REVIEW ARTICLE

Hot bathing has the potential to provide a new means of secreting brain-derived neurotrophic factor

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Abstract

Background: Brain-derived neurotrophic factor (BDNF) promotes and improves neuronal plasticity, suppresses neuronal cell death, induces neural regeneration, and stimulates neuronal survival, particularly in motor and sensory neurons of the peripheral and central nervous systems. Moreover, BDNF plays important roles in memory, learning, mood, food intake, and energy metabolism. Increased BDNF is involved in the prevention and amelioration of brain health and neurological diseases. The factors that increase BDNF include heat stress and exercise-independent pathways. Goekint (2011), demonstrated that exercise at high temperatures increased core temperature and resulted in higher BDNF concentrations than exercise at low temperatures. Based on these results, they concluded that BDNF concentrations was stimulated by an increase in core temperature during exercise. However, there is no information on whether BDNF concentrations change from core temperature increase, independent of exercise. The study was carried out for the following two purposes. In the first study, we examined BDNF concentrations changes in a hot bath to clarify the relationship between an increase in core temperature and an increase in BDNF concentrations. In study 2, an experiment using the additive effect of the warm bath combined with exercise was carried out to efficiently increase BDNF concentrations.

Methods: In study 1, an eight healthy males performed 20 min head-out water immersion (HOI) at 42 °C (hot-HOI) and 35 °C (neutral-HOI). In study 2, a ten healthy young males subjects performed HOI at 40 °C (40 °C HOI) or continuous cycling at 60% of maximal oxygen uptake while immersed in 40 °C (40 °C HOI-ex) or 23 °C water (23 °C HOI-ex) for 15 min. Serum BDNF, cortisol concentrations, and core temperature (T_{core}) were measured pre, immediately post, and 15 and 30 min post-immersion.

Results: From study 1, we demonstrated that a 20-min HOI at 42°C resulted in a significant increase in serum BDNF concentrations in young healthy men. From study 2, we report that the combination of a 15-min HOI in 40°C water with cycling ergometer exercise at 60% VO_2 max increased serum BDNF concentration, while 40°C HOI and 23°C HOI-ex did not.

Conclusion: The combination of exercise and heat stress may provide a time-efficient strategy to elevate serum BDNF concentrations.

INTRODUCTION

Traditional folk remedies have been handed down worldwide, such as the idea that bathing in a hot spring regulates health (Obama, 2013; Yang, 2018). It has been reported that mild hot spring treatments are effective for beauty and various diseases such as bone and joint, neurological, and collagen diseases. In addition, the effect of heat shock protein 70 (HSP 70) is well known (Detanico, 2004; Dokladny, 2015; Fábián, 2009; Kim, 2020). However, the method of bathing (temperature and time) may be carried out by experience, and it is necessary to clarify the medical effect of hot spring and warm bath therapy.

In the 1990s, the effects of sauna treatment on patients with heart failure (Basford, 2009; Tei, 1995) and type II diabetes mellitus (Hooper, 1999) were reported, and the effects of hot springs and hot baths has becoming clear. Brain-derived neurotrophic fac-

tor has been attracting attention as a cytokine that affects glucose and lipid metabolism. BDNF is a key protein that promotes neurogenesis, neuroprotection, neuroregeneration, cell survival, and development, maintains synaptic connections between neurons, and plays a regulatory role in glucose and energy metabolism (Greenberg, 2009; Park, 2013; Pederson, 2009; Wisse, 2003).

In recent years, it has been reported that BDNF, which is increased through pharmacological and endurance exercise, is involved in tissue repair and improvement of motor function around the injury site of cerebral infarction and spinal cord injury, therefore, its application in regenerative medicine is expected (Kim, 2005; Sasaki, 2016). In addition, the increase in BDNF through exercise improves glucose and lipid metabolism (Carroll, 2004; Pederson, 2006; Pederson, 2009; Tonoli, 2012) and is involved in the prevention and improvement mechanisms of brain health (Cotman, 2002; Marquez, 2015; Mattson, 2012) and neurological diseases such as Alzheimer's disease (Buchman, 2012), Parkinson's disease (Ahlskog, 2011), and multiple sclerosis (Schulz, 2004) (Figure 1). Thus, the multifaceted effects of BDNF have been elucidated and are expected to be used for the prevention and treatment of various diseases.

