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# Effects of Mild Hypohydration and Hyperthermia on Cognition and Mood in Obese and Non-Obese Females

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## Abstract

**INTRODUCTION**: Information regarding effects of hypohydration (HY) and hyperthermia (HT) on cognition, mood and development of symptoms is conflicting since the two conditions often confound each other. Further, although obese individuals may have physiological impairments during heat stress, whether psychological impairments occur with HY and HT is unknown. **PURPOSE**: To assess the independent and combined effects of mild HY and HT on cognition, mood, and the development of symptoms in obese and non-obese females. **METHODS**: Twenty-two healthy females (11 non-obese, 22±2y, 61±6kg, 25±4% body fat; 11 obese, 22±2y, 80±18kg, 44±5% body fat) volunteered in two randomized, repeated-measures trials, involving passive heating. 24-h prior to heat stress, females either remained euhydrated (EU) or became HY via fluid restriction. EU individuals received fluid during heating to maintain euhydration. Percent BM change and blood osmolality along with urine osmolality and specific gravity were measured prior to and after heating. Cognition (ImPACT, VAS, NASA-TLX), mood (Brunel Mood Scale; BRUMS), and symptoms (visual analog scales) were measured before and after a 1.0ºC increase in core temperature ($T_C$). **RESULTS:** Participants started the trials within the designated cut-offs for EU and HY. All HY hydration biomarkers were significantly greater than EU at baseline and when hyperthermic ($p<0.05$). Further EU individuals remained EU during heat stress ($p>0.05$). HY at baseline impaired Visual Memory and increased temporal demand, frustration, feelings of Depression, and Total Symptom Score ($p<0.05$). No other variables were impaired during baseline HY ($p>0.05$). HT, while remaining EU, impaired Visual Memory and mental task load and led to negative symptoms and mood ($p<0.05$). HY did not exacerbate any heat-related effects on cognition ($p>0.05$). However, participants reported increased temporal demand, Anger, and negative symptoms ($p<0.05$). Body

composition did not affect any of the above-mentioned variables in any condition ($p>0.05$). **CONCLUSION:** These data indicate that HY and HT independently impair Visual Memory, mental task load, and result in development of negative symptoms. HY did not exacerbate the effects of HT on cognition, but greater impairments to mental task load and mood and development of negative symptoms did occur. Body composition does not have an effect on cognition, mood, or the development of negative symptoms with mild HY, mild HT, or when the stressors are combined.

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## Introduction

Although physiological responses to hyperthermia and hypohydration are well established, psychological responses such as mood and cognition are largely unclear (1, 2). Understanding the independent and combined effects of hyperthermia and hypohydration on cognitive performance and mood is important for individuals who live and work in warm environments, such as the military personnel, but also because free-living individuals often have periods of hypohydration throughout the day without heat stress (3).

There are many methodological factors to consider when assessing the effects of hypohydration and hyperthermia on mood and cognition, such as: magnitude of hypohydration, gender, and method of hypohydration. In general, greater hypohydration is associated with greater impairments in mood, but the minimal level of hypohydration eliciting initial mood impairments is unknown (1, 2, 4). Gender may alter the level at which mood impairments begin, with some data suggesting women have greater mood impairment at low levels of hypohydration compared to males (5, 6). Last, the method used to induce hypohydration may influence the outcomes and interpretation. Using heat stress or exercise to dehydrate individuals can confound the findings, given these stresses independently effect cognition and mood (7, 8). In order to control for the possible extraneous effects of heat stress or exercise on cognition, fluid restriction protocols can be used in order to achieve hypohydration (9). The present study systematically isolates the independent and combined effects of mild hypohydration and hyperthermia on females.

Hypohydration has been associated with structural brain changes, including reduced gray and white matter and increased cerebral spinal fluid and ventricular networks (10). Furthermore, hypohydration is associated with hypernatremia, which causes a change in the osmotic gradient

and leads to cell shrinkage (11). Inducing mild hypohydration to 1-3% body mass loss with fluid restriction has been linked to slower psychomotor processing speed, impaired visual and spatial working memory, and increased errors and impairments in golf-shot distance accuracy in golfers (12-17). However, some studies report no cognitive differences with 2.6% hypohydration when fluid restricted for 36-h (18). The lack of change in cognitive performance with some studies could be linked to an increased mental task load, or cognitive effort. In order to complete a cognitive task, necessary cognitive activation and cerebral perfusion must occur (19, 20). Increasing cognitive effort, as measured with higher cerebral blood flow, could be an explanation for lack of impaired cognitive performance during hypohydration (21). Along with cognitive effort being negatively affected with hypohydration, mood ratings such as feelings of tiredness or fatigue and reduced vigor and confusion are observed with hypohydration (17, 18, 22, 23). Increased headaches and reduced concentration and alertness also occur with hypohydration (17, 18, 22, 23).

As an individual is heat stressed, their body temperature may rise, or become hyperthermic. Although heat stress may not always result in hyperthermia, consistent terminology is used throughout to avoid confusion. Because hypohydration often is coupled with hyperthermia, and has its own independent effects (see above), little is known regarding the independent effect of hyperthermia on cognition and mood (24). Hyperthermia reduces blood flow to caudate, putamen, insula, posterior cingulum, while cerebral metabolic rates in hypothalamus, thalamus, corpus callosum, cingulate gyrus, and cerebellum are increased (25). Regardless of brain blood flow changes (or lack of) when hyperthermic, cognitive activation has shown to be unaltered in working memory task during passive heat stress to a ~1.5 ºC increase in core temperature (21). Conflicting evidence shows that with passive heating of ~1.1ºC, cognitive

impairments to visual processing, pattern recognition memory, and spatial memory are present. Simple tasks like measures of attention are preserved (26). Whether increasing mental effort can preserve cognitive performance, as shown when hypohydrated, is unknown (27-29). Similar to hypohydration, mood changes when hyperthermic include increased feelings of fatigue and decreased vigor (30, 31).

The effect of obesity on cognition and mood is a relatively new area of study but some studies suggest obesity is linked with negative cognitive performance (32-34). Most research has looked at morbidly obese individuals with comorbidities or older obese individuals, but we are unaware of any studies with hypohydrated or hyperthermic obese individuals. However, increased body mass index (BMI) in healthy young individuals is correlated with impaired executive function, attention, problem solving, memory, and mental flexibility (35-37). Differences in cognitive effort or mood between obese and non-obese individuals have not been investigated. Given that obese individuals may have impaired thermoregulation (38, 39), their ability to perform cognitive tasks and mood might be impaired when hyperthermic. Similarly, because obese individuals sweat more (38-40), they may be more susceptible to hypohydration and the associated negative effects. However, to our knowledge, the effects of hyperthermia on cognition and mood have not been investigated in the obese population, especially in females.

**Purpose of the Study**

The purpose of this study is to examine the independent and combined effects of mild hypohydration (i.e., <2% body mass loss) and hyperthermia on cognition and mood in obese and non-obese females. The aims of this study were to: 1) examine the independent effects of mild hypohydration on cognition and mood, 2) examine the independent effect of hyperthermia on cognition and mood, 3) examine the combined effects of hypohydration and hyperthermia on

cognitive and mood and 4) examine the effect of obesity on cognition and mood during mild hypohydration or hyperthermia.

**Hypotheses**

We hypothesized: 1) mild hypohydration induced by 24-h of fluid restriction would cause significant impairments to cognition and mood, 2) hyperthermia would impair cognition and mood in euhydrated females 3) hypohydration would exacerbate hyperthermic related impairments to cognition and mood compared to deficits seen when females were euhydrated and hyperthermic, and 4) obese individuals would have greater decrements in all variables compared to non-obese.

**Operational Definitions**

The following terms and definitions were used in this study:

- Cognition refers to processes of Visual and Verbal Memory, Visual Motor speed, Reaction Time, and Impulse Control as measured by the Immediate Post-Concussion Assessment Testing (ImPACT).
- Mood is defined as the measure of the feelings of Tension, Depression, Anger, Vigor, Fatigue, and Confusion as measured via the Brunel Mood Scale (BRUMS) (41).
- Dehydration is the process of becoming hypohydrated, as measured by increasing values in hydration biomarkers (see hypohydration for clarification on hypohydrated values) (42)
- Hypohydration is defined as negative total body water balance, determined by meeting three of the four biomarkers: percent body mass loss (≥1%), serum osmolality (≥290 mOsmol), urine specific gravity (≥1.020), and/or urine osmolality (≥700 mOsmol) (42, 43).

- Euhydration is defined as optimal total body water balance, determined by meeting three of the four biomarkers: percent body mass loss (<1%), serum osmolality (<290 mOsmol), urine specific gravity (<1.020), and/or urine osmolality (<700 mOsmol) (42, 43).
- Hyperthermia is defined as an elevation in core temperature as measured by a rectal thermometer. In this study it was invoked with passive heat stress.
- Obesity is defined as a body composition of ≥39% body fat as measured by dual x-ray absorptiometry (DXA) (44).

**Assumptions**

Assumptions of this study include compliance to experimental protocol (i.e., 24-h fluid deprivation, low water content food intake), honesty toward exclusion criteria, honest answers for perceptual scales, and effortful attempts on cognition tests.

## Methods

**Participants**

A convenience sample was used to recruit 12 obese and 12 non-obese female adults from 18 to 35 years old from the University of Arkansas community and surrounding area. Prior to participation all participants signed a written informed consent approved by the University's Institutional Review Board. Upon consent, individuals completed a Medical History Questionnaire. Participants were excluded if they had a history of cardiovascular, hematologic, hepatic, gastrointestinal, renal, pulmonary, or endocrine disease that may affect body fluid balance, or if they had a history of psychiatric disease, concussion, dyslexia, or attention-deficit/hyperactivity disorder. Furthermore, participants were excluded if they took drugs influencing body fluid balance (e.g., diuretics) or regularly consumed the caffeine equivalent of

greater than six cups of coffee each day. Women who started or stopped using oral contraceptives within the three months prior to their trial were not able to participate in order to account for the hormonal stabilization. Those who had an irregular menstrual cycle, as well as those using an intrauterine device, nuva ring, or progesterone only form of birth control were excluded due to different hormone concentrations of estrogen and progesterone from normally menstruating females.

**Design**

A randomized, repeated-measures design was used to determine if hypohydration and hyperthermia independently impaired cognitive performance, mental task load, and mood and if hypohydration exacerbates hyperthermic impairments in cognition and mood. Examining differences between obese and non-obese individuals occurred using a cross-sectional design.

Each participant completed a familiarization and two trials: one euhydrated and one hypohydrated. The familiarization visit occurred within two weeks prior to the first trial. For each trial, participants participated in a 24-h hydration intervention prior to having their core temperature increased 1.0ºC via passive heating. Trials occurred during the follicular phase of the menstrual cycle (days 1-7). Menstrual cycle status was based on self-reported start of menses. Trials took place in a randomized order separated by a minimum of 48-h. The layout of each trial is shown in Figure 1.

**Familirization**

Prior to experimental trials, participants underwent a familiarization visit. At this visit we recorded the participant's weight via a scale (Health-o-Meter® digital scale, model: 349KLX, IL, USA), height via a stadiometer (Seca, model: 7701321004, Vogel & Hamburg, Germany), and body composition via dual x-ray absorptiometry (DEXA, General Electric®, Lunar Prodigy

Promo). According to the World Health Organization, obesity corresponds with a body mass index ≥30 (1), which is equivalent to a body fat ≥39%. Non-obese participants were classified similarly as having a body mass index <25, which corresponded to a body fat <32% (45). Participants were classified as obese or non-obese via dual x-ray absorptiometry (DEXA, General Electric®, Lunar Prodigy Promo); participants with a body fat between 32-38% were excluded to avoid the "overweight" category and enhance the ability to detect the effects of obesity on the primary measures when hyperthermic and hypohydrated.

Participants were then familiarized with the perceptual scales for thermal sensation and thirst, the Brunel Mood States (BRUMs) mood assessment, and the Immediate Post-Concussion Assessment Test (ImPACT) (41, 46-48). The Brunel Mood Scale (BRUMS) is validated instrument with 24-item Likert measures evaluating feelings of Tension, Depression, Anger, Vigor, Fatigue, and Confusion (41). ImPACT computerized neurocognitive test battery assesses neurocognitive performance by measuring attention processes, Verbal recognition memory, Visual working memory, Visual processing speed, Reaction time, numerical sequencing ability, and learning. The test has five parallel forms to minimize and control for practice effects (46). Additionally, ImPACT measures the presence of 22 symptoms (i.e headache, memory problems, confusion, fogginess, etc.).

After each cognitive test, participants were familiarized with visual analog scales (VAS) and NASA Task Load (NASA-TLX) (49, 50). The visual analog scale is continuous scale with two extreme statements used as anchors at opposite ends of a 100mm line. The anchors used were “not at all strong(ly)” and “very strong(ly)” for all three questions asked. This scale was used to assess concentration and headache using the statements in respective order “How hard did you have to concentrate to accomplish the tasks successfully?” and “I have a headache.” The

NASA-TLX measures subjective workload for mental demands, physical demands, temporal demands, own performance, effort and frustration. The participant marks on a line, which represents a continuous scale for each of the previously mentioned sub-scales.

Participants were sent home with a scale (Model: IP-TITN-ZJXR, BalanceFrom) to record nude body weight at the same time, relative to the start time of their trials, each day for three consecutive days to establish a baseline body weight. Participants were provided a 250 mL bottle of water as their only fluid consumption for the 24-h prior to their hypohydrated trial.

**Experimental Trials**

For the 24-hr prior to each experimental trial, participants underwent a hydration intervention, where they kept diet and fluid logs; they replicated this diet for the 24-h preceding the subsequent trial. During euhydrated trials participants were encouraged to consume adequate fluid, including an extra 950 ml the night prior to the trial and 475 ml several hours before the trial. During heating for the euhydrated trial, participants consumed 37.5ºC water to maintain euhydration. For the hypohydrated trial participants were given a 250 ml bottle of water and asked to refrain from all other fluids and high moisture content foods (e.g., soup and fruits) for 24-h prior to the start of passive heating.

Four measures of hydration were assessed and used to define hydration state for each experimental trial: body mass change, serum osmolality ($S_{OSM}$), urine osmolality ($U_{OSM}$), and urine specific gravity ($U_{SG}$). Change in body mass was calculated from the baseline weight recorded as the average of the three or more weights obtained by the participant. The percentage of body weight change from baseline was used to determine percent hypohydration at the beginning of each trial (i.e., before heating, after the 24-h hydration intervention) and after each trial (i.e., after heating). Additionally, a urine sample and blood sample were taken before and

after heating of each trial. $U_{SG}$ via refractometer and $U_{OSM}$ via freezing point depression (3D3 Advanced Instruments osmometer) were measured from the urine sample and $S_{OSM}$ from the blood sample. Previously determined cutoffs for body mass change (<1%), $S_{OSM}$ (<290mOsm), $U_{OSM}$ (<700mOsm), and $U_{SG}$ (<1.020) were used to define participants at euhydrated and participants were required to meet three of the four criteria to be classified by the respective hydration status (43).

Prior to each experimental trial participants refrained from alcohol and exercise for 24-h, food for 4-h, and caffeine for 8-h. After the 24-h fluid intervention, and upon arrival to the laboratory, pre-test compliance was verified with a 24-h history questionnaire. Participants then used a private bathroom to obtain a nude weight (Health-o-Meter® digital scale, model: 349KLX, IL, USA), insert a rectal thermometer (Physitemp Inc., Clifton, NJ, Ret-1) 15 cm (~6 inches) past the anal sphinctor to measure core temperature ($T_C$), and provide a urine sample for measures of $U_{SG}$ (clinical refractometer 30005, SPER Scientific, Scottsdale, AZ, USA) and $U_{OSM}$ by freezing point depression (3D3 Advanced Instruments osmometer, Model 3250, Norwood, MA, USA). Participants were then fitted with a blood pressure cuff (Tango+; SunTech Medical, Inc., Morrisville, NC, USA) placed directly on the brachial artery to obtain blood pressure via electrosphygmomanometry, and a monitor around the chest continuously recorded heart rate (HR; Polar Electro Inc., Lake Success, NY, USA).

To record skin temperature ($T_{sk}$), skin thermistors (Omega Engineering, Stamford, CT, USA) were placed on the right anterior thigh (midway between the greater trochanter and lateral condyle), chest (midway between the axilla and areola), lateral calf (midway between the tibial condyle and malleolus), and upper arm. Mean-weighted $T_{sk}$ was calculated as proposed by Ramanathan (51): $T_{sk}$ = 0.3(chest + arm) + 0.2(thigh + calf). Mean body temperature ($T_b$) was

calculated using $T_C$ and $T_{sk}$ (19): $T_b = 0.2(T_{sk}) + 0.8(T_C)$. Participants were then dressed in a water-perfused, tube-lined suit that covers the entire body, except the head, face, hands, and feet (Allen-Vanguard Technologies). The suit permits the control of skin and core temperature by changing the temperature of the water perfusing the suit.

After instrumentation, and prior to heating, participants completed the thermal and thirst perceptual measures, BRUMs mood assessment, ImPACT and Post-Concussion Symptom Scale and mental task load measures (VAS and NASA-TLX). After completing the psychological measures, an intravenous catheter was inserted into the antecubital vein. A blood draw was obtained prior to heating after participants entered an environmental chamber (34.59 ± 0.79°C, 30.92 ± 1.03%, respectively) and lay supine for ~30 minutes. Water at 34°C was perfused through the suit during this period. After this resting period, participants were then exposed to passive heat stress by perfusing 38.5°C water through the suit. HR, $T_{SK}$, $T_B$ and $T_C$ were continuously measured via computer (LabChart7) at 50 Hz. Once achieving a 1.0°C rectal temperature increase, $T_C$ was maintained at this temperature by stabilizing the temperature of the water going through the suit. At the 1.0°C $T_C$ increase a post-heating blood draw was obtained, then participants completed the thermal and thirst perceptual measures, BRUMs mood assessment, ImPACT cognitive test and mental task load measures (VAS and NASA-TLX). Once heating and testing were completed, participants were asked to obtain another nude body mass to calculate total body water loss and also provide another urine sample.

**Data analysis**

Statistical analyses were performed using IBM SPSS Statistics v20.0. A repeated-measures two-way analysis of variance (ANOVA) was used to answer the four main aims of the study.

*Independent effects of mild hypohydration.* For the 1st aim, a two-way ANOVA was used on the baseline raw values (hypohydration and euhydration) to assess effects of hydration status between groups (obese and non-obese). The ANOVA was used to determine if there was a main effect of hydration at pre-test measures and if a main effect of group (body composition) existed. Additionally, it determined if there was an interaction between the main effects of hydration and body composition.

*Independent effect of hyperthemia.* For the 2nd aim, a two-way ANOVA was used to test the effect of hyperthermia during the euhydrated trial to assess the independent effect of hyperthermia between groups (obese vs non-obese). The ANOVA was used to determine if there was a main effect of heating between pre-test (prior to heating) and post-test (1.0°C increase in $T_C$) raw scores and if a main effect of group (body composition) existed. Additionally, it determined if there was an interaction between the main effects of heating and body composition.

