at 0°C did
452 not increase oxygen consumption relative to exercise at 22°C, which means that intense
453 thermoregulatory heat production in cold-exposed rats was substituted by thermogenesis induced
454 by exercise.

455

456 *Two compartments are necessary to explain complex temperature dynamics*

457 In a model with a single compartment, the increase of heat generation would result in an
458 immediate growth of the body temperature. In contrast, we observed a clear delay between the
459 beginning of an exercise and the temperature rise. Therefore, a single compartmental model is
460 not able to accurately describe changes in body temperature during running.

461 It appears that a two-compartment model reproduces experimental data with sufficient level
462 of precision. For simplicity sake we named the additional heat produced from exercise as the
463 heat produced by muscles, P_m . It is important to note that this variable can include heat
464 produced by other organs as well (e.g., the heart or brain), and disregards any heat produced by
465 the muscles at rest. In fact, an immediate increase of brain temperature during exercise (8, 10) is
466 a feature of “muscle temperature” in our model, and indicate that additional heat production in
467 the brain during the exercise contributes to the “muscle” compartment of our model. The rate of
468 the initial temperature change in the second compartment (the core) is defined by the difference
469 between overall heat production in the core and dissipation to the environment, P_0 . Later during
470 the run, the rate of temperature change in the core will be also modified by heat transfer from
471 warmer “muscles” in addition to P_0 . Note again that the use of P_0 is a simplification to refer to

472 any heat produced by the body that is not directly related to additional heat resulting from
473 exercise. It also allows us to disregard different forms of heat dissipation from the body by
474 combining them in a single term.

475

476 *Prior restrictions on model parameters*

477 Parameter confidence regions on the (P_0, P_m) plane appeared to be ellipses strongly extended
478 in a certain direction (see Fig. 3A). As a result, the model parameter estimates had large standard
479 errors and, hence, did not differ statistically significantly between various speeds and ambient
480 temperatures. As described in the methods, our findings became much more conclusive when
481 additional restrictions were applied. Two reasonable hypotheses were formulated and proved to
482 not contradict to the initial estimates of the parameters of the model. First, we hypothesized that
483 in hot environment there was no room to decrease heat generation in the core, supported by the
484 fact that 95% P_0 confidence regions for each speed in $T_a = 32\text{ }^\circ\text{C}$ included 0 (Fig. 3A).

485 Therefore, we assumed that the amount of heat generated in the core body was at its minimum
486 level and it could not be further reduced to compensate the additional heat coming from the
487 muscles. This hypothesis is firmly footed in the concept of a thermoneutral zone (TNZ).

488 $T_a = 32\text{ }^\circ\text{C}$ is just exceeding the estimate of the upper border of the TNZ for rats (34). This
489 means that at $T_a = 32\text{ }^\circ\text{C}$, the rat does not generate heat to keep its core temperature steady.

490 Instead the only heat that the rat generates is in the process of sustaining normal bodily functions.

491 Next, we hypothesized that heat generation in the muscles, P_m , at given running speed is
492 independent of the ambient temperature. Fig. 3C represents the P_m estimates for 0, 6, 12, and 18
493 m/min speed of treadmills at both values of T_a . There is no statistically significant difference
494 between values for matching speeds, which is compatible with the above idea.

495 Using these two restrictions provided more precise estimates of the model parameters
496 (compare Fig.3B and 3C with Fig.3D and 3E). This allowed us to conclude that the heat
497 generated by the muscles increases with the speed, but this increase saturates as speed is
498 increased. As soon as we used the P_m estimates obtained by fixing $P_0 = 0$ for the models with
499 the high ambient temperature ($T_a = 32\text{ }^{\circ}\text{C}$) as priors for the model with the ambient temperature
500 $T_a = 25\text{ }^{\circ}\text{C}$, we were able to calculate changes in the core heat production at $T_a = 25\text{ }^{\circ}\text{C}$ with
501 much better accuracy (Fig.3E). Again the compensatory decrease in the core heat generation
502 appeared statistically significant even when the animal was placed on the idle treadmill, and
503 saturated at the speed of 12 m/min.

