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Sufficiently Elevated Core Body Temperature May Be Necessary to Maintain Cerebral Blood Flow Response throughout the Morning

Ryohei Tanaka-Kanegae*#, Shingo Miyamoto*, Masao Sakurai, Koichiro Hamada

Saga Nutraceuticals Research Institute, Otsuka Pharmaceutical Co., Ltd., Kanzaki, Japan
Email: #Tanaka.Ryohei@otsuka.jp

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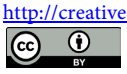

Abstract

In comparison to a carbohydrate-rich breakfast, a nutritionally balanced breakfast reportedly leads to a higher core body temperature because of diet-induced thermogenesis (DIT) and also results in higher task performance. This study aimed to examine the relationships among the core body temperature, blood glucose level, cerebral blood flow, and cognitive performance when the core body temperature is raised to a similar extent as in DIT in the morning. This crossover study included 18 male participants who performed four sets of cognitive tests in the morning with four different foot baths and glucose intake conditions. In elevated body temperature (EBT) conditions, the core body temperature was increased by a foot bath at 42˚C or 39˚C, while in low body temperature (LBT) conditions, it was maintained at 35˚C by a foot bath; the participants received no glucose or two intakes of 20-g glucose for each thermal condition. In addition to the core body temperature measurement, the cerebral blood flow in the dorsolateral prefrontal cortex (DLPFC) was measured using near-infrared spectroscopy. Three blood collections were performed to measure the changes in blood glucose levels. The results revealed that in the EBT conditions, the core body temperature remained 0.3˚C - 0.5˚C higher than that at wake-up time, while the glucose intake conditions increased blood glucose levels which remained higher than those during fasting. No significant between-treatment difference was observed in the results of cognitive tests. However, the blood flow in the DLPFC increased during the second test period in the EBT/glucose and LBT/glucose

*These authors contributed equally to this work.
#Corresponding author.

conditions, whereas during the fourth test period, it increased solely in the EBT/glucose condition. Thus, in addition to the blood glucose level, an elevated core body temperature within the physiological range may be needed for long-term maintenance of the cerebral blood flow response.

Keywords

Elevated Core Body Temperature, Cerebral Blood Flow, Dorsolateral Prefrontal Cortex, Near-Infrared Spectroscopy, Cognitive Function, Morning, Breakfast, Foot Bath

1. Introduction

Breakfast plays a vital role in supplying energy and nutrients as the first meal of the day [1]. Studies have shown various disadvantages of skipping breakfast, including fatigue, decreased body temperature, and decreased work efficiency [2]. Evidence from other studies also suggests that breakfast may improve cognitive function associated with memory, test results, and school attendance [3]. Given that glucose is a major source of energy for the brain, and carbohydrate deficiencies can adversely affect cognitive function [4], it may be reasonable to consider that the significant role of breakfast may be the provision of carbohydrates. However, accumulating evidence suggests that the consumption of carbohydrates alone for breakfast is insufficient to enhance brain function. An fMRI study conducted by Akitsuki *et al.* revealed that the consumption of a nutritionally adjusted balanced food for breakfast led to significant activation of the medial prefrontal cortex and the superior frontal gyrus during cognitive tasks, whereas sugar solution and water did not have the same effects [5]. Moreover, Higuchi *et al.* investigated whether the nutritional quality of breakfast affects cognitive function [6] by evaluating three meals for breakfast; a nutritionally adjusted balanced food, a Western-style bread-based meal (also nutritionally balanced), and rice balls. Their results showed that intellectual work ability (performance on the Uchida-Kraepelin [U-K] test) in the morning increased when participants had the nutritionally adjusted food or the Western-style meal for breakfast; when participants only consumed rice balls, their blood glucose level was higher than that in the other two conditions, but their intellectual work ability was approximately the same as when fasting. These findings suggest that an elevated blood glucose level alone is not enough to improve intellectual work ability in the morning. Interestingly, both nutritionally balanced conditions also showed higher increases in sublingual temperature, approximately 0.3˚C from the baseline. Considering the deep relationship between core body temperature and physiological function [7], Higuchi *et al.* presumed that the improved work ability in the two conditions might be linked to increased core body temperature due to diet-induced thermogenesis (DIT), as higher protein content in a meal

leads to greater DIT [8]. However, this could not be confirmed because the findings could not clarify whether the factors necessary for improved cognitive function were increased body temperature owing to DIT, the nutrients themselves (e.g., protein), or both.

The core body temperature in humans fluctuates within an approximate range of 1˚C during the day, with the lowest temperatures recorded early in the morning and highest in the evening [9]. Notably, in the field of exercise physiology, studies have suggested that lung function and muscle strength are improved, new records are frequently set, and the risk of injury is reduced during the evening hours [10]. Moreover, from the perspective of warming up, raising muscle or core temperature by external means leads to better performance [11]; thus, a high core body temperature within the physiological range may favorably influence physical activity.

The association between brain activity and core body temperature has been primarily investigated in cases showing an excessively high body temperature, such as hyperthermia and heat stroke. Although these extreme conditions cause deterioration of cognitive functions [12] [13], the effects of a core body temperature increase within the physiological range on cognitive function currently remain unclear. Further, the potential relationship between the increase in core body temperature through DIT and cognitive outcomes has not been fully investigated. Therefore, this study aimed to examine the relationship between core body temperature and brain function by raising body temperature to the same extent as in DIT. We decided to use a footbath with varying water temperatures to modulate core body temperature and set an elevated body temperature (EBT) condition as well as a low body temperature (LBT) condition, in which the core body temperature was maintained. The experiment was conducted in the morning, when the body temperature is relatively low and may reflect the effects of thermal load well. We also investigated the effects of glucose intake on brain function in each thermal condition.

2. Materials and Methods

2.1. Participants

Twenty healthy male volunteers aged 20 - 39 years participated in this study. All participants were non-smokers, right-handed, and non-shift workers who habitually ate breakfast for four days or more in a week and did not have any prior medical history of the brain, nerves, psychiatric or mental illness, circulatory system or endocrine function disorders, or color vision deficiencies. All participants provided written informed consent for participation in the study. This study was approved by the ethics committee of Yoga Allergy Clinic (approval number: RD09006HS04) and was conducted according to the principles of the Declaration of Helsinki. Furthermore, the study protocol was registered with the University Hospital Medical Information Network Clinical Trial Registry

(UMIN-CTR; registration number: UMIN000042983).

2.2. Experimental Procedure

We designed an open, randomized two-by-two crossover study, and all experiments were performed in Japan in March (spring season in Japan). The treatments were as follows: 1) LBT (35˚C water-immersion) with water ingestion (LBT/water), 2) LBT with glucose ingestion (LBT/glucose), 3) EBT (42˚C water-immersion) with water ingestion (EBT/water), and 4) EBT with glucose ingestion (EBT/glucose). Each participant performed one trial in each condition, with one-week breaks as wash-out periods between successive trials. The order of the four different treatments was randomly assigned to each participant. During the experimental period, the participants were requested to maintain the same diet quantity and quality as well as the same amount of exercise and sleep as those at the baseline of this study. Moreover, excessive exercise and lack of sleep on the day before the tests were prohibited.