*Combined effects of hypohydration and hyperthermia.* Change scores were calculated to determine if hypohydration exacerbated any cognitive performance, mental task load, or symptom variables during heating. Change scores were determined by subtracting the pre-test scores from the post-test scores (taken after a 1.0°C increase in $T_C$) within the same conditions (i.e., hypohydrated & hyperthermia – hypohydrated baseline vs euhydrated & hyperthermia– euhydrated baseline). Once change scores were calculated, a two-way ANOVA was used to assess if there was a greater impairment with hyperthermic hypohydration compared to hyperthermic euhydration in obese versus non-obese individuals. The ANOVA was used to determine if there was a main effect on the difference between the two hydration conditions and if there was a main effect of body composition, and finally, if there was an interaction between the two main effects. Results were presented as means and standard deviations. For statistically

significant F scores, Bonferroni's post hoc analyses were performed. An alpha of 0.05 defined significance.

## Results

### Participant characteristics

Due to participant attrition and/or missing data, there were 11 obese and 11 non-obese participants used for analysis. Participant characteristics are in Table 1. Age and height were not significantly different between groups (p=0.695, p=0.113, respectively). However, as expected, the obese group had significantly greater body mass and body fat percent than the non-obese (p=0.002, p<0.001, respectively).

### Hydration data

Hydration biomarkers are listed in Table 2. As expected, percent body mass change (%BM), serum osmolality ($S_{OSM}$), urine osmolality ($U_{OSM}$), and urine specific gravity ($U_{SG}$) were all significantly increased with hypohydration (normothermic at baseline) (p=0.002, p<0.001, p<0.001, p<0.001, respectively). $S_{OSM}$ remained unchanged with euhydrated hyperthermia (p=0.181), but hydration biomarkers for %BM, $U_{OSM}$, and $U_{SG}$ were significantly increased with heating during the euhydrated trial; however, they remained within the cut-offs set by the researchers for euhydration (p=0.030, p=0.018, p<0.001, respectively).

During the hypohydrated trial, %BM, $U_{OSM}$, $U_{SG}$, and $S_{OSM}$ were significantly increased after heating (p<0.001, p<0.001, p=0.002, p<0.001, respectively). Hypohydrated hyperthermia, regardless of group, resulted in higher %BM, $U_{OSM}$, $U_{SG}$, and $S_{OSM}$ when compared to euhydrated hyperthermia (all p<0.001). The non-obese group had significantly greater %BM when hypohydrated and hyperthermic compared to the obese group (p=0.003); however, the obese group had significantly greater $U_{OSM}$ (p=0.007) compared to the non-obese group.

**Physiological data**

Physiological data is listed in Table 3. Hypohydration did not affect baseline core temperature ($T_C$), mean body temperature ($T_B$), or heart rate (HR) (p=0.268, p=0.104, p=0.198, respectively) with the exception of mean skin temperature ($T_{SK}$) being higher (p=0.006). Heart rate was significantly higher in obese compared to non-obese at baseline hypohydration (p=0.014). As expected, with heating alone (euhydrated hyperthermia), $T_C$, $T_{SK}$, and $T_B$ increased over the duration of heating (all p<0.001). There was a trend for HR to be higher in obese females compared to non-obese females when heated and euhydrated (p=0.063). However, none of the physiological variables (i.e., $T_C$, $T_{SK}$, and $T_B$) during heating were significantly different between non-obese and obese groups (p=0.776, p=0.581, p=0.718, respectively).

Mild hypohydration accompanied with hyperthermia resulted in higher $T_C$, $T_{SK}$, $T_B$, and HR over the duration of the hypohydrated trial, regardless of group (all p<0.001). Furthermore, HR was significantly increased in obese females compared to non-obese females during hypohydrated hyperthermia (p=0.043). When hyperthermic, hypohydration resulted in a higher $T_C$, $T_{SK}$, and $T_B$, than when hyperthermic and euhydrated (p=0.049, p=0.014, p=0.011, respectively). There was a trend for HR to higher during hypohydrated hyperthermia compared to euhydrated hyperthermia (p=0.087).

**Independent effects of mild hypohydration ("A" panel of each figure)**

*Cognitive performance.* Cognitive performance variables included the composite scores for Verbal Memory, Visual Memory, Visual Motor Score, and Reaction Time. The p-values for all cognitive performance are located in Table 4. Hypohydration did not independently (normothermic at baseline) impair Verbal Memory, Visual Motor score, or Reaction Time (Figure 2a, 4a, 5a; p>0.05, respectively). Visual Memory was impaired while hypohydrated

(Figure 3a; p=0.015). None of the variables depended on grouping (obese versus non-obese; p>0.05).

*Mental task load.* Mental task load was defined with the use of five subscales on the NASA-TLX: effort, mental demand, temporal demand, performance, frustration, and the VAS for concentration (Figure 6a-11a). The p-values for all mental task load variables are located in Table 5. Temporal demand and frustration were significantly higher with hypohydration compared to euhydration at baseline (Figure 7a, 9a, respectively; p=0.023, p=0.007, respectively). All other variables for mental task load: mental demand, performance, effort, and concentration, were not significantly affected (Figure 6a, 8a, 10a, 11a, respectively; p>0.05). None of the variables (see list above) depended on grouping (obese versus non-obese) (p>0.05).

*Symptoms.* Symptoms included: thermal sensation, thirst, total symptom score, and 22 symptoms from the Post-Concussion Symptom Score (see list in table) and p-values for all symptoms can be found in Table 6. Unsurprisingly, thirst was increased with hypohydration (Figure 14a; p<0.001), and thermal sensation at baseline was not significantly different between euhydrated and hypohydrated baseline measurements (Figure 13a; p=0.628). Although total symptom score was increased with mild hypohydration (Figure 12a; p=0.028), no individual symptom was significantly impacted (Table 6; p>0.05). However, there was a trend for several symptoms to be impaired with hypohydration (i.e., headache, difficulty concentrating, feeling foggy, feeling slowed down, and sensitivity to light; Table 6; p<0.07). None of the variables depended on grouping (obese versus non-obese; p>0.05). However, “feeling slowed down” did have a trend to have a main effect of group (p=0.065).

*Mood.* Mood scores were from the six subscales from the Brunel Mood State and measured feelings of Tension, Depression, Anger, Fatigue, Vigor, and Confusion. The p-values

for mood are located in Table 7. Most mood scores were stable during hypohydration. This includes feelings of Tension, Anger, Fatigue, Vigor, Confusion (Figure 16a, 18a-21a; all $p>0.05$). However, Depression was significantly increased with hypohydration (Figure 17a; $p=0.007$). None of the variables depended on grouping (obese versus non-obese; $p>0.05$).

**Independent effect of hyperthermia ("B" panel of each figure)**

*Cognitive performance.* See Table 4 for p-values on cognitive performance when hyperthermic and euhydrated. Hyperthermia did not have an independent effect on cognitive performance variables: Verbal Memory, Visual Memory, Visual Motor Score, and Reaction Time (Figure 2b, 4b, 5b; $p>0.05$). However, Visual Memory was impaired with heating, when maintaining euhydration (Figure 3b; $p=0.044$). None of the variables depended on grouping (obese versus non-obese; $p>0.05$).

*Mental task load.* See Table 5 for p-values on mental task load. Although cognitive performance was relatively stable with heating, mental task load deteriorated. With heating, participants reported higher mental demand, lower perceived performance, higher effort exerted on the task, and difficulty concentrating (Figure 6b, 8b, 10b, 11b, respectively; all $p<0.05$). There were no significant differences with temporal demand or frustration or between groups for any of the variables (Figure 7b, 9b; $p>0.05$). None of the variables depended on grouping (obese versus non-obese; $p>0.05$).

*Symptoms***.** See Table 6 for p-values for symptoms. As expected, thermal sensation values were significantly higher with heating (Figure 13c; $p<0.001$). Hyperthermic, euhydrated individuals had significantly higher thirst than at baseline (Figure 14c; $p=0.038$). Heating independently deteriorated several individual symptoms and there was a trend for total symptom score to be higher when heated ($p=0.089$). Impaired individuals symptoms include: vomiting,

dizziness, fatigue, sensitivity to light, sleeping less than usual, difficulty concentrating, visual problems, headache, increased thirst and increased thermal sensation (Table 6; all $p<0.05$). There was a trend for participants to feel slowed down ($p=0.076$). None of the variables depended on grouping (obese versus non-obese; $p>0.05$).

*Mood.* See Table 7 for p-values for mood. With heating, feelings of Depression increased and feelings of Vigor decreased, which are both associated with negative mood states (Figure 17b, 19b; $p=0.010$, $p=0.017$, respectively). However, all other mood states (Tension, Anger, Fatigue, Confusion) remained stable and group did not play a role in mood (Figure 16b, 18b, 20b, 21b; $p>0.05$). None of the variables depended on grouping (obese versus non-obese; $p>0.05$).

Upon analysis of both independent factors (hypohydration and hyperthermia), we further analyzed if one stressor impacted the variables to a greater degree than the other. The analysis was conducted on variables that were impaired during both hypohydration and hyperthermia (Visual Memory, Depression, Thirst). We did not find that either stressor had a greater impairment on Visual Memory, feelings of Depression, or Thirst when compared to the other ($p=0.454$, $p=0.104$, $p=0.162$, respectively).

**Change in response from baseline to hyperthermia while euhydrated or hypohydrated ("C" panel of each figure)**

*Cognitive performance.* See Table 4 for p-values for cognitive performance differences when hyperthermic and either euhydrated or hypohydrated. Cognitive performance, as determined by the change from baseline to hyperthermic within the same hydration condition, was not exacerbated with hyperthermic hypohydration ($p>0.05$). This included Verbal Memory, Visual Memory, Visual Motor Scores, and Reaction Time (Figure 2c-5c). However, there was a trend for Verbal Memory to decline greater when hypohydrated and hyperthermic than when

euhydrated and hyperthermic (Figure 2c; p=0.062). None of the variables depended on grouping (obese versus non-obese; p>0.05).

*Mental task load.* See Table 5 for p-values for mental task load. Mental task load, effort on a task, mental demand, perceived performance, frustration, and concentration, was not significantly different between hydration statuses when heated (Figure 6c, 8-11c; p>0.05). However, individuals did feel more temporal demand (Figure 7c; p=0.010). None of the variables depended on grouping (obese versus non-obese; p>0.05).

*Symptoms.* See Table 6 for p-values for symptoms. There was a trend for total symptom score to be impaired to a greater degree with hyperthermia when hypohydrated compared to euhydrated (Figure 12c; p=0.057). Participants did not feel a higher thermal sensation hypohydrated and hyperthermic compared to euhydrated and hyperthermic (Figure 13c; p>0.05). However, they did feel more thirst (Figure 14c; p=0.025). Individual symptoms of sensitivity to light, feeling slowed down, and thirst were significantly impaired (Table 6; all p<0.05); while a couple symptoms trended toward impairment—dizziness and drowsiness (Table 6; p<0.09). None of the variables depended on grouping (obese versus non-obese; p>0.05).

*Mood.* See Table 7 for p-values for mood. Subscales of mood for feelings of Tension, Depression, Vigor, Fatigue, and Confusion were not further impaired when hypohydrated versus euhydrated while hyperthermic (Figure 16c, 17c, 19c-21c; p>0.05). However, Anger was significantly increased with heating when hypohydrated compared to euhydrated (Figure 18c; p=0.031). None of the variables depended on grouping (obese versus non-obese; p>0.05).

It should be noted that a one-way ANOVA analyzing the impact of time (i.e., regardless of condition) on the variables did not reveal a significant change over time (i.e., order of

presentation; p=0.353). Indicating the stressors, not time, were the cause for impaired Visual Memory.

## Discussion

The purpose of this study was to examine the independent and combined effects of mild hypohydration and hyperthermia on cognition and mood between obese and non-obese females. The specific aims of this study were to: 1) examine the independent effects of mild hypohydration on cognition and mood, 2) examine the independent effect of hyperthermia on cognition and mood, 3) examine the combined effects of hypohydration and hyperthermia on cognition and mood 4) examine the effect of obesity on cognition and mood during mild hypohydration or hyperthermia. We hypothesized: 1) mild hypohydration induced by 24-h of fluid restriction would cause significant impairments to cognition and mood, 2) hyperthermic, euhydrated females would have significantly impaired cognition and mood, 3) hypohydration would exacerbate hyperthermic related impairments to cognition and mood in hyperthermic, hypohydrated females, and 4) obese individuals would have greater deficits in all variables compared to non-obese.

The results supported some of our hypotheses, but not all. 1) Mild hypohydration decreased Visual Memory and temporal demand and increased frustration, thirst, total symptom score, and feelings of Depression. 2) Hyperthermia impaired Visual Memory, mental demand, perceived performance, task effort, and concentration. Feelings of dizziness, fatigue, sleepiness, light sensitivity, headache, and visual problems also occurred with hyperthermia, along with increased thirst and thermal sensation. When hyperthermic, feelings of Vigor decreased and feelings of Depression increased, which are associated with negative mood. 3) Hypohydration did not exacerbate any cognitive performance variables. However, mental task load showed

increased temporal demand and symptoms increased (increased light-headedness and feeing "slowed down"). Furthermore, thirst perception and feelings of Anger were heighted when hypohydrated and hyperthermic compared to euhydrated and hyperthermic. 4) Body composition did not affect any of the results.

The rationale and hypotheses made for the current study stem from the idea that cognition is defined by Baar's global workspace theory as having limited capacity (52). Baar's suggests cognitive functions are working in competition with each other rather than in parallel. Selected activities dominate cognitive awareness by harnessing executive function. Stressors could increase the need for cognitive function in one area of the brain, compromising other areas of the brain via cognitive load competition (52, 53). Hypohydration and hyperthermia could: 1) primarily be causing hormonal or cellular impairments, which limit cognitive ability, or 2) hypohydration and hyperthemia could indirectly impair cognitive performance by increasing perceived symptoms and impairing mood state, which competes with the brain's ability to delegate properly. Therefore, competition surfaces in executive function, and in order to preserve the cognitive skill being used, the brain exerts more effort toward the skill, sparing cognitive performance impairment. Thus, in order to meet the demands for stable cognitive performance, cognitive effort increases.

**Independent effects of mild hypohydration**

*Cognitive Performance and Mental Task Load.* Theoretically, there is a threshold in which brain resources are exhausted and cognitive function declines. A study using fluid restriction not find cognitive performance deficits with 2.6% body mass loss and suggested that young healthy participants are able to adapt slowly to progressive water deficit (18). However, subjective feelings (i.e., thirst, headache, concentration, tiredness, alertness) were significantly

impaired, indicating the brain was working harder to compensate for cognitive performance. Kempton suggests this phenomenon is fueled by the increase in the fronto-parietal brain activation resulting in unimpaired cognitive scores and a deteriorated mental task load (27). Fluid restrictions studies have demonstrated that although there may be an initial drop in short-term memory after acute hypohydration, cognitive function returned to baseline 3.5 hours later independent of water intake (18, 54). The results of our study support the idea that cognitive function is overall well-preserved with mild hypohydration in obese and non-obese females.

Most cognitive functions could be preserved by a preservation of cerebral metabolic rate. Research shows that even though cerebral blood flow is reduced during dehydration, cerebral metabolic rate is maintained (55). Although Visual Memory was significantly lower (Figure 3a), all other variables: Verbal Memory, Visual Motor, and Reaction Time were preserved (Figure 2a, 4a, 5a). However, the mechanism impairing Visual Memory is unknown. Supporting the idea that cognition is of limited capacity, Visual Memory could be impaired prior to other deteriorations because the brain's limited capacity to store visual information long-term (56). Visual short-term memory can only hold information for a short amount of time (30 seconds or less), is severely limited, and depends on object complexity (56). With the added stress of hypohydration or hyperthermia and the limited ability for Visual Memory recall, this cognitive domain might be more susceptible to impairment compared to other functions. The threshold for other cognitive impairments might start with higher levels of hypohydration, but with ~1% hypohydration only initial cognitive deterioration occurs with Visual Memory.

Cognitive impairment due to hypohydration can be from: 1) hyperosmotic conditions, or 2) hypovolemic conditions in the brain. Hypohydration leads to hyperosmotic conditions in the brain by increasing serum osmolality. Higher serum osmolality creates an osmotic gradient,

shifting water from intracellular stores and shrinking the cells. Furthermore, ventricular enlargement has been seen following hypohydration, and is proportional to body weight lost (27). Hypohydration reduces blood volume thereby reducing brain volume with an associated increase in ventricular volume (57). Research is equivocal as to whether cognitive performance is impaired or not.

*Symptoms and Mood.* As expected, thirst was increased with hypohydration--the brain centers of the hypothalamus sense the higher osmolality. Although no individual symptom was impaired, Total Symptom Score was with hypohydration (Table 6; Figure 12a). This is similar to previous research (5, 6, 28). Participants had undetectable deficits in individual symptoms but cumulatively there was an increased Total Symptom Score (Figure 12a). Mood was preserved for Tension, Anger, Fatigue, Vigor, and Confusion, but not for Depression (Figure16a-21a). Research has shown that mood is more susceptible to deterioration in women with hypohydration (5). However, this study indicated that women would be more hypohydrated at 1.3%. We only had females dehydrate until 1.1% body mass loss and found mood was preserved.

No effects were seen with hypohydration between obese and non-obese individuals. This could be because the sample we investigated was healthy, not morbidly obese, and young individuals compared to previous research (33, 36, 37, 58). It is likely the magnitude of hypohydration was not sufficient to impose markedly higher impairment between groups. Our study suggests in both obese and non-obese females cognitive function, mood, and symptoms are preserved with 1.1% hypohydration.

**Independent effect of hyperthermia**

*Cognitive Performance and Mental Task Load.* We found most cognitive performance variables were stable when hyperthermic and euhydrated. However, Visual Memory and mental

task load (i.e., mental demand, perceived performance perceived effort and concentration,) were impaired (Figure 3b, 6b, 8b, 10b, 11b). With heat stress it is possible the redistributed blood flow to the muscle and skin might limit the ability for the brain to receive proper cerebral blood flow (59, 60). Elevated core temperature at rest can lead to hyperventilation and subsequent hypocapnia (61), Cerebral blood flow is sensitive to these changes and the limited cerebral perfusion could be associated with heighted mental task load and awareness of symptoms, as seen in this study. However, a research study using fMRI to determine cerebral blood flow during heat stress found increased blood flow to portions of the brain and subsequently found cognitive performance was maintained, suggesting the increased in blood flow could be due to necessity to exert more effort (27). Another study found that heat stress does not alter the increase in cerebral perfusion during cognitive task (working memory) and there was no impairment to cognitive task when heated (21).

Another study investigated passive hyperthermia of a 1.5°C increase in core temperature and determined hyperthermia was associated with increased cerebral metabolic rates in hypothalamus, thalamus, corpus callosum, cingulate gyrus, and cerebellum via PET, with significant decreases in caudate, putamen, insula, posterior cingulum (25). Increasing cerebral blood flow and metabolism to certain areas of the brain may limit the function of structures that have reduced blood flow. Furthermore, the increase of metabolic rate and energy demand in select regions of the brain is accompanied with hypoglycemia that accompanies hyperthermia. The glycogen depletion of the brain could play a role in the increased use of glucose metabolism in those brain sections could lead to increased perception of symptoms (62) . However, the relationship between these changes in cerebral metabolism and cognitive processes require further research.

Our findings that Visual Memory is impaired if supported by several research studies (21, 26, 63). Research for heat stress consistently finds cognitive tests involving greater neuronal resources are impaired to a greater extent than less demanding tasks (26, 64-67). Memory is a more complex task than attention. Baddedly's working memory theory states Verbal Memory is controlled by the phonological loop, while the visuo-spatial sketchpad controls Visual Memory (68). These functions are connected and controlled by central executive function. In order to store memory as long-term memory, rehearsal is necessary, and, as stated in an earlier section, Visual Memory is of limited capacity. Referring back to Baar's global workspace theory, if the brain is exerting effort toward storing Verbal Memory, which is an easier task, competition occurs and the brain's ability to store Visual Memory, a more complicated task, is limited. This could be why Visual Memory was impaired and Verbal Memory was not. Following suit of previous studies, speed and attention were maintained during heat stress. Research suggests this is because heating increases arousal to a certain core temperature, which our participants were below (69). Furthermore, the reduction of cerebral blood flow to the caudate could also lead to visual impairments as impairment to this brain structure have been linked to errors on spatial working memory tasks (70).