504

505 *Role of heat dissipation*

506 There are two ways in which the net heat accumulation P_0 can be decreased by exercise. It
507 can be due to suppression of thermogenesis, which is used to keep body temperature constant in
508 relatively cold environment, or it can occur through increased heat dissipation. The major
509 mechanism responsible for controlling heat dissipation in mammals is cutaneous blood flow.
510 Cordeiro et al. (7) recorded the body temperature of rats running at 18 m/min at 5% incline
511 simultaneously with the tail temperature — a marker of heat dissipation. The tail temperature
512 reached its maximum at approximately 20 minutes, and this moment corresponded to the
513 beginning of plateau phase of body temperature at approximately 38.5°C . The experimental set
514 up for our study with the highest speed was the same except we did not impose the incline.
515 Running time in our experiment was 15 min, and we did not observe plateauing of the body
516 temperature. Hasegawa et al.(18) also confirmed that at body temperatures below 38°C , the tail is
517 not dissipating heat effectively. Cutaneous heat loss threshold temperature in running rats is at or

518 above 38.5°C in other studies as well (27, 39). Tanaka et al (38) also demonstrated that the
519 threshold for cutaneous vasodilation increases with increasing speeds and exceeded 39°C when
520 running required above 70% of maximal VO_2 consumption, which approximately corresponds to
521 the maximal running speed in our experiments (40). This let us consider that at least for most of
522 the experimental time we did not reach conditions for significant cutaneous vasodilation
523 resulting in noticeable additional heat loss. As a result, the changes in P_0 account for alterations
524 in the thermoregulatory heat generation.

525

526

527

528 **Biological implications**

529 In this study we quantitatively describe a particular thermoregulatory mechanism
530 concerned with partial compensation of heat overproduction during exercise. This mechanism is
531 based on the reduction of regulatory thermogenesis. We hypothesize that the impairments of this
532 mechanism may lead to faster overheating and hence may underlie heat injury/stroke in some
533 cases.

534 For example, heat illness is typically observed in individuals exercising in a hot
535 environment. However, there are multiple occasions when it affects competitors in events in
536 moderate climate, when a majority of participants appears to be not prone to this medical
537 condition. Surprisingly, the correlation with running speed is often significant but fails to reliably
538 explain more than a fraction of the variability in body temperatures (6, 30). In modeling terms it
539 means that high body temperature is not a direct consequence of extreme amounts of heat due to

540 the intensity of running. Less effective suppression of baseline thermoregulatory heat production
541 could be one of mechanisms which results in overheating in some athletes.

542

543

544 *Are changes in metabolism specific to exercise?*

545 One could interpret the increase in body temperature at the idle belt as a stress response,
546 which remains despite rat familiarization to running on a treadmill. However, one can also notice
547 that this increase is a part of a pattern: the core heat production drops while the heat production
548 in muscles increases. The same phenomenon was observed in rats that were actually running.
549 This allows formulating a hypothesis that muscular metabolic engagement and a compensatory
550 decrease in the core heat production are not a response to exercise per se, but an anticipatory
551 response to the need to run. In some situations this demand may never actualize (when the speed
552 is 0).

553 Can we distinguish between anticipation of exercise and stress? The very definition of
554 general adaptation syndrome by Selye as “a generalized effort of the organism to adapt itself to
555 new conditions” (37), suggests that reactions to exercise could be considered as responses to
556 stress. Noteworthy, stress is typically associated with locomotion, and helps to prime the
557 organism for fight-or-flight (5).

558

559 *Temperature of muscles as a critical physiological end-point*

560 It is difficult to measure muscle temperature in humans and animals while they are exercising
561 without negatively affecting their ability to run. In those experiments where researchers placed
562 temperature probes adjacent to muscles of exercising animals (13), similar values to what we

563 predict (Fig.5) were observed (39°C adjacent to muscles after 15 min at 9 m/min in (13) vs
564 39.5°C in the muscle after same time at 12 m/min in our model at room temperature).

565 In our model the heat was dissipated from muscles to the core only, supposedly by the
566 means of blood flow. This property of the model is based on the fact that in the beginning of the
567 exercise the temperature of the muscles is the same as the temperature of the core, which is
568 supported by experimental observations in rats (13). However, some authors suggest that in
569 certain conditions, such as extreme exposure to cold environment (36) or in specific muscles in
570 humans (23), the dissipation from muscles can occur directly to the environment. To describe
571 such conditions the model needs to be modified appropriately. The physiological parameter,
572 which is most sensitive to such dissipation, will be the temperature of the muscles. Obviously, at
573 ambient temperatures lower than body temperature, it will be shifted down at all times including
574 the baseline.