Figure 1 provides a schematic overview of the experimental protocol. All participants visited the hospital on the evening prior to the designated experiment day. They were instructed to consume a standardized meal in the evening, and after the meal, they fasted until test completion. After dinner, they only drank a minimal amount of water. The participants swallowed the sensor for measuring gastrointestinal temperature after dinner and went to bed by 24:00. On the morning of the experiment, participants were requested to maintain a recumbent position until 7:30. Experiments began at 8:00 with blood collection and were performed in a temperature-controlled environment at 25˚C ± 1.5˚C and approximately 50% relative humidity.

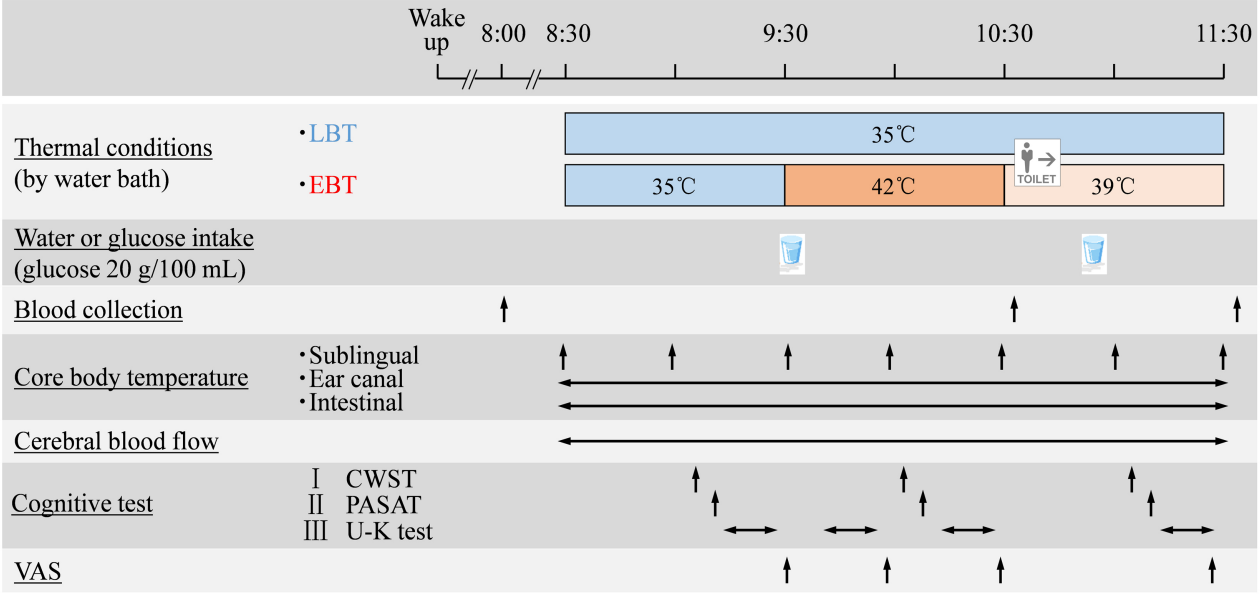

Figure 1. Experimental protocol. LBT: low body temperature, EBT: elevated body temperature, CWST: color-word-matching Stroop task, PASAT: paced auditory serial addition test, U-K: Uchida-Kraepelin, VAS: visual analog scale.

After the first blood collection, all the sensors and probes listed below were attached, and participants started the footbath by immersing both feet in 10-cm-deep water. For both the LBT and EBT conditions, the water temperature was set at 35˚C for the first hour; however, while the temperature was subsequently maintained at 35˚C for 3 h for the LBT condition, in the EBT condition, the water temperature was increased to 42˚C at 1 h after the commencement of the footbath, maintained at 42˚C for 1 h, reduced to 39˚C, and then maintained at 39˚C for another hour. The participants ingested 100 mL of water heated to 35˚C (distilled water; Otsuka Pharmaceutical Co., Ltd.) or 100 mL of 20% glucose solution (Otsuka Pharmaceutical Co., Ltd.) at 1 h and 2 h 25 min after the footbath started. Blood collection was performed 2 and 3 h after the start of the footbath. The participants were allowed to go to the bathroom after the second blood collection (2 h after the start of the footbath). They were instructed to remain awake and maintain a sitting position until each trial ended.

2.3. Measurements

2.3.1. Core Body Temperature

Core body temperature was measured by obtaining sublingual, ear canal, and intestinal temperatures. The sublingual temperature was measured using a thermometer (OMRON MC172L or equivalent) for 5 min before the commencement of the thermal load and before and after the sets of cognitive tests. An infrared sensor (VitThermo VTB-01; Vitarate Inc.) was attached to the ear canal on the morning of the test days, and the ear canal temperature was measured continuously at 30-s intervals. A pill-sized sensor (CorTemp; HQ Inc.) for measuring the gastrointestinal tract temperature was ingested after the dinner prior to the experiment, and the intestinal temperature was continuously measured at 10-s intervals. When the averages of the ear canal and intestinal temperatures during cognitive tests were calculated, we excluded data for the first 30 s while measuring sublingual temperature as well as the first 30 s of each cognitive test to account for noise contamination. Similarly, the average of measurements obtained over 1 min before measuring sublingual temperature was adopted for ear canal temperature instead of the values during the sublingual temperature measurement.

2.3.2. Prefrontal Hemodynamics

Prefrontal cortical hemodynamics were evaluated by near-infrared spectroscopy (NIRS) using NIRO-200NX manufactured by Hamamatsu Photonics K.K. The device measures changes in the chromophore concentrations of oxy-hemoglobin (Hb) and deoxy-Hb (ΔO_2Hb and ΔHHb) via the modified Beer-Lambert law and provides depth-resolved measures of tissue O_2 saturation (total oxygenation index [TOI]) and tissue Hb content (*i.e.*, the relative value of the total Hb normalized to the initial value; nTHI) using the spatially resolved spectroscopy (SRS) method. The SRS-derived NIRS parameters limit contamination from su-

perficial tissue via depth-resolved algorithmic methods [14]. Probes that were enclosed in light-shielding rubber housing and maintained emitter-to-detector optode spacing (4 cm) were positioned between F_{P1} and F_3 according to the landmarks of the international 10 - 20 system for electroencephalography (EEG) electrode placement in order to evaluate the hemodynamics in the left dorsolateral prefrontal cortex (DLPFC). Probes were securely attached to the head, and head tilt was kept constant during measurements of sublingual temperature and cognitive tests with the dedicated table support. Parameters were continuously measured at 1-s intervals and the amounts of O_2Hb and HHb were estimated as TOI × nTHI × tissue hemoglobin at the measurement initiation (Int. THI). Total Hb was calculated as O_2Hb plus HHb.

