Combined stimuli of cold, hypoxia, and dehydration status on body temperature in rats: a pilot study with practical implications for humans

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Abstract
Objective: As human thermoregulatory responses to maintain core body temperature \( T_{core} \) under multiple stressors such as cold, hypoxia, and dehydration (e.g., exposure to high-altitude) are varied, the combined effects of cold, hypoxia, and dehydration status on \( T_{core} \) in rats were investigated. The following environmental conditions were constructed: (1) thermoneutral (24 °C) or cold (10 °C), (2) normoxia (21% \( O_2 \)) or hypoxia (12% \( O_2 \)), and (3) euhydration or dehydration (48 h water deprivation), resulted in eight environmental conditions [2 ambient temperatures (\( T_a \)) \( \times \) 2 oxygen levels \( \times \) 2 hydration statuses]. Each condition lasted for 24 h.

Results: Normoxic conditions irrespective of hypoxia or dehydration did not strongly decrease the area under the curve (AUC) in \( T_{core} \) during the 24 period, whereas, hypoxic conditions caused greater decreases in the AUC in \( T_{core} \), which was accentuated with cold and dehydration (\( T_a \times O_2 \times \) hydration, \( P = 0.040 \) by three-way ANOVA). In contrast, multiple stressors (\( T_a \times O_2 \times \) hydration or \( T_a \times O_2 \) or \( O_2 \times \) hydration or \( T_a \times \) hydration) did not affect locomotor activity counts (all \( P > 0.05 \)), but a significant simple main effect for \( O_2 \) and \( T_a \) was observed (\( P < 0.001 \)). Heat loss index was not affected by all environmental conditions (all \( P > 0.05 \)). In conclusion, decreases in \( T_{core} \) were most affected by multiple environmental stressors such as cold, hypoxia, and dehydration.

Keywords: Area under the curve, Heat loss index, High-altitude, Hypothermia, Metabolism, Set point

Introduction
Most of mammal’s (including human) energy regulates core body temperature \( T_{core} \). Various environmental factors such as heat, cold, hypoxia, humidity, or wind would affect \( T_{core} \). Although physiological adaptation to environmental stressors is often studied in isolation, these stressors are frequently combined outside of laboratory settings. At high altitudes, both barometric pressure and ambient temperature \( T_a \) decreases with an increase in altitude.

Cold exposure reduces human cutaneous blood flow that decreases heat transfer from the core to peripheral tissues [1]. This decreases in skin temperature, which narrows the gradient for cutaneous heat loss, and promotes heat conservation, and is vital to the prevention of hypothermia in cold. Contrariwise, hypoxic conditions reduce metabolism and core body temperature \( T_{core} \) in many small mammals [2], primates [3], and humans [4–7]. In humans, this may be explained by peripheral circulation. Simulated high altitude elicits a cutaneous hyperemia that is mediated at the tissue level [8, 9], suggesting an acceleration in the rate of core cooling. The combined

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effects of cold and hypoxia may have competing effects on cutaneous circulation, and hence, $T_{core}$ changes would be complicated. There are several issues to consider when investigating the combined effects of cold and hypoxia on $T_{core}$ and/or peripheral circulation in humans. Previous human studies of cold and hypoxia have been conducted in a relatively short period (within 2 h) [9–13]. Generally, when humans are exposed to high-altitude such as climbing a mountain, exposure of several hours or a few days can occur. Thus, it may be difficult to expose people to multiple environmental stressors for a longer time due to ethical problems and strain on the participants. Importantly, greater individual variances in response to multiple stressors, i.e., cold and hypoxia, exist in humans [13]. Specifically, changes in $T_{core}$ under exposure to hypoxia in cold stress cannot be explained by only peripheral circulation [13]. An initial step using animal models (considered to have less individual variance), is required.

As heat dissipation caused by fluid redistribution is important in thermoregulation, the hydrations status on $T_{core}$ should also be considered. For example, hyperventilation-induced dehydration is observed at high-altitude [14]. Similarly, inhalation of hypoxic gas causes an increase in urine volume, also suggesting dehydration status [15]. However, little is known about the combined effects of cold, hypoxia, and hypohydration on $T_{core}$ irrespective of whether in humans or animals.

