Journal of Thermal Biology 51 (2015) 47–54

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

# Estimation of the core temperature control during ambient temperature changes and the influence of circadian rhythm and metabolic conditions in mice

Ken Tokizawa [a,b,1,2], Tamae Yoda [c,1], Yuki Uchida [b,3], Kazuyuki Kanosue [a], Kei Nagashima [b,d,*]

[a] Sport Science Center for Active Life, Faculty of Sport Sciences, Waseda University, Tokorozawa, Japan
[b] Body Temperature and Fluid Laboratory (Laboratory of Integrative Physiology), Faculty of Human Sciences, Waseda University, Mikajima 2-579-15, Tokorozawa, Saitama 359-1192 Japan
[c] Faculty of International Liberal Arts, Dokkyo University, Soka, Japan
[d] Institute of Applied Brain Sciences, Waseda University, Tokorozawa, Japan

ARTICLE INFO

*Article history:*
Received 7 October 2014
Received in revised form
10 March 2015
Accepted 10 March 2015
Available online 12 March 2015

*Keywords:*
Thermoregulation
Fasting
Heat balance
Metabolism
Behavioral thermoregulation
Physical activity

ABSTRACT

It has been speculated that the control of core temperature is modulated by physiological demands. We could not prove the modulation because we did not have a good method to evaluate the control. In the present study, the control of core temperature in mice was assessed by exposing them to various ambient temperatures ($T_a$), and the influence of circadian rhythm and feeding condition was evaluated. Male ICR mice ($n=20$) were placed in a box where $T_a$ was increased or decreased from 27 °C to 40 °C or to −4 °C (0.15 °C/min) at 0800 and 2000 (daytime and nighttime, respectively). Intra-abdominal temperature ($T_{core}$) was monitored by telemetry. The relationship between $T_{core}$ and $T_a$ was assessed. The range of $T_a$ where $T_{core}$ was relatively stable (range of normothermia, RNT) and $T_{core}$ corresponding to the RNT median (regulated $T_{core}$) were estimated by model analysis. In fed mice, the regression slope within the RNT was smaller in the nighttime than in the daytime (0.02 and 0.06, respectively), and the regulated $T_{core}$ was higher in the nighttime than in the daytime (37.5 °C and 36.0 °C, respectively). In the fasted mice, the slope remained unchanged, and the regulated $T_{core}$ decreased in the nighttime (0.05 and 35.9 °C, respectively), while the slopes in the daytime became greater (0.13). Without the estimating individual thermoregulatory response such as metabolic heat production and skin vasodilation, the analysis of the $T_a$–$T_{core}$ relationship could describe the character of the core temperature control. The present results show that the character of the system changes depending on time of day and feeding conditions.



## 1. Introduction

Homeothermic animals constantly regulate core temperature against disturbing factors that originate both from inside the body or from the environment. This regulation seems to be changed when animals have homeostatic demands, e.g., fever alters regulation, thus increasing core temperature.

To describe the change in the regulation of core temperature, a "set-point temperature" of the system has been used (Caputa, 2005; Hammel, 1968; Romanovsky, 2004; Werner, 1980). Hammel (1968) considered that core temperature is regulated by a negative feedback system: the effectors of the system start to work when core temperature deviates from the set-point temperature. However, the effectors, such as sweat glands, skin vessels and brown fat, have different threshold core temperatures for activation (Kanosue et al., 1997). Satinoff (1978) proposed that an independent system controls each effector based on neuroanatomical and physiological studies. Thus, "set-point temperature" does not exist in the control of core temperature.

In most physiological conditions, ambient temperature ($T_a$) is a major disturbance of the system. The thermoregulatory system aims to defend core temperature against the disturbance. Two different approaches have been used to describe the control. A thermoneutral zone is defined as the range in which core temperature is controlled only by vasodilation and vasoconstriction of

* Corresponding author at: Body Temperature and Fluid Laboratory (Laboratory of Integrative Physiology), Faculty of Human Sciences, Waseda University, Mikajima 2-579-15, Tokorozawa, Saitama 359-1192, Japan. Fax: +81 4 2947 6918.
*E-mail address:* k-nagashima@waseda.jp (K. Nagashima).
[1] KT and TY equally contributed to the present study.
[2] Present Address: National Institute of Occupational Safety and Health, Kawasaki, Japan.
[3] Present Address: Kochi University, School of Medicine, Kochi, Japan.

http://dx.doi.org/10.1016/j.jtherbio.2015.03.005
0306-4565/

the skin. Romanovsky et al. (2002) described the range in rats by examining the relationship between tail surface temperature and $T_a$. Rudaya et al. (2005) showed that, in rats injected with lipopolysaccharide, the thermoneutral zone changes, depending on the dose. Hensel et al. (1973) showed changes in core temperature during exposure to various $T_a$. Gordon (2005) used the term "range of normothermia (RNT)", which denotes the range of $T_a$, in which an animal can stably control core temperature. Aydin et al. (2011) showed that the RNT decreased in physically restrained rats.