*Symptoms and Mood.* Hyperthermia leads to an increased metabolism in focal areas of the brain and potentially cerebral glucose depletion (62). This could lead to feelings of exhaustion, fatigue, or sleeping more, which were all symptoms we found to be significant with hypohydration and hyperthermia. Participants in our study also reported difficulty concentrating, even though their attention was not impaired. Difficulty concentrating could impair the ability to form long-term memories and affect the learning ability of females during heat stress. This leads to the question--whether the cognitive impairments seen with hyperthermia are related to heat-

associated changes to the brain or secondary to distractions formed from increased symptoms and impaired mood?

Dizziness is to be expected with heat stress, as orthostatic challenge has been repeatedly illustrated in the research (71). During heat stress, cardiac output redistributes to the periphery affecting the baroreceptors and reducing orthostatic tolerance. Visual problems and light sensitivity could be secondary symptoms to dizziness and associated with the reduction of cerebral blood flow. Headache stimulates from a dilation of cerebral arteries, so the increased blood flow to the middle cerebral artery could be responsible for this symptom. The reduction in feelings of Vigor and increased feelings of Depression could be linked to changes in cortisol and serotonin levels in the brain with hyperthermia (62).

Although there was a trend for body composition to affect performance, frustration, concentration, and headache, none of these variables were significant (Table 6). This might indicate that body composition could be affected by heat, but this is not sufficient to impair cognitive performance, mental task load, symptoms, or mood with moderate heating. Although cardiovascular strain occurs with obesity (as evidence by increased HR in this study) (Table 3), the ability to maintain cerebral perfusion during cognitive task between groups is probably unaffected (38).

**Change in response from baseline to hyperthermia while euhydrated or hypohydrated**

*Cognitive Performance and Mental Task Load.* Our results indicate that cognitive performance or mental task load are not exacerbated to a further degree during hyperthermic, hypohydration compared to hyperthermic, euhydration. An fMRI study illustrated higher neuronal firing in the dorsal fronto-parietal network mediating executive functions during heating with hypohydration from baseline tests. However, cognitive performance was unchanged

with 1.1% hypohydration. This indicates that a higher mental effort was needed to exert the same level of performance. However, theoretically, all the resources could be recruited and once a threshold is met, the cognitive performance will decline. No cognitive performance impairment was found with 1.1% hypohydration, but the study suggested this might be because cognitive effort was increased, as shown via increased fronto-parietal activity with hypohydration, post-heating. This is similar to our study, no significant cognitive differences during rest (Figure 2b-5b), but increased impairments to mental task load during heating (Figure 6b-11b). However, the hypohydration trial was not significantly greater than the euhydrated trial with heating (Figure 2c-11c) (27).

*Symptoms and Mood.* The impaired symptoms exacerbated by hyperthermia when hypohydrated could be due to cerebral blood flow reduction, and/or blood redistribution to the periphery. This could lead to feeling light-headed or visual problems (Table 6). Furthermore, the blood shunting from the insula brain structure during heating could be the cause for emotional distress, as it plays a part in emotional control. However, neurotransmitter changes such as serotonin and cortisol could play a role in mood dysfunction and perception of fatigue and feeling slowed down. There was no effect of body composition on any variables between hyperthermic euhydration and hyperthermic hypohydration, suggesting obese individuals are not limited in their ability to increase cerebral blood flow during cognitive task. Furthermore, obese individuals did not have any significant impairment to mood or symptoms when compared to non-obese, indicating, hyperthermia and hypohydration do not result in markedly higher mood or symptom deterioration between groups.

Although some variables (Visual Memory, feelings of Depression, and thirst) were impaired during both independent conditions, they were not impaired to a greater degree when

the two were combined. Two potential causes could explain this. First, given that our sample was young and healthy, physiological mechanisms could compensate in order to stabilize variables after a certain amount of stress. Previous research has suggested that further hypohydration of 37-hr fluid restriction past 24-hr fluid restirciton in young individuals did not exacerbate impairments seen at lower levels of hypohydration, but instead young individuals were able to return back to baseline values (18). Second, it is possible that a floor effect existed for the two variables. As shown by our results, neither condition independently had greater effects than the other. Therefore, a floor effect may have been achieved in which individuals had already met the level of impairment that would be achieved with mild stressors.

**Obesity**

Although we did not see any impacts on obesity with our variables, it should be noted that the mild levels of hypohydration and hyperthermia might not have been great enough to reveal a difference, if one does exist. Furthermore, we studied otherwise healthy, young individuals. With this information we suggest that obesity does not impact cognitive, mood, or perception of symptoms when under mild levels of hypohydration or mild hyperthermia.

**Future Directions**

Future studies should attempt to perform PET scans and MRI scans during cognitive testing during 1) mild hypohydration 2) hyperthermia 3) combined mild hypohydration and hyperthermia, to determine what areas of the brain in females are at a higher neuronal firing rate.

**Limitations**

Mood score only reflected negative feelings and did not give an overall mood state, which may reduce our ability to fully interpret the overall mood state of the females. Furthermore, the cognitive assessment (ImPACT) used was a series of five modules. The

modules are always administered in the same order for each test. Thus, the cognitive domains measured were not counterbalanced. We recognize this as a limitation. The lack of counterbalancing could indicate impairment to one domain might affect a later module and the corresponding domain. However, given the conditions of the experiment were counterbalanced, we are confident in our ability to examine the effect of hydration status on the measured variables. Additionally, we recognize impairment to attention might impair latter modules. Although, most studies using heat stress indicate attention is preserved, studies using hypohydration suggest attention might deteriorate with hypohydration. Furthermore, since we were using a controlled passive heating protocol involving a water-perfusion suit, heat was administered to the skin first and subsequently caused an increase core temperature.

**Delimitations**

Delimitations of this study include that we controlled for only one phase of the menstrual cycle, as it is known the menstrual cycle affects total body water content. The within-participant, repeated measure design prevented specific phase differences from interfering with the analysis. We only measured females under hormone stabilization (i.e., no change, starting or stopping, in medication for at least three months) and, if on medication, hormone ratios equivalent to free-living females. Females under 35 were recruited, as reproductive system changes after that age may confound our variables. We controlled the amount of fluid intake to 250 ml in order to achieve hypohydration without extreme discomfort. The demographics were a convenience sample of females from the Northwest Arkansas area.

**Threats to Internal Validity**

In our opinion, internal validity was well controlled. First, we used a within-participants design to eliminate differences between participants when comparing trials. Using the within-

participant design controlled for differences in physiologies since participants serve as their own control. Between participants (non-obese and obese comparisons) we controlled for physical activity to which eliminates selection. We controlled for confounding variables (i.e., heat stress to induce hypohydration or hypohydration during heating) on cognition and mood by testing individuals at four tightly controlled time points to represent each condition: euhydrated, euhydrated and hyperthermic, hypohydrated, hypohydrated and hyperthermic. Baseline testing represented effects of hydration status on dependent variables. Post-heating testing represented independent effects of heating (when euhydrated) and combined effects of both conditions (when hypohydrated). We controlled for confounding effects of hypohydration during heating during the euhydrated trial by replacing fluid during heating. Utilizing a baseline test during the familiarization, at which time participants are also familiarized to mood and perceptual scales, controls learning effects. Practice effects are controlled by different versions of the test, shown to be reliable for testing participants. All trials took place in an environmental chamber to control for environmental effects.

Some aspects lack control and are threats to internal validity. Although we are replacing fluid, we did not test sweat rate prior to heating, so we did not know the total amount of fluid lost until after the trial, making accurate fluid replacement difficult.

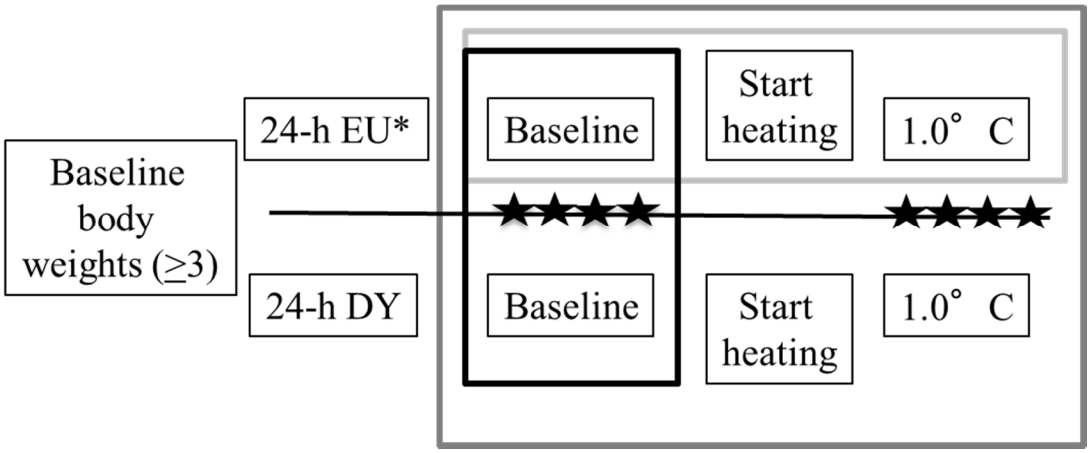
**Threats to External Validity**

Although we have strict controls for internal validity, external validity might be slightly more threatened. First, population validity was reduced. By using a convenience sample we are making ourselves vulnerable to selection bias. Additionally, multiple treatment interference might be another external validity threat. Since we are measuring mood at separate trial days, we might have residual effects from the previous heating, which could impair mood scores.

Furthermore, participants could anticipate the end of the trial, thus mood and perceptual measures toward the end of the trial might not accurately represent the participants feelings of the treatment at the current moment since anticipation could confound their perception. Participants could also have reactive affects, and by knowing what we are testing, bias their effort in the hypohydration and heating trial to yield hypothesized results. However, the participants were not be told specific hypothesize in order to control for this.

## Figures and Tables

**Figure 1.** Schematic for repeated-measures trials.




★ Represents measures for thirst and thermal perception, BRUMS, ImPACT, NASA+VAS, respectively

**Table 1.** Demographic M±SD for obese and non-obese groups.

| | Non-Obese | Obese |
|---|---|---|
| **Age (y)** | 22 ± 2 | 22 ± 2 |
| **Height (cm)** | 165 ± 5.8 | 161.3 ± 4.9 |
| **Mass (kg)** | 60.56 ± 5.76 | 79.79 ± 17.69* |
| **Body Fat (%)** | 25.13 ± 3.93 | 44.24 ± 5.08* |

*Significantly different between groups ($p<0.05$)

**Table 2.** M±SD for hydration variables for all trials.

| | EU baseline | | EU hyperthermia | | HY baseline | | HY hyperthermia | |
|---|---|---|---|---|---|---|---|---|
| | **Non-obese** | **Obese** | **Non-obese** | **Obese** | **Non-obese** | **Obese** | **Non-obese** | **Obese** |
| **Percent BM change (%)†‡§** | 0.30 ± 1.10 | -0.54 ± 1.21 | -0.53 ± 1.08 | -0.86 ± 1.22 | -1.26 ± 0.81 | -0.96 ± .86 | -2.57 ± 0.88 | -1.99 ± 0.80* |
| **$S_{OSM}$ (mOsm/kg)†§** | 285 ± 3 | 283 ± 3 | 285 ± 3 | 281 ± 2 | 289 ± 4 | 287 ± 4 | 295 ± 4 | 291 ± 4 |
| **$U_{OSM}$ (mOsm/kg)†‡§** | 322 ± 201 | 354 ± 194 | 390 ± 96 | 476 ± 243 | 969 ± 109 | 1022 ± 129 | 998 ± 90 | 1126 ± 107* |
| **$U_{SG}$†‡§** | 1.008 ± 0.006 | 1.009 ± 0.005 | 1.010 ± 0.003 | 1.013 ± 0.007 | 1.024 ± 0.004 | 1.026 ± 0.004 | 1.026 ± 0.004 | 1.029 ± 0.003* |

EU: euhydrated, DY: hypohydrated
BM: body mass, $U_{SG}$: urine specific gravity, $U_{OSM}$: urine osmolality $S_{OSM}$: serum osmolality
EU: euhydrated, HY: hypohydrated
*Significantly different between groups at the same time point
†Main effect of hypohydration (normothermic baseline), independent of group
‡Main effect of heating from corresponding hydration condition (normothermic to hyperthermic), independent of group
§Main effect of hydration when hyperthermic (euhydrated hyperthermia v hypohydrated hyperthermia), independent of group

**Table 3.** M±SD physiological variables for non-obese and obese during baseline and hyperthermia while euhydrated and hypohydrated

| | EU baseline | | EU hyperthermia | | HY baseline | | HY hyperthermia | |
|---|---|---|---|---|---|---|---|---|
| | **Non-obese** | **Obese** | **Non-obese** | **Obese** | **Non-obese** | **Obese** | **Non-obese** | **Obese** |
| **Core Temperature (°C)‡§** | 36.78 ± 0.27 | 36.79 ± 0.39 | 37.72 ± 0.26 | 37.76 ± 0.37 | 36.81 ± 0.23 | 36.87 ± 0.43 | 37.80 ± 0.24 | 37.85 ± 0.44 |
| **Increase in Core Temperature (°C)** | -- | -- | 0.95 ± 0.05 | 0.98 ± 0.36 | -- | -- | 1.00 ± 0.16 | 0.98 ± 0.02 |
| **Skin Temperature (°C)†‡§** | 35.10 ± 0.48 | 34.98 ± 0.43 | 37.62 ± 0.68 | 37.79 ± 0.74 | 35.34 ± 0.32 | 35.43 ± 0.58 | 37.92 ± 0.60 | 38.27 ± 0.96 |
| **Mean Body Temperature (°C)‡§** | 36.61 ± 0.25 | 36.61 ± 0.37 | 37.71 ± 0.27 | 37.77 ± 0.39 | 36.66 ± 0.23 | 36.72 ± 0.42 | 37.81 ± 0.24 | 37.90 ± 0.44 |
| **Heart Rate (bpm)‡** | 64 ± 5 | 69 ± 10 | 94 ± 8 | 104 ± 13 | 64 ± 9 | 75 ± 10* | 97 ± 8 | 106 ± 11* |

EU: euhydrated, HY: hypohydrated
*Significantly different between groups at the same time point
†Main effect of hypohydration (normothermic baseline), independent of group
‡Main effect of heating from corresponding hydration condition (normothermic to hyperthermic), independent of group
§Main effect of hydration when hyperthermic (euhydrated hyperthermia v hypohydrated hyperthermia), independent of group

**Table 4.** p-values for cognitive performance for main effects of condition (hypohydration, heating, combination), main effect of group, and interaction between variables.

| | Hypohydrated Baseline while Normothermic | | | Effect of Hyperthermia while Euhydrated | | | Change in Response from Baseline to Hyperthermia while EU or DY | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Main Effect of Hydration Status** | **Main Effect of Body Composition** | **Interaction of Hydration Status Body Composition** | **Main Effect of Heat Stress** | **Main Effect of Body Composition** | **Interaction of Heating on Body Composition** | **Main Effect of Hydration Status on Changes during Heating** | **Main Effect of Body Composition on Changes during Heating** | **Interaction of Body Composition and Hydration Status on Changes during Heating** |
| **Verbal Memory** | 0.442 | 0.492 | 0.905 | 0.199 | 0.971 | 0.971 | 0.062# | 0.595 | 0.401 |
| **Visual Memory** | 0.015† | 0.881 | 0.056# | 0.044‡ | 0.179 | 0.179 | 0.198 | 0.584 | 0.079# |
| **Visual Motor Score** | 0.579 | 0.104 | 0.537 | 0.836 | 0.936 | 0.936 | 0.706 | 0.979 | 0.766 |
| **Reaction Time** | 0.309 | 0.124 | 0.309 | 1.000 | 0.819 | 0.819 | 0.314 | 0.345 | 0.130 |

*Main effect of body composition
†Main effect of hypohydration (normothermic baseline), independent of group
‡Main effect of heating from corresponding hydration condition (normothermic to hyperthermic), independent of group
§Main effect of hydration when hyperthermic (euhydrated hyperthermia v hypohydrated hyperthermia), independent of group
$Interaction of main effect of body composition and main effect of hydration or heating
#Represents variables having a trend toward significance

**Table 5.** p-values for mental task load for main effects of condition (hypohydration, heating, combination), main effect of group, and interaction between variables.

| | Hypohydrated Baseline during Normothermia | | | Effect of Hyperthermia while Euhydrated | | | Change in Response from Baseline to Hyperthermia while EU or DY | | |
|---|---|---|---|---|---|---|---|---|---|
| | Main Effect of Hydration Status | Main Effect of Body Composition | Interaction of Hydration Status Body Composition | Main Effect of Heat Stress | Main Effect of Body Composition | Interaction of Heating on Body Composition | Main Effect of Hydration status on Changes during Heating | Main Effect of Body Composition on Changes during Heating | Interaction of Body Composition and Hydration Status on Changes during Heating |
| **Concentration** | 0.803 | 0.247 | 0.452 | <0.001‡ | 0.549 | 0.715 | 0.113 | 0.425 | 0.383 |
| **Mental Demand** | 0.412 | 0.221 | 0.693 | 0.002‡ | 0.221 | 0.156 | 0.518 | 0.268 | 0.906 |
| **Temporal Demand** | 0.023† | 0.489 | 0.295 | 0.507 | 0.0531 | 0.321 | 0.010§ | 0.879 | 0.263 |
| **Performance** | 0.457 | 0.893 | 0.747 | 0.002‡ | 0.047* | 0.054# | 0.375 | 0.238 | 0.244 |
| **Frustration** | 0.007† | 0.550 | 0.841 | 0.287 | 0.088# | 0.891 | 0.766 | 0.890 | 0.598 |
| **Effort** | 0.102 | 0.219 | 0.638 | 0.002‡ | 0.842 | 0.350 | 0.944 | 0.093 | 0.169 |

*Main effect of body composition
†Main effect of hypohydration (normothermic baseline), independent of group
‡Main effect of heating from corresponding hydration condition (normothermic to hyperthermic), independent of group
§Main effect of hydration when hyperthermic (euhydrated hyperthermia v hypohydrated hyperthermia), independent of group
$Interaction of main effect of body composition and main effect of hydration or heating
#Represents variables having a trend toward significance

**Table 6.** p-values for symptoms for main effects of condition (hypohydration, heating, combination), main effect of group, and interaction between variables.