2.3.3. Cognitive Tests

We employed the color-word-matching Stroop task (CWST) reported by Byun K *et al.* [15]. In the CWST, two color names appeared on the PC monitor, with one in the upper row with colors and another in the lower row. Participants were instructed to answer whether the color of the word written in the upper row corresponded with the meaning of the word written in the lower row. Depending on the answer, they were asked to press the "correspond" or "not correspond" button on the keyboard quickly. The reaction time of button pressing was calculated separately for congruent (*i.e.*, the color of the word written in the upper row corresponds with the meaning of the word), incongruent (*i.e.*, the color of the word written in the upper row does not correspond with the meaning of the word), and neutral (*i.e.*, the upper word is presented as "XXXX") trials. Each session consisted of 30 trials including 10 congruent, 10 incongruent, and 10 neutral trials presented in random order. This test assesses the inhibitory component of selective attention and processing speed ability [16] and the executive function, indicated by Stroop interference calculated as the difference in reaction time between congruent and incongruent trials. Processing speed, flexibility, and calculation ability were assessed by the Paced Auditory Serial Addition Test (PASAT [17]) and U-K test [18]. In the PASAT, a single digit was presented every 2 s for 2 min, and test participants were instructed to add a new digit to the previous one mentally and write down the answers on a paper sheet. The total number of correct answers was calculated. In the U-K test, participants were required to refer to a prepared number sequence and add two adjacent numbers. If the result was a single-digit number, they were instructed to write that number, whereas if it was a two-digit number, they were instructed to only write the last digit. After each announcement at 1-min intervals, they were directed to the next row of digits, and this task was continued for 15 min. The number of correct answers for every 1, 3, 5, and 15 min was calculated. The participants performed the test battery (the CWST, PASAT, and U-K test) three times (every hour, starting from 30 minutes after the commencement of the footbath). The U-K test was additionally performed 75 minutes after the start of

the footbath. The participants performed practice sessions to ensure that they understood the tests and were familiar with each test well before starting the experiment.

2.3.4. Visual Analog Scale

The subjective sensory intensities of concentration and fatigue were recorded before the first test period and after each test period using a 100-mm visual analog scale (VAS) with anchor statements "not at all" on the left and "extremely" on the right.

2.3.5. Heart Rate and Heart Rate Variability

Heart rate and its variability were measured to evaluate autonomic nervous activity, and heart rate was also monitored for safety evaluation during thermal loading. Heart rate was continuously recorded every second with a chest-guided wireless heart rate monitor (Polar Team Pro, Polar Electro Inc.) attached to the chest with a dedicated fixed belt on the morning of the experiment days. As for heart rate variability, the R-R intervals for 30 s at the beginning of each event (e.g., the measurement of sublingual temperature and cognitive tests) were excluded and the values for the subsequent 4 min were analyzed using HRV analysis (Université Jean Monnet, Saint-Étienne) to avoid artifacts. The sympathetic activity was calculated as the ratio of low frequency to high frequency (LF/HF), and the parasympathetic activity was calculated as HF and the standard deviation of normal-to-normal R-R intervals (SDNN) [19]. Because the PASAT finished in 2 min, heart rate variability was not evaluated during this test.

2.3.6. Blood Analyses

Blood samples were collected in tubes containing EDTA-Na and immediately centrifuged (1300 × *g*, 20 minutes, 4˚C). For serum collection, blood samples were left at room temperature for more than 30 min and centrifuged (1600 × *g*, 15 minutes, 4˚C). Plasma and serum samples were placed in polypropylene tubes and cooled to −80˚C.

Plasma glucose levels were measured using an assay kit (product number: 298-65701; FUJIFILM Wako Pure Chemical Corporation). The plasma concentrations of noradrenaline, adrenaline, and dopamine were measured by high-performance liquid chromatography at SRL Inc. (Tokyo, Japan). Plasma cortisol was quantified using the ELISA kit (product number: 50191; Immuno-Biological Laboratories Co., Ltd.). The serum level of brain-derived neurotrophic factor (BDNF) was quantified using the ELISA kit (product number: BEK-2211-1P; Biosensis Pty. Ltd.).

2.3.7. Statistical Analyses

Statistical analyses were performed using SAS software version 9.4 (SAS Institute). Statistical significance was set at $P < 0.05$. Treatment effects were assessed using a linear mixed-effects model with treatment, period, and sequence as fixed

factors and participant as a random factor. Statistical differences within the same treatment were analyzed by two-tailed Dunnett's *post-hoc* test after analysis of variance (ANOVA) for randomized block designs.

3. Results

Although 20 men participated in this experiment, one dropped out because of vasovagal reflex during the first blood collection. In addition, another participant could not complete all of the planned assessments because of an unfavorable health condition. Age, height, weight, and body mass index of the remaining 18 males were 28.0 ± 5.8 years, 170.1 ± 5.4 cm, 63.4 ± 7.9 kg, and 21.9 ± 2.1 kg/m^2, respectively (mean ± SD).

3.1. Core Body Temperature and Blood Glucose

Trends in core body temperature during the experiment are presented in **Figures 2(a)-(c)**. In the LBT conditions, although the core body temperature remained similar to that at the baseline (before the commencement of the footbath), some significant changes from the baseline were observed. Namely, sublingual temperature was significantly lower at the measurement after the third test period and higher at the measurement before the fourth test period when glucose was ingested; ear canal temperature showed significant lower values during the second U-K test with the water or glucose intake, and a lower value at the measurement before the fourth test period with the glucose intake; intestinal temperature was significantly lower since the measurement before the third test period until the measurement after the third test period with the water intake. In the EBT conditions, all the sublingual, ear canal, and intestinal temperatures were significantly higher than those in the LBT conditions from the third test period and remained higher until the end of experiment. After the third test period, both EBT conditions (water and glucose intake) showed the highest core body temperature during the entire experimental period, and the values were 0.3˚C - 0.5˚C higher than those in the LBT conditions. Additionally, ear canal temperature showed a significantly lower value compared to the baseline during the first CWST in the EBT/water condition. There were no significant differences in the sublingual, ear canal, and intestinal temperatures between the LBT/water and LBT/glucose or between the EBT/water and EBT/glucose conditions. Blood glucose levels are shown in **Figure 2(d)**. Compared to the baseline (before the start of thermal load), blood glucose levels in the water intake conditions decreased significantly at the end of experiment, whereas those in the glucose conditions were significantly elevated after two glucose intake sessions in both the LBT and EBT conditions. Blood glucose level at the end of experiment in the EBT/glucose condition was higher than that in the LBT/glucose condition but it did not reach statistical significance ($P = 0.054$).