Accordingly, the main aim of this pilot study was to investigate the combined effect of cold, hypoxia, and dehydration on $T_{core}$ and related factors using animal models. We hypothesized that multiple stimuli (cold, hypoxia, and dehydration) would cause the greatest reductions in $T_{core}$.

**Main text**

**Methods**

**Animals**

Experiments were performed using age-matched (10–14 weeks), male, Wistar rats weighing 250–320 g ($n = 40$). Animals were maintained in a temperature-controlled ambient temperature ($T_a = 24 ^\circ C$) and relative humidity (50%), fed ad libitum, and kept on a 12 h light–dark cycle. All experiments were performed in accordance with the Ethics Committee for Animal Experiments, Mount Fuji Research Institute, Yamanashi Prefecture Government (ECAE-03–2016).

**Experimental procedures**

Environmental conditions were as follows; (1) thermonutral ($24 ^\circ C T_a$) or cold ($10 ^\circ C T_a$), (2) normoxia ($21%O_2$; room air) or normobaric hypoxia ($12%O_2$), (3) euhydration (48 h ad libitum access to water before the experiment) or dehydration (48 h water deprivation before the experiment) (Fig. 1). In previous studies, $5 ^\circ C T_a$ [16, 17] or $10 ^\circ C T_a$ [18], and $10%O_2$ [16, 19, 20] or $7–10–12%O_2$ [21] were used. In our preliminary experiments, a few rats fell into asphyxia condition under $5 ^\circ C$, therefore, we conducted the experiment of $10 ^\circ C T_a$. Regarding to $O_2$ concentration, $O_2$ saturation acutely decrease around below 50–60 torr $PO_2$, at which is almost equivalent to $12%O_2$ based on oxygen–hemoglobin dissociation curve [22]. To produce dehydration status, 48 h water deprivation was enforced before the experiment, and the experiment lasted 24 h, resulted in 72 h water deprivation [23]. Eight experiments ($2 T_a \times 2$ oxygen $\times 2$ hydration status = 8 conditions; $n = 5$ for each) were performed. Ambient temperature ($24 ^\circ C$ or $10 ^\circ C$) was maintained in a climatic chamber (MIR-153; SANYO Electric Co., Ltd, Japan). The oxygen concentrations in the vinyl tent surrounding the climatic chamber were set at normobaric normoxia ($21%O_2$) or hypoxia ($12%O_2$). Hypoxic gas ($12%O_2$) was supplied via a generator (Hypoxico Everest Summit II; Will Co., Ltd., Tokyo, Japan) and oxygen concentration was verified before and after each experiment (AE-300; Minato Medical Science, Osaka, Japan). The rats were weighed before and after the experiment.

**General surgical preparation**

Before the experiment, animals were anesthetized with an anesthetic mixture of medetomidine, midazolam, and butorphanol. For the measurements of $T_{core}$, a radio transmitter ($15 \times 30 \times 8$ mm; Physio Tel TA10TA-F40, Data Sciences International Co., Ltd., St. Paul, MN, USA) was placed in the abdominal cavity of each rat. The rats were allowed to recover for at least one week before
measurements. After each experiment, a radio transmitter was taken under anesthesia, thereafter, exsanguination from a cut through the heart aorta was applied for euthanization.

**Measurements**

In addition to the measurement of $T_{\text{core}}$, infrared thermography (Thermo Shot F30; NEC Avio Infrared Technologies Co., Ltd., Japan) was used to measure tail skin temperature ($T_{\text{tail}}$). Counts of locomotor activity (an indicator of behavior) were recorded every minute with a data collection system, which consisted of a receiver board (model RLA2000, Data Sciences International, Co., LTD, St. Paul, MN, USA) under the cage connected to a personal computer. The locomotor activity counts reflected positional movements but did not show other movements such as grooming or food intake.

**Data analysis**

The area under the curve (AUC; 0–24 h) of $T_{\text{core}}$ was calculated from values measured at 0 h, using data every 1 min and the trapezoidal method. $T_{\text{tail}}$ was the averaged values from the first and second thirds of the tail. Heat Loss Index (HLI) as an indicator of peripheral vasomotor activity, was calculated using the following equation:

$$\text{Heat Loss Index (HLI)} = \frac{(T_{\text{tail}} - T_{\text{a}})}{(T_{\text{core}} - T_{\text{a}})}$$

The value of HLI ranges from 0 (full vasoconstriction) to 1 (full vasodilation).