575 Our modeling shows that the temperature in muscles when running with the fastest speed, 18
576 m/min, reaches 41°C and approaches the levels (41-42°C) at which the muscle tissue can be
577 damaged (24). Avoiding this damage is important for selection of appropriate training regimens,
578 especially in highly competitive athletes. Modeling may serve as an effective tool to determine if
579 specific exercise regimens and conditions can result in thermal injury.

580

581 *Energy production as a marker of substrate utilization*

582 Heat production is a measure of substrate utilization during exercise, and as such is directly
583 linked to weight loss induced by exercise. Therefore, knowing heat production during exercise is
584 important from public health viewpoint. The finding that energy expenditure is relatively
585 independent of the exercise load could have quite far-reaching considerations for fitness

586 practitioners. In our experimental conditions, most important factor associated with calorie
587 expenditure was that exercise was occurring; the intensity of the exercise appears to have had a
588 minor influence.

589

590 **Conclusion**

591 Our study serves as a step towards a comprehensive mathematical model of the thermoregulation
592 during exercise. Inclusion of both thermogenesis and heat dissipation together with
593 corresponding control mechanisms are needed to predict adverse temperature in exercising
594 individuals. In this paper we have studied in rodents an “Activity for free” phenomenon (21),
595 which is a significant thermoregulatory mechanism activated by exercise. The phenomenon is
596 relatively obvious biologically – when additional heat is produced by exercise, the generation of
597 heat aimed at maintaining body temperature can be reduced. The energy which was used for
598 maintaining body temperature is now used for locomotion (thus the name “Activity for free”).
599 However, for the first time we had characterized this phenomenon quantitatively. At room
600 temperature, rats are able to save from a quarter to a half of energy used for running. This
601 compensatory suppression of metabolism is saturated with the low load. At running speeds as
602 low as 12 m/min with zero incline the drop of metabolic activity in the core is already maximal.
603 We predict that in colder conditions, which induce significant levels of metabolic support for
604 homeothermy, this compensation could be more substantial.

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- 735

736 **Figure Legends**

737

738 **Figure 1.** Experimental data: the average and standard deviations of the core body temperature
739 of rats running on a treadmill for 15 min at various speeds in two environments – (A) at 25°C
740 and (B) at 32°C. Rats were in their home cages before the run, transferred to the treadmill at t=0
741 min, and the belt was set to the actual speed in 30 sec.

742

743

744 **Figure 2.** Comparison of the body temperature dynamics (A) and heat accumulation rate (B)
745 between animals at two ambient temperatures which were either running at 18 m/min or staying
746 on the idle belt. Heat accumulation rate was calculated as an average rate of the body
747 temperature change between 6 and 11 min. * indicates statistically significant difference with
748 staying on the idle belt ($p < 0.05$).

749

750

751 **Figure 3.** (A) Confidence regions on the (P_0, P_m)-plane for each of the four studied speeds (0, 6,
752 12, and 18 m/min) at 32 °C; **B-C**: Parameter estimates before the model reduction for two
753 ambient temperature, 25 °C and 32 °C: (B) P_0 vs speed, (C) P_m vs speed; **D-E**: Parameter
754 estimates after model reduction: (D) P_m vs speed at $T_a = 32$ °C after P_0 is set to 0 for the
755 experiments at 32 °C, (E) P_0 estimates at different speeds of running at 25°C with P_m values
756 from panel D under an assumption that heat generation in the muscles does not depend on the
757 ambient temperature.

758

759

760

761

762 **Figure 4.** Comparison of the experimental data (error bars) and the core temperature curves as
763 generated by the model (solid lines) using the parameter estimates represented in Fig. 3D,E. The
764 error bars show the mean \pm one standard deviation of the core body temperature over a group of
765 rats. Rows of graphs correspond to different speeds of running (**A,E:** 0 m/min, **B,F:** 6 m/min,
766 **C,G:** 12 m/min and **D,H:** 18 m/min as marked on the left). Columns of graphs correspond to
767 different ambient temperatures (25°C, first column; 32°C, second column).

768

769

770

771 **Figure 5.** Muscle temperatures as predicted by the model. (**A**) at $T_a = 25 \text{ }^\circ\text{C}$ (**B**) at $T_a = 32 \text{ }^\circ\text{C}$.