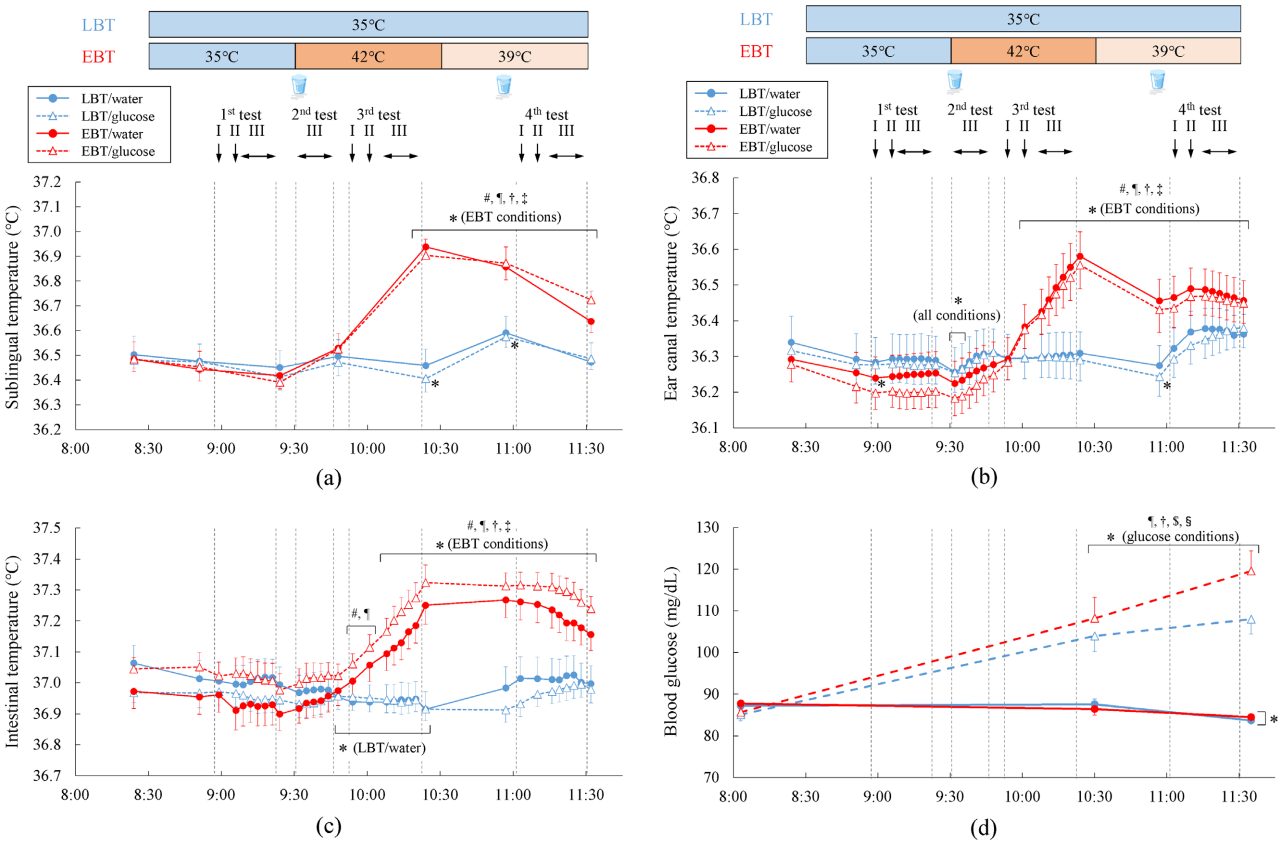

Figure 2. Trends in core body temperature and blood glucose level during the experiment. Sublingual, intestinal, and ear canal temperatures were measured during the experimental period as core body temperature. Sublingual temperature (a) was measured for 5 min before and after the sets of cognitive tests. (b) Ear canal temperature was continuously measured at 10-s intervals throughout the experiment. The data points represent the averages of 1 min before the measurement of sublingual temperature, 3 min during the Stroop test, 2 min during the PASAT, and each 3-min period during the Uchida-Kraepelin test. (c) Intestinal temperature was continuously measured at 30-s intervals. The data points represent the averages of 4.5 min during the measurement of sublingual temperature, 3 min during the Stroop test, 2 min during the PASAT, and each 3-min period during the Uchida-Kraepelin test. Arrows indicate the timing of cognitive tests. (d) Blood was collected at 8:00 (before the start of thermal load), 10:30 (the end of thermal load at 42˚C [EBT conditions]/after the third test period), and 11:30 (the end of thermal load/after the fourth test period), and plasma glucose levels were measured. Data are presented as mean ± SEM (n = 18 for sublingual, intestinal, and ear canal temperatures, and n = 17 for plasma glucose levels). #: $P < 0.05$, LBT/water vs. EBT/water; ¶: $P < 0.05$, LBT/water vs. EBT/glucose; †: $P < 0.05$, LBT/glucose vs. EBT/water; ‡: $P < 0.05$, LBT/glucose vs. EBT/glucose; $: $P < 0.05$, LBT/water vs. LBT/glucose; §: $P < 0.05$, EBT/water vs. EBT/glucose; ∗: $P < 0.05$ vs. Pre.

3.2. Cerebral Hemodynamics

Data for one participant could not be obtained because a sufficient quantity of transmitted light was not detected. Hemodynamic responses in the left DLPFC were measured during the entire experiment, and the percentage changes in the amount of O_2Hb, HHb, and total Hb are shown in **Figures 3(a)-(c)**. The average value over 4.5 min while measuring sublingual temperature before the first test period was used as the baseline. Marked elevations of the O_2Hb value were observed during the first and second test periods in all conditions. Notably, significant elevations of O_2Hb levels were detected during the second test period in the glucose intake conditions regardless of body temperature. The amounts of O_2Hb

during the third and fourth test periods were almost at the same level as the baseline in the LBT/water, LBT/glucose, and EBT/water conditions. However, only in the EBT/glucose condition, the O_2Hb values during the CWST and U-K test in the fourth test period were significantly greater than that at the baseline, and significant differences were observed between the EBT/glucose and LBT/glucose or EBT/water conditions during the CWST ($P = 0.028$) or U-K test ($P = 0.043$), respectively. As for the change in HHb levels, it showed a significant increase during the PASAT in the first test period but no significant differences among the treatments were observed throughout the experiment. The percentage change in total Hb showed significant elevations during the second test period in both the LBT/glucose and EBT/glucose conditions, and during the CWST and U-K test in the fourth test period in the EBT/glucose condition. The total-Hb value in the EBT/glucose condition was higher than that in the LBT/glucose condition during the CWST ($P = 0.028$) and those in the EBT/water condition during the U-K test ($P = 0.044$ and 0.038) in the fourth test period.

3.3. Cognitive Tests

Table 1 presents the results of the CWST, PASAT, and U-K test. There were no significant differences in the reaction time (data not shown) or Stroop interference in the CWST among the four treatments; significant differences from the baseline were not observed, either. Additionally, no significant differences among the treatments or from the baseline were observed in the PASAT results. In assessments of the total number of correct answers in the U-K test over 15 min, the scores of the second to fourth tests were higher than that of the first test in all the four conditions. Although the scores in the fourth test under the LBT/glucose, EBT/water, and EBT/glucose conditions were relatively higher than that under the LBT/water condition, no significant differences were observed among the conditions. Similarly, analyses of the total numbers of correct answers for 1, 3, and 5 min in the U-K test did not show significant differences among the treatments (data not shown).