BDNF is produced in a variety of organs, including the cerebral cortex, limbic system, hippocampus, hypothalamus, vascular endothelial cells, immune cells, and skeletal muscle. Factors that increase BDNF include heat and exercise through independent pathways. BDNF production by heat is produced through HSP and nuclear factor κB. BDNF production by exercise has been reported to be mediated by nuclear factor in activated T-cells.

In the 2010s, research on the effects of Heat stress and BDNF started, but there have been very few reports. The only study, Goekint (2011), performed cycle ergometer exercises at the same load for 60 min at ambient temperatures of 30°C and 18°C. They reported that exercise at 30°C significantly increased serum BDNF levels compared with 18°C, suggesting an association between ambient temperature and BDNF. However, the relationship between hot baths and BDNF has not yet been clarified. Based on the above background, we conducted two studies to clarify the relationship between bathing and the increase in BDNF. We first conducted a study to clarify the relationship between hot baths and the increase in BDNF (Study 1) (Kojima, 2018). In addition, we tested a method to efficiently increase BDNF using the effect of a hot bath (Study 2) (Ohko, 2021).

STUDY 1
Participants

Eight healthy male volunteers (age: 25.4 ± 3.3 years; height: 172.8 ± 0.9 cm; weight: 66.2 ± 3.9 kg; body mass index: 22.2 ± 0.9 kg/m^2) participated in this study. We interviewed the subjects to obtain data regarding their medical history and lifestyle. None of the subjects had participated in any regular exercise for more than one year. Also, no subjects received medical or psychological status and/or medication of smokers. The Research Ethics Committee of Wakayama Medical University approved the study protocol. Informed written consent was obtained from all subjects.

Experimental protocol

The subjects were instructed to refrain from strenuous physical exercise and alcohol consumption the day before the experiment and to refrain from taking any fluids and food after 10pm the day before the
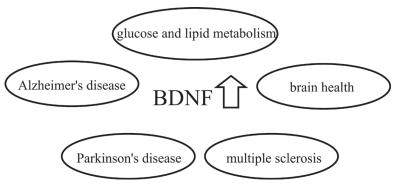

Figure 1. Increased brain-derived neurotrophic factor (BDNF) is involved in metabolic improvement, neuropathic prevention, and improvement mechanisms.

experiment, with the exception of water, until the completion of the experiment. They arrived at the laboratory between 9am and 1pm on the day of the experiment. Each subject wore swimming trunks and rested in a thermoneutral room (28°C) for at least 30 min, then they were prepared for ECG recording, blood pressure (BP) monitoring, and core body temperature (T_{core}) recording. To determine the effects of head-out water immersion (HOI) in hot water at rest, each subject sat in hot water (42°C, hot-HOI) or thermo-neutral water (35°C, neutral-HOI). The first test was completed under either hot-HOI or neutral-HOI conditions, administered in a randomized order, and then the second test was conducted under the other condition at least 7 days later (Figure 2). All subjects were allowed to drink water throughout the study. T_{core} was measured throughout the experiment with a copper–constantan thermocouple inserted into the esophagus at the level of the atrium. ECG was monitored throughout the experiment. Systolic BP (SBP) and diastolic BP (DBP) were measured using a sphygmomanometer three times during each measurement period. After confirmation of stable heart rate (HR) and BP, the above variables were measured in a sitting resting position during a 10-min rest, 20-min HOI, and 30-min recovery (total experimental period = 60 min). The mean arterial pressure (MAP) was calculated as one-third of the pulse pressure plus diastolic pressure. BP was measured at 5, 25, 30, 40, and 55 min after the start of the experiment. Venous blood samples (8 ml each) were collected through an intravascular catheter before, at the end, and 15 and 30 min after the end of the HOI. Blood samples were used for measurements of serum BDNF and S100β (4 ml), plasma cortisol (2 ml), and blood cell counts (2 ml).

Statistical analysis

All results were expressed as mean ± SD. Significant changes in each parameter relative to before HOI, at the end of HOI, 15 and 30 min after the end of HOI were examined by repeated measures ANOVA. Differences between hot-HOI and neutral-HOI were examined for statistical significance using repeated measures ANOVA. A value of p less than 0.05 was considered statistically significant.