772

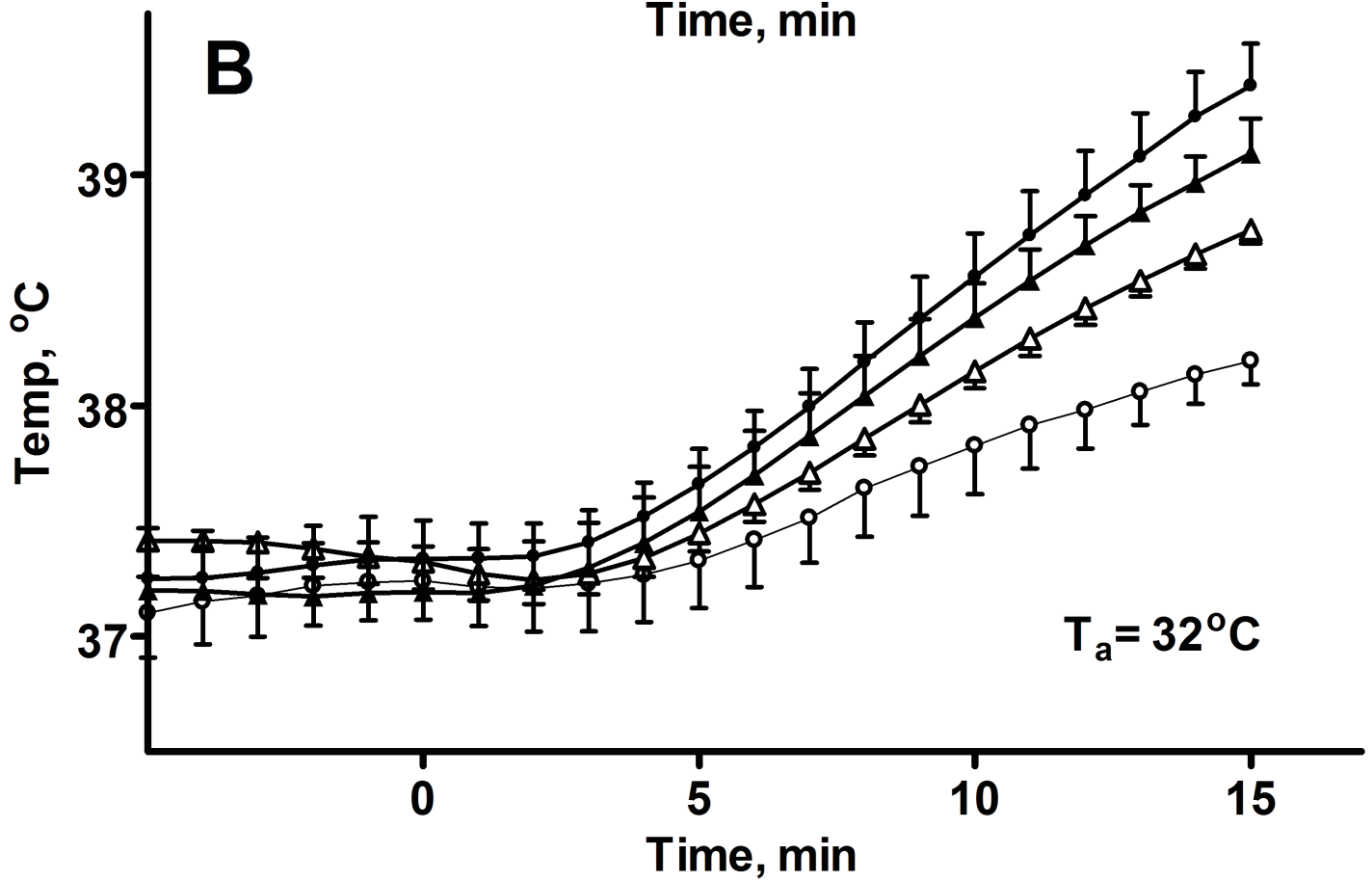
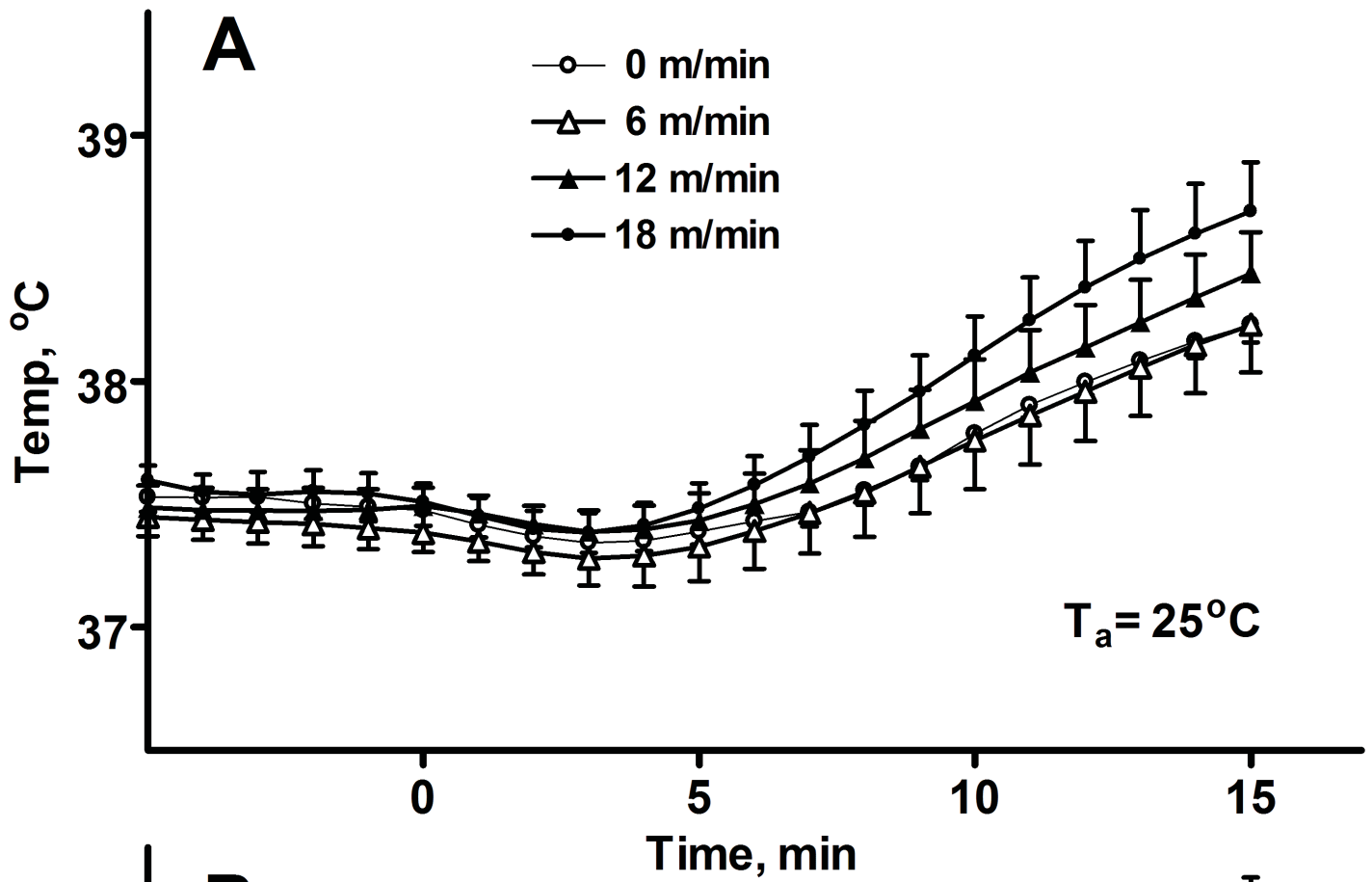
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