Changes in HLI (ΔHLI) were calculated by the difference between pre (time = 0 h)- and post (time = 24 h)- exposure to each environmental condition [24].

**Statistics**

Values are represented as mean± standard deviation. All statistics were performed using a R software (ver. 3.1.3). A three-way ANOVA ($T_a \times O_2 \times$ Hydration) was performed for comparisons of the AUC, HLI, and activity counts. If significant $F$ values were obtained, Bonferroni’s post-hoc test was used for further comparisons. A $P$ value < 0.05 was defined as statistically significant.

**Results**

Dehydration status (irrespective of $T_a$ or $O_2$ conditions) decreased body weight compared with the control conditions (24 °C $T_a$, 21%$O_2$, and euhydration) ($F=95.99$, $P<0.001$) (Additional file 1: Table S1). Conversely, hypoxia or cold per se did not affect body weight changes.

Mean values of $T_{\text{core}}$ in each condition and comparisons of the $T_{\text{core}}$ AUC are shown in Fig. 2. To avoid difficulties of observation in time course changes in $T_{\text{core}}$, mean values without SD are shown (Fig. 2a). Normoxic conditions (irrespective of hypoxia or dehydration) did not decrease the AUC, whereas, hypoxic conditions caused greater decreases in the AUC, and were accentuated with cold and dehydration. A second-order interaction ($T_a \times O_2 \times$ hydration) was observed by three-way ANOVA ($F=4.570$, $P=0.040$, Fig. 2b). Figure 3a shows comparisons of activity in each condition. A three-way ANOVA revealed a trend in a second-order interaction ($T_a \times O_2 \times$ hydration; $F=4.066$, $P=0.052$), while no significant simple interactions were observed ($T_a \times O_2$; $F=0.011$, $P=0.918$, $O_2 \times$ hydration; $F=0.065$, $P=0.800$, $T_a \times$ Hydration; $F=0.250$, $P=0.620$, $O_2 \times$ Hydration; $F=0.085$, $P=0.769$, $T_a \times$ Hydration; $F=0.044$, $P=0.836$).

![Fig. 2](image-url) **Fig. 2** Time course of $T_{\text{core}}$ among different environmental conditions. Values are given as only means (n = 5 for each, a). Mean values of the area under the curve (AUC) with standard deviation (SD) among all conditions throughout the 24 h experimental period (b). Hypoxia significantly decreased the AUC irrespective of cold and dehydration (dashed black lines). In hypoxia, cold environment (10 °C) further decreased the AUC in both euhydration and dehydration conditions (solid gray lines). Dehydration affected the AUC only in both hypoxia and cold (solid black line).
Ta × hydration; F = 0.357, P = 0.554). A simple main effect of O₂ and Ta was observed (Ta; F = 32.30, O₂; F = 56.202, P < 0.001). Comparisons in HLI under all conditions showed no significant differences in the simple main effect, a simple interaction, and second order interaction were observed (all P > 0.05) (Fig. 3b).

**Discussion**

The major findings of this pilot study were two-fold: (1) the AUC of Tcore was mostly affected by multiple stressors (cold, hypoxia, and dehydration), (2) activity counts and HLI were not affected by multiple stressors.