Results

Changes in T_{core}, MAP, and HR

T_{core} increased at the end of hot-HOI (39.5 ± 0.6°C, $p < 0.05$) and at post 15 min ($p < 0.05$) compared with at rest, and returned to the level before HOI at 30 min (Figure 3). The T_{core} at neutral-HOI did not change during the entire study period. T_{core} at hot-HOI, at the end of HOI, and 15 min after the end of HOI were significantly higher than those at neutral-HOI ($p < 0.05$) (Figure 3). The HR increased from 64.9 ± 8.2 before HOI to 112.0 ± 11.3 bpm at the end of hot-HOI ($p < 0.05$) but diminished to 78.8 ± 13.0 bpm at the end of the study. The HR at neutral HOI did not change throughout the study. The HR at hot-HOI was significantly higher at the end of HOI than at neutral-HOI ($p < 0.05$). The MAP remained constant throughout the study of hot-HOI and neutral-HOI.
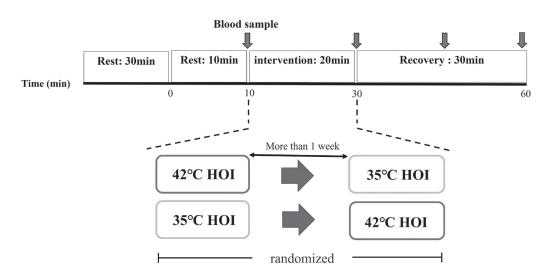

Figure 2. Experimental protocol (Study 1)

Changes in serum BDNF and S100β

Serum BDNF concentration was significantly higher at the end of hot-HOI ($p < 0.05$) and was still high at 15 min later, compared with that before hot-HOI. Whereas, the level 30 min after the end of hot-HOI was similar to that before hot-HOI (Figure 4). No change was observed in serum BDNF concentration in the neutral HOI throughout the study. Serum BDNF of hot-HOI, at the end of HOI, and 15 and 30 min after the end of HOI were significantly higher than that of neutral-HOI ($p < 0.05$) (Figure 4). On the other hand, serum S100β levels did not change throughout the hot-HOI and neutral-HOI studies and were not significantly different between hot-HOI and neutral-HOI at the four time points.

Discussion

The major findings were as follows: (1) body immersion in hot water (42°C), but not thermo-neutral water (35°C), for 20 min significantly increased serum BDNF concentrations; (2) the increase in serum BDNF concentration was associated with a significant increase in T_{core} resulting from immersion in hot water; and (3) the rise in T_{core} and serum BDNF concentrations were not associated with similar changes in serum S100β. The present findings suggest that hyperthermia resulting from immersion in hot water significantly and independently stimulates serum BDNF concentrations in humans.
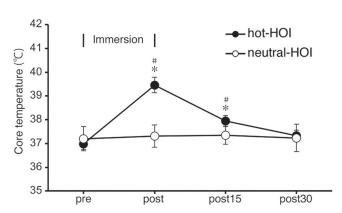
The exercise-induced increase in BDNF concentrations was recently reported to be related to a corresponding increase in T_{core} (Goekint, 2011). In that study, BDNF concentrations were higher during exercise conducted at 30°C, compared to that in a room set at 18°C ($p = 0.02$). Furthermore, it was suggested that the increase in T_{core} enhanced the increase in serum BDNF concentration during exercise. In fact, exercise increased T_{core}, which in turn activated BDNF secretion. However, the present results of increases in BDNF under rest conditions strongly suggest that a high T_{core} acts independently of exercise to increase serum BDNF concentrations.

On the other hand, considering the clinical application, since the load is high in the hot bath of 42 °C for 20 minutes, it is necessary to examine a method for increasing BDNF more efficiently.