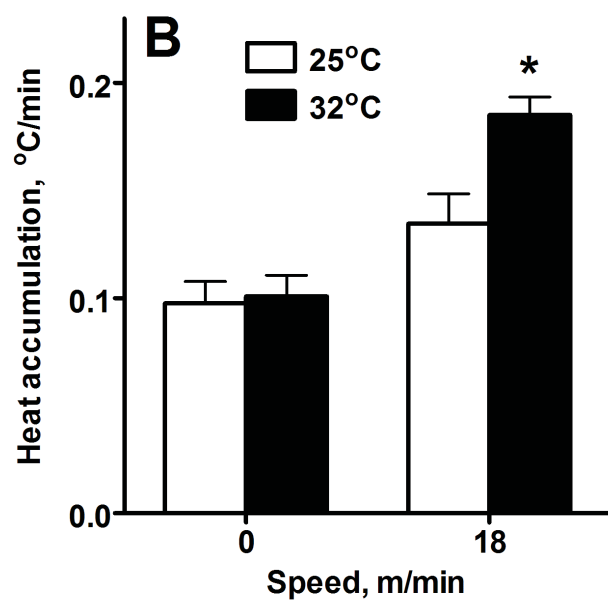
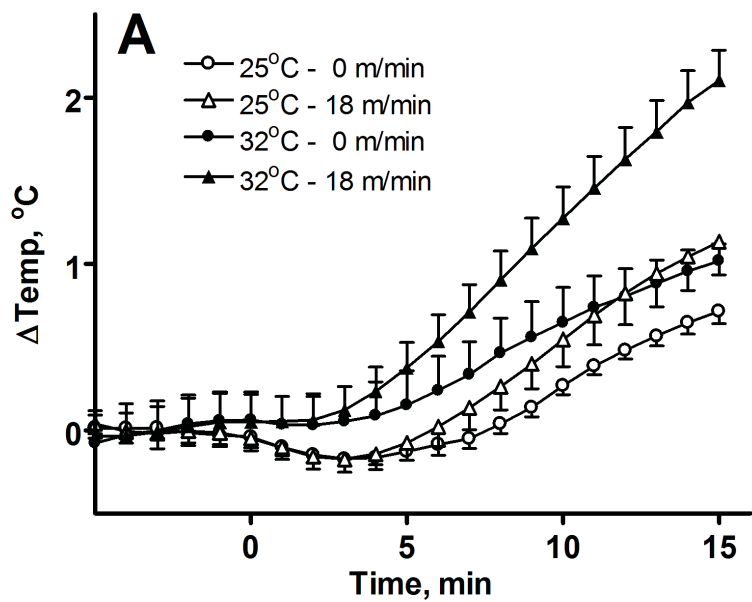
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