3.4. VAS

Subjective concentration was significantly greater since the second test period in the EBT/water condition compared to that at the baseline (before the first test period). Additionally, a significant difference from the baseline was observed after the fourth test period in the EBT/glucose condition. In contrast, the two LBT conditions showed no such significant increase, however, no significant difference was observed between the LBT and EBT conditions. Additionally, no significant differences were observed between the water and glucose intake conditions (**Figure 4(a)**). In all the four conditions, fatigue continued to increase over time and was significantly higher after the fourth test period than that at the baseline. Significant between-treatment differences were not observed at any time point (**Figure 4(b)**).

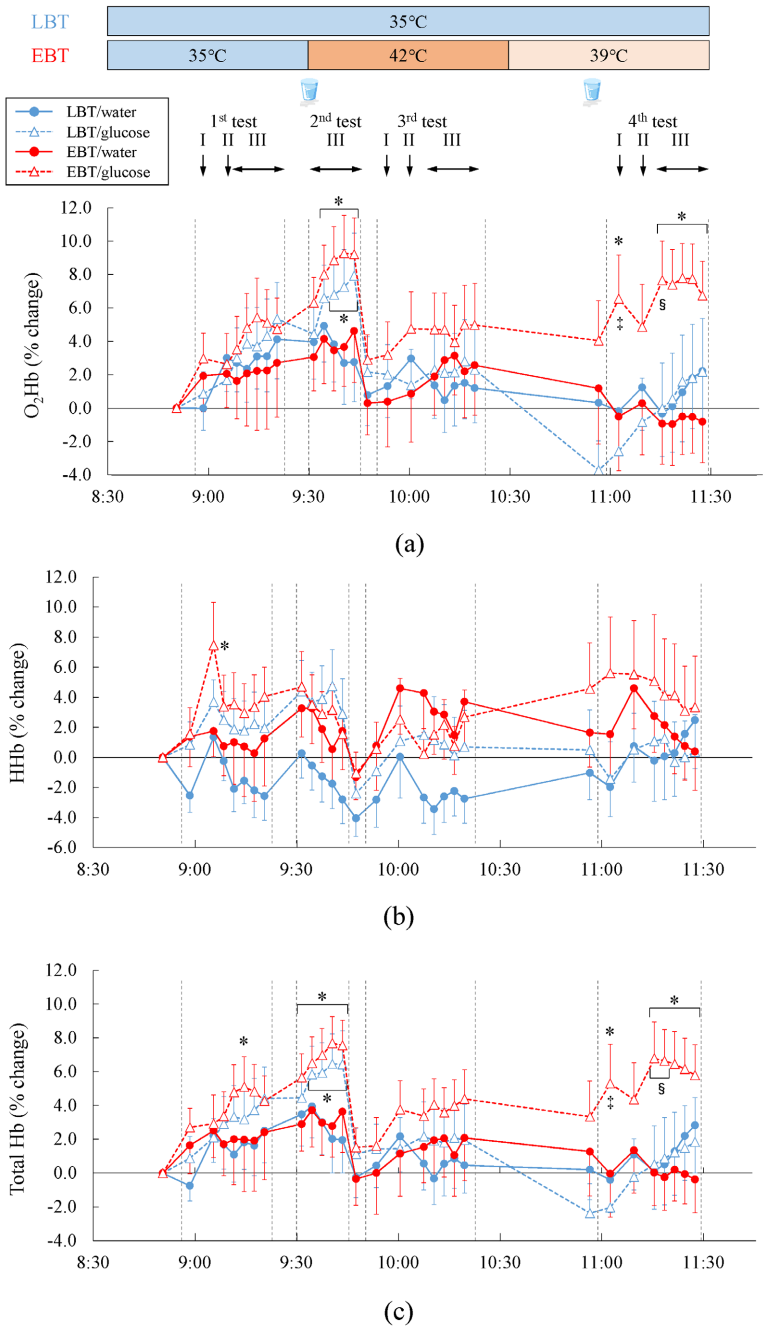

Figure 3. Trends in hemodynamics in the left dorsolateral prefrontal cortex during the experiment. Each parameter was continuously measured at 1-s intervals and the amounts of oxygenated hemoglobin (O_2Hb) and deoxygenated Hb (HHb) were estimated as total oxygenation index × the relative value of Hb normalized to the initial value × tissue hemoglobin at the beginning of measurement. Total Hb was calculated as O_2Hb plus HHb. Percentage changes from the baseline (the average of 4.5 min during the measurement of sublingual temperature before the first test) were calculated for O_2Hb (a), HHb (b), and total Hb (c). Data points represent the averages of 4.5 min during the measurement of sublingual temperature, 3 min during the Stroop test, 2 min during the PASAT, and each 3-min period during the Uchida-Kraepelin test. The arrows indicate the timing of cognitive tests. Data are presented as mean ± SEM (n = 17). ‡: $P < 0.05$, LBT/glucose vs. EBT/glucose; §: $P < 0.05$, EBT/water vs. EBT/glucose; *: $P < 0.05$ vs. Pre.

Table 1. Results of the color-word-matching Stroop task (CWST), the Paced Auditory Serial Addition Test (PASAT), and the Uchida-Kraepelin (U-K) test.

CWST, Stroop interference (sec)

Treatment	Test period		
	1st	3rd	4th
LBT/water	0.153 ± 0.019	0.142 ± 0.018 (−0.010 ± 0.027)	0.190 ± 0.022 (0.037 ± 0.030)
LBT/glucose	0.168 ± 0.026	0.174 ± 0.029 (0.006 ± 0.031)	0.153 ± 0.020 (−0.014 ± 0.028)
EBT/water	0.135 ± 0.026	0.144 ± 0.022 (0.009 ± 0.027)	0.181 ± 0.016 (0.046 ± 0.037)
EBT/glucose	0.162 ± 0.021	0.172 ± 0.022 (0.010 ± 0.027)	0.212 ± 0.021 (0.051 ± 0.025)

PASAT, number of correct answers

Treatment	Test period		
	1st	3rd	4th
LBT/water	58.1 ± 0.7	58.7 ± 0.4 (0.6 ± 0.8)	58.9 ± 0.6 (0.8 ± 0.7)
LBT/glucose	58.4 ± 0.6	59.2 ± 0.3 (0.8 ± 0.6)	59.3 ± 0.2 (0.8 ± 0.6)
EBT/water	59.3 ± 0.3	59.5 ± 0.3 (0.2 ± 0.2)	59.1 ± 0.4 (−0.2 ± 0.3)
EBT/glucose	58.7 ± 0.4	58.6 ± 0.4 (−0.1 ± 0.7)	58.9 ± 0.7 (0.2 ± 0.5)

U-K test, number of correct answers for 15 min

Treatment	Test period			
	1st	2nd	3rd	4th
LBT/water	885.1 ± 47.1	944.3 ± 48.9* (59.2 ± 10.0)	935.9 ± 51.6* (50.8 ± 13.5)	943.7 ± 43.9* (58.6 ± 21.4)
LBT/glucose	885.4 ± 39.5	946.8 ± 40.1* (61.3 ± 14.3)	939.4 ±40.5* (54.0 ± 20.5)	980.8 ±40.9* (95.4 ± 25.8)
EBT/water	898.2 ± 34.5	953.5 ± 38.5* (55.3 ± 13.7)	954.9 ± 43.7* (56.7 ± 20.9)	982.7 ± 41.4* (84.5 ± 18.3)
EBT/glucose	892.3 ± 37.2	943.4 ± 40.7* (51.1 ± 9.8)	948.9 ± 43.6* (56.7 ± 14.2)	978.9 ± 41.5* (86.6 ± 13.8)

The values in parentheses are the amounts of change from the first test. Data are presented as mean ± SEM (n = 18). *: $P < 0.05$ vs. 1st.