STUDY 2

In addition to the hot bath effect, an experiment aiming at the additive effect by combining exercise, which is a route to increase the other BDNF, is introduced. Since Neeper (1996) discovered that BDNF mRNA can be elevated through exercise, the effects of different exercise components on BDNF concentration, such as modality, intensity, and duration, have been investigated. In humans, regular resistance and endurance exercises elevate resting serum BDNF concentrations (Byun, 2016; Frazzitta, 2014; Levinger, 2008; Seifert, 2010; Vedovelli, 2017). Moreover, a
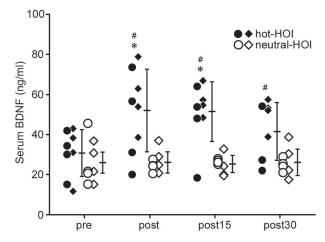

Figure 3. Changes in core body temperature in head-out water immersion (HOI).
Data are presented as mean ± standard deviation. *$p < 0.05$, compared with before immersion. #$p < 0.05$, hot-HOI vs. neutral-HOI. pre: before HOI, post: at the end of HOI, post15: at 15 min after the end of HOI, post30: at 30 min after the end of HOI.

Figure 4. Changes in serum brain-derived neurotrophic factor (BDNF) in head-out water immersion (HOI).
The center of each bar represents the mean value for each time period and the length of the bar represents the standard deviation value. *$p < 0.05$, compared with before immersion. #$p < 0.05$, hot-HOI vs. neutral-HOI. Each figure shows one open symbol (neutral HOI) and one solid symbol (hot-HOI). See Figure 3 for definitions.

single bout of exercise for at least 30 min at an intensity higher than 60% of maximal oxygen uptake (VO_2 max) can acutely elevate serum BDNF concentration (Castellano, 2008; Schulz, 2004). However, the influence of additional factors related to the exercise bout, such as the environment in which it is performed or the subject's characteristics, remains unclear (Hötting, 2016; Santos, 2016).

As introduced in Study 1, Kojima (2018) reported that HOI at 42°C for 20 min increased serum BDNF concentrations. Based on these results, the combined use of a thermal stressor and endurance exercise may elicit a synergistic effect. By combining both stressors, serum BDNF concentration may be elevated by interventions that are shorter than those previously studied. As a lack of time is often cited as a barrier to exercise participation (Felipe, 2007), such a time-efficient strategy would have significant practical implications for the promotion of health in the general population.

Therefore, the aim of this study was to evaluate the acute BDNF response to endurance exercise combined with HOI in 40°C water. The intervention time was determined from observation of core temperature changes during exercise in water at 40°C in a preliminary experiment with 3 subjects. It was hypothesized that 15 min of endurance exercise combined with HOI results in a larger BDNF response compared with both stressors individually.

Participants

Ten young healthy males (age: 23.7 ± 0.8 years; height: 170.1 ± 7.0 cm; weight: 63.6 ± 7.2 kg; body mass index: 21.7 ± 1.7 kg/m^2; VO_2 max: 2.8 ± 0.3 l/min) participated in this study. None of the subjects performed regular exercise for at least 6 months. The exclusion criteria were smoking and use of medication for medical or psychiatric conditions. The study was approved by the Research Ethics Committee of Wakayama Medical University and was performed in accordance with the Declaration of Helsinki. The purpose and risks of the study were explained to all subjects, who provided written informed consent.

Experimental protocol

At least 1 week prior to the experiment, VO_2 max was determined using a stepwise incremental cycle ergometry test (828E; Monark, Varberg, Sweden). O_2 uptake and ventilation were measured using a respiratory metabolic cart (MetaMax 3 B, Cortex, Leipzig, Germany).

For the experimental trials, the subjects visited the laboratory three times. In a randomized order, they performed seated rest in 40°C water (40°C HOI) or cycling ergometer continuous exercise at 60% VO_2 max in 40°C (40°C HOI-ex). In addition, the same subjects were invited for a third control visit, namely, endurance exercise in thermoneutral water (23°C HOI-ex). The visits were separated by a minimum of 1 week (Figure 5). All subjects were instructed to refrain from intense exercise, alcohol, and caffeine consumption the day prior to the experiment and to consume only water after 10pm.