| | Hypohydrated Baseline during Normothermia | | | Effect of Hyperthermia while Euhydrated | | | Change in Response from Baseline to Hyperthermia while EU or DY | | |
|---|---|---|---|---|---|---|---|---|---|
| | Main Effect of Hydration Status | Main Effect of Body Composition | Interaction of Hydration Status Body Composition | Main Effect of Heat Stress | Main Effect of Body Composition | Interaction of Heating on Body Composition | Main Effect of Hydration Status on Changes during Heating | Main Effect of Body Composition on Changes during Heating | Interaction of Body Composition and Hydration Status on Changes during Heating |
| **Total Symptom Score** | 0.028† | 0.505 | 0.812 | 0.089# | 0.962 | 0.962 | 0.057# | 0.831 | 0.765 |
| **Headache** | 0.287 | 0.929 | 0.220 | 0.359 | 0.901 | 0.853 | 0.065# | 0.488 | 0.336 |
| **Nausea** | . | . | . | . | . | . | . | . | . |
| **Vomiting** | 0.374 | 0.374 | 0.826 | 0.026‡ | 0.124 | 0.124 | 0.169 | 0.355 | 0.683 |
| **Balance Problems** | 0.374 | 0.374 | 0.826 | 0.152 | 0.152 | 0.152 | 0.687# | 0.097 | 0.687 |
| **Dizziness** | 0.424 | 0.130 | 0.665 | 0.011‡ | 0.384 | 1.000 | 0.064# | 0.745 | 0.574 |
| **Fatigue** | 0.413 | 0.043* | 0.119 | 0.033‡ | 0.207 | 0.207 | 0.990 | 0.052# | 0.790 |
| **Trouble Falling Asleep** | 0.096 | 0.984 | 0.412 | 0.244 | 0.830 | 0.244 | 0.964 | 0.691 | 0.351 |
| **Sleeping More than Usual** | 0.374 | 0.374 | 0.826 | . | . | . | . | . | . |
| **Sleeping Less than Usual** | 0.397 | 0.116 | 0.999 | 0.019‡ | 0.621 | 0.779 | 0.188 | 0.189 | 0.446 |
| **Drowsiness** | 0.870 | 0.451 | 0.243 | 0.177 | 0.161 | 0.177 | 0.083# | 0.125 | 0.500 |
| **Sensitivity to Light** | 0.070# | 0.181 | 0.672 | 0.040‡ | 0.349 | 0.810 | 0.029§ | 0.780 | 0.780 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Sensitivity to Noise** | . | . | . | . | . | . | . | . | . |
| **Irritability** | 0.096 | 0.552 | 0.683 | 0.329 | 1.000 | 0.329 | 0.287 | 0.360 | 0.686 |
| **Sadness** | 0.102 | 0.172 | 0.682 | 0.254 | 0.605 | 0.254 | 0.845 | 0.124 | 0.845 |
| **Nervousness** | 0.881 | 0.881 | 0.785 | 0.329 | 0.177 | 0.329 | 0.405 | 0.405 | 0.654 |
| **Feeling More Emotional** | 0.638 | 0.075# | 0.344 | 0.152 | 0.499 | 0.152 | 0.532 | 0.766 | 0.532 |
| **Numbness or Tingling** | . | . | . | 0.329 | 0.329 | 0.329 | 0.156 | 0.140 | 0.156 |
| **Feeling Slowed Down** | 0.062# | 0.065# | 0.896 | 0.076# | 0.810 | 0.792 | 0.004§ | 0.686 | 0.179 |
| **Feeling Mentally "Foggy"** | 0.051# | 0.630 | 0.230 | 0.144 | 0.631 | 0.618 | 0.499 | 0.687 | 0.753 |
| **Difficulty Concentrating** | 0.068# | 0.605 | 0.099 | 0.002‡ | 0.386 | 0.083# | 0.858 | 0.124 | 0.858 |
| **Difficulty Remembering** | 0.547 | 0.440 | 0.376 | 0.152 | 0.152 | 0.152 | 0.100 | 0.512 | 0.870 |
| **Visual Problems** | 0.097 | 0.668 | 0.189 | 0.011‡ | 0.362 | 0.362 | 0.225 | 0.343 | 0.953 |
| **Thermal Sensation** | 0.628 | 0.644 | 0.835 | <0.001‡ | 0.473 | 0.942 | 0.096 | 0.837 | 0.714 |
| **Thirst** | <0.001† | 0.552 | 0.677 | 0.038‡ | 0.361 | 0.173 | 0.025§ | 0.107 | 0.126 |
| **Headache** | 0.057# | 0.349 | 0.637 | 0.041‡ | 0.0.78# | 0.080# | 0.636 | 0.259 | 0.373 |

*Main effect of body composition
. No participant reported feelings of symptom at the time point
†Main effect of hypohydration (normothermic baseline), independent of group
‡Main effect of heating from corresponding hydration condition (normothermic to hyperthermic), independent of group
§Main effect of hydration when hyperthermic (euhydrated hyperthermia v hypohydrated hyperthermia), independent of group
$Interaction of main effect of body composition and main effect of hydration or heating
#Represents variables having a trend toward significance

**Table 7.** p-values for symptoms for main effects of condition (hypohydration, heating, combination), main effect of group, and interaction between variables.

| | Hypohydrated Baseline during Normothermia | | | Effect of Hyperthermia while Euhydrated | | | Change in Response from Baseline to Hyperthermia while EU or DY | | |
|---|---|---|---|---|---|---|---|---|---|
| | Main Effect of Hydration Status | Main Effect of Body Composition | Interaction of Hydration Status Body Composition | Main Effect of Heat Stress | Main Effect of Body Composition | Interaction of Heating on Body Composition | Main Effect of Hydration Status on Changes during Heating | Main Effect of Body Composition on Changes during Heating | Interaction of Body Composition and Hydration Status on Changes during Heating |
| **Tension** | 0.339 | 0.078# | 0.652 | 0.389 | 0.743 | 0.772 | 0.338 | 0.880 | 0.654 |
| **Depression** | 0.007† | 0.327 | 0.327 | 0.010‡ | 0.444 | 0.518 | 0.207 | 0.439 | 0.951 |
| **Anger** | 0.090# | 0.207 | 0.090# | 0.173 | 0.952 | 1.000 | 0.031§ | 0.113 | 0.079# |
| **Vigor** | 0.245 | 0.517 | 0.765 | 0.017‡ | 0.830 | 1.000 | 0.185 | 0.628 | 0.185 |
| **Fatigue** | 0.134 | 0.304 | 0.595 | 0.109 | 0.311 | 0.442 | 0.865 | 0.453 | 0.662 |
| **Confusion** | 0.616 | 0.228 | 0.616 | 0.727 | 0.626 | 0.488 | 0.580 | 0.686 | 0.164 |

*Main effect of body composition
†Main effect of hypohydration (normothermic baseline), independent of group
‡Main effect of heating from corresponding hydration condition (normothermic to hyperthermic), independent of group
§Main effect of hydration when hyperthermic (euhydrated hyperthermia v hypohydrated hyperthermia), independent of group
$Interaction of main effect of body composition and main effect of hydration or heating
#Represents variables having a trend toward significance

**For all Figures:**
*Main effect of body composition
†Main effect of hypohydration (normothermic baseline), independent of group
‡Main effect of heating from corresponding hydration condition (normothermic to hyperthermic), independent of group
§Main effect of hydration when hyperthermic (euhydrated hyperthermia v hypohydrated hyperthermia), independent of group

**Figure 2.** Composite scores for Verbal Memory between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 3.** Composite scores for Visual Memory between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 4.** Composite scores for Visual Motor between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 5.** Composite scores for Reaction Time between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 6.** Scores for Mental Demand between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 7.** Scores for Temporal between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 8.** Scores for perceived Performance between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 9.** Scores for Frustration between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 10.** Scores for perceived Effort between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 11.** Scores for Concentration between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 12.** Composite scores for Total Symptom Score between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 13.** Scores for perception of Thermal Sensation between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 14.** Scores for perception of Thirst between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 15.** Scores for Headache between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 16.** Scores for feelings of Tension between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 17.** Scores for feelings of Depression between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 18.** Scores for feelings of Anger between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 19.** Scores for feelings of Vigor between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated..

**Figure 20.** Scores for feelings of Fatigue between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated.

**Figure 21.** Scores for feelings of Confusion between non-obese and obese. a) Raw values when euhydrated and hypohydrated during normothermic pre-test, b) raw values during 1.0°C increase in core temperature when euhydrated, c) change values during hyperthermia when euhydrated

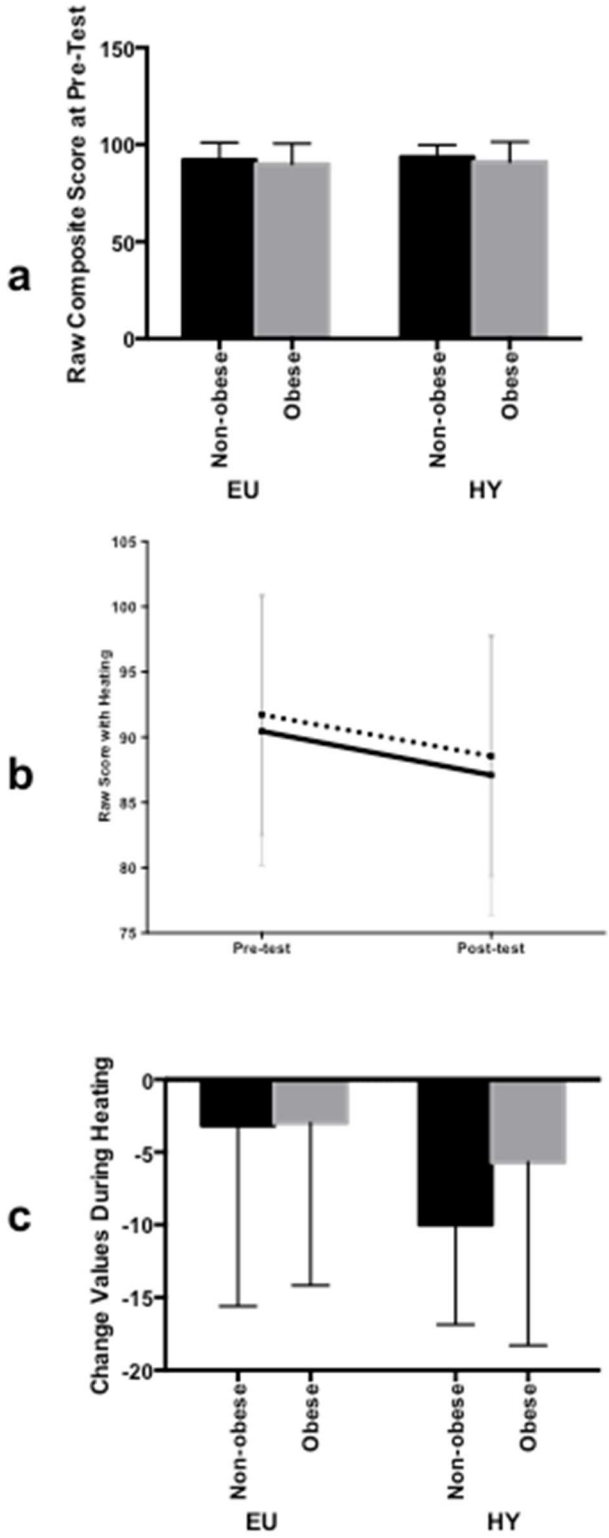

a
Raw Composite Score at Pre-Test
150
100
50
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
105
100
95
90
85
80
75
Pre-test
Post-test
c
Change Values During Heating
0
-5
-10
-15
-20
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 2.**

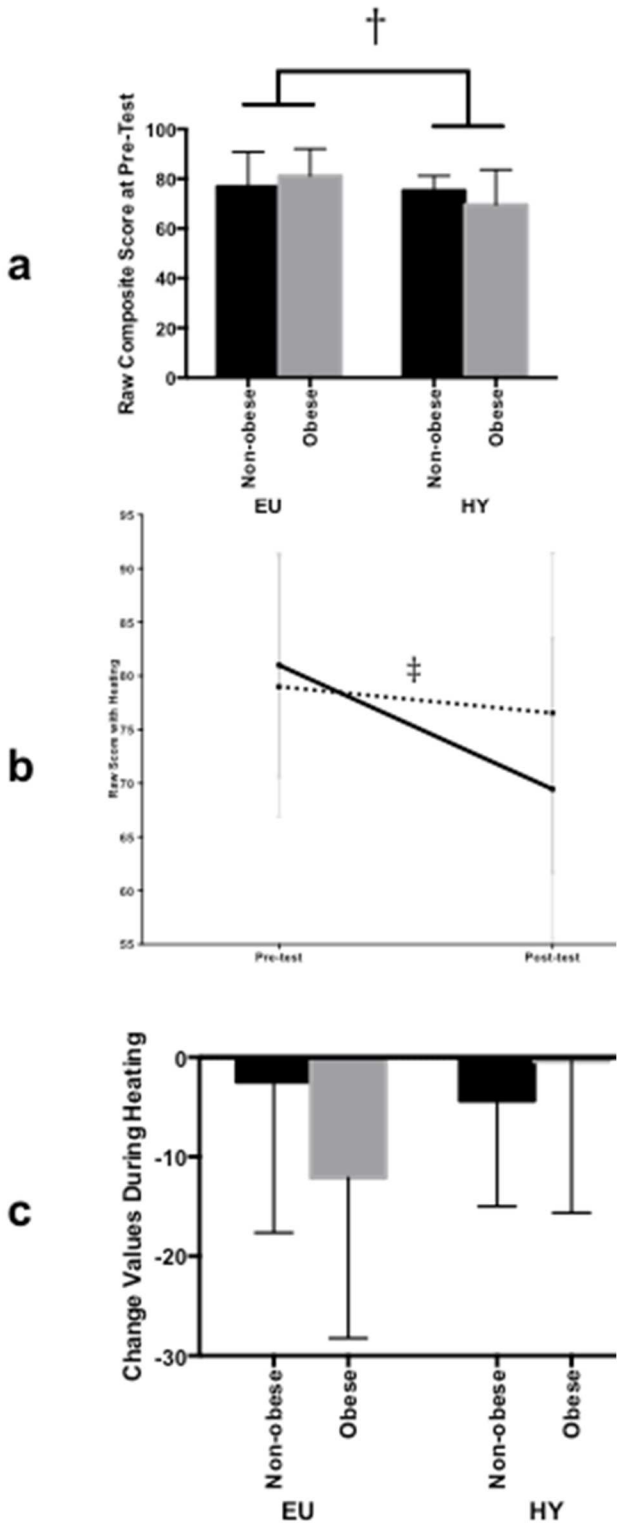

a
†
Raw Composite Score at Pre-Test
100
80
60
40
20
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
‡
Pre-test
Post-test
c
Change Values During Heating
0
-10
-20
-30
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 3.**

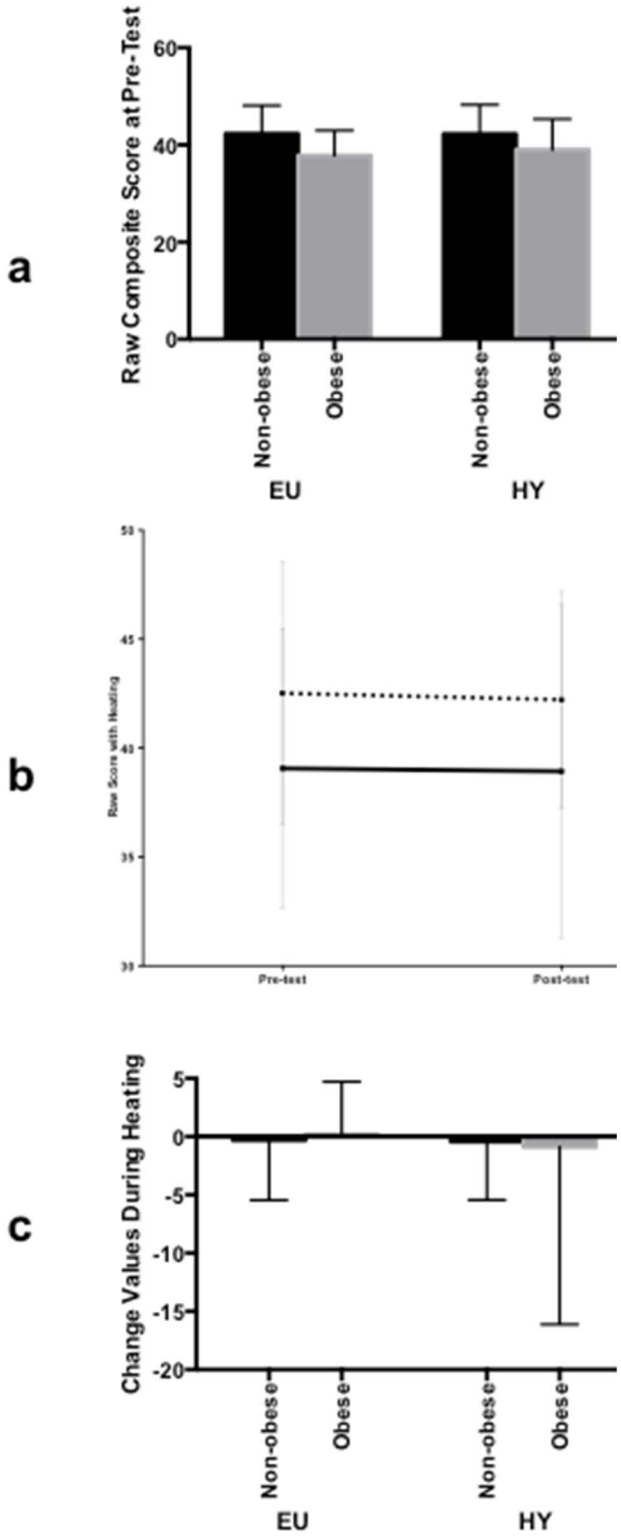

a
Raw Composite Score at Pre-Test
60
40
20
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
45
40
35
Pre-test
Post-test
c
Change Values During Heating
5
0
-5
-10
-15
-20
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 4.**

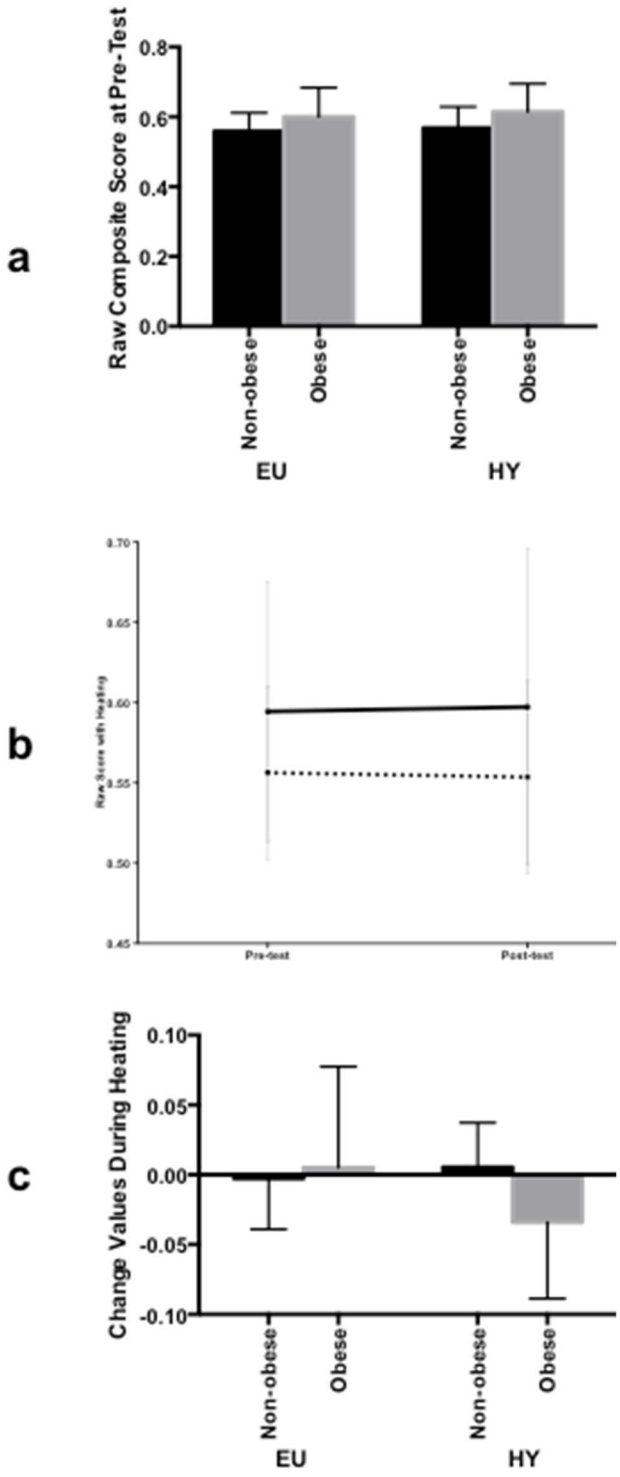

a
Raw Composite Score at Pre-Test
0.8
0.6
0.4
0.2
0.0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
0.70
0.65
0.60
0.55
0.50
0.45
Pre-test
Post-test
c
Change Values During Heating
0.10
0.05
0.00
-0.05
-0.10
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 5.**