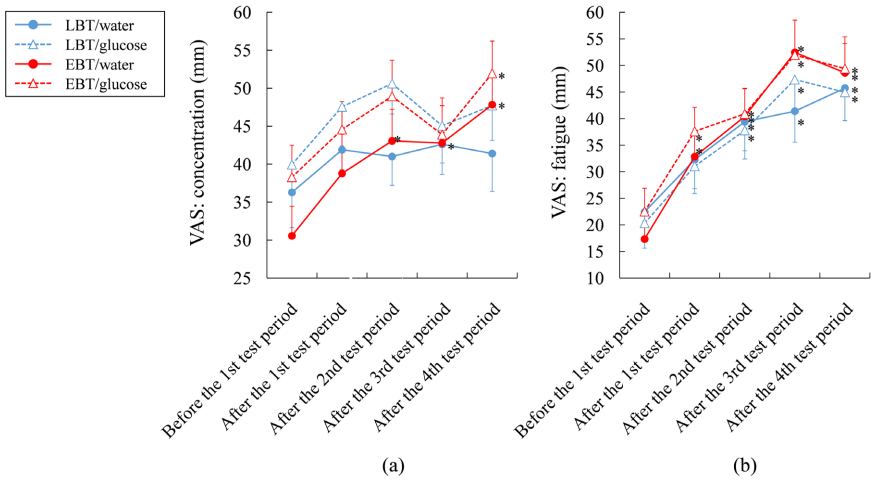

Figure 4. Trends in subjective concentration and fatigue. The subjective sensory intensities of concentration (a) and fatigue (b) were recorded using a 100-mm visual analog scale (VAS) from "not at all" to "extremely" before the first test period and after each test period. Data are presented as mean ± SEM (n = 18). *: $P < 0.05$ vs. Pre.

3.5. Heart Rate and Heart Rate Variability

In the two LBT conditions, although significant increases from the baseline (when the sublingual temperature was measured before the first test) were detected after the first U-K test, heart rate was approximately maintained throughout the experiment. In contrast, it continued to increase after the temperature of the water bath was changed to 42˚C in the EBT conditions. During the third test period, heart rate was significantly higher in the two EBT conditions than in the two LBT conditions. After the hot bath temperature was changed to 39˚C, heart rate decreased in the two EBT conditions but remained higher than that in the two LBT conditions. Additionally, during the third and fourth test periods, significant differences were observed between the EBT/water and EBT/glucose conditions and between the LBT/water and LBT/glucose conditions (**Figure 5(a)**). LF/HF ratio increased significantly from the baseline during the second CWST in the LBT/glucose condition and the value at the time was higher than that in the LBT/glucose condition. Similarly, LF/HF increased significantly during the third CWST in the EBT/glucose condition and the value was higher than that in the LBT/water condition at the time (**Figure 5(b)**). HF decreased significantly during the second and fourth U-K tests in the LBT/water condition, during the second U-K test in the LBT/glucose condition, during the third and fourth U-K tests in the EBT/water condition, and during all the four U-K tests in the EBT/glucose condition. Additionally, significant differences in HF were observed between the LBT and EBT conditions during the third and fourth test periods (**Figure 5(c)**). The SDNN was significantly lower during all the four U-K tests in the two EBT conditions and significant differences between the LBT and EBT conditions were observed during the third and fourth test periods (**Figure 5(d)**).

