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Effect of Hydration Status on Thermoregulatory Responses in Non-Obese and Obese Males

Matthew Tucker
University of Arkansas, Fayetteville

Effect of Hydration Status on Thermoregulatory Responses in Non-Obese and Obese Males

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by

Matthew Alan Tucker
University of Canterbury
Bachelor of Sports Coaching, 2008
Indiana State University
Master of Arts in Exercise Science, 2011

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University of Arkansas

This dissertation is approved for recommendation to the Graduate Council.

Dr. Matthew Ganio
Dissertation Director

Dr. Stavros Kavouras
Committee Member

Dr. Brendon McDermott
Committee Member

Dr. Ronna Turner
Committee Member

Dr. Tyrone Washington
Committee Member

Abstract

During heat stress the human body thermoregulates via cutaneous vasodilation and sweating. Hypohydration can impair thermoregulatory responses that stem from the central nervous system (CNS), but it is unknown if impairments also occur post-synaptically in the microcirculation. Moreover, obese individuals may have impaired thermoregulation, possibly due to microvascular dysfunction. **Purpose:** The purpose of these studies was two-fold: 1) to determine if obese (OB) individuals exhibit impairments in thermoregulatory responses during exercise heat-stress (centrally-mediated) and intradermal infusion of vasoactive substances (peripherally-mediated) versus non-obese (N-OB), and 2) to determine if hypohydration subsequently affects these thermoregulatory responses differently between groups. **Methods:** Twenty-one healthy, college-age males were classified as either N-OB (n = 11, body fat [BF] <20%) or OB (n = 10, BF >26%) and completed a comprehensive 2-day protocol. In a randomized, counter-balanced order, subjects performed 60 min of cycling in a hot environment while either euhydrated (EU) or hypohydrated (HY) (Study 1). Changes in rectal temperature (ΔT_{rec}), cutaneous vascular conductance (CVC), and local sweat rate (LSR) were recorded. Following exercise, subjects maintained a EU or HY condition and returned 24-h later to undergo cutaneous microdialysis (MDS) of the forearm (Study 2). Dose-response curves comparing CVC and LSR responses were compared while sub-cutaneously perfusing the endothelium-dependent vasodilator methacholine chloride (MCh) and the endothelium-independent vasodilator sodium nitroprusside (SNP). **Results:** In Study 1, compared to EU, HY increased end-exercise ΔT_{rec} in N-OB (0.47 ± 0.37°C, $p < 0.01$) but did not in OB (-0.06 ± 0.29°C, $p > 0.05$). LSR and CVC were not different between groups or hydration condition ($p > 0.05$). In study 2, OB subjects had a higher Log EC_{50} versus N-OB for endothelium-independent

CVC (-1.69 ± 0.17 vs. -2.13 ± 0.06 Log [SNP] M, $p = 0.014$) when EU. There were no differences between groups in endothelium-dependent CVC or LSR responses in either hydration condition (all $p > 0.05$). **Conclusions:** These data suggest that hydration status affects the core body temperature response differently in N-OB and OB males during exercise heat-stress. In addition, OB individuals appear to have impaired post-synaptic endothelium-independent CVC, but similar endothelium-dependent CVC and LSR versus N-OB.

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CHAPTER ONE

Introduction

The ability to dissipate heat and maintain appropriate thermal homeostasis is vital for humans, both at rest and during conditions of physiological stress (i.e., exercise and/or heat stress). Heat dissipation occurs primarily through increases in sweating (evaporative heat loss) and skin blood flow (convective heat loss). Impairments in these thermoregulatory responses can lead to drastic increases in core body temperature when individuals are exposed to high environmental temperatures and/or during exercise. Changes in thermoregulatory function are mediated through two primary avenues: 1) the central nervous system (CNS, "centrally mediated") and 2) at the level of the skin ("peripherally mediated"). The hypothalamus is responsible for maintaining thermal homeostasis, and as such, will respond to increases in body temperature, bringing about a cascade of events that lead to increases in sweating and a redistribution of blood flow from the core to the skin in an effort to dissipate heat. In addition, changes in sweating and skin blood flow can also be modified by changes in skin temperature (15) and/or infusion of certain drugs (8, 11), independent of changes in core body temperature or neural signaling. Thus, impairments in thermoregulation may be present in more than one location of the body. For example, the CNS could misinterpret changes in skin/core temperature and not send the appropriate response signal to the skin. Alternatively, temperature changes may be interpreted and an appropriate CNS response initiated, but impairments at the level of the skin (i.e., blood vessels and/or sweat glands) result in an impaired response (i.e., post-synaptic impairment).

Obesity is often suggested to be a contributing factor to increased core temperature (i.e., hyperthermia) and epidemiological data support the hypothesis that obese individuals are at increased risk for developing heat illness (4, 6). Early research suggested that during exercise, obese individuals have lower heat tolerance, lower sweat production, and less heat activated sweat glands versus non-obese individuals (2, 3). Further, the largest influence on body temperature during exercise has been suggested to be body fat and the surface area to mass ratio (12). Vroman et al. demonstrated that obese individuals have lower forearm blood flow (an index of skin blood flow) when exercising in a hot environment compared to their non-obese counterparts (18). This decreased skin blood flow was thought to be attributed to increased sympathetic vasoconstrictor activity to the obese skin circulation. In contrast, more recent investigations, accounting for differences in relative exercise intensity through controlling metabolic heat production, have shown no differences in thermoregulatory responses between obese and non-obese males (13) and females (1) during low intensity aerobic exercise. Conflicting observations in the current literature may be due to differences in study design (i.e., relative versus absolute exercise workloads) or the amount of physiological stress imposed during the investigation. The type of workload is important when comparing populations with differing levels of body fatness (7, 10). The use of a relative metabolic heat production workload (e.g., Watts per kg body mass) allows for fair comparisons between groups since it accounts for individual differences in body mass and subsequent power generation (i.e., wattage) while cycling. Further, exercise protocols that induce a greater amount of physiological stress (i.e., higher intensity physical activity) are more likely to elicit differences in thermoregulatory function between various populations, as observed recently with older individuals (17).

It is also possible that hydration status influences thermoregulatory responses differently in obese versus non-obese individuals. Dehydration has been shown to have a significant effect on thermoregulatory function in non-obese individuals during exercise. Dehydration prior to and during exercise leads to increased core body temperature secondary to reductions in skin blood flow and sweating, and these effects are directly related to the magnitude of dehydration (14, 16). During exercise, the thermoregulatory system is somewhat at odds with the renin-angiotensin-aldosterone-system (RAAS) in that increases in fluid losses (i.e., sweating) must occur to preserve thermal homeostasis, but RAAS is functioning to maintain body water homeostasis (i.e., fluid conservation). In obese individuals, increased basal plasma renin activity and angiotensin II levels are associated with increased sympathetic nerve activity (SNA) and subsequent increased vasoconstriction (5). Given the complexity of maintaining body fluid balance, particularly under conditions of physiological stress, it is possible that dehydration affects the relationship between RAAS and thermoregulatory function to a greater degree in obese versus non-obese individuals during exercise. Therefore, impairments in thermoregulatory function in obese individuals that may be present when euhydrated could be further exacerbated when dehydrated versus non-obese counterparts.

During exercise heat stress, local sweat rate and skin blood flow increase as body temperature increases. Increases in these parameters are a result of changes in core and skin temperature, and as such, are driven from a combination of central and peripheral inputs to the hypothalamus. More recently, the use of cutaneous microdialysis has been used to isolate post-synaptic thermoregulatory function in a number of different populations (8, 9, 11). That is, outcomes from microdialysis provide insight into whether or not there is an impaired thermoregulatory responses at the level of the skin (i.e., blood vessels and/or sweat glands). The

technique allows for assessment of cutaneous active vasodilation and sweating by sub-cutaneously infusing known vasodilator and neurotransmitter substances that induce localized increases in skin blood flow and sweating. Since this drug infusion occurs at the level of the skin, localized changes in thermoregulatory function are not driven by the CNS (i.e., they are mediated peripherally, post-synaptically, at the level of the skin).

In order to test the hypothesis that differences in thermoregulatory function between obese and non-obese individuals are modified by hydration status, subjects completed a comprehensive protocol while either euhydrated or hypohydrated. This study involved an exercise protocol to assess whole-body thermoregulatory responses and a skin microdialysis procedure to assess cutaneous thermoregulatory responses. The two-day protocol was performed twice; once while euhydrated and once while hypohydrated. This allowed for a thorough investigation of possible differences between obese and non-obese individuals in centrally mediated (whole-body heat stress) versus peripherally mediated (microdialysis) thermoregulatory function and how the magnitude of these differences were modified affected by hydration status.

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CHAPTER TWO

Study 1:

Effect of Hypohydration on Thermoregulatory Responses in Obese and Non-Obese Males Exercising in the Heat

Effect of Hypohydration on Thermoregulatory Responses in Obese and Non-Obese Males Exercising in the Heat

Matthew A. Tucker, Aaron R. Caldwell, Cory L. Butts, Forrest B. Robinson, Haley C. Reynebeau, Stavros A. Kavouras, Brendon P. McDermott, Tyrone A. Washington, Ronna C. Turner, & Matthew S. Ganio

Department of Health, Human Performance, and Recreation, University of Arkansas, Fayetteville, AR 72701, USA

Address for Correspondence:

Matthew S. Ganio, Ph.D.
Human Performance Laboratory
University of Arkansas
155 Stadium Dr.
HPER 321
Fayetteville, AR 72701
479-575-2517
msganio@uark.edu

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Abstract

Obesity may be associated with impaired cutaneous vasodilation and sweating responses to exercise heat stress. Hypohydration (HY) impairs thermoregulatory responses in non-obese (N-OB) individuals, but it is unknown if HY affects these responses differently in obese (OB) versus N-OB individuals compared to a euhydrated (EU) condition. **Purpose:** To test the hypothesis that OB males have impaired thermoregulatory responses to exercise heat-stress versus N-OB, and HY further exacerbates these responses. **Methods:** N-OB (n = 11, BM 73.9 ± 8.5 kg, BF% 13.6 ± 3.8) and OB (n = 9, BM 89.6 ± 6.9 kg, BF% 30.2 ± 4.1) males, in a randomized cross-over design, performed 60 min of upright cycling in a hot environment (40.3 ± 0.4°C, relative humidity 32.5 ± 1.9%) at a metabolic heat production of 6 W/kg BM while either euhydrated (EU) or HY. Change in rectal temperature (ΔT_{rec}), local sweat rate (ΔLSR), and cutaneous vascular conductance [expressed as percent of maximum, $\%CVC_{max}$]) from pre-exercise baseline were collected throughout. **Results:** When EU, both N-OB had similar CVC and LSR responses ($p > 0.05$); however, N-OB had a lower ΔT_{rec} versus OB (0.92 ± 0.35 vs. 1.31 ± 0.32°C, $p = 0.021$). Compared to EU, HY increased end-exercise ΔT_{rec} in N-OB (0.47 ± 0.37°C, $p < 0.01$) but did not in OB (-0.06 ± 0.29°C, $p > 0.05$). ΔLSR and ΔCVC were not different between groups or hydration condition ($p > 0.05$). **Conclusions:** These data suggest that hypohydration affects cutaneous vasodilatory and sweating responses to exercise heat-stress in a similar manner in N-OB and OB males. However, hypohydration increases rectal temperature versus a euhydrated condition in N-OB but not OB males.

Introduction

The ability to effectively dissipate heat during exercise is critical to preventing excessive increases in core body temperature and subsequent heat illness (23). Obesity continues to be a significant global health concern (33) and data suggest that this population may be at an increased risk of developing heat illness (7, 14). Previous studies suggest that obese individuals may have impaired thermoregulatory responses versus non-obese counterparts during sustained aerobic exercise. During exercise heat stress, obese individuals have been shown to have lower heat tolerance, sweat production, and less heat activated sweat glands versus non-obese individuals (5, 6). Similarly, decreased forearm blood flow (an index of skin blood flow) has been observed in obese males exercising in a hot environment (51).

Despite these initial findings regarding thermoregulation in obese individuals, recent work examining how best to compare individuals of different body size suggests earlier work in this area may be systematically flawed (16, 18). Thus, the question of whether obese individuals truly have impairments in thermoregulatory responses, particularly during exercise, remains unclear. Assigning metabolic heat production relative to body mass has been accepted as the correct method to compare thermoregulatory responses between groups (16). Dervis et al. observed a higher core body temperature during an aerobic exercise bout in high- versus low-body fat groups matched for body mass when cycling at a workload of ~6 W/kg body mass (18). This was despite no differences in local or whole body sweating responses. However, exercise was performed in a compensable environment (28°C, 26% relative humidity); thus, it is unclear how these responses may differ in a physiologically uncompensable environment. Further evidence suggests that individuals of large body mass can have significantly higher local and whole body sweat rates versus those with lower body mass during exercise, independent of

differences in metabolic heat production normalized to body surface area (17). This suggests that individuals with greater body mass may have an impaired capacity for heat dissipation during exercise heat stress versus individuals of lower body mass (i.e., obese versus non-obese).

Acute and chronic dehydration (herein referred to as hypohydration) have been shown to have a significant influence on core temperature, sweating, and skin blood flow responses during exercise in non-obese individuals (34-36, 46). Moreover, the degree of hypohydration is directly related to the magnitude of reduction in these responses (35, 46). However, it is unknown if the increased thermal strain accompanying hypohydration in non-obese affects obese individuals to the same extent during exercise. In addition, in much of the previous work investigating thermoregulatory differences between groups with large differences in body mass and/or body fat, hydration status was either not controlled during exercise or was not reported (5, 6, 16, 18, 51). Since obesity is associated with increased basal plasma renin activity and angiotensin II levels, which is associated with increased sympathetic nerve activity (SNA) (3, 11, 30, 42), obese individuals may exhibit an impaired vasodilatory response during exercise, and thus, reduced convective heat loss versus non-obese. Further, under conditions of increased physiological stress (i.e., hypohydration), any potential reductions in convective heat loss that are present when euhydrated may be further exacerbated. Given the complexity of maintaining body fluid balance, particularly under conditions of physiological stress, it is possible that hypohydration affects the relationship between body fluid balance mechanisms (i.e., the renin-angiotensin-aldosterone-system) and thermoregulatory function to a greater degree in obese versus non-obese individuals during exercise. Therefore, impairments in thermoregulatory function in obese individuals that may be present when euhydrated could be further exacerbated when dehydrated versus non-obese counterparts.

Therefore, the aim of the present study was two-fold: 1) to determine if impairments in thermoregulatory responses are present in obese (OB) versus non-obese (N-OB) males during an exercise heat stress bout in a physiologically uncompensable environment while euhydrated, and 2) to determine if hypohydration subsequently affects thermoregulatory responses differently in OB versus N-OB males during exercise heat stress. We hypothesized that OB subjects would have impairments in thermoregulatory responses while euhydrated, and this impairment would be further exacerbated while hypohydrated versus their N-OB counterparts.

Materials and Methods

Subjects

Twenty healthy adult males from the University and surrounding community volunteered to participate in the study. In order to be considered eligible, subjects were required to have a stable body weight (i.e., not actively trying to lose or gain weight), be willing to abstain from caffeine and alcohol on lead-in and testing days, be free of any medications or supplements that may affect body weight or fluid balance, be free of any metabolic and/or cardiovascular disorders, and abstain from physical activity beyond normal activities of daily living during a 48-h experimental period. All subjects were required to provide written informed consent prior to participation through signing a document that was approved by the University's Institutional Review Board.

An *a priori* power calculation was performed using SigmaPlot v. 12 (Systat Software Inc., San Jose, CA) with an α of 0.05, a β of 0.20, and an estimated smallest significant difference (0.35°C) in the primary outcome variable (rectal temperature change; ΔT_{rec}) from

previous studies using similar protocols (16, 18, 25), adequate power could be achieved with a sample of nine subjects per group.

During a screening visit, body fat (BF) percentage, fat-free mass (FFM), and lean body mass (LBM) were determined via dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, General Electric®, Madison, WI). Subjects with a BF <18% were classified as non-obese (N-OB; n = 11) and those ≥26% obese (OB; n = 9) (27). Subject demographic data are presented in Table 1. Subjects had their height measured using a standard stadiometer (Seca 216 stadiometer, Chino, CA) and nude body mass (BM) using a platform scale (Health-O-Meter, Model 349KLX, Alsip, IL). These values were used to calculate body surface area (BSA) according to Dubois and Dubois (20) and body mass index (BMI). Mean specific heat of the body (C_p) was estimated using a previously described formula (28). A digital bathroom scale was provided to subjects (High Accuracy Bathroom Scale, BalanceFrom LLC, China) to measure morning euhydrated BM during a 3-day baseline period to use for calculations of BM change with various hydration states (13). Subjects completed the International Physical Activity Questionnaire (9) to assess habitual physical activity levels. Peak oxygen uptake (VO_{2peak}) was measured via indirect calorimetry (TrueOne® 2400, Parvo Medics, Sandy, UT) with subjects completing an incremental graded exercise test on a mechanically-braked cycle ergometer (Veletron, RacerMate Inc., Seattle, WA). Starting at a resistance of 50 W, resistance increased 25 W every two minutes until either volitional fatigue or a reduction in cadence below 30 rpm occurred. Values of absolute VO_2 (L/min) and respiratory exchange ratio (RER) from this test were used to calculate appropriate starting external resistance during the subsequent experimental trials.

Experimental Procedures

To assess differences in thermoregulatory function during exercise in the heat between N-OB and OB males, in a randomized, counter-balanced order, subjects completed 60 min of cycling in a hot environment (ambient temperature 40.3 ± 0.4°C, relative humidity 32.5 ± 1.9%) while either euhydrated (EU) or hypohydrated (HY). For both trials, 24-h prior to subjects' arrival at the laboratory for testing, they were required to abstain from physical activity outside of activities of daily living and were restricted to drinking water only. During this same period, subjects recorded all food intake that could then be replicated for the second trial. For EU trials, euhydration was achieved through prescribing 24-h water intake of 45 ml·kg^{-1} and hypohydration was achieved in the HY trials via 24-h fluid restriction of 237 ml (~8 oz).

Following instrumentation and collection of baseline urine and blood measures, subjects entered an environmental chamber wearing athletic shorts and shoes and rested on the cycle saddle for ~20 min to allow body fluid compartments to stabilize. Baseline perceptual and physiological measures were made before a blood sample was taken. Subjects then began exercise starting at a pre-determined external wattage with a target relative metabolic heat production (MHP; W·kg^{-1}) of 6 W·kg^{-1}. Absolute VO_2 (L/min) and RER were assessed every 5-10 min during exercise and external wattage was adjusted accordingly to ensure the target MHP was maintained. Metabolic heat production was calculated by subtracting external work performed (Watts) from metabolic energy expenditure. Metabolic energy expenditure (M) was calculated from VO_2 and RER during exercise using the formula $M = VO_2[(((RER - 0.7)/0.3) \times e_c) + (((1 - RER)/0.3) \times e_f)]$, where e_c is the caloric equivalent per liter of oxygen for the oxidation of carbohydrates (21.13 kJ), and e_f is the caloric equivalent per liter of oxygen of fat (19.62 kJ) (39). Heat storage (S; W·m^{-2}) was calculated as $S = (0.965 \cdot BM)((0.9 \cdot \Delta T_{rec})+(0.1 \cdot \Delta T_{sk}))/BSA$, where BM is pre-exercise body mass (kg), ΔT_{rec} is the

change in rectal temperature (T_{rec}; °C), ΔT_{sk} is the change in mean skin temperature (T_{sk}; °C), and BSA is body surface area (m^2) (2).