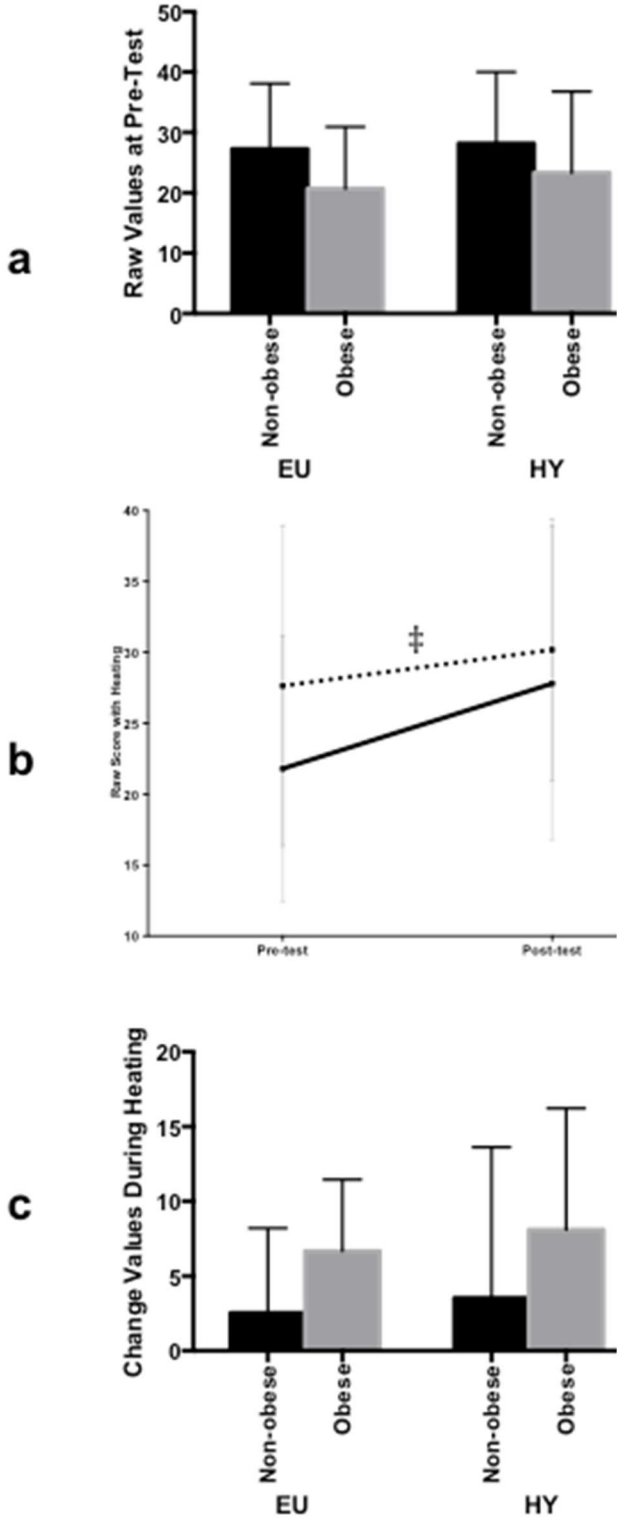

a
Raw Values at Pre-Test
50
40
30
20
10
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
40
35
30
25
20
15
10
++
Pre-test
Post-test
c
Change Values During Heating
20
15
10
5
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 6.**

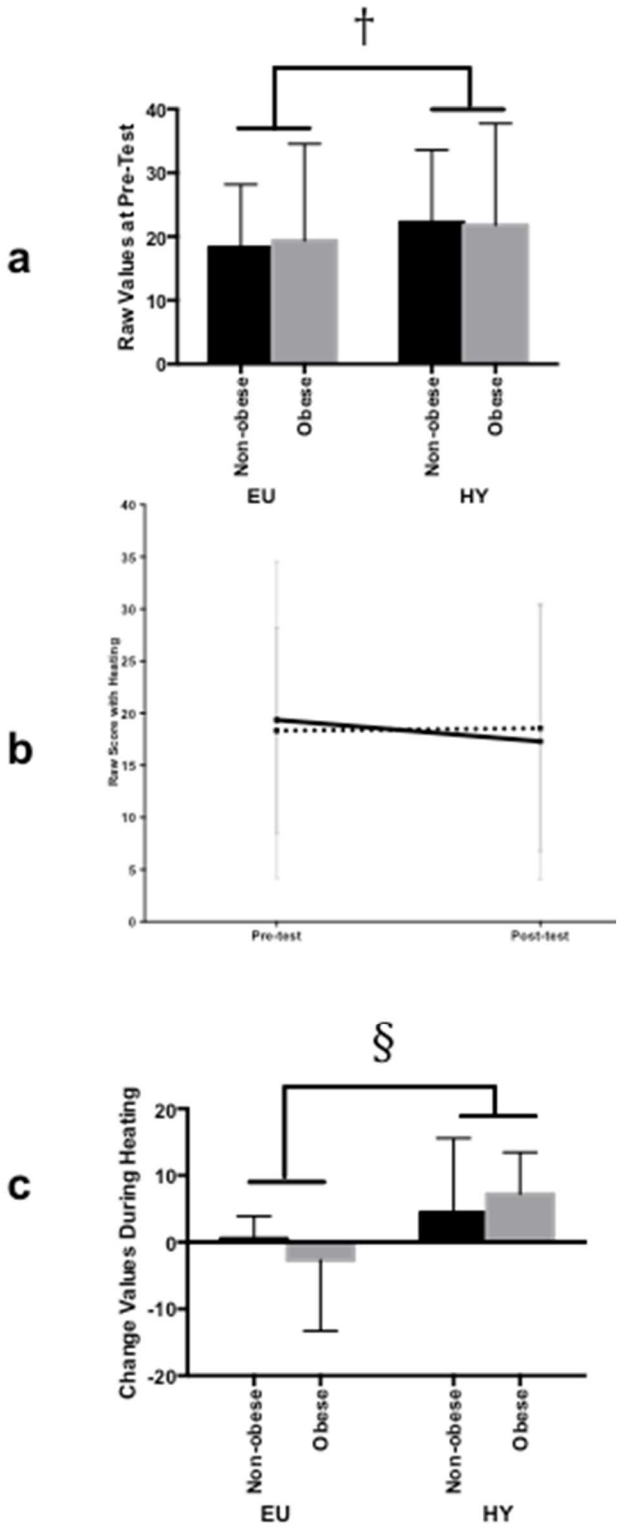

a
†
Raw Values at Pre-Test
40
30
20
10
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
40
35
30
25
20
15
10
5
0
Pre-test
Post-test
c
§
Change Values During Heating
20
10
0
-10
-20
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 7.**

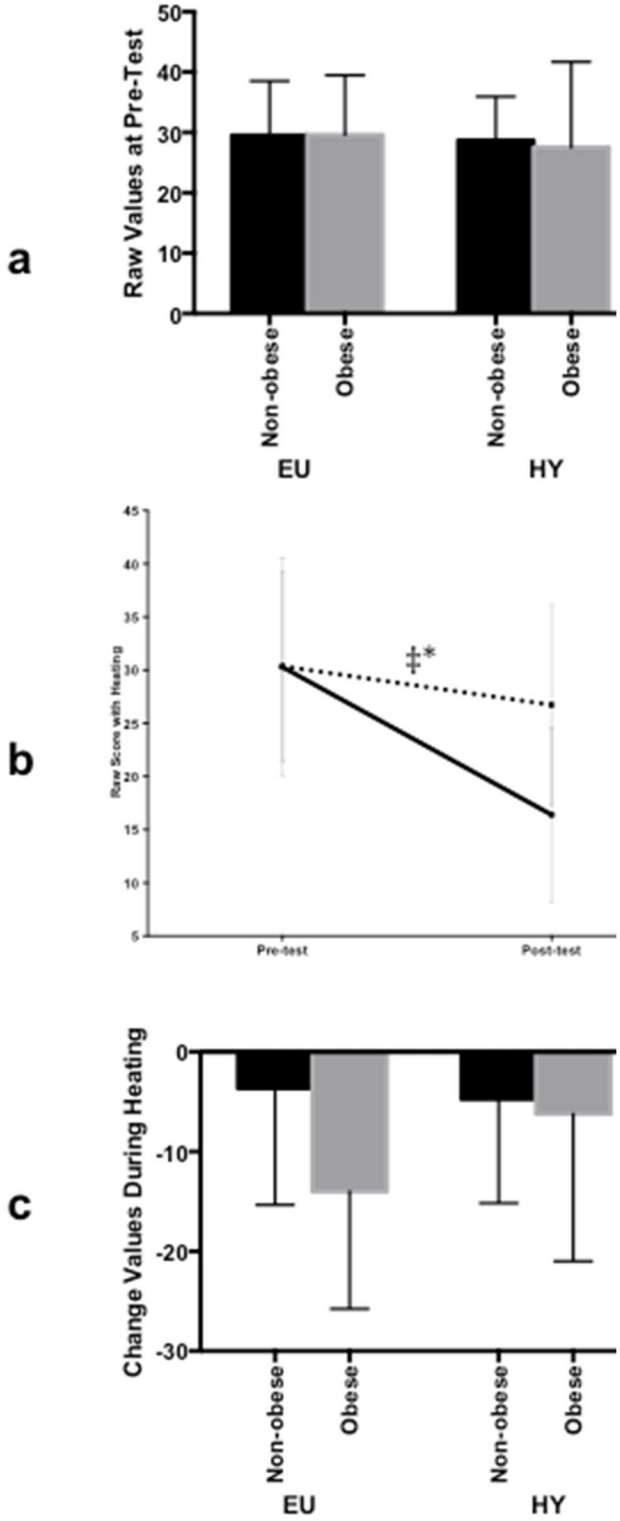

a
Raw Values at Pre-Test
50
40
30
20
10
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
45
40
35
30
25
20
15
10
5
‡*
Pre-test
Post-test
c
Change Values During Heating
0
-10
-20
-30
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 8.**

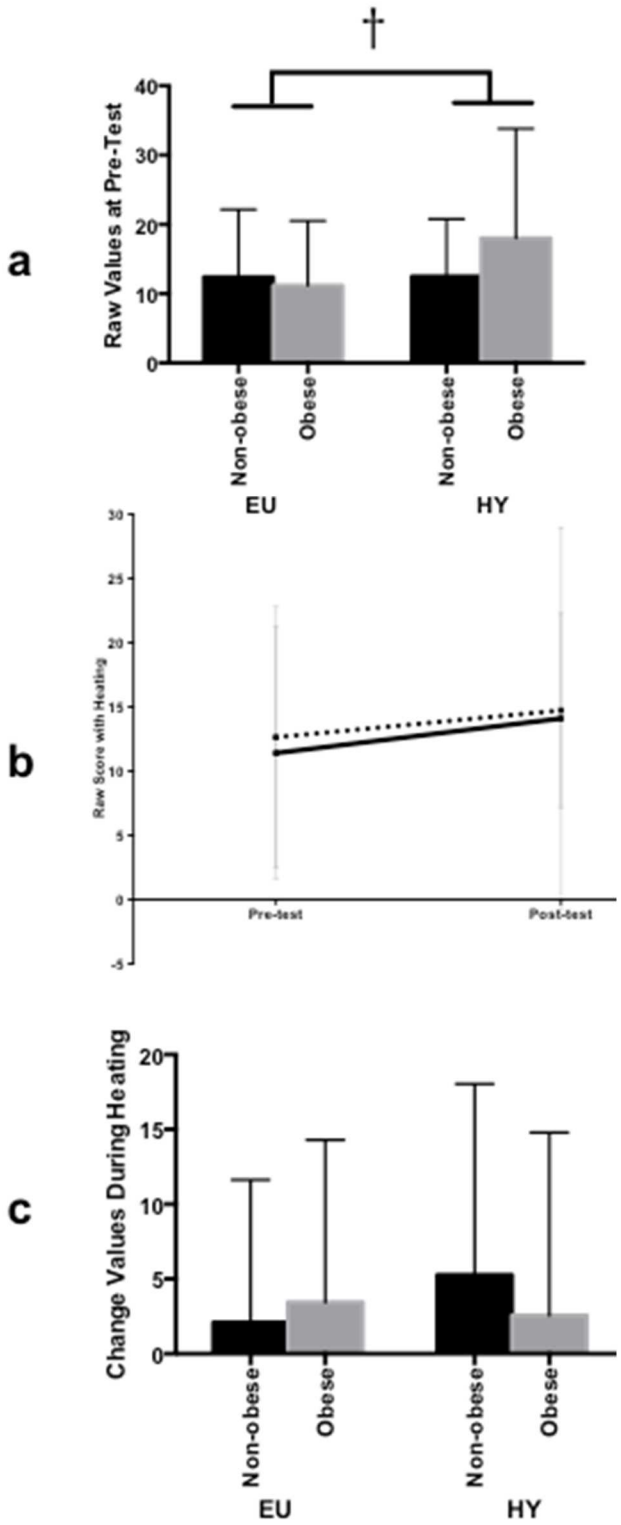

a
†
Raw Values at Pre-Test
40
30
20
10
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
30
25
20
15
10
5
0
-5
Pre-test
Post-test
c
Change Values During Heating
20
15
10
5
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 9.**

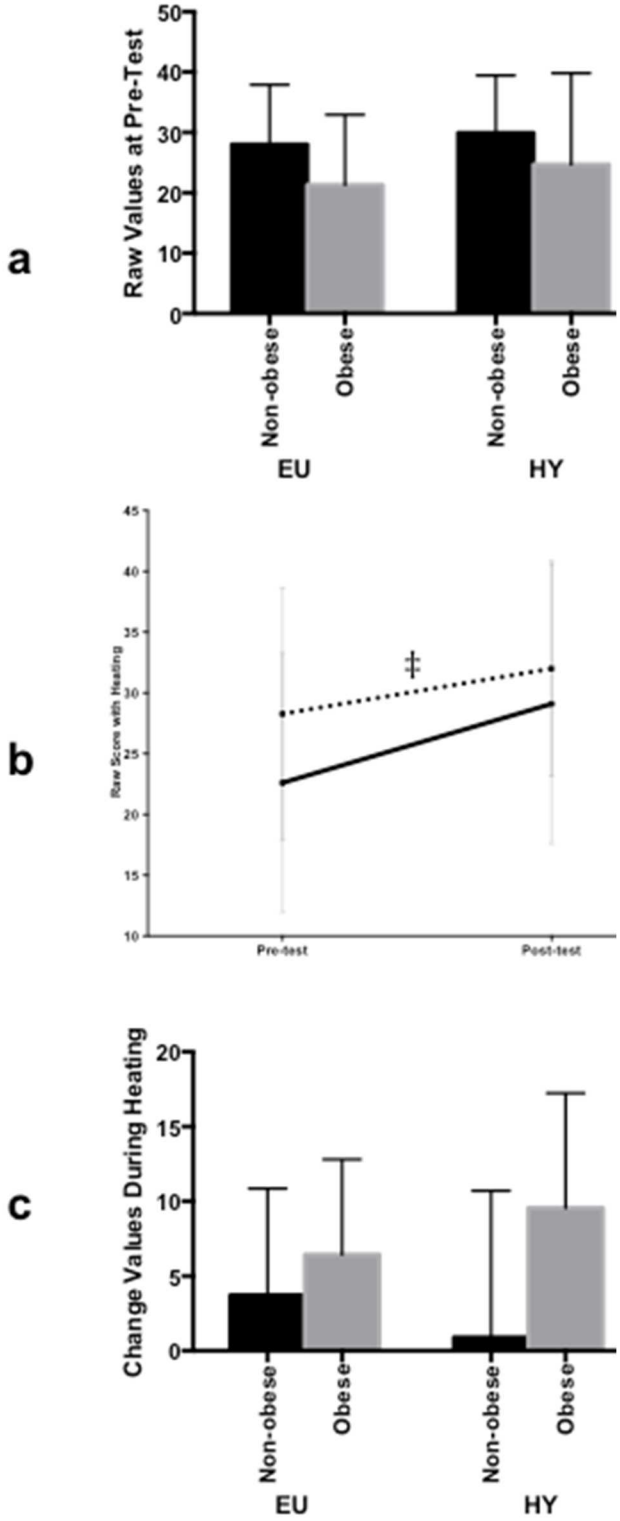

a
Raw Values at Pre-Test
50
40
30
20
10
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
45
40
35
30
25
20
15
10
‡
Pre-test
Post-test
c
Change Values During Heating
20
15
10
5
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 10.**

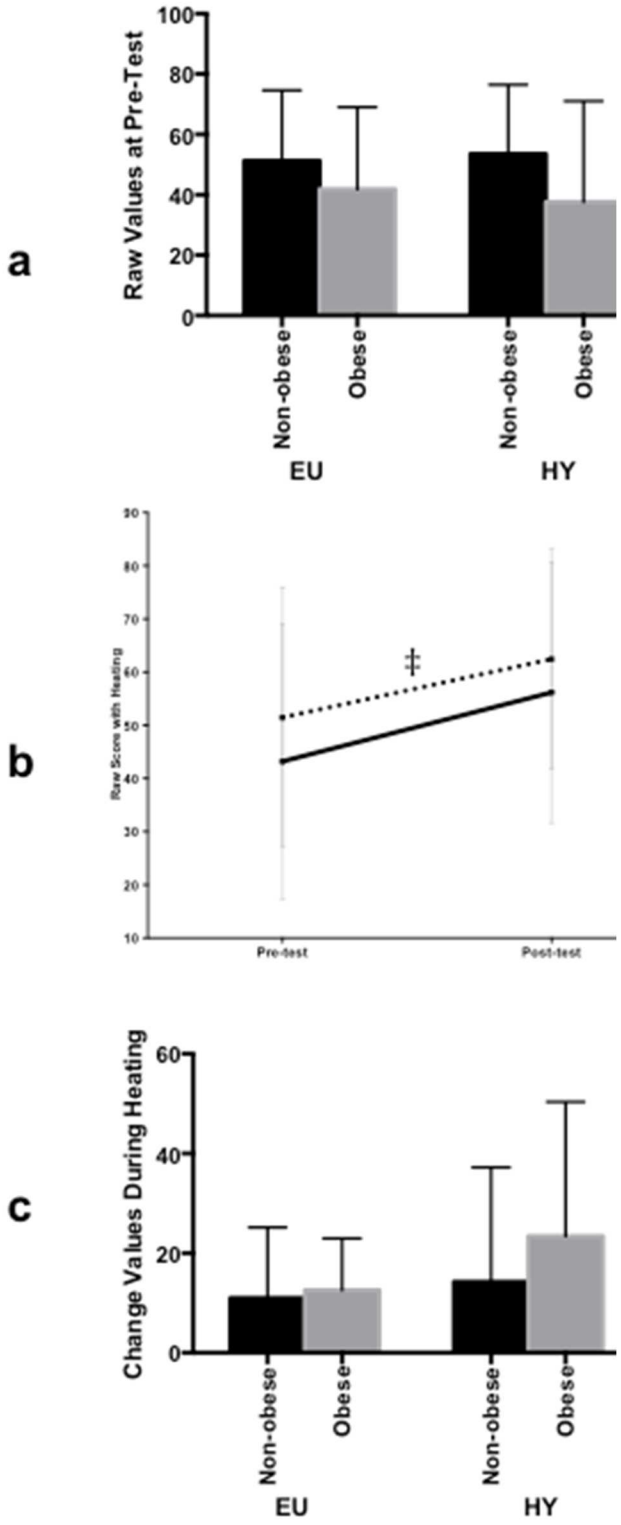

a
Raw Values at Pre-Test
100
80
60
40
20
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
90
80
70
60
50
40
30
20
10
‡
Pre-test
Post-test
c
Change Values During Heating
60
40
20
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 11.**