In the present study, we hypothesized that circadian rhythm and/or fasting changes thermoregulatory control in mice, which is reflected by the RNT. Core temperature follows the circadian rhythm. Although physical activity is a passive influence, circadian core temperature change is thought to be regulated (Aschoff, 1983; Gander et al., 1986; Nagashima et al., 2005; Tokizawa et al., 2009). Fasting decreases core temperature in rats, mice and pigeons (Graf et al., 1989; Nagashima et al., 2003; Rashotte et al., 1995; Sakurada et al., 2000; Székely et al., 1997; Tokizawa et al., 2009; Yoda et al., 2000). Nagashima et al. (2003) showed that the reduction of core temperature occurs during the light phase of rats. To obtain the RNT, mice were exposed to gradually changing $T_a$, and the relationship between $T_a$ and core temperature was assessed. In addition, by examining metabolic rate, spontaneous activity, and behavioral responses, we assessed how such thermoregulatory and non-thermoregulatory responses affect the RNT.

## 2. Materials and methods

### 2.1. Animals

Twenty male mice from Institute of Cancer Research (ICR; age, 12–16 weeks; and body weight, 35–45 g) were used in the present study. They were housed individually in a plastic cage ($45 \times 25 \times 20$ cm$^3$) with free access to water and food. $T_a$ was maintained at $27 \pm 0.5$ °C, and the lighting cycle was 12 h of lights on (300 lx at the eye level, lights on at 0700) and 12 h of complete darkness. The Institutional Animal Care and Use Committee of Waseda University approved all experimental procedures in the present study.

### 2.2. Surgery

A radio transmitter device for measuring intra-abdominal temperature ($T_{core}$) and spontaneous activity ($17 \times 10 \times 8$ mm$^3$; Physiotel, model TA10TA-F40, DataScience, St. Paul, MN) was implanted using a sterile technique under inhaled 2% isoflurane anesthesia (Sigma Aldrich, Tokyo, Japan). Penicillin G (1000 U, Meiji Pharmaceutical, Tokyo, Japan) was intramuscularly injected to minimize postsurgical infection. The mice were allowed to recover for at least 10 days before the experiment.

### 2.3. Experiment 1 (comparison of thermoregulation between daytime and nighttime)

We used 10 mice that showed clear circadian core temperature and activity rhythms. Each mouse was moved to an experimental box and then placed in a climate chamber (Program Incubator IN602W, Yamato Scientific, Tokyo, Japan) set at 27 °C 12 h before following the protocol. At 0800 or 2000, $T_a$ was decreased or increased from 27 °C to −4 °C (cold trial, 207 min) or to 39 °C (heat trial, 80 min) at a rate of 0.15 °C/min. Each mouse was subjected to four different experimental trials (cold and heat exposure in the daytime and nighttime) and a control trial (27 °C all day). Each trial was conducted on a different day, and the interval was at least 1 week. The order of the trials was randomized. Food and water were available during the experiments. The experiment was stopped when $T_{core}$ reached 33 °C or 40 °C.

### 2.4. Experiment 2 (comparison of thermoregulation between fed and fasted conditions)

We conducted the same exposure protocol as in *Experiment 1* in 10 other mice. In this experiment, food was withheld for 24 h before the heat or cold exposure until the end, but water was freely available. For example, when the cold or heat exposure started at 0800, the fasting started at 0800 1 day before the exposure.

### 2.5. Measurements

$T_{core}$, spontaneous activity, and oxygen consumption ($\dot{V}O_2$) were continuously measured. Signals from the radio-transmitter device were obtained through a receiver board (model CTR86, DataScience, St. Paul, MN) underneath the box and were stored in a personal computer every 1 min. Spontaneous activity was estimated from changes in the intensity of the telemetry signal.

Mice were placed in an experimental box (semi-enclosed Plexiglas metabolic box; $20 \times 20 \times 20$ cm$^3$) that was attached to an airflow system with a constant flow rate of 1500 ml/min. Paper bedding was placed at the bottom to absorb urine. The difference between the air's oxygen tension in the inlet and that in the airflow outlet continuously monitored using an electrochemical oxygen analyzer (model LCJ-700, Toray, Tokyo, Japan), and the data were stored in a personal computer every 10 s. The air was filtered and dried with silica gel (Wako Pure Chemical, Osaka, Japan). $\dot{V}O_2$ was calculated as the product of the difference between the oxygen tension and the flow rate (indirect calorimetry). The value was standardized: per kg body weight, an air temperature of 0 °C, atmospheric pressure of 760 mmHg, and 0% humidity. The temperature of the air in the outlet of the metabolic box was measured with a thermocouple, and this value was used as the $T_a$ value in the analysis.

The behavior of the mice was monitored using an infrared camera and was recorded for past-analysis. One person who did not know the aim of the present study counted the number of grooming behaviors i.e., paw licking and face washing, during the heat exposure. The duration was not considered (the behavior usually lasted from 3 to 20 s). In the cold, the mice stayed still in a curled-up posture. Occasionally, a mouse showed the posture for a short period, but the posture did not seem to be a specific response to the cold; thus, when the posture lasted more than 5 min, we considered that the posture was induced by the cold. The data were expressed as the percentage of mice showing the posture. At the end of the experiments, mice were killed by pentobarbital overdose via intra-abdominal injection (50 mg/kg body weight).

### 2.6. Statistics

Differences in the means of $T_{core}$, $\dot{V}O_2$ and activity and number of grooming behaviors were analyzed using three-way analysis of variance: phase (daytime vs. nighttime) × feeding condition (fed vs. fasted) × $T_a$. A post hoc test to identify a significant difference at a specific time-point was performed by the Newman–Keuls procedure. Regression analysis for $T_a$ and $T_{core}$ was conducted using the least-squares method, and the regression line equation was estimated. Details of further analysis are shown in Section 3. The null hypothesis was rejected at $p < 0.05$.