At 15, 30, 45, and 60 min, perceptual data, blood pressure, and sweat gland activation (SGA) were collected. Heart rate, T_{rec}, T_{sk}, SkBF, and LSR were recorded continuously throughout the protocol using data acquisition software (LabChart 7, ADInstruments, Colorado Springs, CO) at a frequency of 50 Hz. Reported values for these variables were taken during ~20-30 s periods at the specific time points (i.e., 15 min intervals) during blood pressure measurement. During the EU trials, subjects were provided warm drinking water (~38°C) at 15, 30, and 45 min in ~5 $ml \cdot kg^{-1}$ boluses to maintain euhydration. This fluid intake volume was chosen following pilot testing and was adequate to ensure euhydration (assessed via %BM change) for all subjects. Fan created airflow was not used during exercise; however, subjects' skin was regularly wiped down with a towel to facilitate evaporative heat loss. Immediately following exercise, a body mass was taken after subjects were toweled dry and had removed shoes and a urine sample was collected.

Experimental Measures

Blood pressure (Tango+, SunTech Medical, Morrisville, NC) was measured at the right brachial artery via electrosphygmomanometry, and HR was measured using a standard Polar® heart rate monitor (Polar Electro, Lake Success, NY). Mean arterial pressure (MAP) was calculated as MAP = (1/3·systolic blood pressure) + (2/3·diastolic blood pressure).

Rectal temperature (T_{rec}) was measured using a rectal thermistor (Physitemp Instruments Inc., Clifton, NJ) inserted at least 15 cm beyond the anal sphincter. Skin temperature (T_{sk}) was measured using four Type T thermocouples (Omega Engineering, Stamford, CT) placed on the

left 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 using the formula T_{sk} = 0.3(chest) + 0.3(upper arm) + 0.2(thigh) + 0.2(calf) (41). Rectal and mean skin temperatures were used to calculate mean body temperature (T_b) using the formula $T_b = 0.1(T_{sk}) + 0.9(T_{rec})$ (49).

Red blood cell flux, an index of skin blood flow (SkBF), was assessed by laser-Doppler flowmetry (38) on the left dorsal forearm with a probe (laser-Doppler perfusion monitor and Probe 2b, Moor Instruments, Wilmington, DE) held in place by a local heater (Perflux System 5000, Perimed, Ardmore, PA) and attached to the skin surface with adhesive tape. Changes in cutaneous vasomotor activity, expressed as cutaneous vascular conductance (CVC), were calculated by dividing red blood cell flux by MAP and reported as a percentage of maximal CVC (%CVC_{max}). Maximal CVC was determined at the end of the trial by locally heating the skin at 44°C for 30 minutes or until a plateau occurred (12).

Adjacent to the laser-Doppler probe, local sweat rate (LSR) was measured using a 2.85 cm^2 ventilated capsule held on the skin by adhesive tape. Dry nitrogen gas was supplied through the capsule at a rate of 0.3 $l \cdot min^{-1}$. The absolute humidity (g/m^3) and ambient temperature from the effluent air of the capsule were monitored by a humidity and temperature sensor (HMT333, Vaisala, Woburn, MA) and LSR ($mg \cdot cm^{-2} \cdot min^{-1}$) was calculated as LSR = ([flow rate in mg^3/min·absolute humidity in gm/^{3}] / [capsule surface area in cm^2])·1000. Whole body sweat rate (WBSR; L/h) and percent BM loss were calculated using nude BM measures pre- and immediately post-exercise, accounting for any fluid ingested during the protocol, respiratory water loss, blood loss from sampling during exercise, and urine formation.

SGA was measured by wiping the skin dry and lightly applying a 2.85 cm^2 circular piece of iodine impregnated paper to a site immediately adjacent to the local sweat rate capsule for ~5 s (26). Two consecutive samples were collected at 15, 30, 45, and 60 min during exercise. All images were immediately scanned and analyzed as previously described (26). Relative SGA (glands/cm^2) was determined by dividing the number of active sweat glands by 2.85 cm^2.

Sweat output per gland (SGO; $\mu g \cdot gland^{-1} \cdot min^{-1}$) was calculated by dividing LSR at the time of measurement by the corresponding number of activated sweat glands. Methodologically, it is not possible to collect SGA samples underneath the sweat capsule during measurement of LSR (ambient humidity would render the LSR inaccurate). However, to confirm that the same number of sweat glands were activated underneath the sweat capsule as the adjacent site (where SGA was measured throughout exercise), SGA was collected under the sweat capsule during the last minute of exercise (~60 min). Differences between the LSR and adjacent sites were 35 ± 43 and 4 ± 45 in the EU trials and 31 ± 43 and 4 ± 22 glands/cm^2 in the HY trials in N-OB and OB groups, respectively. Due to technical difficulties, several subjects were unable to be included in the final analysis. Data for SGA and SGO are reported as N-OB (n = 8) and OB (n = 6).

Throughout the exercise protocol, subjects were asked to provide ratings of perceived exertion (RPE; range 6-20), thermal sensation (TS; 0.5-10), muscle pain (MP; 0.0-10), thirst (TH; 0-9), comfort (COM; 1-5), and motivation to continue (MOT; 1-5) (10, 15, 22, 50). Results of these measures are provided in Appendix A.

Urine and Blood Analysis

Upon arriving at the laboratory, subjects provided a urine sample which was analyzed for U_{SG} using a hand-held refractometer (Master-SUR/NM, ATAGO, Japan), osmolality (U_{osm}), and

color. Urine osmolality was measured in duplicate using freezing point depression osmometry (Model 3250, Advanced Instruments Inc., Norwood, MA). Color was assessed in a well-lit room using the eight-level color scale where the sample was in a glass tube against a plain white background (4, 31). The same measures were performed on a post-exercise urine sample and samples were weighed to the nearest 5 g (i.e., 5 ml) (OHAUS Catapult 1000, Pine Brook, NJ) to determine volume.

Baseline blood samples (collected outside the environmental chamber) were collected via an intravenous catheter (SurFlash®, Terumo Corporation, Tokyo, Japan) placed in a superficial forearm vein while subjects were in a reclined position in a phlebotomy chair for at least 20 min. Whole blood was drawn into a 6 ml Vacutainer collection tube with EDTA additive for analysis of hemoglobin (Hb), hematocrit (Hct), and carboxyhemoglobin concentration (HbCO%). A 4 ml clot activator tube was used for analysis of serum osmolality (S_{osm}). Osmolality was measured in duplicate fresh samples using freezing point depression osmometry (Model 3250, Advanced Instruments Inc., Norwood, MA). Hemoglobin was measured in triplicate 10 µl samples using a HemoCueHb 201+ analyzer (HemoCue AB, Angelholm, Sweden). Hematocrit was analyzed in triplicate 35 µl samples drawn into microcapillary tubes, spun down in a microcentrifuge for three minutes at 12,000 rpm (UNICO model C-MH30, Dayton, NJ), and values measured on a Damon Micro-Capillary Reader (Needham Heights, MA).

Determination of Red Cell Volume, Plasma Volume, Blood Volume, and Plasma Volume Change

Red cell volume (RCV), plasma volume (PV), and blood volume (BV) were determined using the optimized CO-rebreathing method described by Schmidt and Prommer (47). A baseline

blood sample was drawn and analyzed for HbCO% using an ABL80 FLEX OSM-3 co-oximeter (Radiometer, Denmark). Additional blood samples were drawn and analyzed at 6 and 8 minutes after beginning the rebreathing procedure, with an average of these measures (i.e., 7 minutes) being used for the calculation of ΔHbCO%. A portable CO analyzer with parts-per-million sensitivity (Pac 7000, Dräger Safety AG & Co. KGaA, Lübeck, Germany) was used to monitor potential gas leaks during the rebreathing procedure and to determine CO concentration remaining in the lungs and breathing bag following completion of the procedure. RCV, PV, and BV were then calculated using baseline values of [Hb] and Hct (described previously) using the following formulas (29):

$$RCV = BV * Hct$$

$$PV = BV - RCV$$

$$BV = RCV / [Hb]$$

Relative PV and BV (PV_{rel} and BV_{rel}, respectively) were calculated by dividing PV and BV by pre-exercise BM. Due to technical difficulties, results are presented as N-OB (n = 9) and OB (n = 6) for RCV, PV, BV, PV_{rel} and BV_{rel}.

Statistical procedures

Statistical analyses were performed using SPSS v. 23 for Windows (IBM Corporation, Somers,, NY). Two-way repeated measures ANOVA were used to compare changes in HR, MAP, T_{rec}, T_{sk}, $\%CVC_{max}$, and LSR during exercise when each subject group was EU. To test how hypohydration differentially affected these measures *between* groups, a difference valuable was calculated by subtracting EU from HY values at each time point. Results of analyses comparing groups only when HY are provided in Appendix B. Differences in hydration

measures, WBSR, external workload, MHP, heat storage, and exercise intensity were compared within and between groups using dependent and independent t-tests, respectively.

While all N-OB subjects completed the full exercise protocol in both hydration conditions, three OB subjects were unable to finish the full 60 min when euhydrated (exercise times of 50, 53, and 53 min, respectively), and two OB subjects were unable to finish while hypohydrated (40 and 53 min). A two-way repeated measures ANOVA was used to compare changes values of HR, T_{rec}, T_{sk}, $\%CVC_{max}$, and LSR from pre-exercise baseline using all subjects up to 45 min of exercise. The 60 min time-point represents the end-exercise value for all subjects and was compared using independent t-tests. Raw values of HR and MAP were also compared in the same manner.

Data are reported as mean ± standard deviation (SD). An alpha level of 0.05 defined significance for all tests. Greenhouse-Geisser corrections and follow up tests were performed as necessary.

Results

External Workload, Metabolic Heat Production (MHP), Heat Storage, and Exercise Intensity

Values of average external workload, MHP (expressed in absolute and relative [per kg body mass] Watts), heat storage ($W \cdot m^2$), and exercise intensity (expressed as $\%VO_{2peak}$) for both groups in each trial are presented in Table 2. As expected, OB subjects required a higher external workload versus N-OB subjects to achieve the same MHP in watts per kg body mass in both the EU ($p = 0.016$) and HY ($p = 0.020$) trials. Consequently, relative exercise intensity was also higher for OB vs. N-OB subjects in the EU ($p = 0.002$) and HY ($p < 0.001$) trials. Likewise, absolute MHP was higher in OB versus N-OB subjects for both the EU ($p = 0.001$) and HY trials ($p < 0.001$). Importantly, external workload, and relative exercise intensity were not different

between the EU and HY trials within groups (all $p > 0.05$). Similarly, MHP ($W \cdot kg^{-1}$) was not different within or between groups in either hydration condition ($p > 0.05$). Heat storage was not different between EU or HY trials in OB subjects ($p = 0.807$), but was significantly lower in N-OB when EU versus HY ($p = 0.006$). While groups had similar levels of heat storage when HY ($p = 0.525$), it was significantly lower in N-OB versus OB when EU ($p = 0.022$).

Hydration Status Measures

Pre- and post-exercise measures of ΔBM, U_{SG}, U_{osm}, U_{col}, and S_{osm} are presented in Table 3. As intended, there was a main effect of hydration condition, with greater pre-exercise ΔBM, U_{SG}, U_{osm}, U_{col}, and S_{osm} in the HY versus EU trials, independent of group (all $p < 0.001$).

Independent of group, pre-exercise, 15, and 60 min S_{osm} was higher in the HY versus EU trials (all $p < 0.001$). During EU trials no differences in S_{osm} were present between N-OB versus OB subjects at pre-exercise (288 ± 4 vs. 288 ± 4), 15 (294 ± 5 vs. 293 ± 4), or 60 min (288 ± 5 vs. 288 ± 4 $mOsm \cdot kg^{-1}$) (all $p > 0.05$). Similarly, in the HY trials no differences were present at pre-exercise (292 ± 4 vs. 293 ± 4), 15 (298 ± 3 vs. 299 ± 5), or 60 min (300 ± 4 vs. 299 ± 5 $mOsm \cdot kg^{-1}$) (all $p > 0.05$).

There were no baseline differences in RCV, absolute PV, or absolute BV between N-OB and OB subjects in either the EU or HY trials (Table 4; all $p > 0.05$). Independent of hydration condition, N-OB subjects had significantly higher PV_{rel} and BV_{rel} versus OB subjects (both $p < 0.001$).

Rectal (T_{rec}), Mean Skin Temperature (T_{sk}), and Mean Body Temperature (T_b) Responses

In the EU trials, ΔT_{rec} increased over time in both groups ($p < 0.001$), but there was no interaction between time and group ($p = 0.150$; Figure 1A). N-OB subjects had a lower ΔT_{rec} versus OB subjects at 45 (0.82 ± 0.31 vs. 1.15 ± 0.26°C) and 60 (0.92 ± 0.35 vs. 1.33 ± 0.34°C) min (both $p < 0.05$).

The effect of hypohydration on ΔT_{rec} during exercise for both groups is presented in Figure 2A. There was a significant interaction between group and time ($p = 0.016$). When hypohydrated, ΔT_{rec} was elevated in N-OB versus OB subjects at 45 and 60 min (0.39 ± 0.39 vs. -0.07 ± 0.33 and 0.47 ± 0.37 vs. -0.06 ± 0.29°C, respectively, both $p < 0.05$) versus the EU condition.

In the EU trials, ΔT_{sk} decreased over time in both groups ($p < 0.001$), but there was no interaction between time and group ($p = 0.645$; Figure 1B). There were no differences between groups at any time point (all $p > 0.05$).

The effect of hypohydration on ΔT_{sk} during exercise for both groups is presented in Figure 2B. During exercise, the interaction between group and time was not significant ($p = 0.197$). However, at 60 min, ΔT_{sk} was further decreased when HY in N-OB (-0.77 ± 1.18°C), but elevated in OB (1.05 ± 0.72°C) compared to the EU condition ($p < 0.001$).

In the EU trials, ΔT_b increased over time in both groups ($p < 0.001$), but there was no interaction between time and group ($p = 0.221$; Figure 1C). N-OB subjects tended to have a lower ΔT_b verus OB subjects at 15 (0.20 ± 0.13 vs. 0.37 ± 0.22°C, $p = 0.066$) and 60 (0.68 ± 0.35 vs. 1.02 ± 0.34°C, $p = 0.054$) min. ΔT_b was lower in N-OB versus OB subjects at 30 and 45 min (0.43 ± 0.20 vs. 0.63 ± 0.19 and 0.57 ± 0.31 vs. 0.89 ± 0.29°C, respectively, $p < 0.05$).

The effect of hypohydration on ΔT_b during exercise for both groups is presented in Figure 2C. During exercise, there was a trend towards a significant interaction between group and time ($p = 0.080$). When hypohydrated, ΔT_b was elevated in N-OB versus OB subjects at 45 and 60 min (0.33 ± 0.30 vs. -0.02 ± 0.33 and 0.38 ± 0.34 vs. 0.05 ± 0.28°C, respectively, both $p < 0.05$) versus the EU condition.

Cutaneous Vascular Conductance (CVC)

For ΔCVC (expressed as a percent of maximum CVC [$\%CVC_{max}$]) in the EU trials, there was a trend towards a main effect of time ($p = 0.076$), but the interaction between group and time was not significant ($p = 0.887$; Figure 3A). There were no differences between groups at any time point (all $p > 0.05$).

The effect of hypohydration on ΔCVC for both groups is presented in Figure 4A. The interaction between group and time was not significant ($p = 0.862$) and there were no differences between groups in ΔCVC at any time point when HY versus EU (all $p > 0.05$).

Local Sweat Rate (LSR), Whole Body Sweat Rate (WBSR), Sweat Gland Activation (SGA) and Sweat Gland Output (SGO)

In the EU trials, there was a main effect of time for LSR ($p < 0.001$), but the interaction between group and time was not significant ($p = 0.395$; Figure 3B). There were no differences between groups at 15, 30, or 45 min (all $p > 0.05$); however, N-OB subjects tended to have a higher LSR versus OB subjects at 60 min (0.90 ± 0.25 vs. 0.70 ± 0.18 $mg \cdot cm^2 \cdot min^{-1}$, $p = 0.062$).

The effect of hypohydration on LSR for both groups is presented in Figure 4B. The interaction between group and time was not significant ($p = 0.939$) and there were no differences between groups in LSR at any time point when HY versus EU (all $p > 0.05$).

There were no differences between N-OB and OB subjects in WBSR for either hydration condition (EU: 1.02 ± 0.29 vs. 1.03 ± 0.64, $p = 0.978$; HY: 0.77 ± 0.23 vs. 0.82 ± 0.33 L/h; Figure 5). Independent of group, WBSR was lower in the HY versus EU trials (i.e., main effect of hydration condition, $p < 0.001$).

SGA (glands/cm^2) did not change over time in the EU trials ($p = 0.529$), nor was there a significant interaction between time and group ($p = 0.864$; Figure 6A). There were no differences between groups at 15 min ($p > 0.05$); however, N-OB tended to have higher SGA versus OB at 30, 45, and 60 min (110 ± 93 vs. 35 ± 42, $p = 0.072$; 134 ± 113 vs. 43 ± 26, $p = 0.060$; and 146 ± 137 vs. 44 ± 22 glands/cm^2, $p = 0.074$).

The effect of hypohydration on SGA for both groups is presented in Figure 7A. The interaction between group and time was not significant ($p = 0.619$) and there were no differences between groups in SGA at any time point when HY versus EU (all $p > 0.05$).

SGO ($\mu g \cdot gland^{-1} \cdot min^{-1}$) did not change over time in the EU trials ($p = 0.084$), nor was there a significant interaction between time and group ($p = 0.243$; Figure 6B). There were no differences between groups at 15, 30, or 60 min (all $p > 0.05$); however, OB tended to have higher SGO versus N-OB at 45 min (23 ± 11 vs. 11 ± 10 $\mu g \cdot gland^{-1} \cdot min^{-1}$, $p = 0.061$).

The effect of hypohydration on SGO for both groups is presented in Figure 7B. The interaction between group and time was not significant ($p = 0.745$) and there were no differences between groups in SGO at any time point when HY versus EU (all $p > 0.05$).

Heart Rate (HR) and Mean Arterial Pressure (MAP)

In the EU trials, there was a significant interaction between group and time for HR from pre-exercise baseline to 45 min ($p = 0.001$; Figure 8A). OB subjects' HR was higher versus N-OB at 15, 30, and 45 min (165 ± 23 vs. 140 ± 26; 166 ± 20 vs. 145 ± 22; 165 ± 25 vs. 140 ± 21 bpm; all $p < 0.05$). At 60 min, there was a tendency for OB subjects to have a higher HR versus N-OB (159 ± 29 vs. 137 ± 19 bpm, $p = 0.097$).

The effect of hypohydration on changes in HR for both groups is presented in Figure 9A. Hypohydration did not affect the change over time ($p = 0.473$), nor was there a significant interaction between group and time ($p = 0.450$). When hypohydrated, HR was not significantly elevated in either group at 15, 30, or 45 min (all $p > 0.05$); however, there was a trend towards higher HR in N-OB versus OB subjects at 60 min (14 ± 15 vs. 3 ± 10 bpm, $p = 0.062$).

In the EU trials, there was a significant main effect of time for MAP ($p < 0.001$), non-significant effect of group ($p = 0.114$), and a trend towards a significant interaction between group and time from pre-exercise baseline to 45 min ($p = 0.058$; Figure 8B). MAP was lower in OB versus N-OB subjects at 30 min (79 ± 9 vs. 87 ± 6 mmHg, $p = 0.032$), but there were no other differences between groups at any other time point (all $p > 0.05$).

The effect of hypohydration on MAP for both groups is presented in Figure 9B. The interaction between group and time was not significant ($p = 0.640$) and there were no differences between groups in MAP at any time point when HY versus EU (all $p > 0.05$).