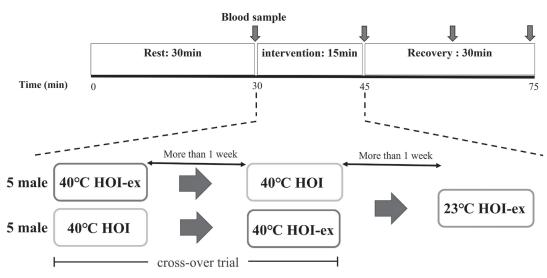On the day of the experiment, subjects arrived

Figure 5. Experimental protocol (Study 2)
HOI, head-out water immersion

at the laboratory between 9am and 0pm, with the starting time kept the same for each subject. The subjects changed into swimming trunks and rested in a thermoneutral room (28°C) for a minimum of 30 min. HR was monitored using an electrocardiogram (BSM-2401; Nihon Kohden, Tokyo, Japan). SBP and DBP were measured at the brachial artery using an inflatable cuff. Measurements were taken every 5 min during the experimental period, and the mean value was used for each period: at baseline, immediately after completing the immersion protocol, and 15 and 30 min after. T_{core} was measured using a copper-constantan thermocouple probe, which was inserted through the nasal cavity, with the tip of the probe at the atrial level of the esophagus. T_{core} was continuously measured from the beginning to the end of the experiment. After 30 min of seated rest, subjects entered the immersion tank at 40°C HOI, 40°C HOI-ex, or 23°C HOI-ex, and were immersed up to the neck (Figure 6).

The water temperature was continuously monitored using digital and mercury thermometers. For 40°C HOI-ex or 23°C HOI-ex, subjects were instructed to maintain a pedaling speed of 50 to 60 rpm, with adjustments made when needed to maintain an intensity of 60% VO_2 max. During HOI-ex, recumbent cycling was performed while the subjects were immersed in water and secured to the seat using a waist belt. After completion of the session, the subjects rested

while seated in the laboratory for 30 min.

Venous blood samples were obtained from an antecubital vein at four time points: after the 30-min rest period (before the session, pre), immediately after the completion of the 15-min session (post), and 15 (post 15) and 30 (post 30) min after the completion of the 15-min immersion session. Blood samples (8 mL each) were used to measure serum BDNF (2 mL), P-selectin (2 mL), plasma lactate (1 mL), plasma cortisol concentration (1 mL), and blood cell counts (2 mL).

Statistical analysis

Data normality was examined using the Shapiro–Wilk test. Data are expressed as mean ± SD, except when noted otherwise. Differences between 40 °C HOI, 40 °C HOI-ex, and 23 °C HOI-ex were examined using a 2-way repeated measures analysis of variance. When significance was detected, Bonferroni-corrected pairwise comparisons were made at each time point as a post-hoc test. The significance level for all analyses was set at $p < 0.05$.

Results
Serum BDNF and core body temperature

The serum BDNF concentration significantly increased immediately after 40°C HOI-ex (26.7 ± 2.2 to 34.1 ± 3.2 ng/mL; $p = 0.003$) compared with at rest, and remained significantly increased 15 min after the

Figure 6. Illustration of the experimental set-up during both head-out water immersion (HOI)-ex conditions.

session (30.8 ± 3.1 ng/mL; p = 0.003). Serum BDNF concentration 30 min post (28.9 ± 3.2 ng/mL) was not statistically different from that before the intervention (Figure 7). No changes in serum BDNF concentrations were observed at 40°C HOI and 23°C HOI-ex. Immediately post-session, serum BDNF concentration was significantly higher in 40°C HOI-ex than in 40°C HOI (p = 0.004) and 23°C HOI-ex (p = 0.006).

T_{core} was significantly increased immediately after 40°C HOI-ex (40.2°C ± 0.2°C; p = 0.001) compared with at rest (37.2°C ± 0.1°C), and remained elevated 15 (38.9°C ± 0.2°C; p = 0.001) and 30 (38.3°C ± 0.1°C; p = 0.002) min after the 40°C HOI-ex intervention (Figure 8). T_{core} was also significantly increased immediately after 40°C HOI (38.8°C ± 0.1°C; p = 0.001) compared with at rest (37.1°C ± 0.1°C), and remained elevated 15 (37.9°C ± 0.1°C; p = 0.001) and 30 min after the session (37.9°C ± 0.1°C; p = 0.002). No changes in T_{core} concentrations were observed at 23°C HOI-ex. T_{core} was significantly higher at 40°C HOI-ex than at 40°C HOI and 23°C HOI-ex immediately after and 15 min after the session (both p = 0.001).