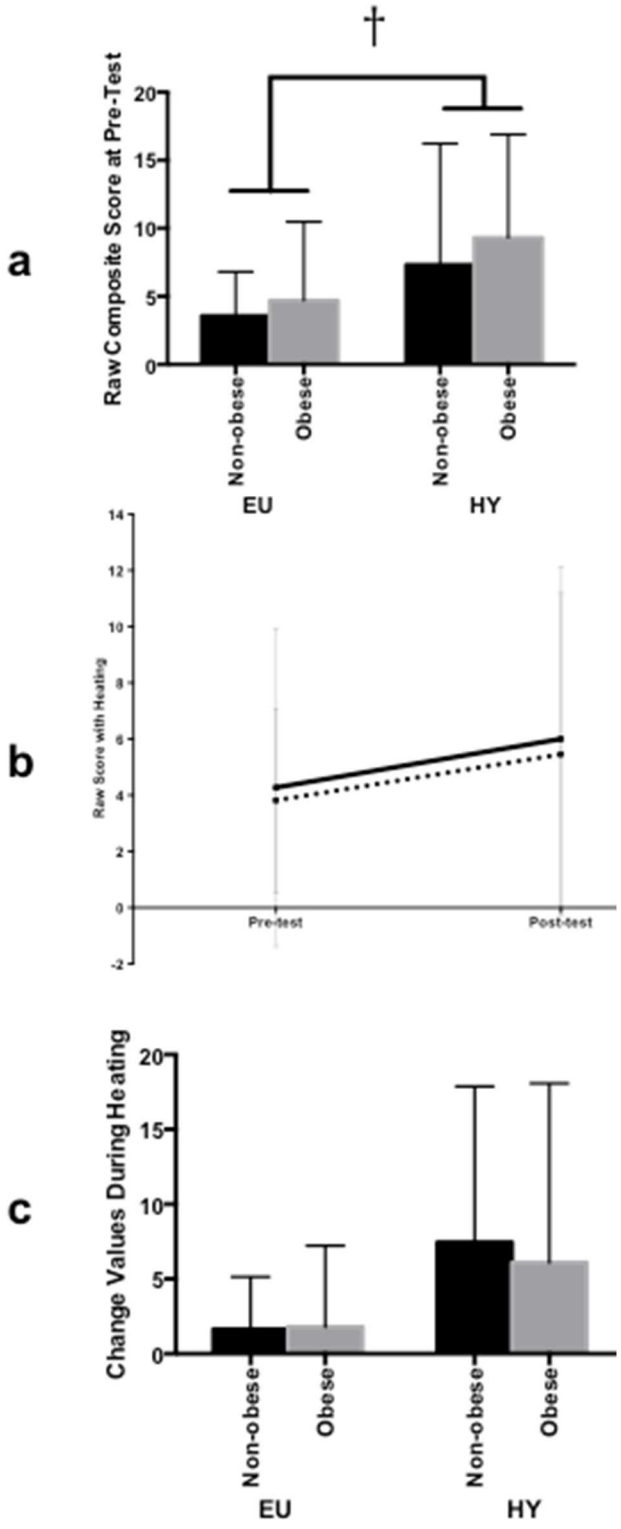

a
†
Raw Composite Score at Pre-Test
20
15
10
5
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
14
12
10
8
6
4
2
0
-2
Pre-test
Post-test
c
Change Values During Heating
20
15
10
5
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 12.**

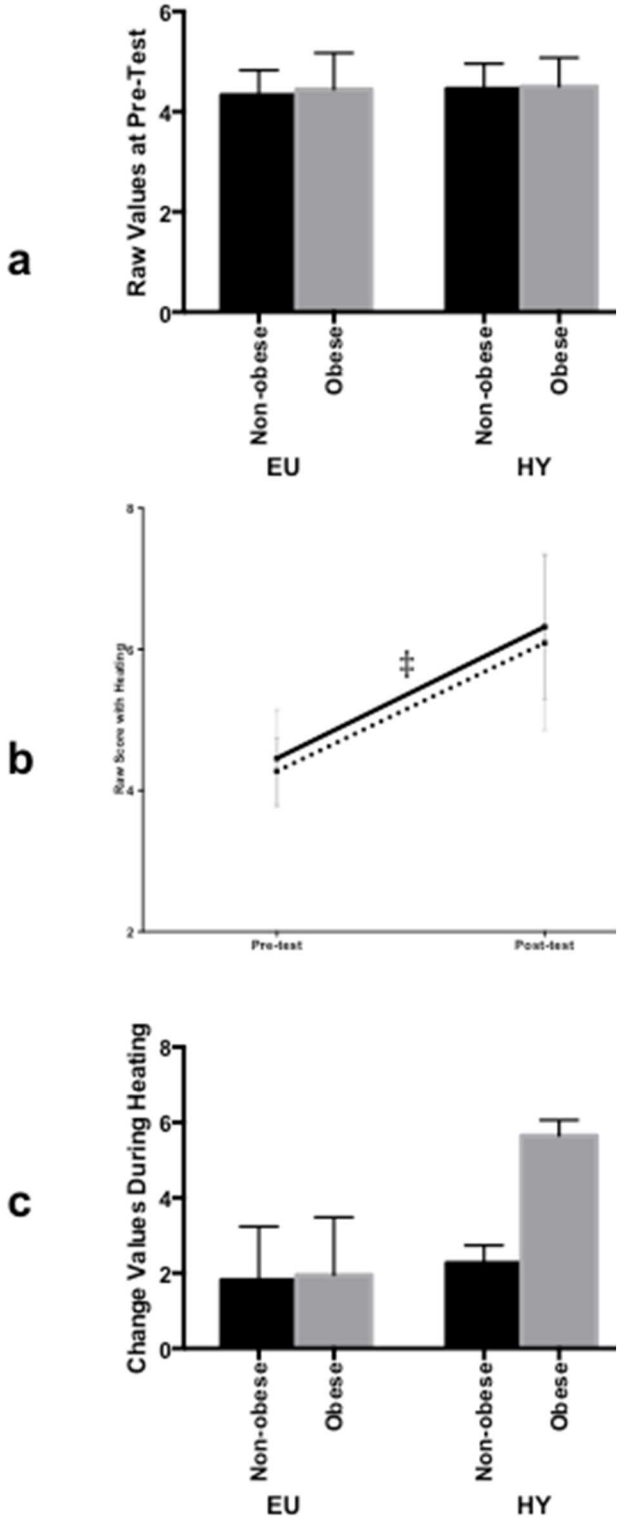

a
Raw Values at Pre-Test
6
4
2
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
4
2
Pre-test
Post-test
c
Change Values During Heating
8
6
4
2
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 13.**

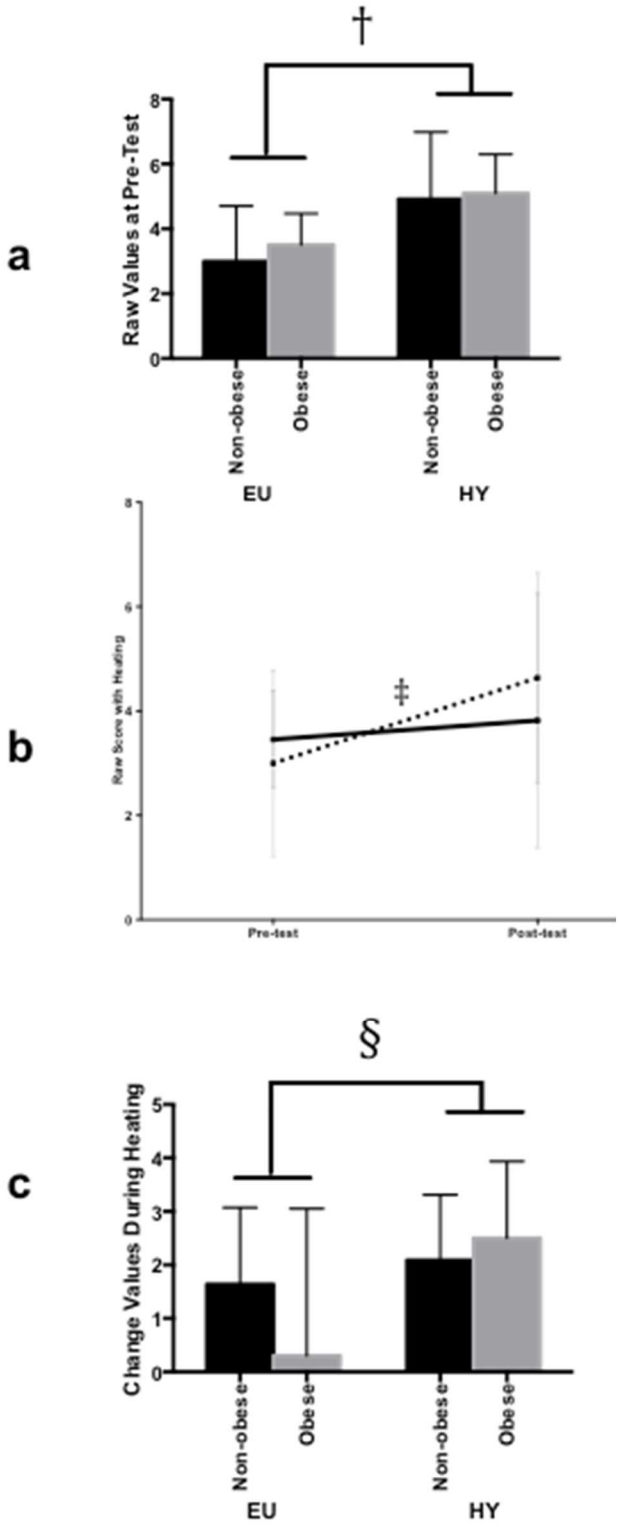

a
†
Raw Values at Pre-Test
8
6
4
2
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
‡
Pre-test
Post-test
c
§
Change Values During Heating
5
4
3
2
1
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 14.**

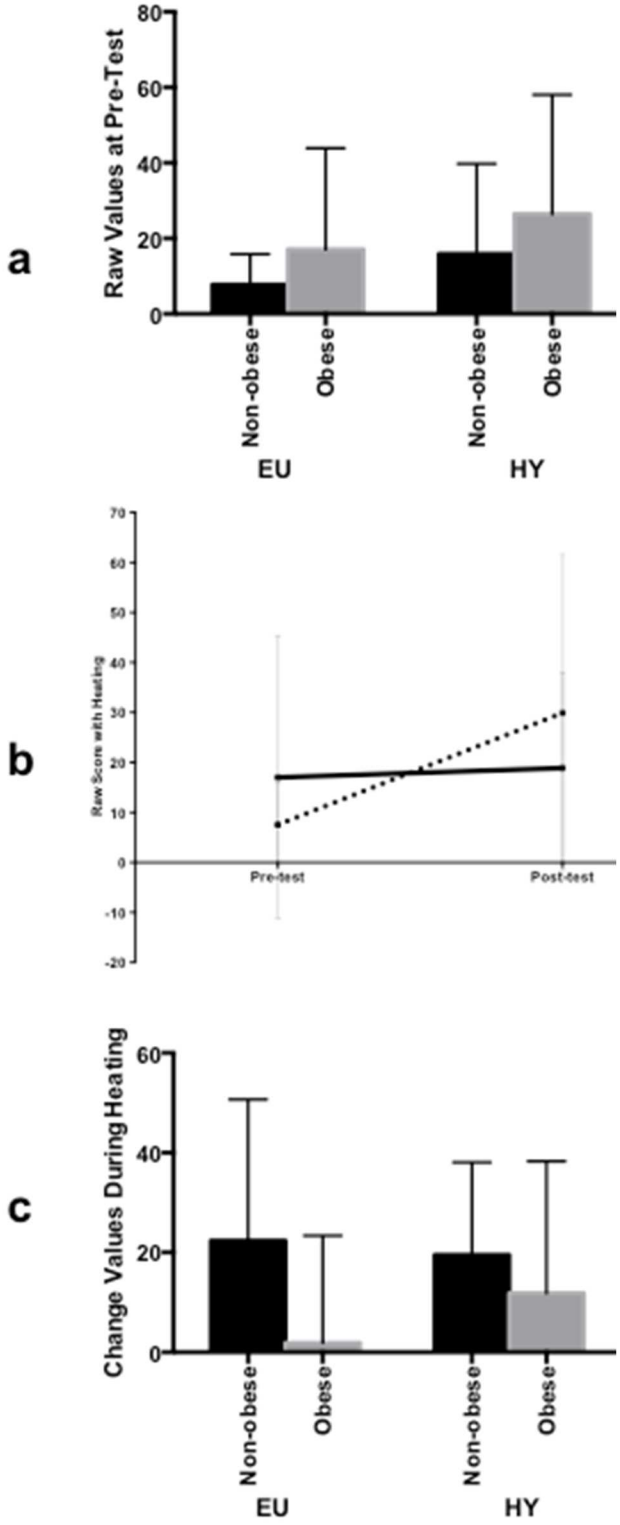

a
Raw Values at Pre-Test
80
60
40
20
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
70
60
50
40
30
20
10
0
-10
-20
Pre-test
Post-test
c
Change Values During Heating
60
40
20
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 15.**

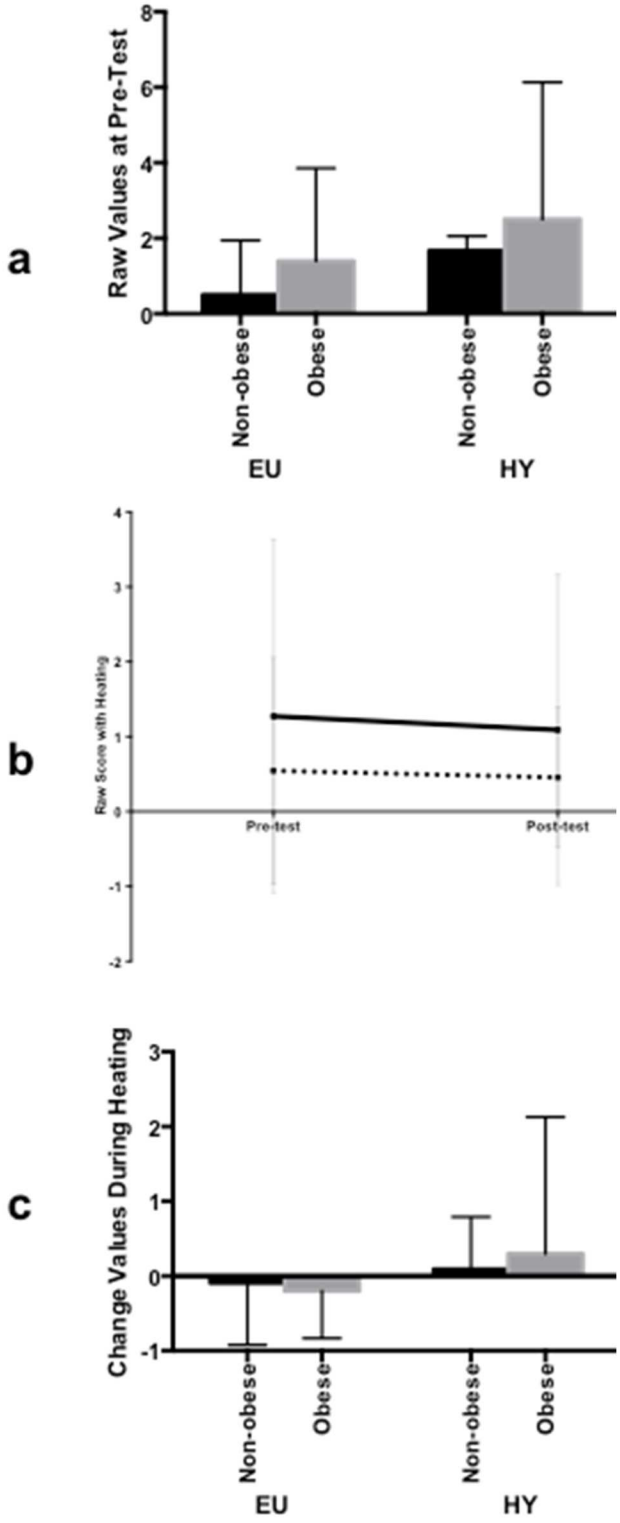

a
Raw Values at Pre-Test
8
6
4
2
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
4
3
2
1
0
-1
-2
Pre-test
Post-test
c
Change Values During Heating
3
2
1
0
-1
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 16.**

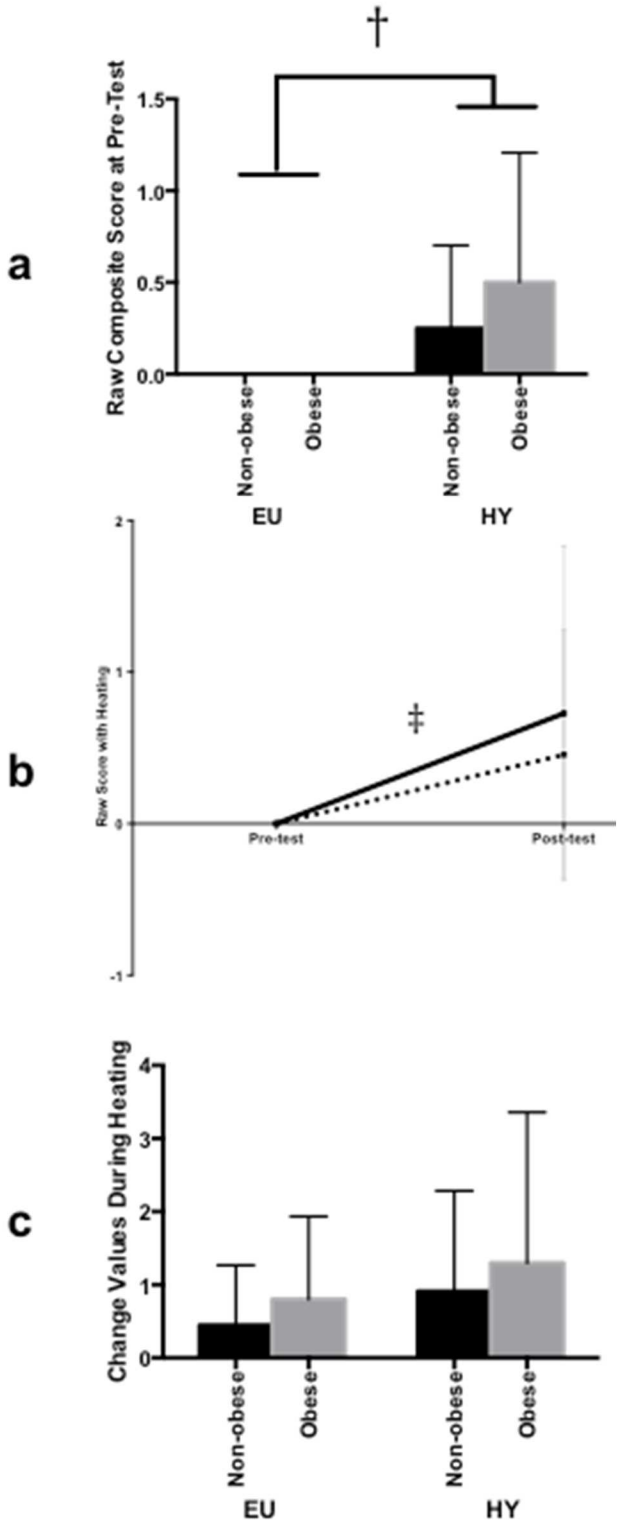

a
†
Raw Composite Score at Pre-Test
1.5
1.0
0.5
0.0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
‡
Raw Score with Heating
2
1
0
-1
Pre-test
Post-test
c
Change Values During Heating
4
3
2
1
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 17.**