Discussion

In the present study, we investigated thermoregulatory responses in non-obese (N-OB) and obese (OB) males to exercise heat stress while either euhydrated (EU) or hypohydrated

(HY). It was hypothesized that OB males would exhibit impaired thermoregulatory responses and hypohydration would further exacerbate these responses versus N-OB. Our results demonstrate similar cutaneous vasodilation and sweating responses between groups, independent of hydration status. When euhydrated, OB subjects had a significantly greater change in rectal temperature versus N-OB (Figure 1A), despite no differences in CVC, LSR, or whole body sweat rate (WBSR) (Figures 5). Interestingly, the elevated rectal temperature response typically observed when HY versus EU during exercise was present in N-OB but not OB subjects (Figure 2A). These data suggest that euhydration is unable to prevent rectal temperature from increasing to levels achieved when hypohydrated in OB males during exercise heat-stress.

Effect of obesity on thermoregulatory responses while euhydrated

Optimal functioning of thermoregulatory mechanisms are critical to maintaining appropriate thermal balance and preventing the onset of heat illness during exercise (23). This is primarily achieved via redistributing central blood volume to the periphery (i.e, skin) for heat dissipation via convection and increasing sweat output for evaporative heat loss (44). Vroman et al. reported significantly lower forearm blood flow (FBF; an index of skin blood flow) in obese versus lean subjects during exercise in a hot environment, despite similar esophageal and skin temperatures (51) and without manipulation of hydration status. It was hypothesized that alterations in FBF in the obese group may have been related to increased sympathetic vasoconstrictor activity to the skin circulation. Indeed, previous work has shown that a reduction in mean arterial pressure (MAP) induced via lower-body negative pressure leads to a baroreflex-mediated skin vasoconstrictor response (8). Moreover, a considerable amount of evidence suggests that obesity is associated with increased basal sympathetic activation (3, 11, 30, 42) and altered baroreflex sensitivity (19, 48). In the present study, MAP while euhydrated was the same

in both N-OB (91 ± 10) and OB (91 ± 11 mmHg) groups at 15 min into the exercise bout, but decreased significantly between 15 and 30 min in OB (-12 ± 8) but not N-OB subjects (-4 ± 8 mmHg; Figure 8B). Thus, it is possible that the significant reduction in MAP may have influenced baroreceptor-mediated vasoconstrictor activity in OB, although not to the extent where CVC was significantly impaired (Figure 3A).

In contrast with previous work (5, 6, 51), we did not observe differences between N-OB and OB in cutaneous vasodilation or sweating when euhydrated. Several recent investigations, however, reported similar findings to the current study (1, 18, 32). Possible discrepancies in the literature may be due to differences in physiological strain imposed by the environmental conditions (i.e., compensable versus uncompensable). Dervis et al. recently reported a higher core temperature in high- versus low-body fat male subjects during exercise in a compensable (~28°C) environment, with no differences in sweat rate between groups (18). Similarly, we also observed a greater change in rectal temperature in OB versus N-OB subjects, coupled with similar local and whole body sweat rates and cutaneous vasodilation. However, the explanation for similar cutaneous vasodilatory and sweating responses between groups, despite large differences in body temperature, is unclear. Given that skin blood flow and sweating increase to their maximum capacity concomitantly with increases in body temperature in order to maintain thermal homeostasis, OB should have exhibited greater CVC and/or sweat rate versus N-OB given their higher rectal temperature. Interestingly, this did not occur, which lead to OB storing considerably more heat versus N-OB (42.1 ± 14.5 vs. 25.8 ± 13.7 $W \cdot m^{-2}$), ultimately leading to a higher rectal temperature (Figure 1A). This suggests the possibility of impaired efferent and/or afferent signaling between the hypothalamus and the effector organ(s), impaired post-synaptic responses to appropriate efferent signaling from the hypothalamus, or some combination of

centrally- and peripherally-mediated responses leading to reduced maximal vasodilatory and sweating responses versus N-OB.

In addition to significant differences between subject groups in body mass and body fat, N-OB had higher specific heat capacity versus OB (Table 1), owing to the large differences between groups in adiposity. The higher proportion of lean mass in N-OB versus OB may have allowed for more efficient heat conductance from the core to the periphery, leading to improved capability of convective heat exchange. However, given the high, uncompensable ambient temperature used (~40°C), convective heat transfer would likely have made a minimal contribution to overall thermal balance due to the large negative thermal gradient between the skin and environment (~4-5°C). Thus, given similar metabolic heat production relative to body mass between groups, coupled with similar vasodilatory and sweating responses, the explanation for higher rectal temperature in OB versus N-OB when euhydrated remains unclear.

Effect of hypohydration on thermoregulatory responses

While several studies have compared thermoregulatory responses between groups of dissimilar body mass and/or body fat during exercise (1, 5, 16, 18), this is the first known investigation to simultaneously assess the influence of hydration status. In non-obese individuals, the influence of hypohydration during exercise heat stress has been repeatedly demonstrated to lead to a higher core temperature versus a euhydrated condition (35, 40, 45, 46). This is likely due to perturbations in thermoregulatory mechanisms (i.e., skin blood flow and sweat rate) that typically accompany hypohydration (34, 36, 43). Interestingly, our data demonstrate a significant difference between groups in the rectal temperature response to hypohydration. The end exercise rectal temperature was significantly higher when HY (38.92 ± 0.36°C) versus EU (38.35 ± 0.38°C) in N-OB, but not OB (38.72 ± 0.45 vs. 38.72 ± 0.45°C) subjects. Importantly, this is

despite both N-OB and OB groups starting and finishing the exercise protocol at the same level of hypohydration, confirmed via multiple variables (Table 2). This suggests that hypohydration appears to affect the rectal temperature response to a greater extent in N-OB versus OB. The reason(s) behind this varied response are not abundantly clear, but may be related to aforementioned possible impairments in centrally- and/or peripherally-mediated thermoregulatory responses in OB versus N-OB.

Consistent with previous investigations using non-obese subjects (21, 24), our results showed reductions in WBSR accompanying hypohydration, independent of group (Figure 5). Similar values of WBSR between groups are consistent with recent investigations comparing groups with vast differences in body fat (1, 18). Similarly, the change in LSR and CVC attributed to hypohydration was not different between groups versus a euhydrated condition. This suggests that during aerobic exercise in the heat, the added physiological stress of hypohydration is tolerated equally well in OB subjects compared to N-OB counterparts. It should be noted, however, that this investigation used subjects with significant differences in body mass and body surface area, in addition to large differences in body fat. Thus, the degree to which each of these characteristics independently influenced WBSR, LSR, or CVC during exercise heat stress cannot be deduced.

Mean skin temperature (T_{sk}) significantly decreased throughout the exercise protocol in both groups and under both hydration conditions. Interestingly, hypohydration had a substantially different effect on this response between groups, with a lower T_{sk} accompanying hypohydration in N-OB subjects (-0.77 ± 1.18°C) compared to an increase in OB subjects (1.05 ± 0.72°C) at 60 min versus a euhydrated state (Figure 2B). While the decrease in T_{sk} over time is consistent with other investigations (32, 35, 37), it is unclear as to why T_{sk} in these groups

responded in opposite fashion when comparing their EU and HY trials. It could be assumed that the reduction in T_{sk} during exercise is representative of evaporative heat loss occurring, gradually cooling the skin's surface. Given that rectal temperature was significantly elevated in hypohydrated N-OB versus EU, the lower T_{sk} when hypohydrated may be indicative of greater reliance on evaporative heat loss. In the OB, however, rectal temperature was not different between hydration conditions. Thus, the inability of T_{sk} to reach similar levels versus EU in OB may suggest differences between N-OB and OB in the manner hypohydration affects the balance between evaporative and convective modes of heat dissipation in uncompensable exercise heat-stress.

Conclusions

The present investigation examined thermoregulatory responses in N-OB and OB males during exercise in the heat while euhydrated and hypohydrated. Using an exercise protocol that allowed for equal amounts of metabolic heat production (Watts per kg body mass) between groups, our results indicate that starting and maintaining euhydration during exercise leads to a lower end-exercise rectal temperature for N-OB but not OB subjects. Hypohydration appears to affect N-OB to a greater extent versus OB, with N-OB subjects exhibiting a significantly higher rectal temperature when HY versus EU, but no difference between hydration conditions in OB. In addition, the added physiological stressor of hypohydration does not appear to affect cutaneous vasodilatory and sweating responses differently between N-OB and OB. These data suggest that OB males have some degree of impairment in thermoregulatory responses to increases in body temperature during exercise heat-stress when euhdyrated versus N-OB. While hypohydration leads to a higher rectal temperature in N-OB, it does not appear to further increase this in OB versus a euhydrated condition. Further investigation into the influence of hydration

status on thermoregulatory responses during exercise should seek to examine females, as well as other clinical populations that may be at increased risk of developing heat illness (i.e., individuals with Type II Diabetes).

Conflicts of interest

There are no conflicts of interest to declare.

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Figure Captions

Figure 1. Changes in rectal temperature (T_{rec}; A), mean skin temperature (T_{sk}; B) and mean body temperature (T_b; C) in non-obese (N-OB) and obese (OB) subjects while euhydrated. *significant difference between groups ($p < 0.05$).

Figure 2. Changes in rectal temperature (T_{rec}; A), mean skin temperature (T_{sk}; B) and mean body temperature (T_b; C) in non-obese (N-OB) and obese (OB) subjects calculated as the difference between hypohydrated (HY) versus euhydrated (EU) conditions. *significant difference between groups ($p < 0.05$).

Figure 3. Changes in cutaneous vascular conductance (CVC; A) and local sweat rate (LSR; B) in non-obese (N-OB) and obese (OB) subjects while euhydrated. No significant differences between groups at any time point (all $p > 0.05$).

Figure 4. Changes in cutaneous vascular conductance (CVC; A) and local sweat rate (LSR; B) in non-obese (N-OB) and obese (OB) subjects calculated as the difference between hypohydrated (HY) versus euhydrated (EU) conditions. No significant differences between groups at any time point (all $p > 0.05$).

Figure 5. Whole body sweat rate (WBSR) in non-obese (N-OB) and obese (OB) subjects while euhydrated (EU) and hypohydrated (HY). *significantly different from HY condition, independent of group ($p < 0.05$).

Figure 6. Changes in sweat gland activation (SGA; A) and sweat gland output (SGO; B) in non-obese (N-OB) and obese (OB) subjects while euhydrated. No significant differences between groups at any time point (all $p > 0.05$).

Figure 7. Changes in sweat gland activation (SGA; A) and sweat gland output (SGO; B) in non-obese (N-OB) and obese (OB) subjects calculated as the difference between hypohydrated (HY) versus euhydrated (EU) conditions. No significant differences between groups at any time point (all $p > 0.05$).

Figure 8. Changes in heart rate (HR; A) and mean arterial pressure (MAP; B) in non-obese (N-OB) and obese (OB) subjects while euhydrated. *significant difference between groups ($p < 0.05$).

Figure 9. Changes in heart rate (HR; A) and mean arterial pressure (MAP; B) in non-obese (N-OB) and obese (OB) subjects calculated as the difference between hypohydrated (HY) versus euhydrated (EU) conditions. *significant difference between groups ($p < 0.05$).

Table 1. Subject demographic data by group.

	N-OB	OB
Age (y)	24 ± 4	26 ± 5
Height (cm)	179 ± 5	176 ± 7
Mass (kg)	73.9 ± 8.5	89.6 ± 6.9**
BMI ($kg \cdot m^2$)	23.0 ± 3.0	28.9 ± 2.5**
BSA (m^2)	1.92 ± 0.10	2.06 ± 0.11*
Body fat (%)	13.6 ± 3.8	30.2 ± 4.1**
FFM (kg)	65.2 ± 6.7	64.2 ± 6.2
LBM (kg)	61.8 ± 6.3	60.4 ± 5.9
C_p ($kJ \cdot kg^{-1} \cdot °C^{-1}$)	3.73 ± 0.05	3.58 ± 0.04*
VO_{2peak} ($ml \cdot min \cdot kg^{-1}$)	49.0 ± 8.2	36.1 ± 4.0**
VO_{2peak} ($ml \cdot min \cdot kgLBM^{-1}$)	58.4 ± 8.6	53.5 ± 6.1
Physical activity ($MET\text{-}min \cdot wk^{-1}$)	4447 ± 3336	3939 ± 1529

N-OB = non-obese; OB = obese; BMI = body mass index; BSA = body surface area; FFM = fat-free mass; LBM = lean body mass; C_p = specific heat; *significantly different versus opposite group ($p < 0.05$); **significantly different versus opposite group ($p < 0.01$).

Table 2. Average external work, metabolic heat production (MHP), and relative exercise intensity for euhydrated (EU) and hypohydrated (HY) trials in non-obese (N-OB) and obese (OB) subjects.

	EU		HY	
	N-OB	OB	N-OB	OB
External Work (W)	94 ± 18	113 ± 13*	90 ± 15	110 ± 19*
MHP (W)	454 ± 54	541 ± 47**	434 ± 56	541 ± 46**
Relative MHP ($W \cdot kg^{-1}$)	6.1 ± 0.2	6.1 ± 0.1	6.0 ± 0.1	6.1 ± 0.1
Heat Storage ($W \cdot m^{-2}$)	25.8 ± 13.7	42.1 ± 14.5*	38.8 ± 15.0†	43.2 ± 14.2
Relative Intensity ($\%VO_{2peak}$)	45.7 ± 9.9	59.3 ± 6.4**	44.3 ± 8.9	59.4 ± 7.0**

*significantly higher versus N-OB ($p < 0.05$), significantly higher versus N-OB ($p < 0.01$), †significantly higher versus EU in N-OB ($p < 0.01$).

Table 3. Hydration biomarkers for euhydrated (EU) and hypohydrated (HY) trials in non-obese (N-OB) and obese (OB) subjects before and after exercise.

	EU		HY	
	N-OB	OB	N-OB	OB
ΔBM (%)				
Pre	-0.2 ± 0.8	-0.2 ± 0.8	-1.8 ± 1.1†	-1.1 ± 1.3†
Post	0.3 ± 1.2	0.3 ± 0.9	-2.5 ± 1.1*†	-1.7 ± 1.5*†
U_{SG} ($g \cdot ml^{-1}$)				
Pre	1.011 ± 0.006	1.009 ± 0.006	1.025 ± 0.003†	1.025 ± 0.003†
Post	1.014 ± 0.006	1.009 ± 0.005	1.026 ± 0.002†	1.026 ± 0.003†
U_{osm} ($mOsm \cdot kg^{-1}$)				
Pre	464 ± 234	391 ± 241	1010 ± 118†	1024 ± 110†
Post	526 ± 193	353 ± 179	1005 ± 86†	1032 ± 93†
U_{col}				
Pre	2 ± 1	2 ± 1	4 ± 1†	4 ± 1†
Post	2 ± 1	2 ± 1	4 ± 1†	4 ± 1†
S_{osm} ($mOsm \cdot kg^{-1}$)				
Pre	287 ± 3	286 ± 4	293 ± 3†	292 ± 3†
Post	288 ± 5	288 ± 4	300 ± 4†	300 ± 6†

ΔBM = percent body mass change (post-exercise versus 3-day euhydrated baseline); U_{SG} = urine specific gravity; U_{osm} = urine osmolality; U_{col} = urine color; S_{osm} = serum osmolality. †main effect of hydration status, independent of group on ΔBM, U_{SG}, U_{osm}, U_{col}, and S_{osm} (all $p < 0.05$). *significantly higher versus Pre, independent of group ($p < 0.01$). No other differences between groups or hydration condition for ($p > 0.05$).

Table 4. Values of red cell volume (RCV), absolute and relative plasma volume (PV and PV_{rel}, respectively) and absolute and relative blood volume (BV and BV_{rel}) for non-obese (N-OB) and obese (OB) subjects in euhydrated (EU) and hypohydrated (HY) trials.

	N-OB			OB		
	EU	HY	Change (HY-EU)	EU	HY	Change (HY-EU)
RCV (ml)	2917 ± 256	2906 ± 296	-10 ± 152	2942 ± 319	2823 ± 290	-119 ± 216
PV (ml)	3941 ± 466	3831 ± 472	-110 ± 290	3651 ± 462	3634 ± 367	-17 ± 139
PV_{rel} ($ml \cdot kg^{-1}$)	52.3 ± 6.1*	51.7 ± 6.9*	-0.6 ± 4.0	39.7 ± 2.6	39.9 ± 1.8	0.2 ± 1.6
BV (ml)	6858 ± 663	6738 ± 692	-120 ± 410	6593 ± 721	6456 ± 614	-136 ± 318
BV_{rel} ($ml \cdot kg^{-1}$)	91.0 ± 8.0*	90.9 ± 9.7*	0.0 ± 5.6	71.7 ± 3.9	70.9 ± 3.8	-0.8 ± 3.4

*significantly higher versus OB subjects, independent of hydration condition ($p < 0.001$). No other differences between groups or condition (all $p > 0.05$

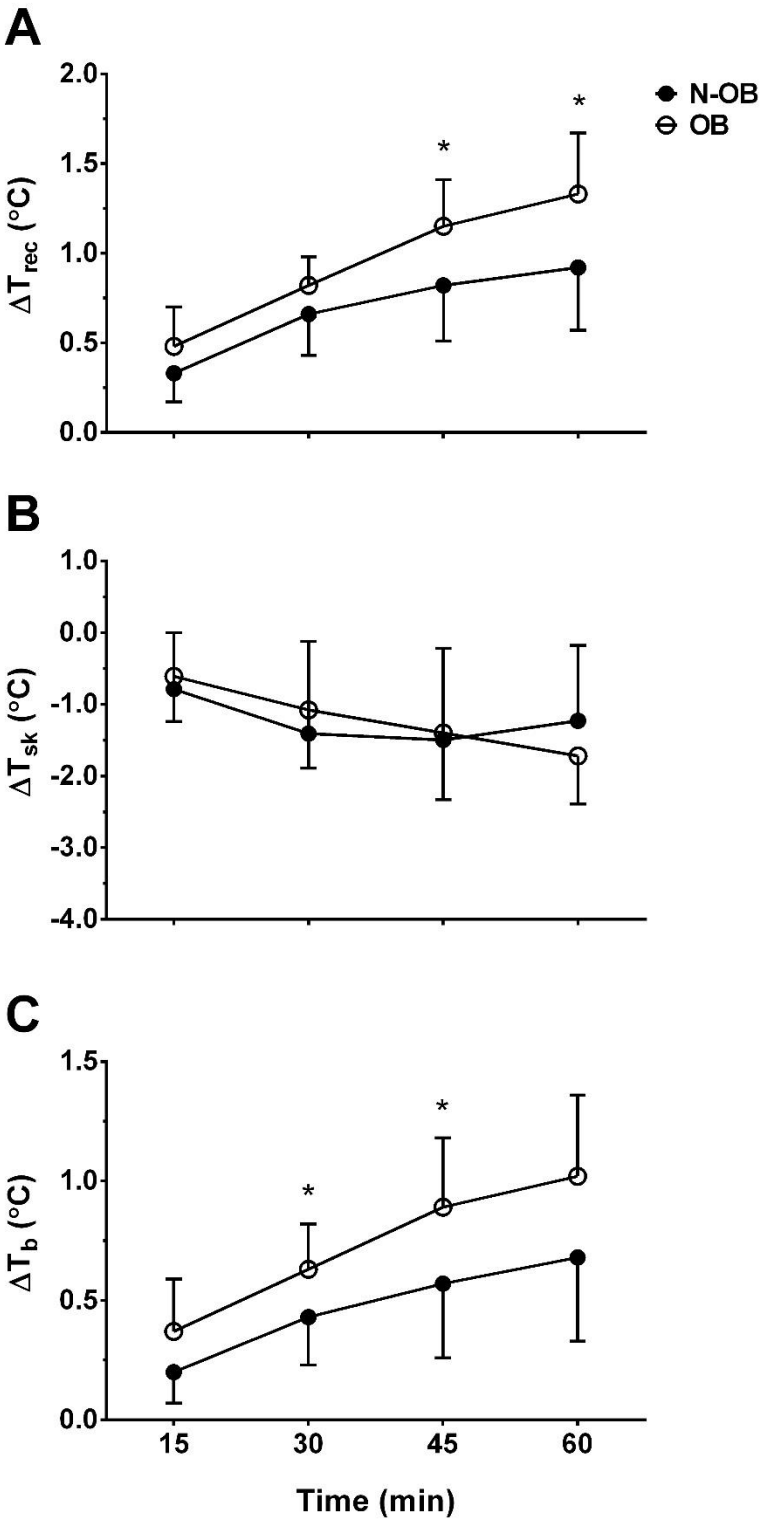
A
B
C
N-OB
OB
ΔTrec (°C)
ΔTsk (°C)
ΔTb (°C)
2.0
1.5
1.0
0.5
0.0
-1.0
-2.0
-3.0
-4.0
*
15
30
45
60
Time (min)

Figure 1.