HR, MAP, hemoglobin, and hematocrit

HR was significantly increased immediately after 40°C HOI-ex (183.5 ± 16.7 bpm; p = 0.001) compared with at rest (67.6 ± 10.0 bpm), and remained elevated 15 (122.8 ± 17.1 bpm; p = 0.001) and 30 min thereafter (113.6 ± 20.4 bpm; p = 0.001). During 40°C HOI, HR was significantly increased immediately after (102.5 ± 19.1 bpm; p = 0.001) compared

with at rest (66.1 ± 10.7 bpm), and remained elevated 15 (81.1 ± 17.1 bpm; p = 0.001) and 30 (77.1 ± 12.7 bpm; p = 0.046) min after the session. HR was significantly increased immediately after 23°C HOI-ex (76.4 ± 10.1 bpm; p = 0.001) compared with at rest (65.8 ± 7.7 bpm), and remained elevated 15 (82.5 ± 9.8 bpm; p = 0.001) and 30 min thereafter (76.4 ± 10.1 bpm; p = 0.003). HR was significantly higher immediately after (p = 0.001), 15 min after (p = 0.001), and 30 min after (p = 0.001) 40°C HOI-ex compared with 40°C HOI and 23°C HOI-ex (p = 0.001). SBP at 40°C HOI-ex (118.5 ± 6.4; 114.6 ± 11.3; 111.2 ± 8.0; 107.0 ± 6.2 mmHg), 40°C HOI (116.8 ± 9.3; 119.1 ± 8.2; 116.4 ± 5.9; 118.4 ± 4.7 mmHg), and 23°C HOI-ex (119.8 ± 5.5; 127.8 ± 15.0; 125.9 ± 12.0; 121.8 ± 11.6 mmHg) remained unchanged throughout. DBP was significantly lowered immediately after (59.3 ± 8.7 mmHg; p = 0.001), 15 min after (59.0 ± 5.7 mmHg; p = 0.001), and 30 min after (59.8 ± 6.1 mmHg; p = 0.001) 40°C HOI-ex compared with baseline (77.8 ± 5.0 mmHg). DBP in the 40°C HOI (87.1 ± 7.7; 71.0 ± 5.2; 65.4 ± 6.5; 73.1 ± 7.6 mmHg) and 23°C HOI-ex (76.6 ± 4.8; 80.6 ± 5.7; 80.5 ± 6.9; 82.4 ± 8.0 mmHg) remained unchanged throughout. DBP was significantly lower in the 40°C HOI-ex group than in the 40°C HOI and 23°C HOI-ex groups immediately and 15 min after the session (both p = 0.004). No changes in hemoglobin or hematocrit were observed in 40°C HOI, 40°C HOI-ex, and 23°C HOI-ex. There was no significant difference in body mass before and after the intervention, or amount of water consumed during the
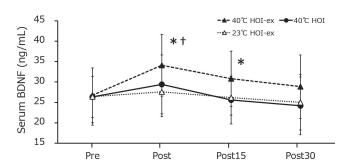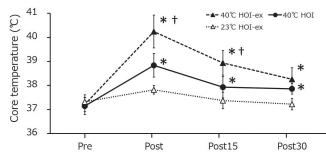

Figure 7. Changes in serum brain-derived neurotrophic factor (BDNF) concentration in response to the three experimental conditions.
Data are presented as mean ± standard deviation. *p < 0.01, compared to before immersion (pre). †p < 0.01, compared with 40°C head-out water immersion (HOI) and 23°C HOI-ex. 40°C HOI, head-out water immersion at 40°C; 40°C HOI-ex, cycling ergometer exercise in combination with head-out water immersion at 40°C; 23°C HOI-ex, cycling ergometer exercise in combination with head-out water immersion at 23°C; pre, 30-min rest period prior to immersion; post, immediately after the completion of 15-min immersion protocol; post15, 15 min after the immersion protocol; post30, 30 min after completion of the immersion protocol.