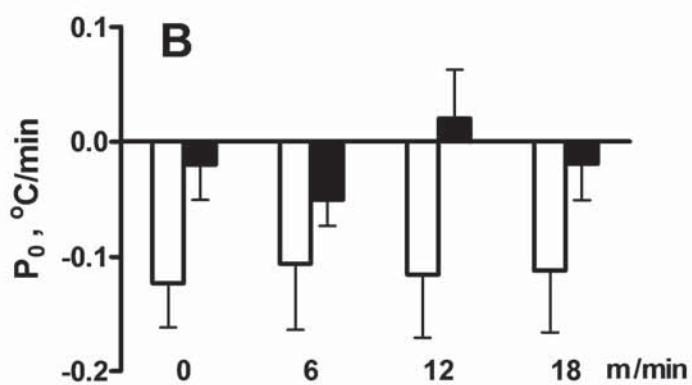
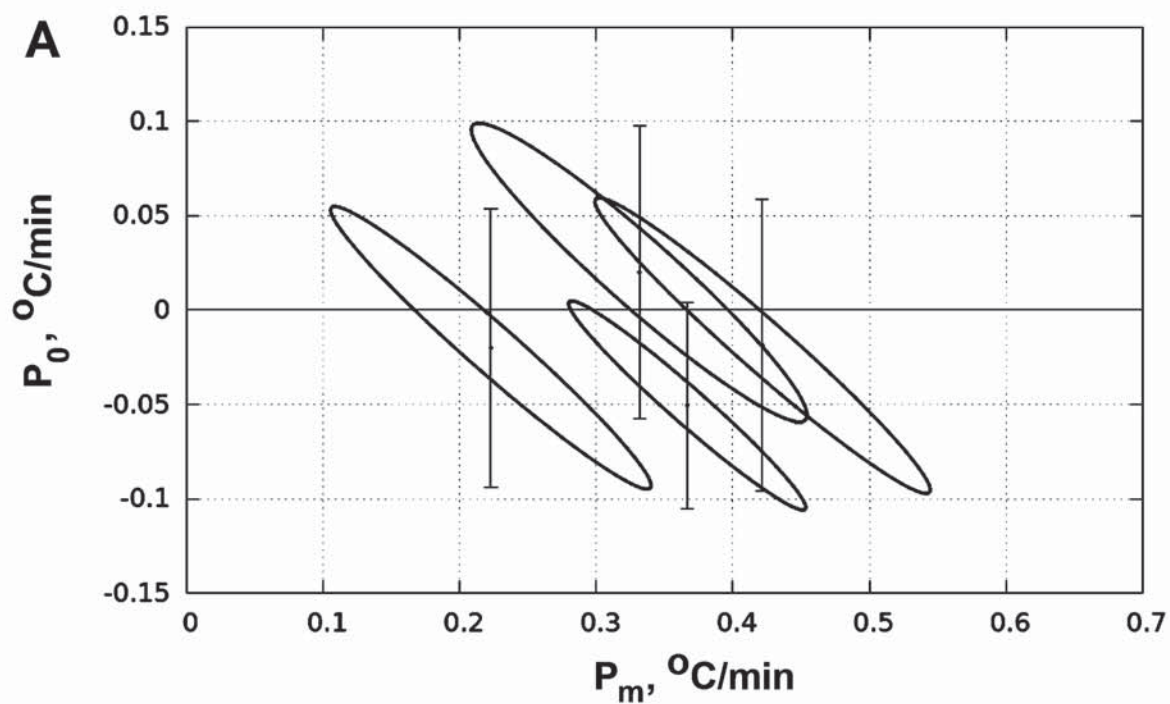
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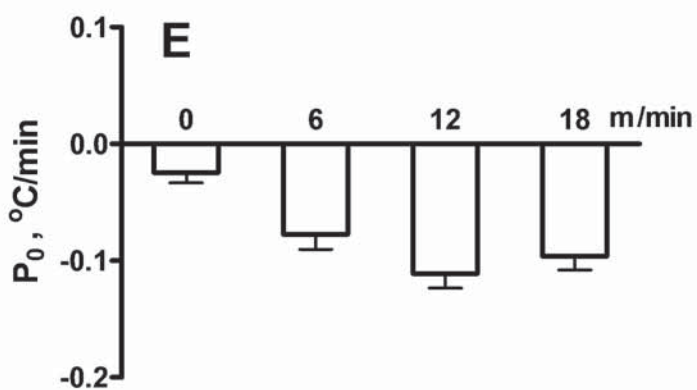