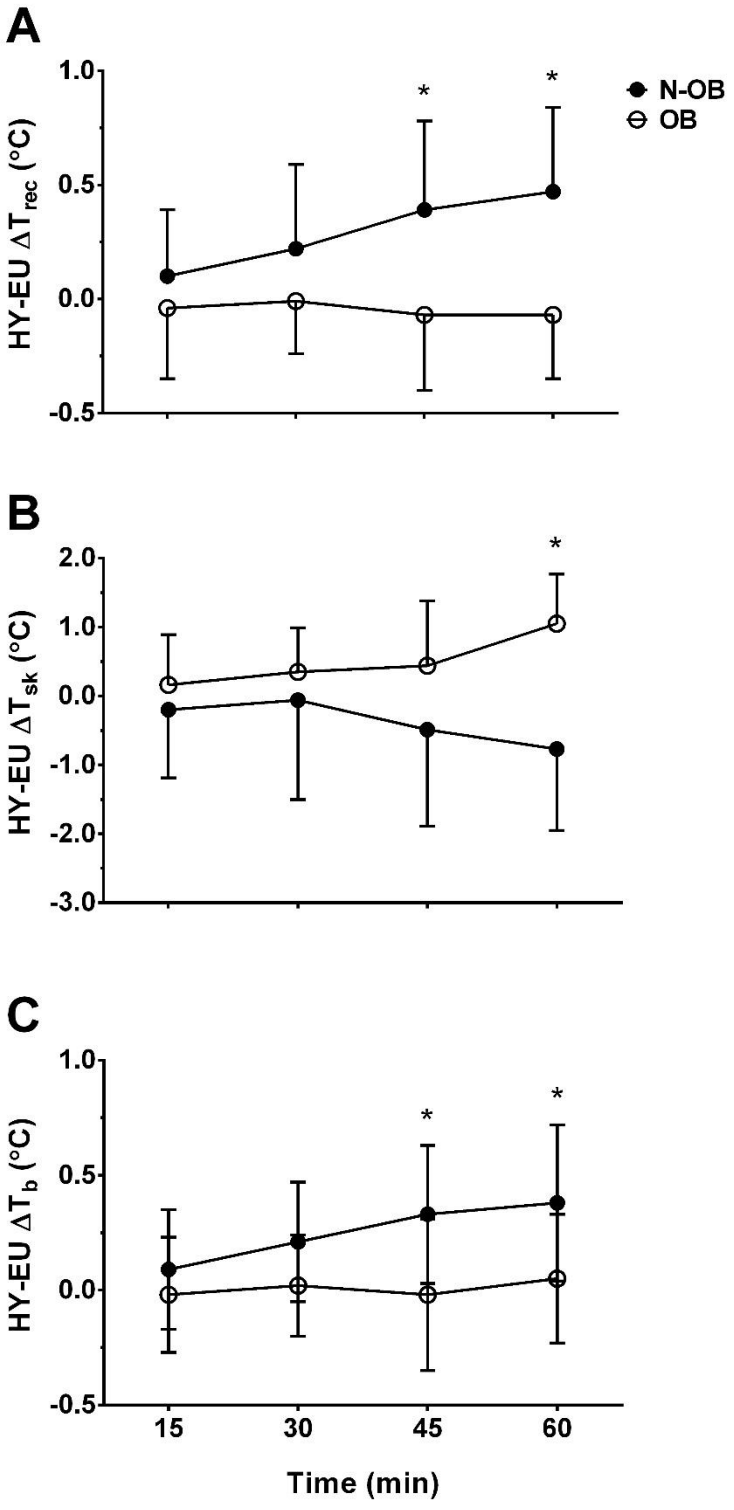
A
B
C
N-OB
OB
HY-EU ΔT_rec (°C)
HY-EU ΔT_sk (°C)
HY-EU ΔT_b (°C)
1.0
0.5
0.0
-0.5
2.0
-1.0
-2.0
-3.0
*
15
30
45
60
Time (min)

Figure 2.

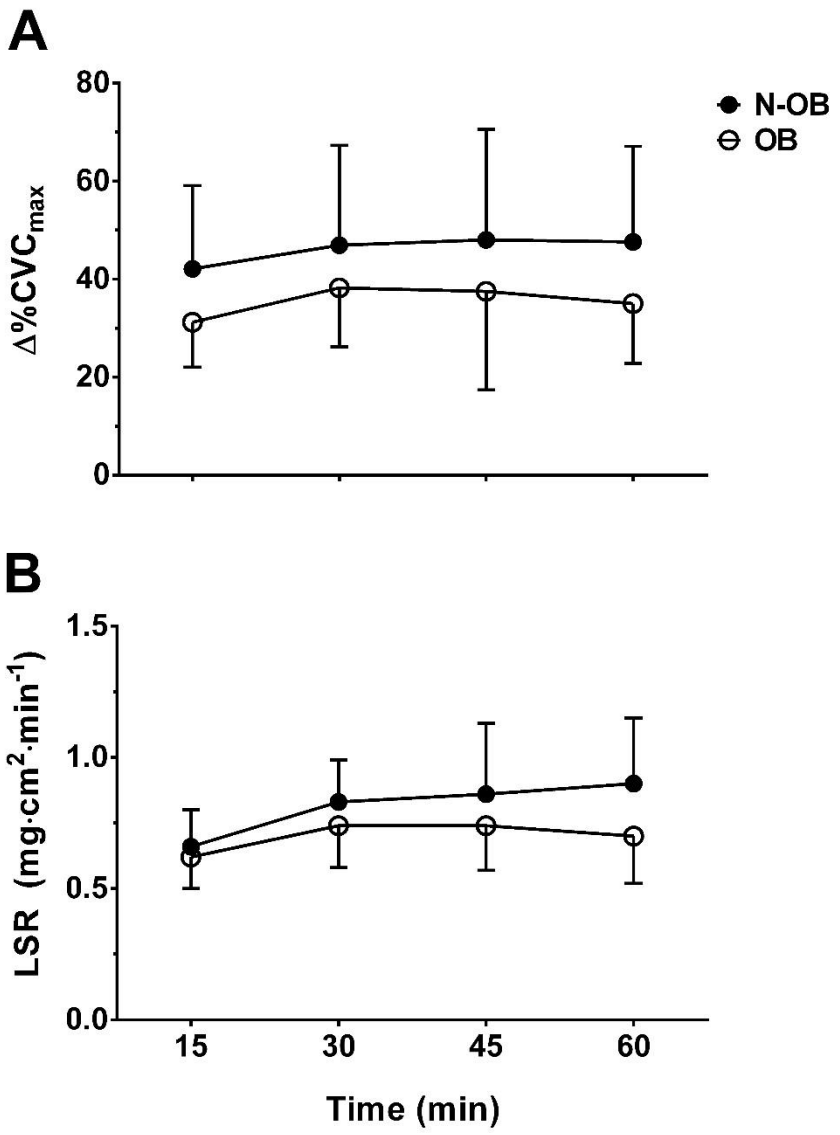
A
80
60
40
20
0
Δ%CVC$_{max}$
N-OB
OB
B
1.5
1.0
0.5
0.0
LSR (mg·cm^2·min^{-1})
15
30
45
60
Time (min)

Figure 3.

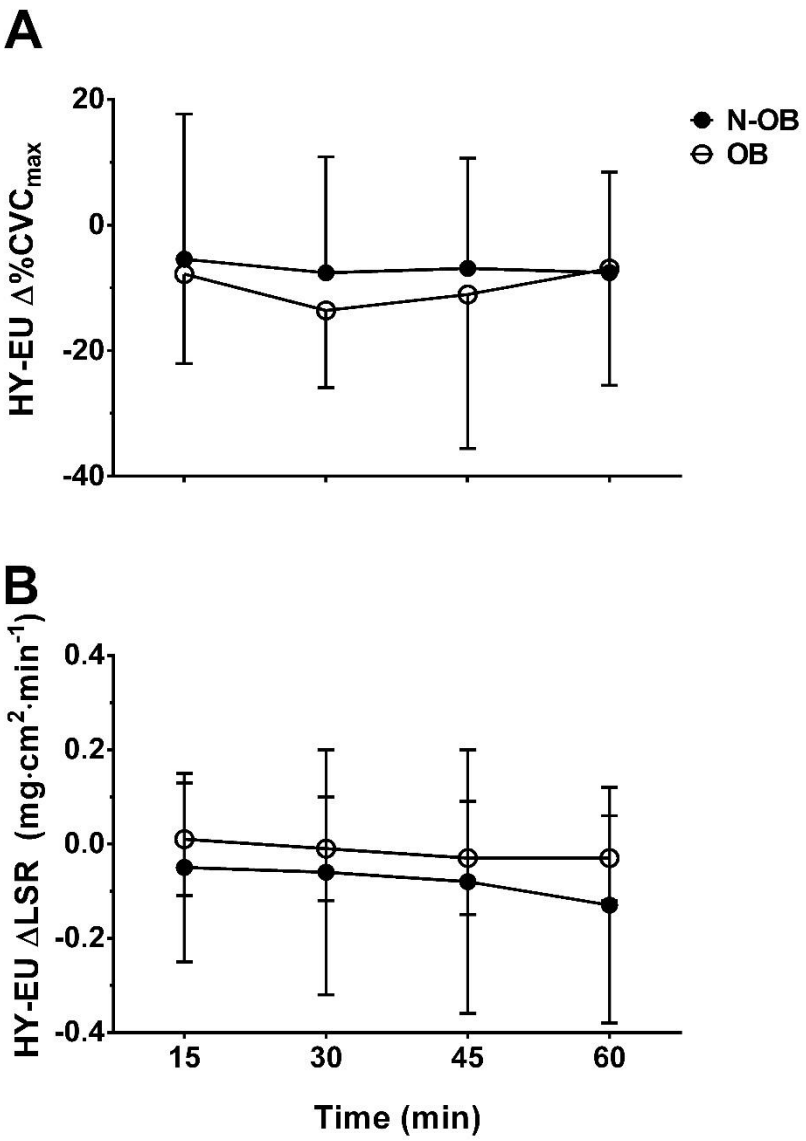
A
20
0
-20
-40
HY-EU Δ%CVCmax
N-OB
OB
B
0.4
0.2
0.0
-0.2
-0.4
HY-EU ΔLSR (mg·cm2·min-1)
15
30
45
60
Time (min)

Figure 4.

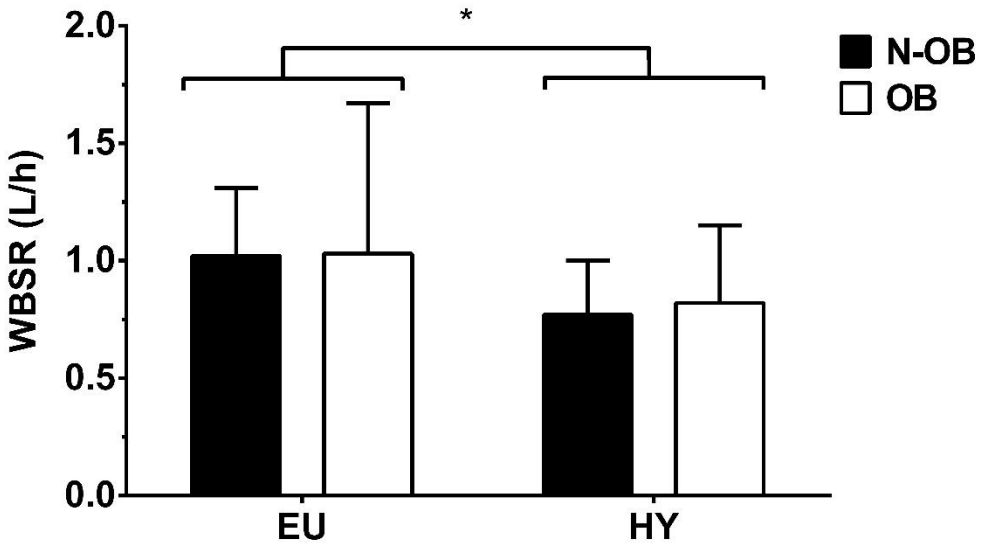

Figure 5.

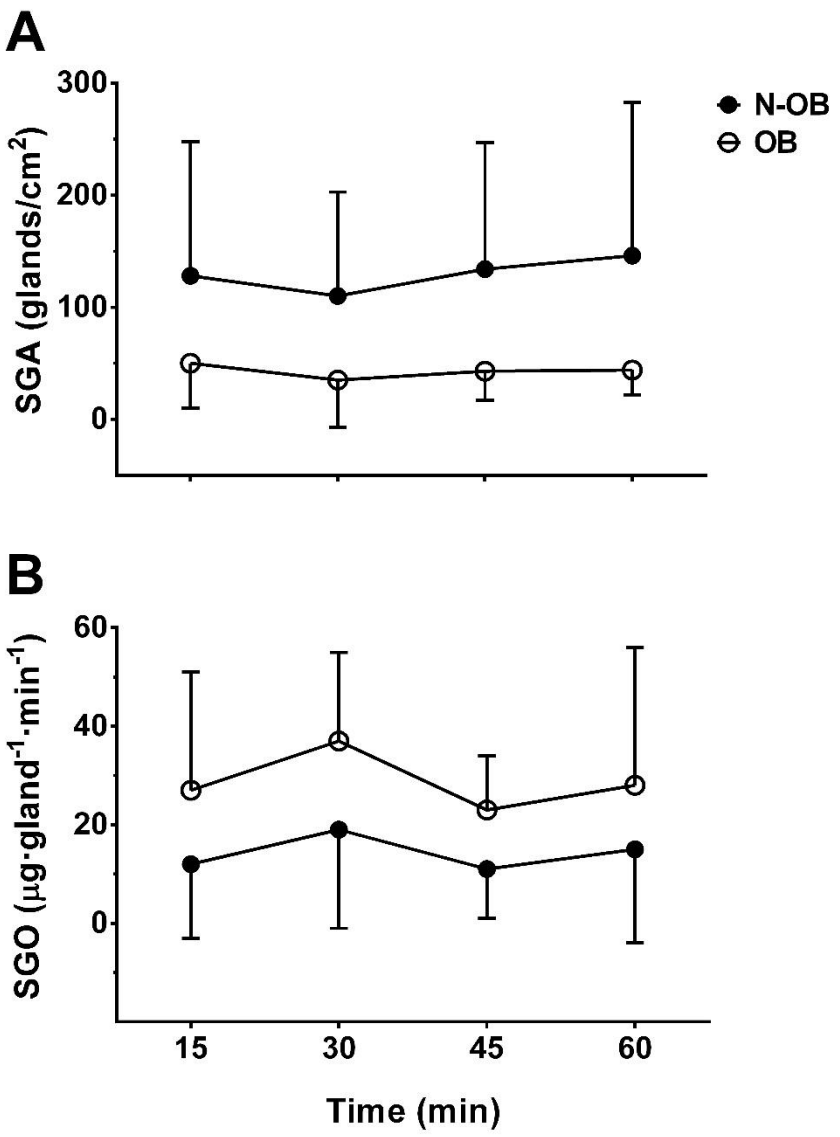
A
300
200
100
0
SGA (glands/cm^2)
N-OB
OB
B
60
40
20
0
SGO (μg·gland^{-1}·min^{-1})
15
30
45
60
Time (min)

Figure 6.

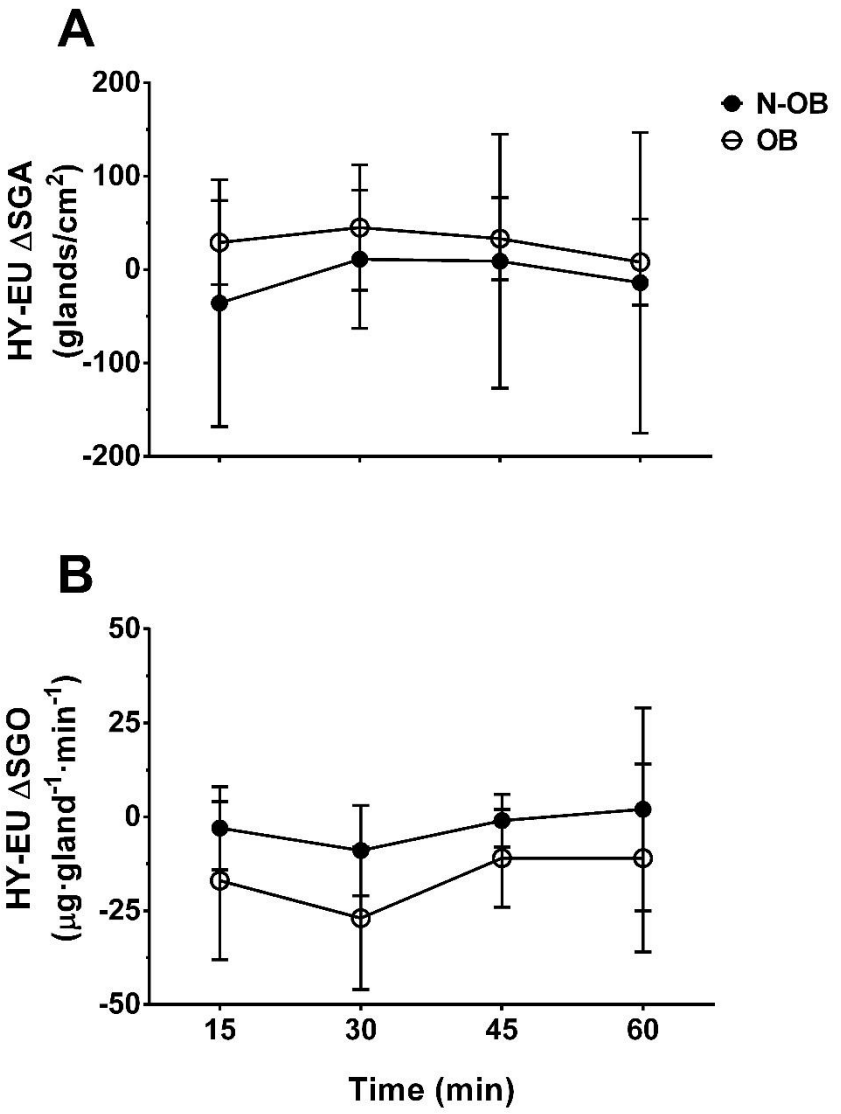
A
200
100
0
-100
-200
HY-EU ΔSGA
(glands/cm²)
N-OB
OB
B
50
25
0
-25
-50
HY-EU ΔSGO
(μg·gland⁻¹·min⁻¹)
15
30
45
60
Time (min)

Figure 7.