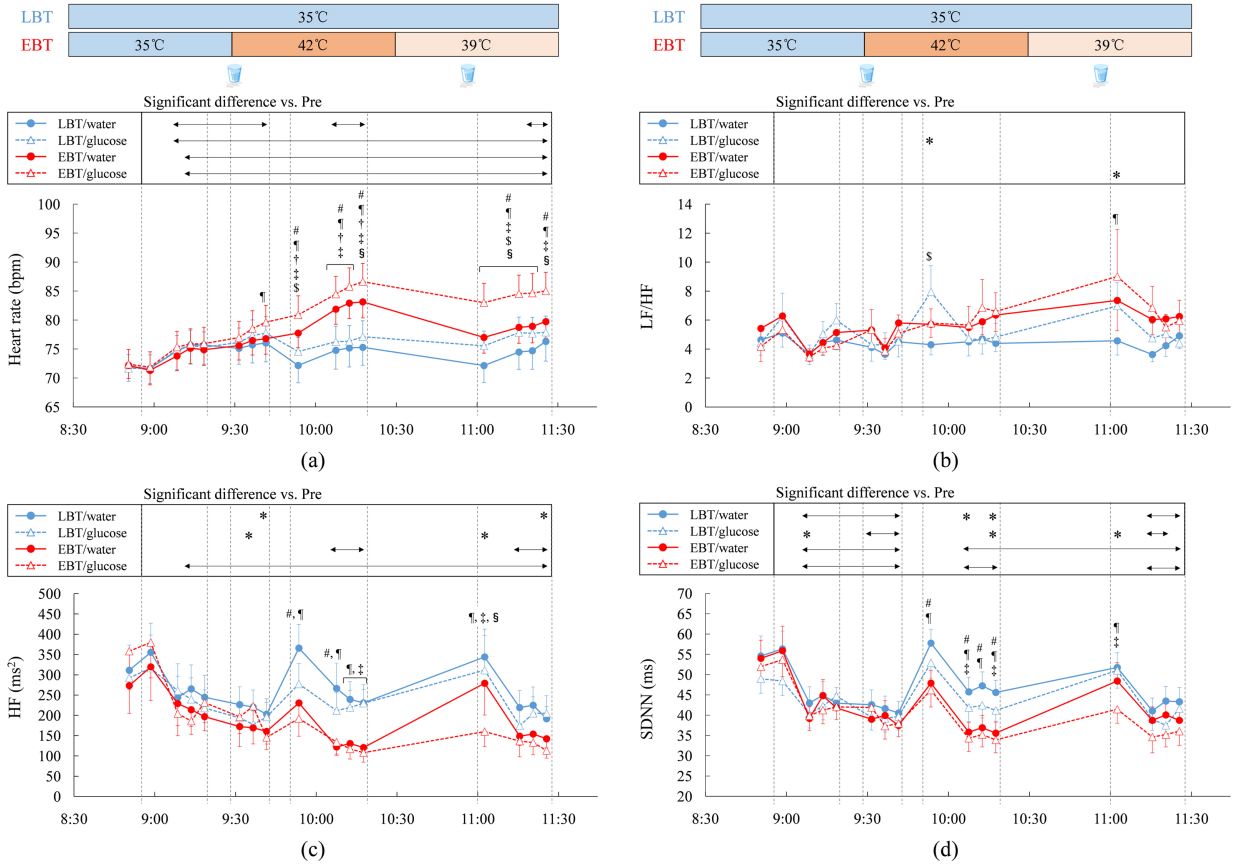

Figure 5. Trends in the heart rate and its variability during the experiment. Heart rate was continuously recorded every second with a chest-guided wireless heart rate monitor (a). Heart rate variability was analyzed using the R-R intervals for 4 min and the ratio of low frequency to high frequency (LF/HF, (b)) was calculated as the sympathetic activity, and HF (c) and the standard deviation of normal-to-normal R-R intervals (SDNN, (d)) were calculated as the parasympathetic activity. Data are presented as mean ± SEM (n = 18). #: $P < 0.05$, LBT/water vs. EBT/water; ¶: $P < 0.05$, LBT/water vs. EBT/glucose; †: $P < 0.05$, LBT/glucose vs. EBT/water; ‡: $P < 0.05$, LBT/glucose vs. EBT/glucose; $: $P < 0.05$, LBT/water vs. LBT/glucose; §: $P < 0.05$, EBT/water vs. EBT/glucose; ∗ and arrows: $P < 0.05$ vs. Pre.

3.6. Concentrations of Plasma Catecholamine, Cortisol, and Serum BDNF

In addition to the participant who dropped out from the study, one experienced a vasovagal response during blood collection. The participant completed the study without undergoing any further blood collection. The blood levels of catecholamine, cortisol, and BDNF in the remaining participants are shown in Figure 6. Adrenaline levels increased significantly at 10:30 (after the third test period/the end of thermal load at 42˚C) in the two EBT conditions and at 11:30 (after the fourth test period/the end of thermal load at 39˚C) in the EBT/water condition; at these time points, significant differences between the LBT and EBT conditions were observed. Noradrenaline levels showed significant increases at 11:30 in the two LBT conditions and significant differences between the LBT/glucose and EBT/glucose and between the LBT/water and EBT/glucose were observed at the time. Dopamine in most samples was under the limit of quantification (5 pg/mL) and could not be determined. In all the four condi-

tions, cortisol levels decreased significantly over time but no significant differences among the treatments were observed. BDNF levels showed significant decreases from the baseline at 11:30 in the LBT/water, EBT/water, and EBT/glucose conditions but did not show any between-treatment difference.

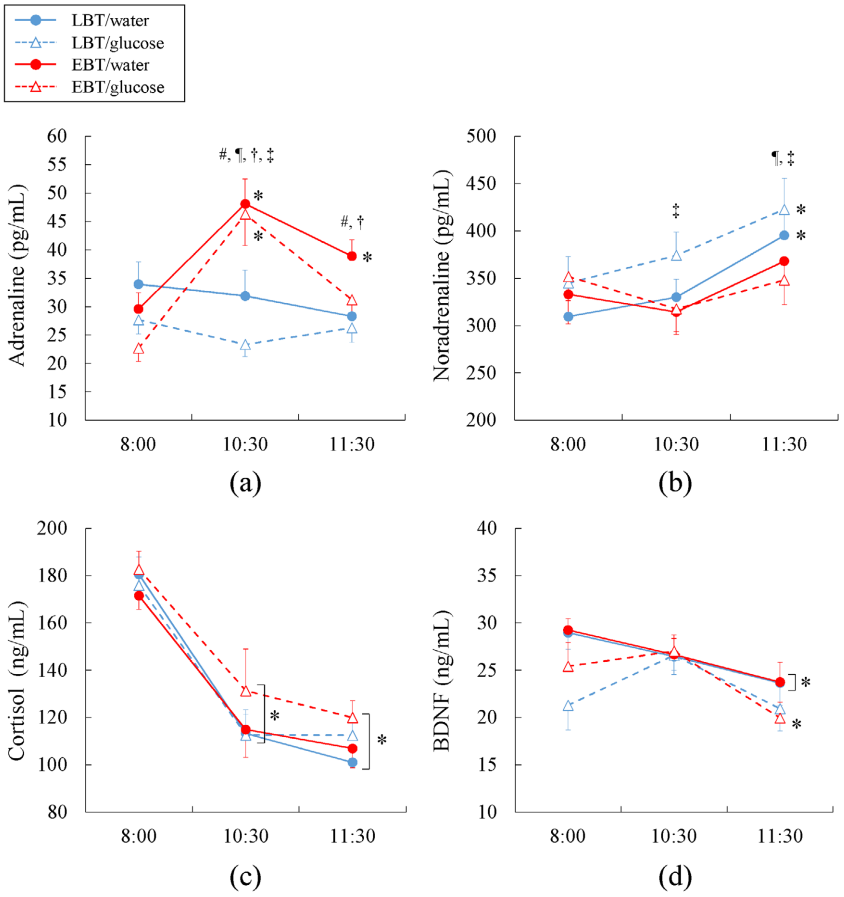

Figure 6. Trends in the plasma catecholamine, cortisol, and serum BDNF concentrations. Blood was collected at 8:00 (before the start of thermal load), 10:30 (the end of thermal load at 42˚C [EBT conditions]/after the third test period), and 11:30 (the end of thermal load/after the fourth test period). Plasma adrenaline (a), noradrenaline (b), dopamine, cortisol (c), and serum BDNF (d) concentrations were measured. Dopamine concentration was under the quantification limit (5 pg/mL). Data are presented as mean ± SEM (n = 17). #: $P < 0.05$, LBT/water vs. EBT/water; ¶: $P < 0.05$, LBT/water vs. EBT/glucose; †: $P < 0.05$, LBT/glucose vs. EBT/water; ‡: $P < 0.05$, LBT/glucose vs. EBT/glucose; ∗: $P < 0.05$ vs. Pre.