The Tcore decreased slightly within a few hours in normoxia (about -0.1 to -0.5 °C decrease per hour); however, these initial reductions in the Tcore were markedly greater under hypoxia (about -1 to -2.5 °C decrease per hour). During acute exposure to a cold environment, shivering thermogenesis is activated to augment heat production [18, 25, 26], compensating for the increase in heat loss that occurs at low Tcore. In rats, shivering thermogenesis is gradually replaced by non-shivering thermogenesis during cold acclimation (four weeks) [27], which allows the animal to maintain high body temperatures under low Ta conditions. In the present experiment, as exposure time was 24 h, both shivering and non-shivering thermogenesis may have contributed to maintain Tcore during the 24 h period under normoxic conditions, irrespective of Ta and dehydration, although the precise mechanisms are unclear. In hypoxia, a similar initial decrease in Tcore has been observed [20, 28], and it is generally a consequence of a decrease in the Tcore set-points, known as hypoxia-induced anapyrexia [29–31]. Therefore, these greater reductions in set-point in hypoxia may be associated with non-recovery in Tcore after a 24 h period. A continuously lower Tcore after an initial phase of 24 h may be explained by several candidates, such as hypoxic-induced hypometabolism [32–34], or nitric oxide pathway-involved mechanisms on thermoregulation [35, 36]. Moreover, effects of acute exposure to hypoxia on Tcore has also been demonstrated to be strongly affected by Tcore [19, 34]. Yet, it is still unclear why combined effects of cold, hypoxia, and dehydration had the most impact on the lowest AUC of Tcore compared with other seven environmental conditions. One possible explanation is a loss of plasma volume with 48 h water deprivation before the main experiment. A previous study reported an initial increase in body surface temperature during hypoxia, and hence an initial increase in peripheral blood flow during hypoxia may indicate the shifting of heat away from the core to the periphery to facilitate cooling [34]. If this hypothesis were true despite dehydration status in this experiment, blood flow redistribution from core to peripheral tissues might dominate peripheral tissues to core transfer for protection in Tcore decreases, and hence, Tcore further decreases under combined conditions (cold, hypoxia, and dehydration). This hypothesis is speculative, and further experiments are needed.

Regarding to activity counts, significant main effects were found for oxygen and Ta. Specifically, Ta of 24 °C or a hypoxic condition showed significantly lower values for activity counts. These results cincture with a previous study, showing activity counts decreased with Ta increase and an observation of hypoxic-induced lower locomotor activity counts [19]. Unexpectedly, none of the
environmental conditions affect HLI. Some methodological consideration should occur. HLI was evaluated at the start and the end of the 24 h period, and therefore, continuous assessment of HLI will be investigated in future studies.

Our findings may be informative for populations who are working and performing in severe environmental conditions (i.e., cold and hypoxia). Specifically, humans cannot control environmental conditions; however, as dehydration status may cause further reductions in $T_{\text{core}}$, an appropriate beverage intake could be effective tactics.

In summary, multiple environmental stressors (cold, hypoxia, and dehydration) have the most impact on the lower $T_{\text{core}}$ as observed in the rats in this experiment. Moreover, locomotor activity counts and HLI do not affect this lower $T_{\text{core}}$.

Limitations
There are several limitations in the study. Firstly, the small size ($n = 5$ for each) should be re-considered. A pos-hoc power analysis for pairwise comparisons with significant difference (i.e., $T_{\text{core}}$ AUC) was used as the standard of 80% power with a two-sided significance level of 0.05 (G Power 3.1). When considering each simple main effect of cold, hypoxia, or dehydration, a sample size of three in each group was necessary to achieve the appropriate statistical power for significant comparisons (Fig. 2b). However, more than 20 for each condition would have been necessary for other non-significant comparisons. Second, to clarify the underlying precise mechanisms of the lowest $T_{\text{core}}$ with the combined effects, the metabolic rate (oxygen consumption), and HLI should have been measured continuously and other influencing factors on thermoregulation or body fluid regulatory hormones (e.g., thyroid hormone, noradrenaline, glucagon, renin, aldosterone, and vasopressin) should have been measured.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s13104-020-05375-w.

Additional file 1. Supplemental table. Body weight (BW) changes 48 hours before the experiment and just before the main experiment (24 h exposure) in each condition.

Abbreviations
AUC: Area under the curve; HLI: Heat loss index; $T_{A}$: Ambient temperature; $T_{\text{core}}$: Core temperature.

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Authors’ contributions
TU, TH, and MH conceived the design and concept. TU performed all experiments. TU, TH and MH analyzed and interpreted the data. TU prepared table and figures. TU and MH drafted the first manuscript. TU, TH and MH edited and revised the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
All experiment procedures were approved by the Animal Experiment Ethics Committee at Mount Fuji Research Institute.

Consent for publication
Not appreciable.

Competing interests
The authors declare that they have no competing interests.

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