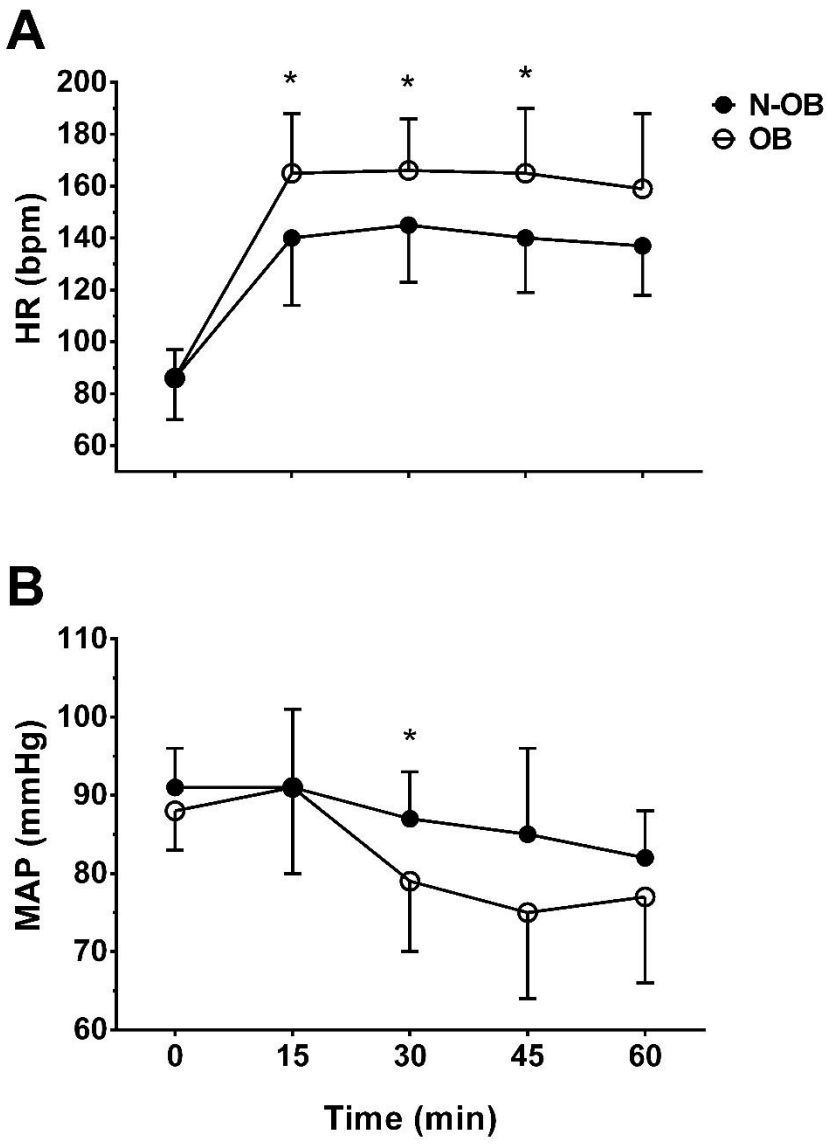
A
200
180
160
140
120
100
80
60
HR (bpm)
*
*
*
N-OB
OB
B
110
100
90
80
70
60
MAP (mmHg)
*
0
15
30
45
60
Time (min)

Figure 8.

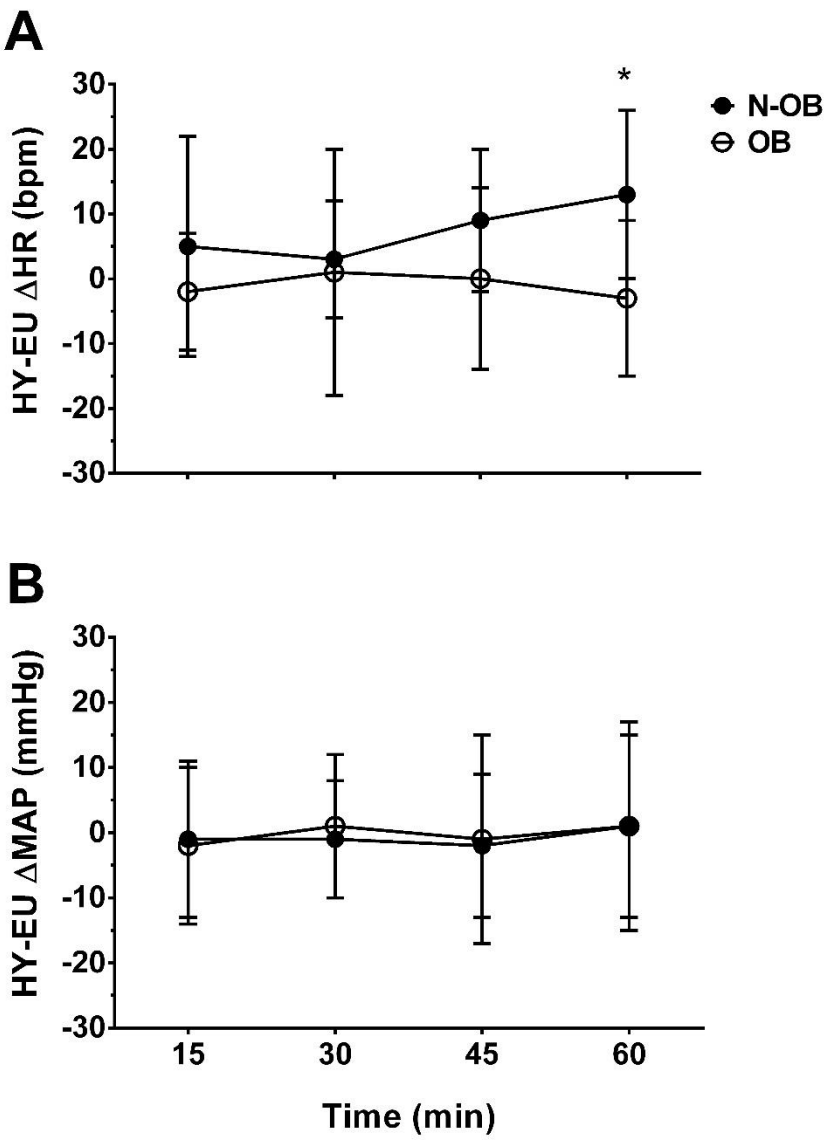
A
B
HY-EU ΔHR (bpm)
HY-EU ΔMAP (mmHg)
30
20
10
0
-10
-20
-30
*
N-OB
OB
15
30
45
60
Time (min)

Figure 9.

CHAPTER THREE

Study 2:

Effect of Hypohydration on Post-Synaptic Cutaneous Vasodilatory and Sweating Responses in Obese and Non-Obese Males

Effect of Hypohydration on Post-Synaptic Cutaneous Vasodilatory and Sweating Responses in Obese and Non-Obese Males

Matthew A. Tucker, Aaron R. Caldwell, Cory L. Butts, Forrest B. Robinson, Stavros A. Kavouras, Brendon P. McDermott, Tyrone A. Washington, Ronna C. Turner, & Matthew S. Ganio

Department of Health, Human Performance, and Recreation, University of Arkansas, Fayetteville, AR 72701, USA.

Address for Correspondence:

Matthew S. Ganio, Ph.D.
Human Performance Laboratory
University of Arkansas
155 Stadium Dr.
HPER 321
Fayetteville, AR 72701
479-575-2517
msganio@uark.edu

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Abstract

Obesity is associated with microvascular dysfunction, which can precede hypertension and cardiovascular disease. During whole-body heat stress hypohydration attenuates cutaneous vasodilation and sweating responses, but it is unknown if this generalized response is due to post-synaptic dysfunction. Further, it is unknown how thermoregulatory responses may be altered in hypohydrated obese (OB) versus non-obese (N-OB) individuals. **Purpose:** To determine the effect of hypohydration on post-synaptic cutaneous vasodilatory and sweating responses in OB and N-OB males when euhydrated (EU) and hypohydrated (HY). **Methods**: In a randomized design, 10 N-OB (body fat [BF] 30.1 ± 39%) and 10 OB (BF 14.3 ± 3.3%) males were instrumented for forearm microdialysis when EU and HY. Changes in cutaneous vascular conductance (CVC) in response to incremental intradermal infusion of the endothelium-independent vasodilator sodium nitroprusside (SNP, 5×10^{-8} to 5×10^{-2} M) and the endothelium-dependent vasodilator methacholine chloride (MCh, 1×10^{-7} to 1×10^{-1} M) were assessed by laser Doppler flowmetry. Local sweat rate (LSR) was simultaneously assessed at the MCh site via ventilated capsule. At the end of the 7th dose, maximal CVC was elicited by delivering a maximal dose of SNP for 30 min to both sites with simultaneous local heating (~44°C) at the SNP site. Dose-response curves were compared between groups and hydration condition using an extra sum of squares F-test. **Results:** When EU, Log EC_{50} of MCh-mediated CVC was not different between N-OB versus OB subjects (-3.04 ± 0.12 vs. -2.98 ± 0.19 Log [MCh] M, $p = 0.841$). Within each group, EU and HY MCh-mediated Log EC_{50} did not differ ($p > 0.05$). Log EC_{50} of SNP-mediated CVC was higher in EU OB versus N-OB (-1.69 ± 0.17 vs. -2.13 ± 0.06 Log [SNP] M, $p = 0.014$). EU and HY SNP-mediated Log EC_{50} did not differ within groups ($p > 0.05$). LSR response did not differ between group or hydration condition ($p > 0.05$). **Conclusion:**

Impaired endothelium-independent vasodilation was present in OB versus N-OB males, with no differences between groups in endothelium-dependent vasodilation or sweating responses. Hypohydration tended to impair endothelium-independent vasodilation in N-OB but not OB.

Introduction

Increases in body and skin temperature are accompanied by subsequent increases in cutaneous vasodilation and sweating in efforts to dissipate heat and maintain thermal homeostasis (3, 26). While these responses are primarily mediated via sensory inputs to the hypothalamus, the post-synaptic (i.e., peripherally-mediated) contribution to these responses is less understood. Thus, the relative influence of microvascular function on cutaneous vasodilatory and sweating responses is unclear.

Microvascular dysfunction has been associated with cardiovascular disease, stroke, and glucose intolerance (14, 23). Further, significant changes in macro- and micro-circulation are known to accompany obesity (33). Previous investigations have shown attenuated endothelium-dependent (8, 15, 31) and endothelium-independent (24) responses to various methods of assessment in obese versus non-obese counterparts. However, the effect of obesity on these responses has not been explored to the same extent in the microcirculation.

Whole-body heat stress allows for assessment of cutaneous vasodilation and sweating in response to increases in body and skin temperature (i.e., mediated via efferent signaling of the central nervous system [CNS]). However, onsets for cutaneous vasodilatory and sweating can be modified in the absence of known CNS modulation (19, 29), suggesting the possibility that these responses may be modified by localized changes at the effector organ (10). Hypohydration is typically characterized by a reduction in plasma volume leading to plasma hyperosmolality (25, 27). Growing evidence suggests a prominent role for chronic hypohydration in the development of medical maladies including chronic kidney disease and nephrolithiasis (5, 30, 32). Moreover, acute hypohydration attenuates cutaneous vasodilatory and sweating responses during whole-

body heat stress (22, 27). However, it is unknown if these attenuated responses with hypohydration are due to altered post-synaptic function.

More recently, the use of intra-dermal microdialysis has been used for assessment of cutaneous vasodilatory and sweating responses (i.e., microvascular function) in a variety of healthy and clinical populations (6, 7, 11) During an assessment of microvascular function in obese and non-obese males, Patik et al. reported an attenuated endothelium-independent response in obese compared to non-obese, while endothelium-dependent vasodilation was preserved (24). However, the study did not compare local sweat rate (LSR) responses between groups, nor did it control for hydration status as a potential modifying variable.

Therefore, the aim of the present study was two-fold: 1) to determine if impairments in cutaneous vasodilatory and sweating responses are present in obese (OB) versus non-obese (N-OB) males while euhydrated, and 2) to determine how these responses may be altered in hypohydrated OB versus N-OB males during incremental intradermal infusion of endothelium-dependent and endothelium-independent vasodilators. We hypothesized that impairments in cutaneous vasodilatory and sweating responses present in OB subjects while euhydrated would be further exacerbated while hypohydrated versus their N-OB counterparts.

Materials and Methods

Subjects

Twenty healthy adult males from the University and surrounding community volunteered to participate in the study. In order to be considered eligible, subjects were required to have a stable body weight (i.e., not actively trying to lose or gain weight), be willing to abstain from caffeine and alcohol on lead-in and testing days, be free of any medications or supplements that may affect body weight or fluid balance, be free of any metabolic and/or cardiovascular

disorders, and abstain from physical activity beyond normal activities of daily living during a 48-h experimental period. All subjects were required to provide written informed consent prior to participation through signing a document that was approved by the University's Institutional Review Board.

During a screening visit, body fat (BF) percentage was determined via dual-energy X-ray absorptiometry (DXA; Lunar Prodigy, General Electric®, Madison, WI). Subjects with a BF <18% were considered non-obese (N-OB; n = 10) and those ≥26% obese (OB; n = 10) (12). Subject demographic data are presented in Table 1. Subjects had their height measured using a standard stadiometer (Seca 216 stadiometer, Chino, CA) and nude body mass (BM) using a platform scale (Health-O-Meter, Model 349KLX, Alsip, IL). These values were used to calculate body mass index (BMI). A digital bathroom scale was provided to subjects (High Accuracy Bathroom Scale, BalanceFrom LLC, China) to measure morning euhydrated BM during a 3-day baseline period to use for later calculations of BM change (4).

Experimental Design

Subjects completed two trials separated by a minimum of 72 h. In a randomized order, skin microdialysis (MD) was performed while subjects were either euhydrated (EU) or hypohydrated (HY). In order to facilitate hypohydration, 48-h before reporting to the laboratory for MD, subjects were restricted to 237 ml of drinking water intake. Twenty-four hours prior to MD, subjects completed 60 min of moderate intensity cycling on a mechanically-braked cycle ergometer (Veletron, RacerMate Inc., Seattle, WA) in a hot environment. Fluids were not provided during the exercise protocol and subjects remained fluid restricted following completion of exercise, receiving a further 237-473 ml of drinking water until returning to the laboratory the following day for MD. For the EU trial, subjects consumed an adequate amount of

drinking water (45 ml·kg^{-1}) 48-h prior to MD, and were provided fluids throughout the exercise protocol in order to replace sweat losses. Following exercise, subjects were encouraged to drink fluids to maintain euhydration for MD the next day.

Hydration Status Assessment

Upon arrival to the laboratory for the MD protocol, nude body mass was measured (Health-o-meter digital scale, model 349KLX, Pelstar LLC, Alsip, IL, USA). Body mass change (ΔBM) was calculated as the difference between subjects' 3-day euhydrated BM baseline and BM upon arrival to the laboratory. Subjects provided a urine sample which was analyzed for U_{SG} using a hand-held refractometer (Master-SUR/NM, ATAGO, Japan), osmolality (U_{osm}), and color (U_{col}). Urine osmolality was measured in duplicate using freezing point depression osmometry (Model 3250, Advanced Instruments Inc., Norwood, MA). Color was assessed in a well-lit room using the eight-level color scale where the sample was in a glass tube against a plain white background (1, 18).

Blood samples were collected via an intravenous catheter (SurFlash®, Terumo Corporation, Tokyo, Japan) placed in a superficial forearm vein while subjects were in a reclined position in a phlebotomy chair for at least 20 min. Whole blood was drawn into a 6 ml Vacutainer collection tube with EDTA additive for analysis of hemoglobin (Hb), hematocrit (Hct), and carboxyhemoglobin concentration (HbCO%). Serum osmolality (S_{osm}) was measured in duplicate fresh samples using freezing point depression osmometry (Model 3250, Advanced Instruments Inc., Norwood, MA). Hemoglobin was measured in triplicate 10 µl samples using a HemoCueHb 201+ analyzer (HemoCue AB, Angelholm, Sweden). Hematocrit was analyzed in triplicate 35 µl samples drawn into microcapillary tubes, spun down in a microcentrifuge for

three minutes at 12,000 rpm (UNICO model C-MH30, Dayton, NJ), and values measured on a Damon Micro-Capillary Reader (Needham Heights, MA).

Red cell volume (RCV), plasma volume (PV), and blood volume (BV) were determined using the optimized CO-rebreathing method described by Schmidt and Prommer (28). A baseline blood sample was drawn and analyzed for HbCO% using an ABL80 FLEX OSM-3 co-oximeter (Radiometer, Denmark). Additional blood samples were drawn and analyzed at 6 and 8 minutes after beginning the rebreathing procedure, with an average of these measures (i.e., 7 minutes) being used for the calculation of ΔHbCO%. A portable CO analyzer with parts-per-million sensitivity (Pac 7000, Dräger Safety AG & Co. KGaA, Lübeck, Germany) was used to monitor potential gas leaks during the rebreathing procedure and to determine CO concentration remaining in the lungs and breathing bag following completion of the procedure. RCV, PV, and BV were then calculated using baseline values of [Hb] and Hct (described previously) using the following formulas (13):

$$RCV = BV * Hct$$

$$PV = BV - RCV$$

$$BV = RCV / [Hb]$$

Relative PV and BV (PV_{rel} and BV_{rel}, respectively) were calculated by dividing PV and BV by pre-trial BM. Due to technical difficulties, data were unable to be collected on some subjects. Results are presented as N-OB (n = 8) and OB (n = 6).

Microdialysis Protocol

Prior to inserting the microdialysis membranes, an ice pack was placed on the subject's arm for ~10 minutes to minimize hyperemia (16). Two intradermal MD probes (MD-2000, BASi® Inc., West Lafayette, IN) were then inserted into the left dorsal forearm by advancing a 23-gauge needle 15-20 mm through the dermal layer, 1-2 mm below the skin's surface. The probe was passed through the lumen of the needle and the needle was then withdrawn, leaving in place a 1 cm dialysis membrane (6). The MD probes were perfused with 0.9 % NaCl saline at a rate of 4.0 $\mu l \cdot min^{-1}$ via a perfusion pump (Pump 11 Pico Plus Elite, Harvard Apparatus, Holliston, MA) for 90 minutes while the hyperemic response from insertion subsided. During this time, the proximal MD site was instrumented for measurement of skin blood flow flux (SkBF; via laser-Doppler flowometry), with the laser being held in place by a ventilated capsule (for simultaneous measurement of local sweat rate [LSR; described below]). The distal MD site was instrumented for SkBF with the laser being held in place by a local heater (Perflux System 5000, Perimed, Ardmore, PA).

Following this initial 90 minute period, the drug infusion protocol commenced. At the proximal MD site, the endothelium-dependent vasodilator methacholine chloride (MCh) was perfused through the membrane starting at a concentration of 1×10^{-7} M and increasing in 10-fold increments to a maximal dose of 1×10^{-1} M. At the distal MD site, the endothelium-independent vasodilator sodium nitroprusside (SNP) was perfused through the membrane starting at a concentration of 5×10^{-8} M and increasing in 10-fold increments to a maximal dose of 5×10^{-2} M. The drugs were infused for 1 minute at a rate of 100 $\mu l \cdot min^{-1}$ before being switched to a rate of 4 $\mu l \cdot min^{-1}$ for an additional 4 minutes so that each dose was administered for at least 5 minutes (6). Beginning at 4 minutes into each dose, blood pressure was measured in duplicate via automated auscultation of the right brachial artery (Tango+; SunTech Medical, Inc.,

Morrisville, NC). Mean arterial pressure (MAP) was later calculated ([1/3*systolic blood pressure] + [2/3*diastolic blood pressure]) for subsequent calculations of cutaneous vascular conductance (CVC).

Skin blood flow flux was continuously recorded through the protocol via an integrated laser-Doppler flowmeter probe (Moor Instruments Inc., Wilmington, DE) at both sites. Changes in cutaneous vasomotor activity, expressed as CVC, were calculated by dividing skin blood flow flux by MAP and reported as a percentage of maximal CVC (i.e., $\%CVC_{max}$). At the end of the maximum dose of each drug, maximal vasodilation was elicited by delivering a maximal dose (5 x 10^{-2} M) of SNP for 30 minutes at both sites. Maximal CVC at the SNP site was taken from the highest SkBF flux/MAP recorded during the maximal SNP dose with simultaneous local heating of the skin for at least 30 minutes (until a plateau occurred) at ~44°C (2). Maximal CVC at the MCh site was taken from the highest SkBF flux/MAP recorded over the protocol.