4. Discussion

We were motivated to investigate the possibility that a DIT-induced increase in core body temperature improves cognitive function. Therefore, the temperature of the footbath water was set to elevate the core body temperature to a similar extent as in DIT. Resultantly, core body temperature (sublingual, ear canal, and gastrointestinal temperature) under the EBT conditions increased 0.3˚C - 0.5˚C from the baseline, whereas it remained stable in the LBT conditions (**Figures**

2(a)-(c)). The increases in core body temperature under the EBT conditions are within the previously reported range for physiological fluctuations of core body temperature [9] and the increase in sublingual temperature is similar to that after eating a balanced diet, as reported by Higuchi *et al.* [6]. Moreover, this difference is consistent with the finding of another study that the presence or absence of breakfast resulted in approximately a 0.3˚C difference in core body temperature [20]. Furthermore, the amount of glucose intake in this study was also set considering diet, and the participants received two intakes of 20-g glucose. This amount is equivalent to that of carbohydrates in the nutritionally balanced food used in the trial of Higuchi *et al.* In the present study, blood glucose levels in the glucose intake conditions significantly increased and remained higher than those in the water intake conditions (approximately 20 - 30 mg/dL). Notably, the blood glucose level at the end of experiment in the EBT/glucose condition showed a tendency to be higher than that in the LBT/glucose condition ($P = 0.054$, **Figure 2(d)**). This may be due to a mechanism wherein high body temperature induced by an external thermal load leads to reduced muscular glucose uptake, resulting in the maintenance of high blood glucose levels [21]. Additionally, the thermal load might have been recognized as mild stress and led to the activation of the sympathetic nervous system [22], although plasma cortisol levels remained low throughout the trial (**Figure 6(c)**). This is probably because blood cortisol levels peak after waking up due to the circadian rhythm [23]. The heart rate in the EBT conditions increased significantly due to the thermal load and remained higher than that in the LBT conditions after the third test period (**Figure 5(a)**). Moreover, the EBT/glucose treatment significantly increased LF/HF during the third test period, whereas the LBT conditions did not (**Figure 5(b)**). Furthermore, HF decreased significantly in the EBT conditions and significant differences were observed between the LBT and EBT conditions after the third test period (**Figure 5(c)**). In addition, plasma adrenaline levels increased significantly in the EBT conditions but not in the LBT conditions (**Figure 6(a)**). Therefore, the synthesized adrenaline following the mild activation of the sympathetic nervous system may have stimulated both glycogenolysis and gluconeogenesis [22] [24]. Another possibility for the higher glucose levels in the EBT/glucose condition is that the sympathetic nervous system activation may have suppressed the secretion of insulin [25].

We chose to examine changes in cerebral blood flow over the morning, and this study evaluated blood flow in the left DLPFC. The DLPFC is involved in various cognitive functions (e.g., attention, executive function, and memory retrieval), and the Hb levels in the left DLPFC reportedly increase following a nutritional intervention [26]. The present study showed significantly increased O_2Hb in the left DLPFC during the second U-K test in the two glucose intake conditions (**Figure 3(a)**). Given that there was no significant difference in core body temperature between the two glucose intake conditions during the test (**Figures 2(a)-(c)**), and that the blood glucose levels during the test seemed to be

increasing because glucose had been ingested before the test started, glucose, recognized as a nutrient for the brain, may lead to increased cerebral blood flow during the cognitive task [27]. Notably, the LBT/water, LBT/glucose, and EBT/water conditions did not increase the percentage change in O_2Hb levels during the fourth test period, whereas the EBT/glucose condition did (**Figure 3(a)**). Since there was no significant difference between the core body temperatures in the EBT/water and EBT/glucose conditions (**Figures 2(a)-(c)**), the increase in cerebral blood flow may not have been only because of heat dissipation. The adoption of the SRS method to evaluate hemodynamics reinforces this presumption because the estimated values with this method are less affected by the superficial blood flow [14]. However, blood glucose levels alone cannot also explain the differences in cerebral blood flow response because the blood flow during the fourth test period (after the second glucose intake) in the LBT/glucose condition remained similar to that at the baseline (**Figure 3(a)**). Another plausible mechanism by which the cerebral blood flow increased in the EBT condition may be the recognition of the thermal load as mild stress, leading to increased vigilance among test participants [28], but the absence of the blood flow response during the fourth test period in the EBT/water condition does not support this possibility (**Figure 3(a)**). The disappearance of the cerebral blood flow response before noon in the three conditions other than the EBT/glucose condition may be due to mental fatigue. The PASAT and U-K test, which have been reported to induce mental stress [29] [30], and the CWST were performed three or four times in this study. In all four conditions, the degrees of fatigue sensation after the fourth test period were significantly higher than those at the baseline (**Figure 4(b)**), and the SDNN decreased during the U-K tests (**Figure 5(d)**). Reduced cerebral blood flow has been previously reported in patients with chronic fatigue syndrome [31]. Therefore, long-term cognitive load that causes fatigue may fade the cerebral blood flow response. Consequently, we propose, for the first time, that an elevated core body temperature within the physiological range combined with adequate cerebral glucose supply may prevent the reduction in cerebral blood flow response due to mental fatigue. Although we did not observe significant effects of the treatments on results of the cognitive tasks we adopted, the maintained response of cerebral blood flow owing to the elevated core body temperature and blood glucose levels might coincide with the improvement in cognitive performance. Two previous studies separately reported greater cerebral blood flow [5] and enhanced cognitive performance [6] during a fatigue-inducing cognitive task after having a nutritionally balanced breakfast. In both studies, not only were the blood glucose levels elevated but core body temperature must also have been raised by DIT, at least to some extent (Higuchi *et al.* measured the temperature and observed its increase [5] but Akitsuki *et al.* did not [6]), and the test participants in both studies reported the sensation of fatigue with assigned cognitive tasks.

This study had the following limitations. First, the results showed significant

between-treatment differences in the cerebral blood flow during the cognitive tests. However, there was no significant between-treatment difference in the cognitive performance at any time point. Future studies should examine the type or load level of cognitive tasks such that the task performance will be correlated with the cerebral blood flow response. Second, we could not exclude the possibility that activation of the sympathetic nervous system is involved in the mechanism underlying increased cerebral blood flow during the footbath. Future studies should examine the effects of sympathetic activity by activating the sympathetic nervous system without increasing core body temperature. Additionally, since the increase in core body temperature in this study was approximately equivalent to that after breakfast, a comparison of the sympathetic activity under the presented experimental condition with that after consuming a balanced meal for breakfast may yield interesting insights. Moreover, although this study was conducted in the morning, when the core body temperature remains low, future studies should be conducted in the afternoon or evening with core body temperature modulated by interventions to further investigate the relationship between core body temperature and the cerebral blood flow response. Furthermore, for the present study, we recruited male participants aged between 20 and 39 years to exclude the possible effects of aging and menstrual cycle on core body temperature and the cognitive outcomes. Additional studies should be conducted with test participants with different attributes, such as older and/or female participants, to ensure consistency of the results.

5. Conclusion

This study used an experimental procedure that simulated the increase in core body temperature and blood glucose levels after breakfast. The cognitive load during the entire morning led to a loss of cerebral blood flow response before noon under low core body temperature and/or low blood glucose level conditions. However, an appropriate blood flow response was observed when the core body temperature and blood glucose levels were kept elevated. Since reduced cerebral blood flow is reportedly associated with mental fatigue, reduced performance, and various diseases, these negative consequences may be prevented or improved by appropriately increasing not only the blood glucose level but also the core body temperature. By the same mechanisms, a nutritionally balanced breakfast may maintain a continuous cerebral blood flow response throughout the morning, and further studies are warranted to investigate this possibility.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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