Figure 8. Changes in core temperature during the three experimental conditions.
Data are presented as mean ± standard deviation. *p < 0.01, compared to before immersion (pre). †p < 0.01, compared with 40°C HOI and 23°C HOI-ex. 40°C HOI, head-out water immersion at 40°C; 40°C HOI-ex, cycling ergometer exercise in combination with head-out water immersion at 40°C; 23°C HOI-ex, cycling ergometer exercise in combination with head-out water immersion at 23°C; pre, 30-min rest period prior to immersion; post, immediately after the completion of the 15-min immersion protocol; post15, 15 min after the immersion protocol; post30, 30 min after completion of the immersion protocol.

intervention, in either condition.

Changes in platelet count and P-selectin level

The platelet counts in 40°C HOI, 40°C HOI-ex, and 23°C HOI-ex remained unchanged throughout. The P-selectin concentration was significantly increased immediately after 40°C HOI-ex ($p = 0.002$) and remained elevated at 15 min post-session ($p = 0.01$). The P-selectin concentration in 40°C HOI and 23°C HOI-ex remained unchanged throughout the experiment. The P-selectin concentration immediately after 40°C HOI-ex was significantly higher than that in 40°C HOI and 23°C HOI-ex ($p = 0.001$).

Changes in plasma cortisol and plasma lactate

Plasma cortisol concentration was significantly increased immediately after 40°C HOI-ex and 40°C HOI (both $p = 0.001$) and remained elevated at 15 and 30 min post-session (both $p = 0.001$). Plasma cortisol concentration was significantly increased immediately after 23°C HOI-ex ($p = 0.001$) and remained elevated 15 min post-session ($p = 0.001$). Plasma cortisol concentration was higher in 40°C HOI-ex than in 40°C HOI and 23°C HOI-ex immediately after, 15 min after, and 30 min after the session (all $p = 0.001$). Plasma lactate concentration was significantly increased immediately after 40°C HOI-ex and remained elevated at 15 and 30 min thereafter (all $p = 0.001$). The plasma lactate concentration significantly increased immediately after 23°C HOI-ex and remained elevated 15 min after the session. No changes in plasma lactate concentrations were observed during 40°C HOI. Plasma lactate concentration was significantly higher in 40°C HOI-ex than in 40°C HOI and 23°C HOI-ex immediately after, 15 min after, and 30 min post-session (all $p = 0.001$).

GENERAL DISCUSSION

This study showed that 15 min of exercise in 40°C water increased serum BDNF concentration, while the same duration of 40°C HOI alone or 23°C HOI-ex did not elevate BDNF concentration. The BDNF response after 40°C HOI-ex was accompanied by an increase in cortisol and lactate concentrations.

In this study, both T_{core} and serum BDNF concentrations increased in 40°C HOI-ex. Kojima (2018, study1) found that increasing T_{core} to 39.5°C ± 0.6°C by a 20 min HOI in 42°C water increases BDNF concentration, suggesting that heat stress can induce a BDNF response independently of exercise. However, while the passive 40°C HOI condition increased the T_{core} to 38.8°C ± 0.1°C, it did not increased serum BDNF concentrations. Considering that a reduced

heat exposure duration in the study by Kojima (2018, study1) could elicit an increased BDNF response, it may be that the time spent at an elevated Tcore was too short in our study to elevate BDNF concentration. In contrast, 40°C HOI-ex increased T_{core} to 40.2°C ± 0.2°C and did result in a significant BDNF response, suggesting that the exercise itself or the additional increase in T_{core} may have contributed to the difference between the two conditions. In support for the use of combined exercise and heat stress, Van Cutsem (2015) reported that T_{core} increased to 38.7°C and the serum BDNF concentration increased from 21.8 ± 1.3 to 26.5 ± 2.1 ng/mL after exercising on a cycling ergometer at 75% maximal power output for 30 min. Although BDNF concentration increased in these studies, the increase in T_{core} was the same as that observed after 15-min HOI at 40°C in our study (38.8°C ± 0.1°C). It is possible that BDNF concentration may have increased due to the additional effect of exercise, even when the increase in T_{core} was below the threshold required to increase serum BDNF concentration.