LSR was measured at the MCh site using a 2.85 cm^2 plastic ventilated capsule held on the skin by adhesive tape. Dry nitrogen gas was supplied through the capsule at a rate of 0.3 $l \cdot min^{-1}$. The absolute humidity (g/m^3) and ambient temperature from the effluent air of the capsule were monitored by a humidity and temperature sensor (HMT333, Vaisala, Woburn, MA) and LSR ($mg \cdot cm^{-2} \cdot min^{-1}$) was calculated as LSR = ([flow rate in mg^3/min·absolute humidity in $gm/^3$] / [capsule surface area in cm^2])·1000.

Heart rate (HR) was recorded prior to blood pressure measurement at the end of each dose via a 3-lead electrocardiogram (Tango+; SunTech Medical, Inc., Morrisville, NC, USA). Local skin temperature was at an exposed area of the forearm between the MD sites (T_{sk}) using a ~1 mm diameter thermocouple (Physitemp, Clifton, NJ).

Statistical Procedures

During each trial, SkBF, LSR, and T_{sk} were continuously recorded using data acquisition software (LabChart 7, ADInstruments, Colorado Springs, CO) at a frequency of 50 Hz. Values of these variables at each dose of MCh and SNP were determined from an average of two separate 30 second averages of data recorded during blood pressure measurement (between ~4-5 minutes of each dose). Dose response curves were created using the $\%CVC_{max}$ calculated at each dose and compared via an extra sum of squares F-test (GraphPad Prism 6, GraphPad Software, La Jolla, CA). The dose that elicited half of the maximal response (Log EC_{50}) at each site was analyzed between group and between hydration condition curves. Thus, expressing the data as $\%CVC_{max}$ isolates the independent effect of hydration status and obesity. Paired and independent t-tests were used to compare hydration biomarkers, baseline, and maximal CVC/LSR responses within and between groups.

One subject in the OB group was excluded from analyses at the MCh site due to a technical error with the microdialysis membrane at this site and a different OB subject was excluded from analyses at the SNP site for the same reason.

A 2 (hydration) x 8 (time) repeated measures ANOVA was used to compare changes in T_{sk} within groups between hydration conditions. All ANOVA and t-test analyses were performed using SPSS v. 23 (IBM Corporation, Somers, NY). Greenhouse-Geisser corrections and follow up tests were performed as necessary.

Descriptive data and hydration measures are reported as mean ± SD. CVC and LSR data are reported as mean ± SE. An alpha less than 0.05 defined significance for all tests.

Results

Hydration Status Measures

Measures of ΔBM, U_{SG}, U_{osm}, U_{col}, and S_{osm} are presented in Table 2. As intended, there was a main effect of hydration condition, with greater ΔBM, U_{SG}, U_{osm}, U_{col}, and S_{osm} in the HY versus EU trials, independent of group (all $p < 0.001$). There were no differences between groups within hydration conditions (all $p > 0.05$).

Measures of RCV, PV, and BV are presented in Table 3. Relative PV and BV (PV_{rel} and BV_{rel}, respectively) were both higher in N-OB versus OB subjects, independent of hydration condition ($p < 0.05$). There were no differences between groups in RCV, PV, or BV in either hydration condition (all $p > 0.05$). Likewise, the HY-EU change was not different between or within groups.

Endothelium-dependent Cutaneous Vascular Conductance (CVC)

Baseline ($\%CVC_{max}$) and maximal CVC was not different between the EU versus HY condition in N-OB (Base: 30.7 ± 4.7 vs. 38.9 ± 6.9%; Max: 76.4 ± 5.9 vs. 78.8 ± 5.8%) or OB (Base: 25.9 ± 5.3 vs. 26.4 ± 4.4%; Max: 82.0 ± 7.6 vs. 83.7 ± 3.9%) (all $p > 0.05$). Within each hydration condition, baseline and maximal CVC were not different between groups ($p > 0.05$).

Percentage of maximum CVC responses to incremental doses of MCh in the EU trial were not different between groups, with a similar Log EC_{50} in N-OB and OB (Figure 1A). Log EC_{50} values when EU versus HY within N-OB and OB groups were also similar (Figures 2A and 2B, respectively).

Endothelium-independent Cutaneous Vascular Conductance (CVC)

Baseline ($\%CVC_{max}$) and maximal CVC was not between the EU versus HY condition within OB subjects (Base: 24.1 ± 7.8 vs. 19.5 ± 3.7%; Max: 48.3 ± 9.2 vs. 44.2 ± 9.5%, both $p > 0.05$) but tended to be lower in N-OB when EU versus HY (Base: 21.8 ± 2.7 vs. 30.7 ± 3.9%, $p = 0.061$; Max: 48.8 ± 6.8 vs. 65.9 ± 6.2%, $p = 0.064$).

In the EU condition, OB subjects had a higher Log EC50 versus N-OB in response to incremental doses of SNP (Figure 3). Hydration status appeared to affect groups differently, with a trend towards a higher Log EC_{50} when EU versus HY ($p = 0.062$) in N-OB but not OB subjects ($p = 0.242$) (Figures 4A and 4B, respectively).

Local Sweat Rate (LSR)

Baseline and maximal LSR was not different between the EU versus HY condition in OB (Base: 0.14 ± 0.01 vs. 0.14 ± 0.01 $mg \cdot cm^2 \cdot min^{-1}$; Max: 0.23 ± 0.02 vs. 0.21 ± 0.02 $mg \cdot cm^2 \cdot min^{-1}$) (all $p > 0.05$). Baseline LSR was not different between EU versus HY in N-OB (Base: 0.16 ± 0.01 vs. 0.15 ± 0.01 $mg \cdot cm^2 \cdot min^{-1}$, $p = 0.424$) but there was a trend towards lower maximal LSR when EU versus HY (0.25 ± 0.03 vs. 0.30 ± 0.03 $mg \cdot cm^2 \cdot min^{-1}$, $p = 0.050$). Within the EU and HY conditions, baseline LSR was not different between groups ($p > 0.05$); however, N-OB had a higher maximal LSR versus OB when HY ($p = 0.020$).

In the EU condition, LSR responses to incremental doses of MCh were not different between groups (Figure 5). Similarly, there were no differences in Log EC_{50} between groups when EU ($p = 0.299$) or HY ($p = 0.179$; Figures 6A and 6B, respectively).

Local Skin Temperature (T_{sk})

There were small increases in T_{sk} over time in the EU ($p = 0.004$) trial in both N-OB and OB subjects (Base: 32.2 ± 1.5 and 32.8 ± 1.7°C; End: 32.8 ± 1.2 and 33.6 ± 1.1°C, respectively); however, this change was not dependent on group ($p = 0.490$). Similarly, T_{sk} increased over time in the HY trial ($p < 0.001$) in both N-OB and OB subjects (Base: 32.3 ± 1.7 and 32.0 ± 1.2°C; End: 33.0 ± 1.0 and 32.6 ± 1.4°C) but this change was not dependent on group ($p = 0.606$). T_{sk} was not different between groups at any time point in either the EU or HY condition (all $p > 0.05$).

Discussion

In this study we investigated post-synaptic cutaneous vasodilatory and sweating responses to intradermal infusion of vasoactive compounds in non-obese (N-OB) and obese (OB) males while euhydrated (EU) and hypohydrated (HY). We hypothesized that OB males would have attenuated responses versus N-OB counterparts while EU, and that these attenuated responses would be further impaired when HY. Our data demonstrate similar endothelium-dependent cutaneous vascular conductance (CVC) between N-OB and OB subjects, independent of hydration status, in response to intradermal infusion of MCh. The higher Log EC_{50} in response to SNP suggests attenuated endothelium-independent CVC in OB versus N-OB subjects when EU but hypohydration only affected this response in N-OB. Neither body type or hydration status affected endothelium-dependent sweating responses, with similar LSR in all conditions. These data suggest that hypohydration affects endothelium-independent vasodilation in N-OB but not OB males.

Effect of obesity on post-synaptic cutaneous vasodilation and sweating while euhydrated

Obesity is associated with impairments in microvascular function, with previous investigations demonstrating attenuated endotheium-dependent (8, 15, 31) and independent (24) vasodilatory responses versus non-obese individuals using various methods of drug delivery. Until recently, however, differences in post-synaptic responses between obese and non-obese had not been investigated. Patik et al. compared vasdodilatory (CVC) responses between N-OB and OB males, reporting similar endothelium-dependent CVC between groups but impaired endothelium-independent CVC in OB subjects (24). Results from the current study further support this early study, as OB individuals required a significantly higher dose of SNP to elicit 50% of the maximal CVC response versus N-OB, suggesting impaired endothelium-independent

vasodilation (Figure 3), with no differences between groups in endothelium-dependent CVC (Figure 1). Given that SNP acts as a nitric oxide (NO) donor, impaired endothelium-independent vasodilation in the OB subjects suggests modification of NO action on the microvasculature, possibly attenuating the release of NO from the endothelium. However, contrary to some other investigations (8, 15, 31), we did not observe impaired endothelium-dependent vasodilation in OB subjects. This suggests that preserved endothelium-dependent vasodilation in the present study (despite impaired endothelium independent vasodilation) may have been modified through the release of other vasoactive substances (24), such as prostaglandins or endothelial derived hyperpolarizing factor (EDHF) (17, 20, 21). In addition to cutaneous vasodilation, we also investigated potential differences between groups in sweating (LSR) responses to intradermal infusion of MCh. The Log EC_{50} and maximal LSR was similar between groups when EU, suggesting obesity does not affect post-synaptic control of sweating while euhydrated.

Effect of hypohydration on post-synaptic cutaneous vasodilation and sweating

Attenuated cutaneous vasodilatory and sweating responses at the whole-body level accompanying hypohydration have been previously observed (22, 27). However, this is the first known investigation to directly assess the effect of hypohydration on microvascular function. While MCh-mediated CVC was unaffected by hypohydration in either group (Figures 3A and 3B), there was a trend towards a higher Log EC_{50} for SNP in hypohydrated versus euhydrated N-OB (Figure 4A) but not OB subjects. This suggests that hypohydration attenuates the endotheium-independent vasodilatory response in N-OB but not OB individuals. Importantly, both groups were at similar levels of hypohydration as indicated by multiple urinary and circulatory indices (Table 2). For example, both groups also had similar absolute plasma and blood volumes, with similar changes in these parameters accompanying hypohydration (Table

3). The tendency of higher baseline and maximal CVC in hypohydrated N-OB subjects versus their euhydrated state is contrary to our hypothesis, but is similar to other findings. For example Gagnon et al. observed elevated CVC following intradermal infusion of hypertonic saline (10). Infusion of hypertonic saline likely lead to hyperosmolality of the interstitial fluid space, mimicking the effect of whole-body hypohydration that was induced in the current study. Interestingly, this finding from Gagnon et al. was in contrast to a previous investigation showing attenuated cutaneous vasodilation during local heating following intradermal infusion of hypertonic saline (9). In the current study, the mechanism(s) behind elevated baseline and maximal CVC response to SNP infusion in hypohydrated N-OB subjects remains unclear. If elevated osmolality of the interstitium occurred as a result of hypohydration-induced plasma hyperosmolality, it is unknown why this would affect cutaneous vasodilation in N-OB, but not similarly hypohydrated OB individuals. Given that we observed an attenuated endothelium-independent CVC response in euhydrated OB, it is possible that the apparent differences in microvascular function between groups subsequently affected the response to hypohydration. Further investigation into the mechanism(s) behind attenuated SNP-mediated vasodilation accompanying hypohydration is warranted.

In addition to examining the effect of hypohydration on cutaneous vasodilation in N-OB and OB, we simultaneously compared the influence of hypohydration on post-synaptic sweating responses. Endothelium-dependent (i.e., MCh-mediated) LSR responses were similarly affected by hypohydration in N-OB and OB, with similar Log EC_{50} values between EU and HY trials in each group (Figures 5 and 6). However, within the HY condition, N-OB had a significantly higher maximal LSR versus OB (0.30 ± 0.03 vs. 0.21 ± 0.02 $mg \cdot cm^2 \cdot min^{-1}$). The exact

mechanism(s) behind a reduced maximal LSR in hypohydrated OB versus N-OS is unclear and warrants further investigation.

Limitations

Skin temperature can modify cutaneous vasodilatory and sweating responses independent from any changes in core body temperature. Given that we were examining changes in these parameters without modifying core body temperature, skin temperature should ideally be the same when comparing different populations under different physiological conditions. Dual assessment of both CVC and LSR at the MCh site limited our ability to clamp skin temperature (T_{sk}) at the drug delivery sites at a fixed point. However, T_{sk} prior to starting drug infusion and subsequent changes throughout the protocol were not different between group or hydration condition, suggesting that this likely had negligible influence on CVC and LSR responses.

While our OB subjects could certainly be classified as such given their high body mass and fat percentage, it should be noted that they were still a relatively young and healthy sample, free of any cardiovascular or metabolic disorders. Due to the exercise-heat stress trial the subjects performed 24-h prior to reporting for the present study, we required individuals with sufficient aerobic fitness to be able to complete the protocol. In doing so, this eliminated potential subjects with higher adiposity and lower aerobic fitness that may have otherwise qualified. Future studies should seek to examine the relationship between obesity, microvascular function, and hydration status in individuals with higher levels of adiposity (i.e., morbid obesity).

Conclusions

We hypothesized that OB individuals would have impaired cutaneous vasodilatory and sweating responses when euhydrated in response to intradermal infusion of MCh and SNP versus N-OB counterparts. Moreover, we hypothesized that hypohydration exacerbate these

impairments to a greater extent in OB versus N-OB. No differences were present between groups or hydration condition in MCh-mediated CVC, suggesting body type and hydration status did not influence endothelium-dependent cutaneous vasodilation. In contrast, OB individuals required a significantly higher dose of SNP to elicit 50% of the maximal CVC response versus N-OB, suggesting impaired endothelium-independent vasodilation. Interestingly, hypohydration tended to attenuate endothelium-independent CVC in N-OB but not OB individuals versus a euhydrated condition. This provides direct evidence for the added influence of whole-body hypohydration on endothelium-independent cutaneous vasodilation. Future studies should continue to examine the effect of hypohydration on microvascular function in healthy and other clinical populations with known microvascular dysfunction (i.e., individuals with type II diabetes and/or hypertension).

Conflicts of interest

There are no conflicts of interest to declare.

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Figure Captions

Figure 1. Cutaneous vascular conductance (CVC, expressed as a percentage of maximum) in non-obese (N-OB) and obese (OB) subjects at baseline (Base) and in response to incremental sub-cutaneous infusion of methacholine chloride (MCh) while euhydrated.

Figure 2A. Cutaneous vascular conductance (CVC, expressed as a percentage of maximum) in non-obese subjects at baseline (Base) and in response to incremental sub-cutaneous infusion of methacholine chloride (MCh) while euhydrated (EU) and hypohydrated (HY).

Figure 2B. Cutaneous vascular conductance (CVC, expressed as a percentage of maximum) in obese subjects at baseline (Base) in response to incremental sub-cutaneous infusion of methacholine chloride (MCh) while euhydrated (EU) and hypohydrated (HY).

Figure 3. Cutaneous vascular conductance (CVC, expressed as a percentage of maximum) in non-obese (N-OB) and obese (OB) subjects at baseline (Base) in response to incremental sub-cutaneous infusion of sodium nitroprusside (SNP) while euhydrated.

Figure 4A. Cutaneous vascular conductance (CVC, expressed as a percentage of maximum) in non-obese subjects at baseline (Base) and in response to incremental sub-cutaneous infusion of sodium nitroprusside (SNP) while euhydrated (EU) and hypohydrated (HY).

Figure 4B. Cutaneous vascular conductance (CVC, expressed as a percentage of maximum) in obese subjects at baseline (Base) and in response to incremental sub-cutaneous infusion of sodium nitroprusside (SNP) while euhydrated (EU) and hypohydrated (HY).

Figure 5. Local sweat rate (LSR) in non-obese (N-OB) and obese (OB) subjects at baseline (Base) and in response to incremental sub-cutaneous infusion of methacholine chloride while euhydrated.

Figure 6A. Local sweat rate (LSR) in non-obese subjects at baseline (Base) and in response to incremental sub-cutaneous infusion of methacholine chloride while euhydrated (EU) and hypohydrated (HY).

Figure 6B. Local sweat rate (LSR) in obese subjects at baseline (Base) and in response to incremental sub-cutaneous infusion of methacholine chloride while euhydrated (EU) and hypohydrated (HY).

Table 1. Subject demographic data by group.

	N-OB	OB
Age (y)	24 ± 4	26 ± 5
Height (cm)	179 ± 5	176 ± 7
Mass (kg)	74.1 ± 9.0	90.1 ± 6.7*
BMI ($kg \cdot m^2$)	23.1 ± 3.1	29.1 ± 2.4*
Body fat (%)	14.3 ± 3.3	30.1 ± 3.9*
Physical activity ($MET\text{-}min \cdot wk^{-1}$)	4417 ± 3514	4839 ± 3055

N-OB = non-obese; OB = obese; *significantly different between groups ($p < 0.001$).

Table 2. Baseline hydration biomarkers for euhydrated (EU) and hypohydrated (HY) trials in non-obese (N-OB) and obese (OB) males.

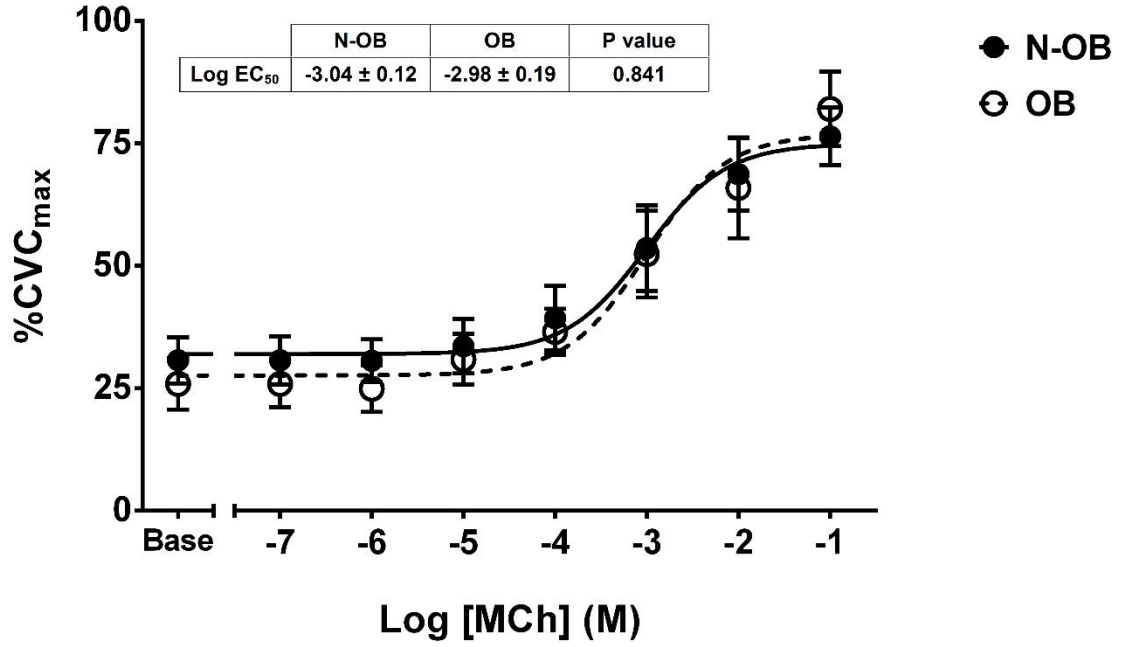
	EU		HY	
	N-OB	OB	N-OB	OB
ΔBM (%)[a]	-0.2 ± 0.7	0.2 ± 0.8	-2.9 ± 1.3*	-1.7 ± 1.4*
U_{SG} ($g \cdot ml^{-1}$)	1.010 ± 0.007	1.011 ± 0.007	1.028 ± 0.003*	1.030 ± 0.004*
U_{osm} ($mOsm \cdot kg^{-1}$)	398 ± 252	439 ± 275	1075 ± 88*	1154 ± 120*
U_{col}	2 ± 1	2 ± 1	4 ± 1*	4 ± 1*
Hb ($g \cdot dl^{-1}$)	14.2 ± 0.9	14.5 ± 0.8	14.8 ± 1.1*	14.8 ± 1.0
Hct (%)	41.9 ± 1.7	42.2 ± 2.1	43.0 ± 2.3	42.6 ± 2.6
S_{osm} ($mOsm \cdot kg^{-1}$)[a]	287 ± 3	287 ± 4	297 ± 5*	295 ± 7*

ΔBM = percent body mass change; U_{SG} = urine specific gravity; U_{osm} = urine osmolality; U_{col} = urine color; Hb = hemoglobin; Hct = hematorcrit; S_{osm} = serum osmolality. [a]n = 9 in OB group. *significantly different versus EU condition ($p < 0.01$).