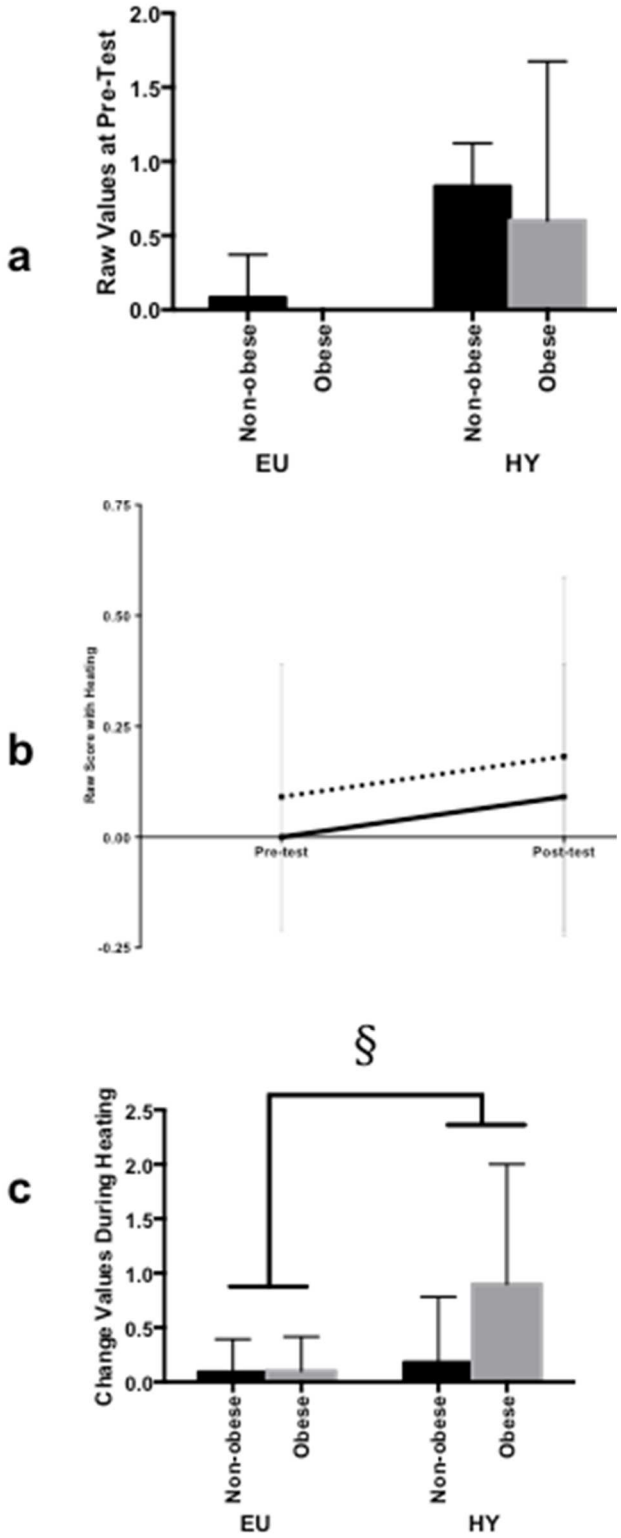

a
Raw Values at Pre-Test
2.0
1.5
1.0
0.5
0.0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
0.75
0.50
0.25
0.00
-0.25
Pre-test
Post-test
c
§
Change Values During Heating
2.5
2.0
1.5
1.0
0.5
0.0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 18.**

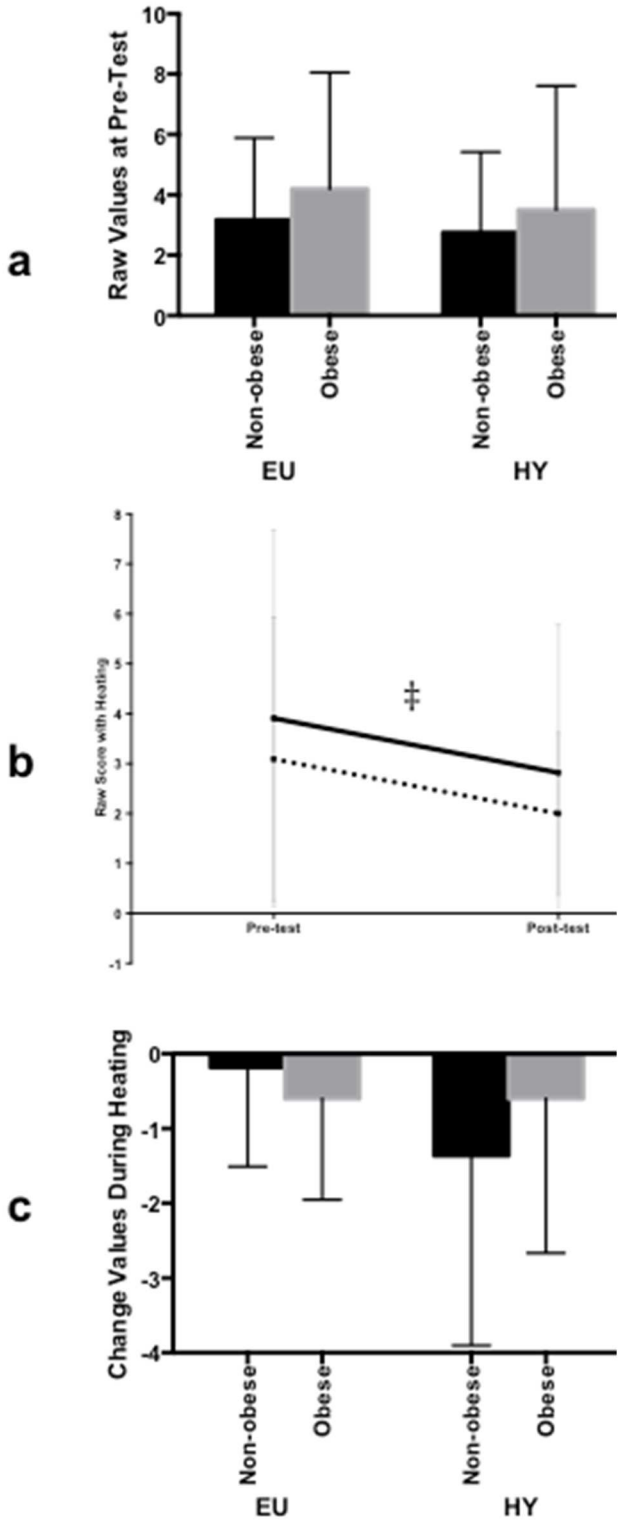

a
Raw Values at Pre-Test
10
8
6
4
2
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
Pre-test
Post-test
c
Change Values During Heating
0
-1
-2
-3
-4
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 19.**

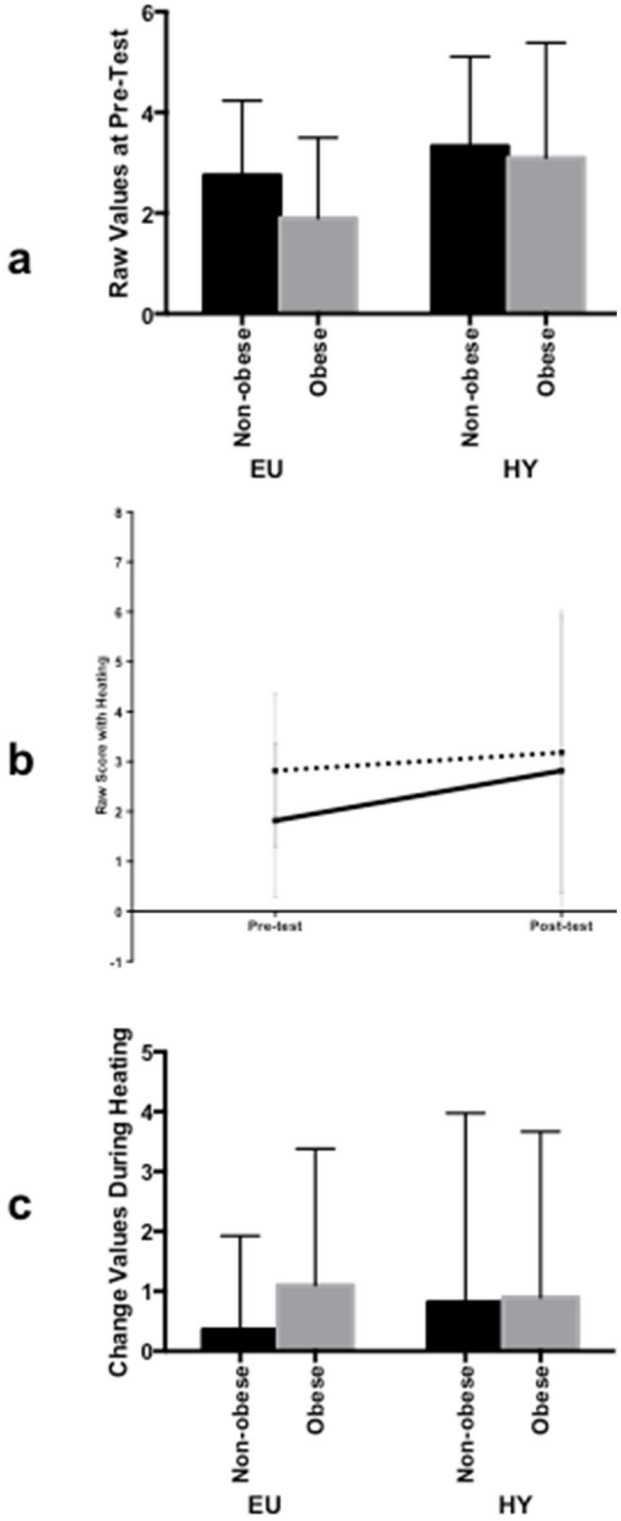

a
Raw Values at Pre-Test
6
4
2
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
Pre-test
Post-test
c
Change Values During Heating
5
4
3
2
1
0
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 20.**

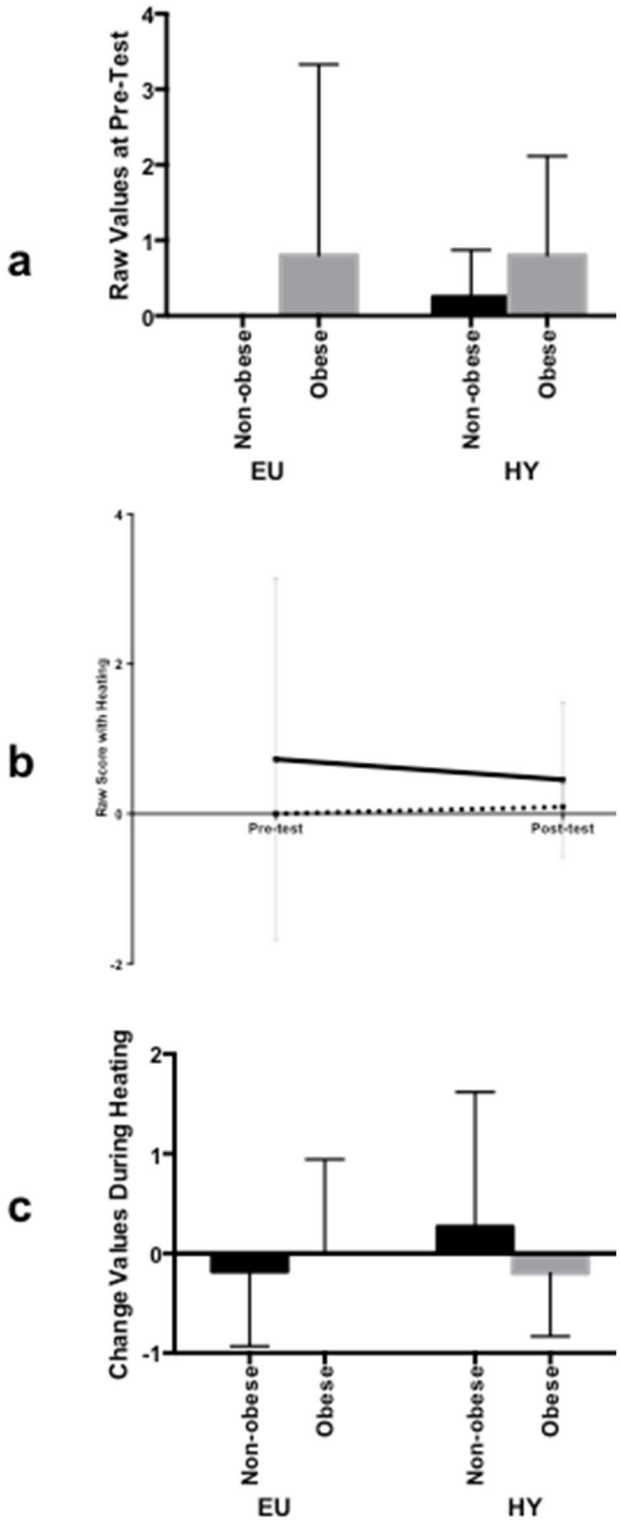

a
Raw Values at Pre-Test
4
3
2
1
0
Non-obese
Obese
Non-obese
Obese
EU
HY
b
Raw Score with Heating
Pre-test
Post-test
c
Change Values During Heating
2
1
0
-1
Non-obese
Obese
Non-obese
Obese
EU
HY

**Figure 21.**

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# Appendices

## Appendix A: Medical History

### HUMAN PERFORMANCE LABORATORY MEDICAL HISTORY QUESTIONNAIRE

**PLEASE ANSWER ALL OF THE FOLLOWING QUESTIONS AND PROVIDE DETAILS FOR ALL "YES" ANSWERS IN THE SPACES AT THE BOTTOM OF THE FORM.**

| YES | NO | | |
|---|---|---|---|
| yes | no | 1. | Has your doctor ever said that you have a heart condition and that you should only do physical activity recommended by a doctor? |
| yes | no | 2. | Has your doctor ever denied or restricted your participation in sports or exercise for any reason? |
| yes | no | 3. | Do you ever feel discomfort, pressure, or pain in your chest when you do physical activity? |
| yes | no | 4. | In the past month, have you had chest pain when you were not doing physical activity? |
| yes | no | 5. | Do you lose your balance because of dizziness or do you ever lose consciousness? |
| yes | no | 6. | Does your heart race or skip beats during exercise? |
| yes | no | 7. | Has a doctor ever ordered a test for you heart? (i.e. EKG, echocardiogram) |
| yes | no | 8. | Has anyone in your family died for no apparent reason or died from heart problems or sudden death before the age of 50? |
| yes | no | 9. | Have you ever had to spend the night in a hospital? |
| yes | no | 10. | Have you ever had surgery? |

11. Please check the box next to any of the following illnesses with which you have ever been diagnosed or for which you have been treated.

| | | | | | |
|---|---|---|---|---|---|
| ☐ | High blood pressure | ☐ | Elevated cholesterol | ☐ | Diabetes |
| ☐ | Asthma | ☐ | Epilepsy (seizures) | ☐ | Kidney problems |
| ☐ | Bladder Problems | ☐ | Anemia | ☐ | Heart problems |
| ☐ | Coronary artery disease | ☐ | Lung problems | ☐ | Chronic headaches |

| YES | NO | | |
|---|---|---|---|
| yes | no | 12. | Have you ever gotten sick because of exercising in the heat? (i.e. cramps, heat exhaustion, heat stroke) |
| yes | no | 13. | Have you had any other significant illnesses not listed above? |
| yes | no | 14. | Do you currently have any illness? |
| yes | no | 15. | Do you know of any other reason why you should not do physical activity? |
| yes | no | 16. | Have you ever been diagnosed with diverticulitis, abdominal adhesions or have a history of abdominal obstructions or abdominal surgeries |

17. Please list all medications you are currently taking. Make sure to include over-the-counter medications and birth control pills.

| Drugs/Supplements/Vitamins | Dose | Frequency (i.e. daily, 2x/day, etc.) |
|---|---|---|
| | | |
| | | |
| | | |
| | | |
| | | |
| | | |

**DETAILS:** _____

18. Please list all allergies you have.

| Substance | Reaction |
|---|---|
| | |
| | |
| | |
| | |

| YES | NO | 19. Have you smoked? | If yes, #/day | Age Started | If you've quit, what age? |
|---|---|---|---|---|---|
| yes | no | Cigarettes | | | |
| yes | no | Cigars | | | |
| yes | no | Pipes | | | |

| YES | NO | 20. Do you drink alcoholic beverages? | If yes, how much? | How often? |
|---|---|---|---|---|
| yes | no | | | |

21. Do you have a family history of any of the following problems? If yes, note who in the space provided.

| | | | |
|---|---|---|---|
| ☐ High blood pressure | | ☐ Heart disease | |
| ☐ High cholesterol | | ☐ Kidney disease | |
| ☐ Diabetes | | ☐ Thyroid disease | |

22. Please check the box next to any of the following body parts you have injured in the past and provide details.

| | | | | | |
|---|---|---|---|---|---|
| ☐ Head | | ☐ Hip | | ☐ Calf/shin | |
| ☐ Neck | | ☐ Thigh | | ☐ Shoulder | |
| ☐ Upper back | | ☐ Knee | | ☐ Upper arm | |
| ☐ Lower back | | ☐ Ankle | | ☐ Elbow | |
| ☐ Chest | | ☐ Foot | | ☐ Hand/fingers | |

| YES | NO | |
|---|---|---|
| yes | no | 23. Have you ever had a stress fracture? |
| yes | no | 24. Have you ever had a disc injury in your back? |
| yes | no | 25. Has a doctor ever restricted your exercise because of an injury? |
| yes | no | 26. Do you currently have any injuries that are bothering you? |

27. Do you consider your occupation as?
- ☐ Sedentary (no exercise)
- ☐ Inactive-occasional light activity (walking)
- ☐ Active-regular light activity and/or occasional vigorous activity (heavy lifting,
- ☐ Heavy Work-regular vigorous activity

28. List your regular physical activities

| Activity | How often do you do it? | How long do you do it? | How long ago did you start? |
|---|---|---|---|
| | | | |
| | | | |
| | | | |
| | | | |

**ADDITIONAL DETAILS:**

**Appendix B: Menstrual History**

**Menstrual History Questionnaire**

We are interested in your personal history. Since some of these questions concern events that may have occurred more than a decade ago, it is possible that your memory of these events is inaccurate. For this reason, next to many of the questions we ask that you rate how certain you are of your memory: Absolutely accurate = 1, generally accurate = 2, probably inaccurate = 3, completely unsure = 4

| Question | Answer | Degree of certainty |
|---|---|---|
| **Exact age of first menstruation** | | |
| **Number of periods missed in 2007** | | |
| **…2006** | | |
| **... 2005** | | |
| **... 2004** | | |
| **... 2003** | | |
| **... 2002** | | |
| **... 2001** | | |
| **... 2000** | | |
| **... 1999** | | |
| **... 1998** | | |
| **... 1997** | | |
| **... 1996** | | |

**Regularity of menstrual cycles:**

Do you have menstrual periods now? (Check one)

___ Yes, regularly every month

___ Yes, but I skip a month once in a while

___ Yes, but not very often (for example, once in six months)

___ No, I have not had a period in at least six months

___ No, I am post-menopausal, have had a hysterectomy, or am pregnant

**Estrogen use:**

Do you currently use birth control (OC) pills?