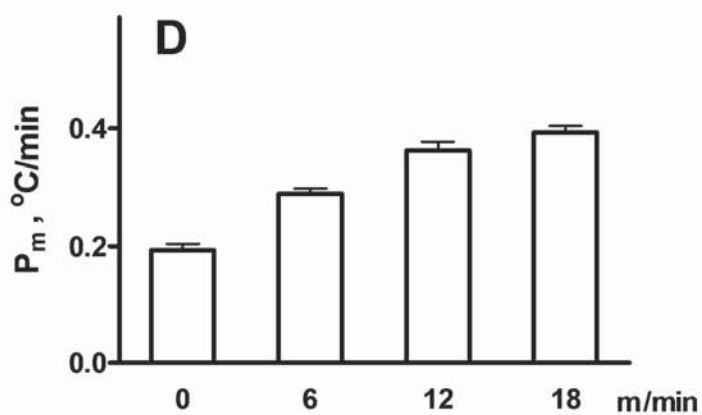
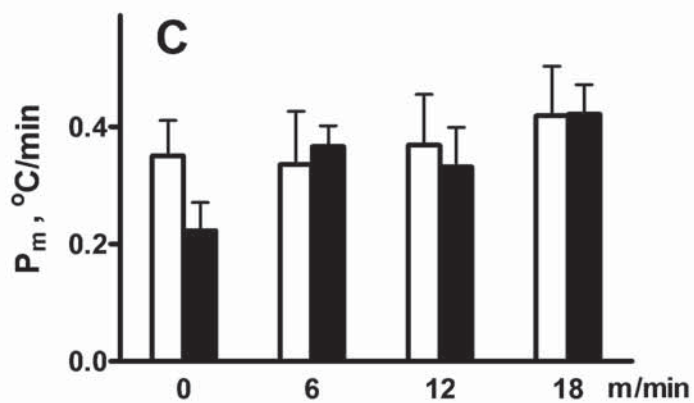
777







□ 25 °C ■ 32 °C



25 °C

32 °C