Table 3. Red cell volume (RCV), absolute and relative plasma volume (PV and PV_{rel}, respectively) and absolute and relative blood volume (BV and BV_{rel}) for non-obese (N-OB) and obese (OB) subjects in euhydrated (EU) and hypohydrated (HY) trials.

	N-OB			OB		
	EU	HY	Change (HY-EU)	EU	HY	Change (HY-EU)
RCV (ml)	2792 ± 283	2871 ± 225	78 ± 189	2825 ± 321	2785 ± 293	-40 ± 81
PV (ml)	3894 ± 432	3839 ± 550	-55 ± 304	3863 ± 445	3731 ± 267	-132 ± 437
PV_{rel} ($ml \cdot kg^{-1}$)	51.4 ± 6.8*	52.1 ± 8.1*	0.7 ± 4.6	43.1 ± 2.1	42.6 ± 5.0	-0.5 ± 5.2
BV (ml)	6686 ± 669	6710 ± 732	24 ± 436	6688 ± 713	6516 ± 453	-172 ± 510
BV_{rel} ($ml \cdot kg^{-1}$)	88.2 ± 9.7*	91.0 ± 11.3*	2.9 ± 6.5	74.6 ± 1.6	74.3 ± 5.8	-0.3 ± 6.2

*significantly higher versus OB subjects, independent of hydration condition ($p < 0.05$). No other differences between groups or condition (all $p > 0.05$).


N-OB
OB
P value
Log EC50
-3.04 ± 0.12
-2.98 ± 0.19
0.841
100
75
50
25
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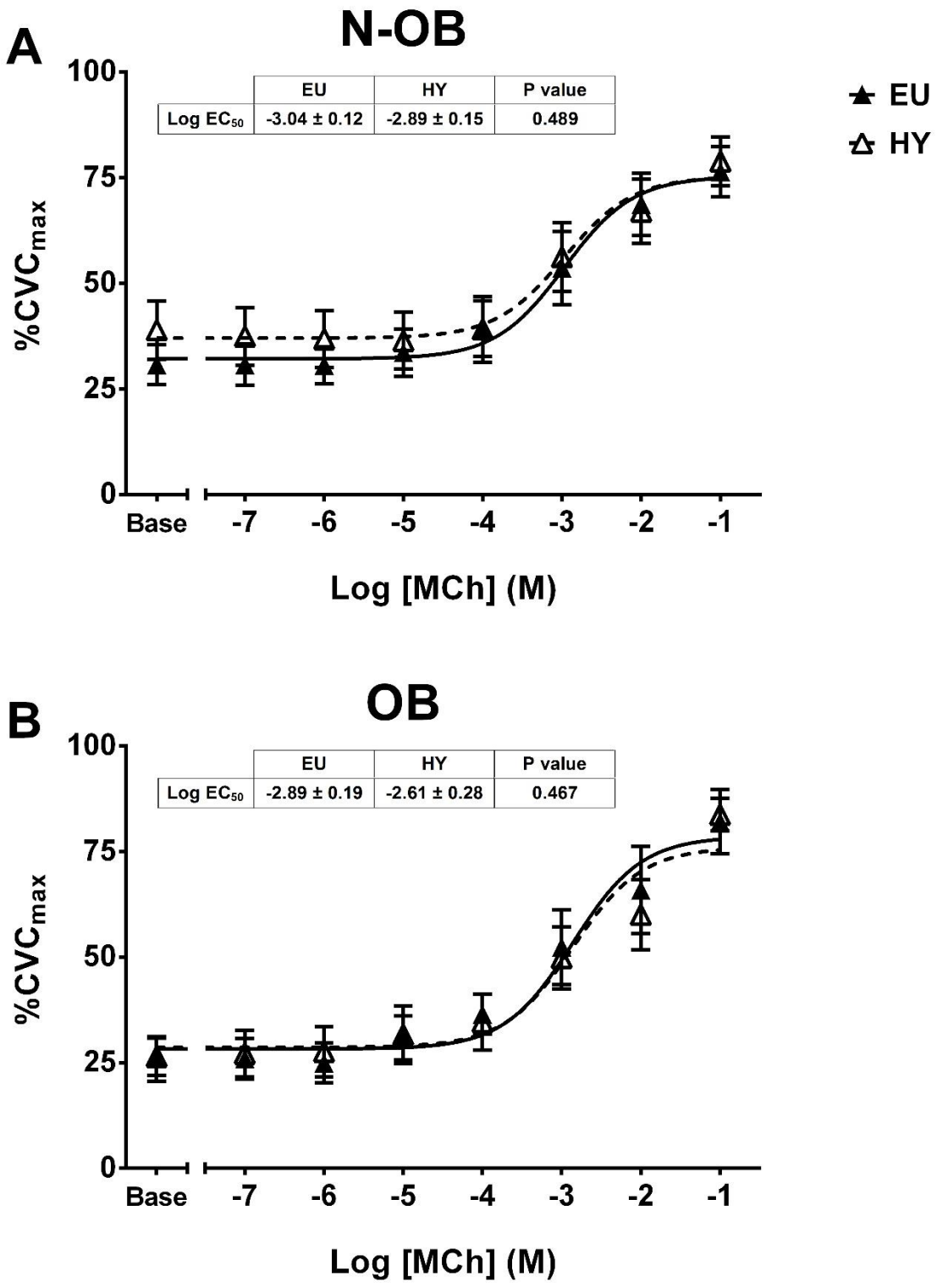
%CVCmax
Base
-7
-6
-5
-4
-3
-2
-1
Log [MCh] (M)
N-OB
OB

Figure 1.


A
N-OB
EU
HY
P value
Log EC50
-3.04 ± 0.12
-2.89 ± 0.15
0.489
%CVCmax
100
75
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25
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Base
-7
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-5
-4
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-2
-1
Log [MCh] (M)
EU
HY
B
OB
EU
HY
P value
Log EC50
-2.89 ± 0.19
-2.61 ± 0.28
0.467
%CVCmax
100
75
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Base
-7
-6
-5
-4
-3
-2
-1
Log [MCh] (M)

Figure 2.

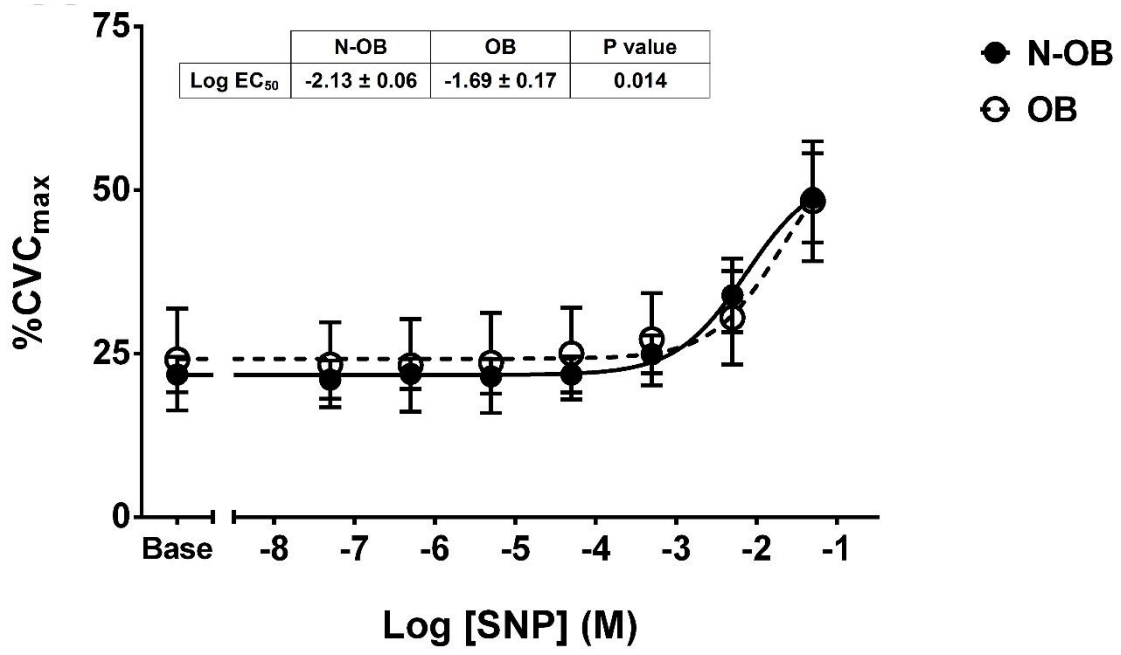
N-OB
OB
P value
Log EC50
-2.13 ± 0.06
-1.69 ± 0.17
0.014
75
50
25
0
%CVCmax
Base
-8
-7
-6
-5
-4
-3
-2
-1
Log [SNP] (M)

Figure 3.

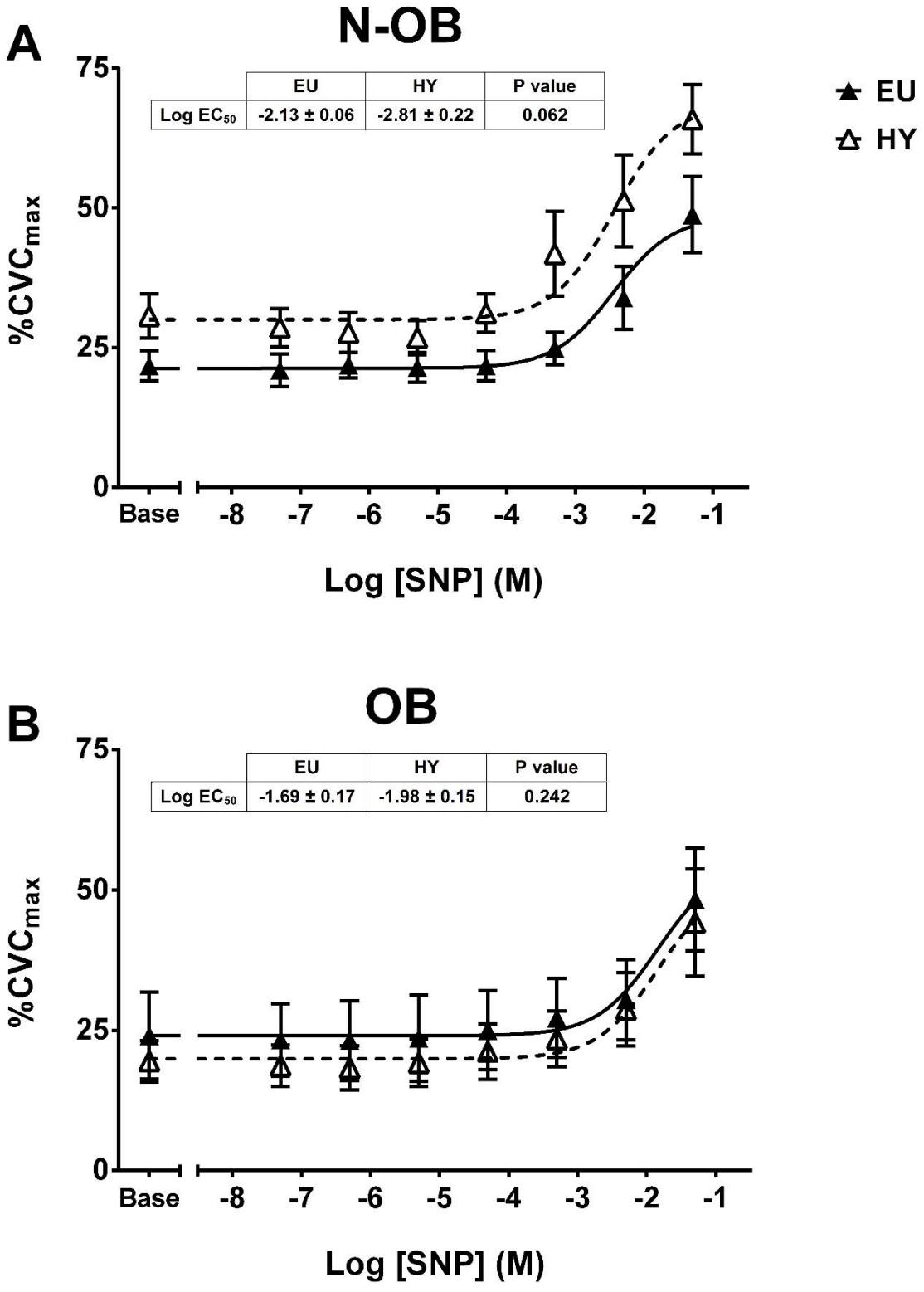
A
N-OB
EU
HY
P value
Log EC50
-2.13 ± 0.06
-2.81 ± 0.22
0.062
EU
HY
%CVCmax
75
50
25
0
Base
-8
-7
-6
-5
-4
-3
-2
-1
Log [SNP] (M)
B
OB
EU
HY
P value
Log EC50
-1.69 ± 0.17
-1.98 ± 0.15
0.242
%CVCmax
75
50
25
0
Base
-8
-7
-6
-5
-4
-3
-2
-1
Log [SNP] (M)

Figure 4.

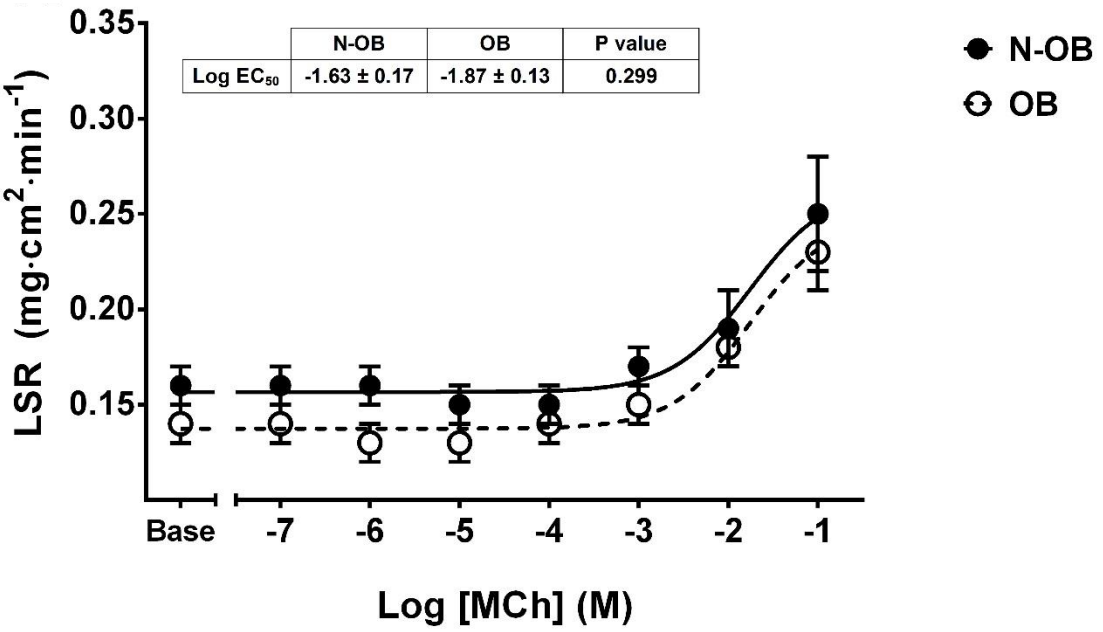
N-OB
OB
P value
Log EC50
-1.63 ± 0.17
-1.87 ± 0.13
0.299
LSR (mg·cm2·min-1)
0.35
0.30
0.25
0.20
0.15
Base
-7
-6
-5
-4
-3
-2
-1
Log [MCh] (M)

Figure 5.

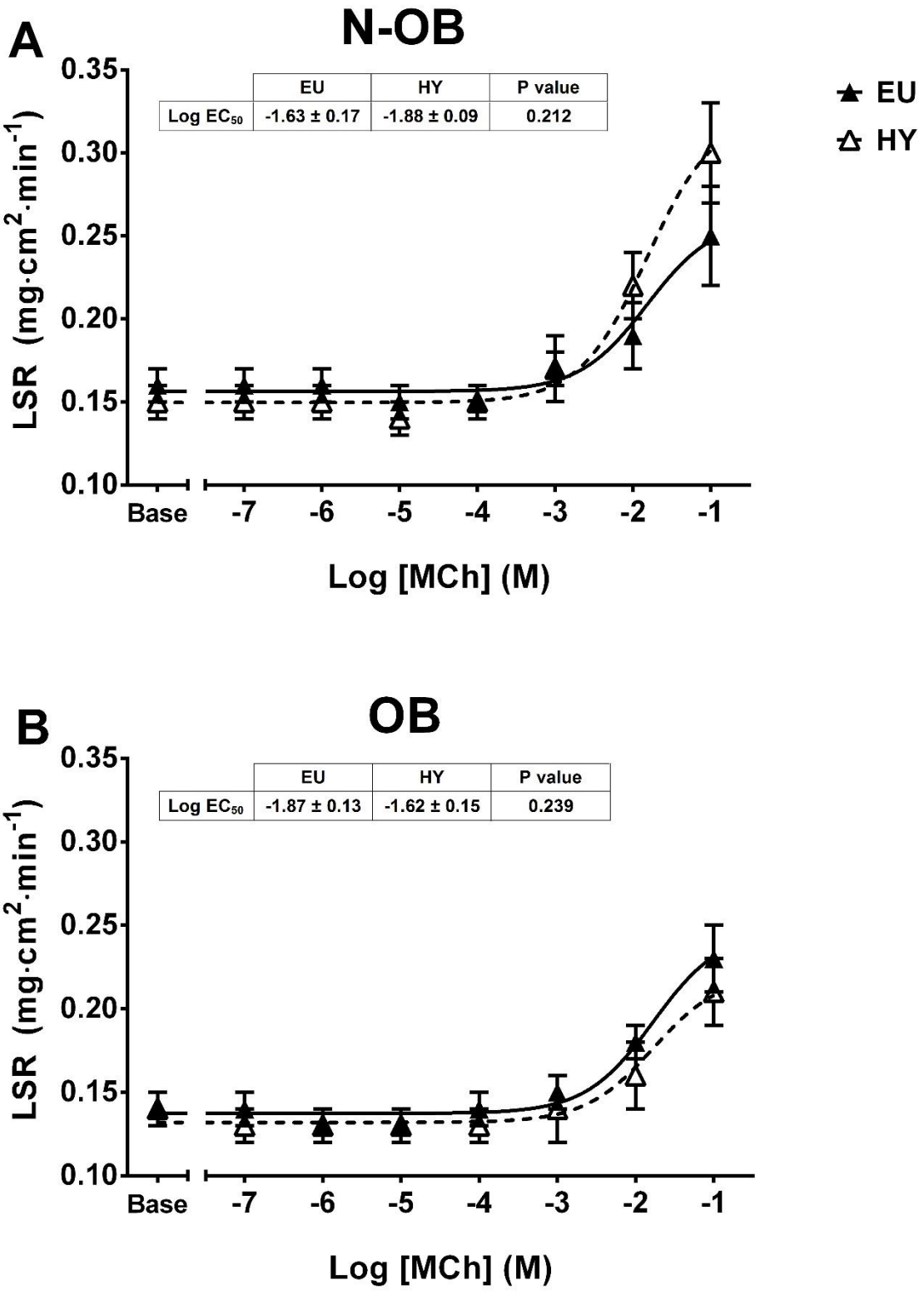
A
N-OB
EU
HY
P value
Log EC50
-1.63 ± 0.17
-1.88 ± 0.09
0.212
EU
HY
LSR (mg·cm2·min-1)
0.35
0.30
0.25
0.20
0.15
0.10
Base
-7
-6
-5
-4
-3
-2
-1
Log [MCh] (M)
B
OB
EU
HY
P value
Log EC50
-1.87 ± 0.13
-1.62 ± 0.15
0.239
LSR (mg·cm2·min-1)
0.35
0.30
0.25
0.20
0.15
0.10
Base
-7
-6
-5
-4
-3
-2
-1
Log [MCh] (M)

Figure 6.