If so, what is the primary reason (circle): Contraception To regulate cycle Both

At what age did you begin using birth control pills?

Estimate total number of months that you have used birth control pills (e.g. 6 months one year plus 3 months in another and 12 months in a third year would equal 12 months):

Name the type (i.e. brand) of birth control pill that you use:

Is this a constant dose, bi-phasic, or tri-phasic pill?

Do you use any other estrogen or progesterone supplements (describe)?

**Current menstrual status:**

If using birth control pills, what day of your pill-pack is today?

If not using birth control pills, when did your last period start?

If not using birth control pills, how long is your average period and your average cycle (i.e. 7 days/ 28 days)?

Have you had unprotected intercourse within the last 60 days? Is there any chance you might be pregnant?

_____ Yes _____ No

## Appendix C: International Physical Activity Questionnaire

### INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

**_____ days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

**This is the end of the questionnaire, thank you for participating.**

**Appendix D: 24-hours History**

24-Hour History

24-Hour History

ID _____

Treatment _____ Treatment # _____

Date _____ Time _____

1. How many hours of sleep did you get last night? (please circle one)
   1 2 3 4 5 6 7 8 9 10 11 12
   >12

2. How many hours of sleep do you normally get? (please circle one)
   1 2 3 4 5 6 7 8 9 10 11 12
   >12

3. How many hours has it been since your last meal or snack? (please circle one)
   1 2 3 4 5 6 7 8 9 10 11 12
   13 >14

   List what you had here: _____
   _____

4. When did you last have:

   · a cup of coffee or tea? _____
   · drugs (including aspirin)? _____
   · alcohol? _____
   · herbal or dietary supplements? _____

5. How many 8 oz. glasses of water or other beverages have you consumed in the last 24 hours?
   1 2 3 4 5 6 7 8 9 10 11 12
   13 14

6. When did you last consume water or another beverage? _____How much? _____ (glasses)

7. What sort of physical activity did you perform yesterday?

8. What sort of physical activity have you performed today?

9. What was the date of the start of your last menstrual cycle?

10. Describe how you feel right now by checking one of the following:

| | | |
|---|---|---|
| _____excellent | _____good | _____very bad |
| _____very, very good | _____neither good nor bad | _____very, very bad |
| _____very good | _____bad | _____terrible |

**Appendix E: Thermal Perception**

## Thermal Perception (1-8 pt.)

| | |
|---|---|
| 0.0 | Unbearably Cold |
| 0.5 | |
| 1.0 | Very Cold |
| 1.5 | |
| 2.0 | Cold |
| 2.5 | |
| 3.0 | Cool |
| 3.5 | |
| 4.0 | Comfortable |
| 4.5 | |
| 5.0 | Warm |
| 5.5 | |
| 6.0 | Hot |
| 6.5 | |
| 7.0 | Very Hot |
| 7.5 | |
| 8.0 | Unbearably Hot |

Source: Young AJ, Sawka MN, Epstein Y, Decristofano B, and Pandolf KB. Cooling different body surfaces during upper and lower body exercise. J Appl Physiol 63:1218-1223, 1987.

## Appendix F: BRUNEL PROFILE OF MOOD STATES (BRUMS)

### Circle the answer which describes HOW YOU FEEL RIGHT NOW

| | Not at all | A little | Moderately | Quite a bit | Extremely |
|---|---|---|---|---|---|
| **Panicky** | 0 | 1 | 2 | 3 | 4 |
| **Lively** | 0 | 1 | 2 | 3 | 4 |
| **Confused** | 0 | 1 | 2 | 3 | 4 |
| **Worn out** | 0 | 1 | 2 | 3 | 4 |
| **Depressed** | 0 | 1 | 2 | 3 | 4 |
| **Downhearted** | 0 | 1 | 2 | 3 | 4 |
| **Annoyed** | 0 | 1 | 2 | 3 | 4 |
| **Exhausted** | 0 | 1 | 2 | 3 | 4 |
| **Mixed- up** | 0 | 1 | 2 | 3 | 4 |
| **Sleepy** | 0 | 1 | 2 | 3 | 4 |
| **Bitter** | 0 | 1 | 2 | 3 | 4 |
| **Unhappy** | 0 | 1 | 2 | 3 | 4 |
| **Anxious** | 0 | 1 | 2 | 3 | 4 |
| **Worried** | 0 | 1 | 2 | 3 | 4 |
| **Energetic** | 0 | 1 | 2 | 3 | 4 |
| **Miserable** | 0 | 1 | 2 | 3 | 4 |
| **Muddled** | 0 | 1 | 2 | 3 | 4 |
| **Nervous** | 0 | 1 | 2 | 3 | 4 |
| **Angry** | 0 | 1 | 2 | 3 | 4 |
| **Active** | 0 | 1 | 2 | 3 | 4 |
| **Tired** | 0 | 1 | 2 | 3 | 4 |
| **Bad tempered** | 0 | 1 | 2 | 3 | 4 |
| **Alert** | 0 | 1 | 2 | 3 | 4 |
| **Uncertain** | 0 | 1 | 2 | 3 | 4 |

**Appendix G: Thirst Scale**

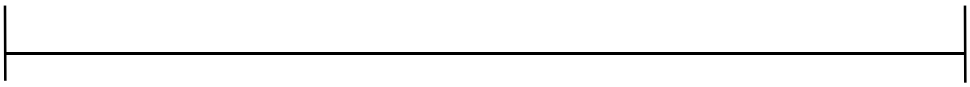
## THIRST SCALE (1-9 pt)

| | |
|---|---|
| 1 | Not Thirsty At All |
| 2 | |
| 3 | A Little Thirsty |
| 4 | |
| 5 | Moderately Thirsty |
| 6 | |
| 7 | Very Thirsty |
| 8 | |
| 9 | Very, Very Thirsty |

**Appendix H: Visual Analog Scale (VAS)**

**VAS**

**How hard did you have to concentrate to accomplish the tasks successfully?**

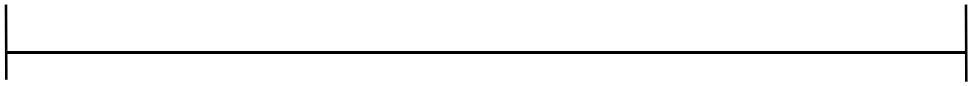


**not at all strong(ly)**

**very strong(ly)**

**VAS**

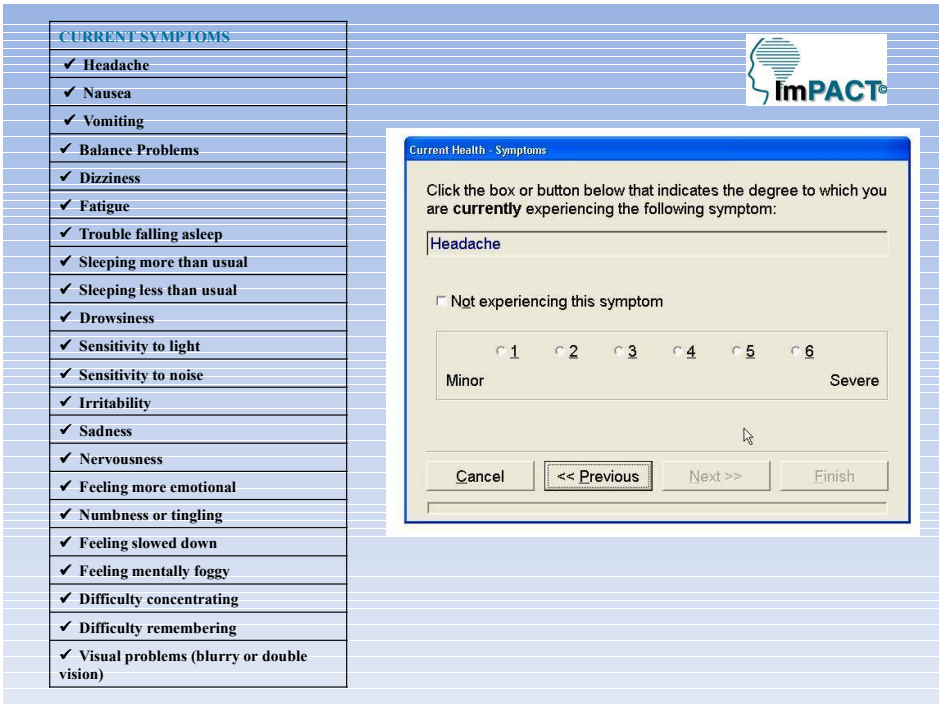
**I have a headache.**

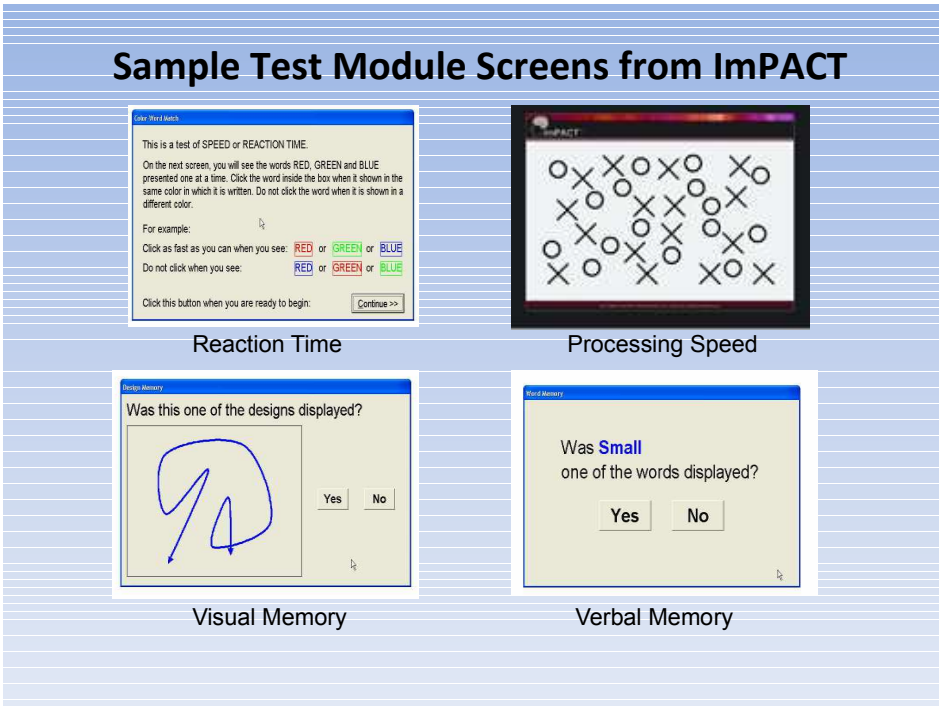


**not at all strong(ly)**

**very strong(ly)**

# Appendix I: Immediate Post-Assessment Concussion Testing (ImPACT)



**Appendix J: NASA Task Load Index (NASA-TLX)**

# NASA TASK LOAD INDEX

NAME: _____ DATE: _____ TIME: _____

---

PLACE A MARK ON EACH SCALE THAT REPRESENTS THE MAGNITUDE OF EACH FACTOR IN THE TASK YOU JUST PERFORMED

---

MENTAL DEMAND How much mental and perceptual activity was required (e.g., thinking, deciding, calculating, remembering, looking, searching, etc)? Was the task mentally easy or mentally demanding, simple or complex, exacting or forgiving?

LOW [——————————] HIGH _____

TEMPORAL DEMAND How much time pressure did you feel due the rate or pace at which the tasks or task elements occurred? Was the pace slow and leisurely or rapid and frantic?

LOW [——————————] HIGH _____

PERFORMANCE How successful do you think you were in accomplishing the goals of the task set by the experimenter? How satisfied were you with your performance in accomplishing these goals?

POOR [——————————] GOOD _____

FRUSTRATION LEVEL How insecure, discouraged, irritated, stressed and annoyed versus secure, gratified, content, relaxed and complacent did you feel during the task?

LOW [——————————] HIGH _____

EFFORT How hard did you have to work (mentally and physically) to accomplish your level of performance?

LOW [——————————] HIGH _____

## Appendix K: Diet Logs

### Directions for Keeping a 24-h Diet Log

For the 24-h preceding the start of the first trial, please write down everything you eat (meals, snacks, beverages)

Use as much space as you need.

1. Write down the date and day at the top of the form.

2. Write down the first foods you ate for that day. Write down:

    a. The time of day you ate the food(s).

    b. Each food that you ate.

    c. How the food was prepared (baked, boiled, fried, microwaved).

    d. How much you ate (cup, ½ cup, pieces, tablespoons, teaspoons).

3. It is important to describe each food you eat in detail.

For example:

    Write down brand names for each food you ate if you know them.

    Write down the type of milk (whole, 2%, or skim) and bread (write, wheat, etc.)

    Write down if the food was fresh, frozen, or canned.

    If you ate a casserole or a salad, write down the foods that were in it and amounts.

    If you add things like butter, jelly, sugar, honey, or cream to foods or beverages, please write them down with the amounts.

4. Do you drink whole ____, 2% ____, 1% ____, or skim ____ milk?

5. Do you use white ____ or whole-wheat ____ bread?

6. What is the complete name and brand name of bread that you eat most often?

_____

7. About how many glasses of water do you drink each day? _____

## ***GOOD*** DIET RECORD

Date of Record ____12/14/03_____ Subject #

Day of Week (circle): MON (TUES) WED THURS FRI SAT SUN

**NOTE**: Please be as specific as possible with recordings. Include detail about the meal such as the brand of the product and what the product is specifically called that you are consuming. If possible measure out amounts consumed. Measuring the volume of frequently used dishware such as cups and bowls is the easiest way to do this.

Food and Beverage Consumed

| Meal | What Did You Eat?<br>What Brand? | Amount | Cooking Method | Time of Day | Activity While Eating |
|---|---|---|---|---|---|
| Example | "Eggland's Best" Eggs | 2 med. | Fried | 7:30 a.m. | Talking w/friends |
| BREAKFAST | Kellogg's Raisin Bran Cereal<br>Skim Milk<br>Egg and American Cheese Omelet<br>Orange Juice, Tropicana- Calcium Fortified | 3/4 cup<br>8 oz<br>3 eggs, 2 slices cheese<br>8 oz | Fried w/ pam | 8:30 a.m. | |
| SNACK | | | | | |
| LUNCH | Wheat Bread (Arnold's)<br>Sliced Turkey- Deli Meat<br>Apple, medium, diced, no skin<br>Soup- Minestrone (Campbells)<br>Crackers (Ritz)<br>Soda, Coca-Cola | 2 slices<br>3 thin slices<br>1<br>1 ½ cups<br>4<br>12 oz | Canned | 12:30 a.m. | |
| SNACK | Yogurt (Breyer's), Low-Fat, Smooth and Creamy<br>Coffee w/ cream | 8 oz<br>12 oz, 1 tsp cream | | 3:00 p.m. | |
| DINNER | Chicken Breast, boneless, skinless<br>Baked Potato w/ skin<br>Cheddar Cheese<br>Broccoli Pieces<br>Salad- Romaine Lettuce<br>Tomato<br>Salad Dressing (Kraft), French, Low-fat<br>Skim Milk (Skim Plus) | 4 oz<br>1 large<br>2 oz<br>¼ cup<br>1 cup<br>¼ cup<br>2 TBSP<br>8 oz | Grilled, no marinade<br><br><br>Steamed<br>Fresh<br>Fresh | 7:00 p.m. | |
| SNACK | Ice Cream (Edy's), Chocolate | ¾ cup | | 9:30 p.m. | |

DIET RECORD

Date of Record _____ Subject # _____

Day of Week (circle): MON TUES WED THURS FRI SAT SUN

**NOTE:** Please be as specific as possible with recordings. Include detail about the meal such as the brand of the product and what the product is specifically called that you are consuming. If possible measure out amounts consumed. Measuring the volume of frequently used dishware such as cups and bowls is the easiest way to do this.

Food and Beverage Consumed

| Meal | What Did You Eat? What Brand? | Amount | Cooking Method | Time of Day | Activity While Eating |
|---|---|---|---|---|---|
| Example | "Eggland's Best" Eggs | 2 med. | Fried | 7:30 a.m. | Talking w/friends |
| BREAKFAST | | | | | |
| SNACK | | | | | |
| LUNCH | | | | | |
| SNACK | | | | | |
| DINNER | | | | | |
| SNACK | | | | | |

UNIVERSITY OF ARKANSAS

*Office of Research Compliance*
*Institutional Review Board*

May 16, 2014

MEMORANDUM

| | |
|---|---|
| TO: | Nicole Moyen<br>Jenna Burchfield<br>Matthew Tucker<br>AZ Satterfield<br>Ashley Six<br>R.J. Elbin<br>Matthew Ganio |
| FROM: | Ro Windwalker<br>IRB Coordinator |
| RE: | New Protocol Approval |
| IRB Protocol #: | 14-05-719 |
| Protocol Title: | *Effect of Dehydration and Heat Stress on Cognitive and Thermoregulatory Responses in Females of Various Body Sizes* |
| Review Type: | ☐ EXEMPT ☐ EXPEDITED ☒ FULL IRB |
| Approved Project Period: | Start Date: 05/16/2014 Expiration Date: 05/15/2015 |

Your protocol has been approved by the IRB. Protocols are approved for a maximum period of one year. If you wish to continue the project past the approved project period (see above), you must submit a request, using the form *Continuing Review for IRB Approved Projects*, prior to the expiration date. This form is available from the IRB Coordinator or on the Research Compliance website (http://vpred.uark.edu/210.php). As a courtesy, you will be sent a reminder two months in advance of that date. However, failure to receive a reminder does not negate your obligation to make the request in sufficient time for review and approval. Federal regulations prohibit retroactive approval of continuation. Failure to receive approval to continue the project prior to the expiration date will result in Termination of the protocol approval. The IRB Coordinator can give you guidance on submission times.

**This protocol has been approved for 50 participants.** If you wish to make *any* modifications in the approved protocol, including enrolling more than this number, you must seek approval *prior to* implementing those changes. All modifications should be requested in writing (email is acceptable) and must provide sufficient detail to assess the impact of the change.

If you have questions or need any assistance from the IRB, please contact me at 210 Administration Building, 5-2208, or irb@uark.edu.