Despite the additive effects of exercise and heat stress, the increase in serum BDNF after 40°C HOI-ex was smaller than in Kojima (2018, study1). Specifically, in the study, when HOI was performed at 42°C for 20 min, serum BDNF increased ~1.7 fold, whereas in the present study it increased ~1.3 fold. As previously mentioned, session duration may be a potential reason for the absence of BDNF response after 40°C HOI. Indeed, the response to BDNF and other myokines has been reported to be largely dependent on the intervention duration (Gold SM, 2003; Wahl P, 2012; Wahl P, 2015; Walsh JJ, 2018). In this study, after 15 min of 40°C HOI-ex, T_{core} was elevated to 40.2°C ± 0.2°C. In the experiment by Kojima et al., T_{core} after 20 min of 40°C HOI was 39.5°C ± 0.6°C.

Peak T_{core} is, therefore, unlikely to be the reason for the difference in BDNF responses between the study1 and study2. Furthermore exercise duration is also a likely explanation for the lack of a BDNF response following the 23°C HOI-ex condition. Indeed, a minimum of 30 min at a moderate intensity has been suggested to elevate BDNF concentration (Ferris, 2007; Sapolsky, 2000).

The second reason is that the combined use of exercise and heat stress in this study resulted in higher physiological stress compared with exercise at thermoneutral temperatures. An increase in cortisol levels, a stress hormone (Issa, 2010), may have caused the downregulation of serum BDNF (Knaepen, 2010; Rothman, 2013). Exercise in this study was per-

formed using 60% VO2 max, which is the suggested minimum exercise intensity required to increase serum BDNF concentration. In addition, HR and lactate increased markedly during 40°C HOI-ex, suggesting that the physiological stress imposed by this condition was larger than that of land-based exercise at a similar intensity. The minimum HR that increases BDNF concentration after exercise may be approximately ≥70% of the maximal HR (Rothman, 2013). The HR immediately after 40°C HOI-ex was 90% of the maximal HR, which was sufficient to increase BDNF concentration. Furthermore, exercise-induced lactate and peripheral BDNF concentrations have been reported to be correlated (Sapolsky, 2000; Sobral-Monteiro-Junior, 2019), which is supported by the results of this study. Previous studies have reported a negative correlation between cortisol and BDNF, with higher cortisol levels leading to lower BDNF concentrations (Issa, 2010; Knaepen, 2010). In our study, high-intensity exercise was performed for 15 min continuously at 40°C HOI-ex; thus, it was possible that cortisol increased, causing the serum BDNF levels to decrease.

With respect to the origin of BDNF, it has been reported that BDNF stored in platelets is released in response to exercise (Fujimura, 2002; Heber, 2015; Matthews, 2009; Tang, 2008). In our study, there was no change in the platelet count immediately after and 15 min after the 40°C HOI-ex. Nonetheless, the increased serum BDNF levels were likely to be derived from platelets, given that P-selectin, an indicator of platelet activity, was significantly increased.

Limitations and recommendations

Since this was the first study to investigate changes in BDNF during 40°C HOI combined with endurance exercise, we decided to prescribe moderate-intensity exercise (i.e., 60% VO_2 max). However, HR, lactate, and cortisol concentrations increased, resulting in an exercise session with physiological stress akin to high-intensity exercise. Therefore, it is possible that the increase in serum BDNF concentration would have been higher with lower-intensity exercise. Considering the potential implications of this finding, we recommend future studies on the effects of session duration, temperature, and intensity to optimize new health interventions. In addition, bathing habits may also affect perceived exercise intensity and physiological stress. Based on these points, a limitation of this study was that we did not collect subjects' bathing habits in the weeks prior to the study. Finally, given that the subjects in this study were healthy young men, further studies are required in other populations, such as women, the elderly, and clinical populations.

CONCLUSION

The present study demonstrated that 20-min hot-HOI resulted in a significant increase in serum BDNF levels. These results suggest that elevation of T_{core} is an independent factor responsible for the increase in serum BDNF levels in humans. The combination of 15-min HOI in 40°C water with cycling ergometer exercise at 60% VO_2 max acutely increased serum BDNF concentration, while 40°C HOI and 23°C HOI-ex did not. Therefore, the combination of exercise and heat stress may provide a time-efficient strategy to acutely elevate serum BDNF concentrations. Further investigations with multiple exercise protocols, temperatures, and time points, in addition to subjects from a wider population in terms of health, age, and sex, are required to design more widely applicable novel interventions.

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