CHAPTER FOUR

Conclusions

These studies sought to determine if impairments in thermoregulatory responses were present in obese males via both centrally- and peripherally-mediated mechanisms compared to non-obese. In order to investigate this, we compared thermoregulatory responses in non-obese and obese males during exercise heat-stress (i.e., centrally-mediated) and in response to intradermal infusion of vasoactive substances (i.e., peripherally-mediated). Further, we compared these groups while subjects were euhydrated and hypohydrated in order to examine potential differences between groups in the physiological modification of thermoregulation attributed to hypohydration. During exercise-heat stress, cutaneous vasodilation and sweating responses were similar between groups when euhydrated. However, obese subjects had a greater increase in rectal temperature versus non-obese. Hypohydration affected cutaneous vasodilatory and sweating responses in a similar magnitude in both groups but compared to the euhydrated condition, hypohydration increased the end-exercise rectal temperature in non-obese but not obese subjects. This suggests that, despite a similar ability of obese subjects to increase cutaneous vasodilation and sweating compared to non-obese when euhydrated, obese were unable to prevent rectal temperature from increasing to levels observed when hypohydrated.

During intradermal drug infusion, both non-obese and obese males exhibited similar endothelium-dependent vasodilation and sweating responses when either euhydrated or hypohydrated. However, obese showed attenuated cutaneous vasodilation in response to the nitric oxide donor sodium nitroprusside (SNP) versus non-obese when euhydrated, suggesting

impaired endothelium-independent vasodilation. Hypohydration did not affect endothelium-dependent, -independent, or sweating responses differently between groups.

These data suggest that during exercise-heat stress, obese males are not able to appropriately increase cutaneous vasodilation and sweating in response to an elevated rectal temperature versus non-obese when euhydrated. Moreover, hypohydration appears to affect non-obese to a greater extent versus obese, with non-obese subjects exhibiting a significantly higher rectal temperature when hypohydrated versus euhydrated, but no difference between hydration conditions in obese. In addition, obese males exhibit impaired peripherally-mediated cutaneous vasodilation in response to intradermal infusion of an endothelium-independent vasodilator, independent of hydration status. The exact mechanism(s) for this peripheral impairment are not clear, but may be related to microvascular dysfunction associated with obesity. Future studies should seek to examine the relationship between thermoregulatory responses and hydration status in other populations that may have impaired centrally- and/or peripherally-mediated vasodilatory and sweating responses (i.e., females, older individuals, individuals with Type II diabetes mellitus).

Appendix A

Table. Perceptual ratings during euhydrated (EU; top) and hypohydrated (HY; bottom) trials in non-obese (N-OB) and obese (OB) subjects.

	EU									
	Baseline		15 min		30 min		45 min		60 min	
	N-OB	OB	N-OB	OB	N-OB	OB	N-OB	OB	N-OB	OB
RPE	-	-	10 ± 2	11 ± 2	11 ± 3	13 ± 2*	11 ± 2	14 ± 3*	11 ± 3	14 ± 5
TS	4.9 ± 0.4	4.9 ± 0.5	5.3 ± 0.3	5.7 ± 0.4	5.5 ± 0.5	5.8 ± 0.7	5.5 ± 0.7	6.1 ± 0.7	5.6 ± 0.9	6.3 ± 1.2
MP	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.6	1.3 ± 1.0*	0.8 ± 1.2	2.5 ± 1.7*	1.0 ± 1.3	3.8 ± 2.6*	0.9 ± 1.0	3.8 ± 3.2*
TH	3 ± 1	2 ± 1	4 ± 1	4 ± 1	3 ± 1	3 ± 2	3 ± 1	3 ± 2	2 ± 2	2 ± 2
COM	1 ± 1	1 ± 0	2 ± 0	2 ± 1	2 ± 1	3 ± 1	2 ± 1	3 ± 1	2 ± 1	3 ± 1
MOT	4 ± 1	5 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1
	HY									
	Baseline		15 min		30 min		45 min		60 min	
	N-OB	OB	N-OB	OB	N-OB	OB	N-OB	OB	N-OB	OB
RPE	-	-	11 ± 2	12 ± 2	12 ± 2	14 ± 2	13 ± 3	15 ± 4	12 ± 3	16 ± 3*
TS	5.1 ± 0.4	5.0 ± 0.4	5.5 ± 0.7	5.7 ± 0.4	5.8 ± 0.6	6.4 ± 0.4*	6.0 ± 0.5	6.6 ± 0.6*	6.1 ± 0.7	6.6 ± 0.8
MP	0.0 ± 0.0	0.2 ± 0.4	0.5 ± 0.9	2.2 ± 1.9*	0.8 ± 1.1	3.6 ± 2.4*	0.8 ± 1.0	4.7 ± 3.1**	1.0 ± 1.1	4.6 ± 2.5**
TH	5 ± 1	5 ± 2	6 ± 1	6 ± 1	7 ± 1	7 ± 1	8 ± 1	8 ± 1	8 ± 1	8 ± 1
COM	2 ± 1	1 ± 1	2 ± 1	2 ± 1	2 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1	3 ± 1
MOT	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1	4 ± 1**	4 ± 1	4 ± 1**

RPE = rating of perceived exertion (range 6-20); TS = thermal sensation (0.5-10); MP = muscle pain (0.0-10); TH = thirst (0-9); COM = comfort (1-5); MOT = motivation to continue (1-5). *significantly different between groups ($p < 0.05$), **significantly different between groups ($p < 0.01$).

Results

Thirst (TH): in the EU trials, there was a main effect of time ($p = 0.004$) but no interaction between group and time ($p = 0.518$). Hypohydration did not affect groups differently, with both groups showing increased TH over time ($p = 0.003$) with no interaction ($p = 0.596$).

Thermal sensation (TS): in the EU trials, TS increased over time ($p < 0.001$) but there was no interaction between group and time ($p = 0.142$). Hypohydration did not affect groups differently, with both groups showing increased TS over time ($p = 0.022$) with no interaction ($p = 0.288$).

Muscle pain (MP): in the EU trials, MP increased over time ($p < 0.001$) and there was a significant interaction between group and time ($p = 0.004$). OB reported higher values of MP at 15, 30, 45, and 60 min versus N-OB (all $p < 0.05$). Hypohydration did not affect the change over time versus EU ($p = 0.177$), nor was there a significant interaction between time and group ($p = 0.137$).

Motivation to continue (MOT): in the EU trials, MOT increased over time ($p = 0.047$) and there was a significant interaction between group and time ($p = 0.043$). N-OB reported lower values of MOT at 45 and 60 min versus OB when HY (both $p < 0.01$). Hypohydration did not affect the change over time versus EU ($p = 0.910$), nor was there a significant interaction between time and group ($p = 0.205$).

Comfort (COM): in the EU trials, COM increased (Note: higher COM indicates greater *discomfort*) over time ($p < 0.001$) and there was a significant interaction between group and time ($p = 0.041$). Hypohydration did not affect groups differently, with both groups showing no change over time ($p = 0.728$) versus EU and no interaction between group and time ($p = 0.459$).

Rating of perceived exertion (RPE): in the EU trials, RPE increased over time ($p < 0.001$) and there was a significant interaction between group and time ($p = 0.004$). RPE was significantly higher in OB versus N-OB at 30 and 45 min (both $p < 0.05$). Hypohydration did not affect groups differently, with both groups showing no change over time ($p = 0.593$) versus EU and no interaction between group and time ($p = 0.957$).

Appendix B

Figure Captions

Figure 1. Changes in rectal temperature (T_{rec}; A), mean skin temperature (T_{sk}; B) and mean body temperature (T_b; C) in non-obese (N-OB) and obese (OB) subjects while hypohydrated. *significant difference between groups ($p < 0.05$).

Figure 2. Changes in cutaneous vascular conductance (CVC; A) and local sweat rate (LSR; B) in non-obese (N-OB) and obese (OB) subjects while hypohydrated. *significant difference between groups ($p < 0.05$).

Figure 3. Changes in sweat gland activation (SGA; A) and sweat gland output (SGO; B) in non-obese (N-OB) and obese (OB) subjects while hypohydrated. *significant difference between groups ($p < 0.05$).

Figure 4. Changes in heart rate (HR; A) and mean arterial pressure (MAP; B) in non-obese (N-OB) and obese (OB) subjects while hypohydrated. . No significant differences between groups at any time point (all $p > 0.05$).

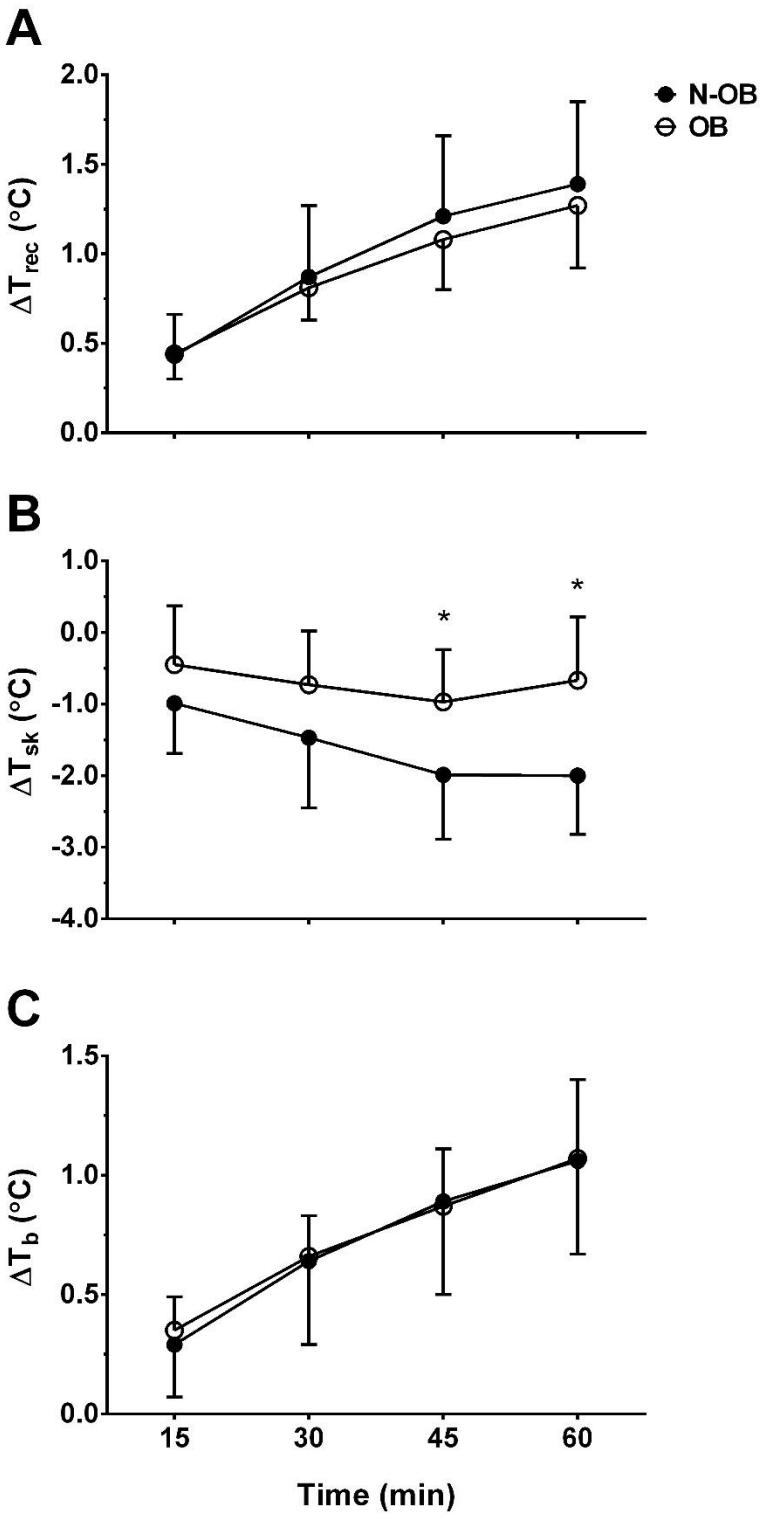
A
B
C
N-OB
OB
ΔTrec (°C)
ΔTsk (°C)
ΔTb (°C)
2.0
1.5
1.0
0.5
0.0
-1.0
-2.0
-3.0
-4.0
*
*
15
30
45
60
Time (min)

Figure 1.

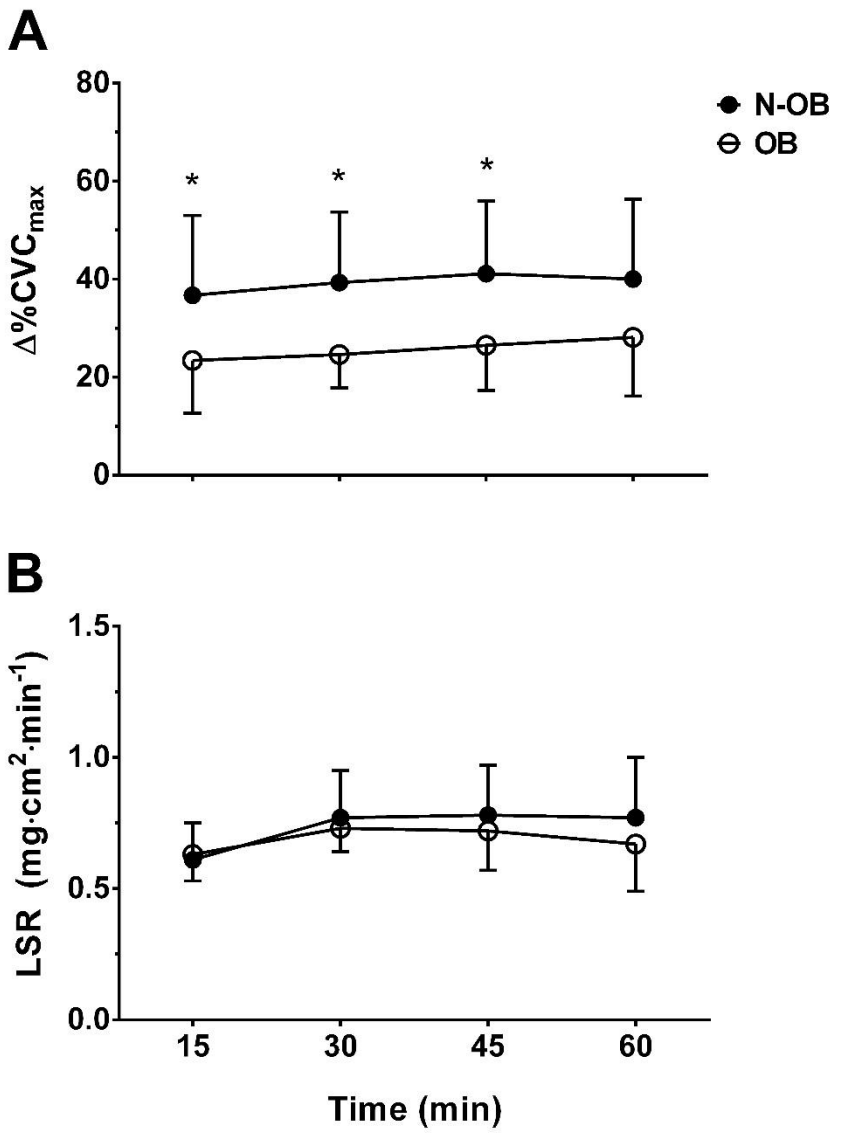
A
80
60
40
20
0
Δ%CVC$_{max}$
*
*
*
N-OB
OB
B
1.5
1.0
0.5
0.0
LSR (mg·cm^2·min^{-1})
15
30
45
60
Time (min)

Figure 2.

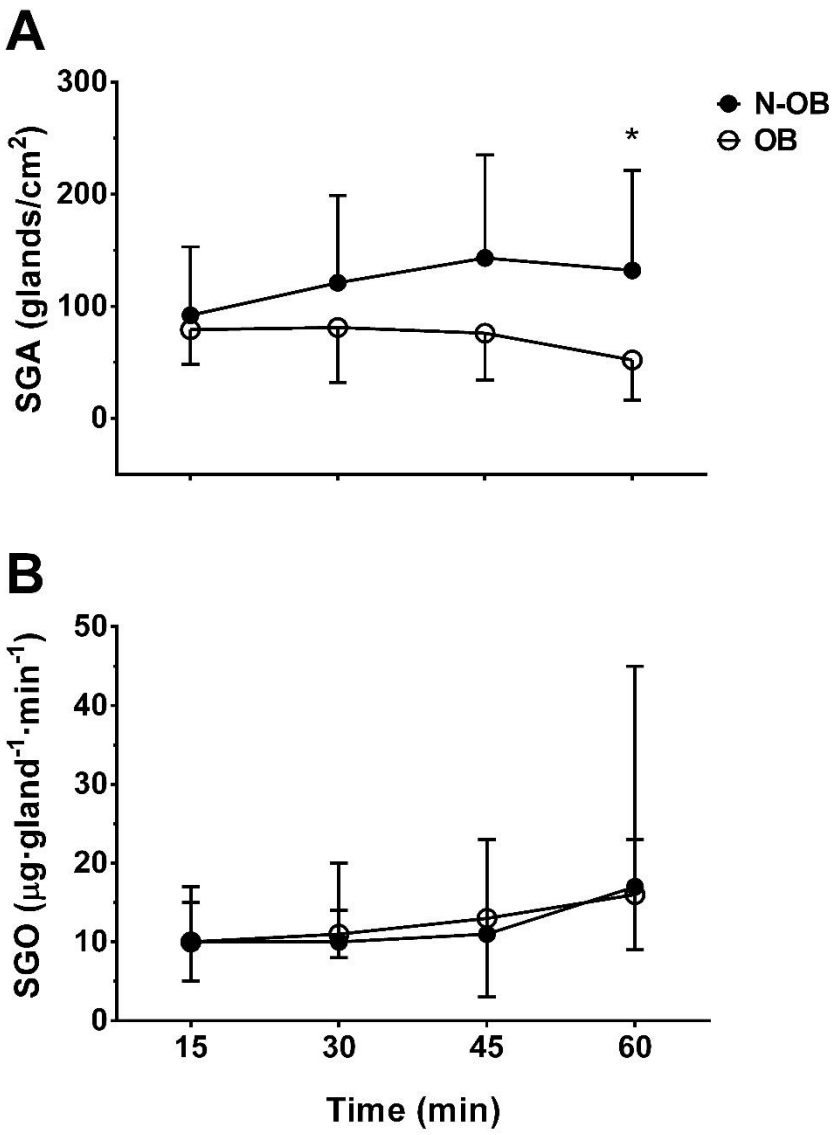
A
300
200
100
0
SGA (glands/cm^2)
*
N-OB
OB
B
50
40
30
20
10
0
SGO (µg·gland^{-1}·min^{-1})
15
30
45
60
Time (min)

Figure 3.

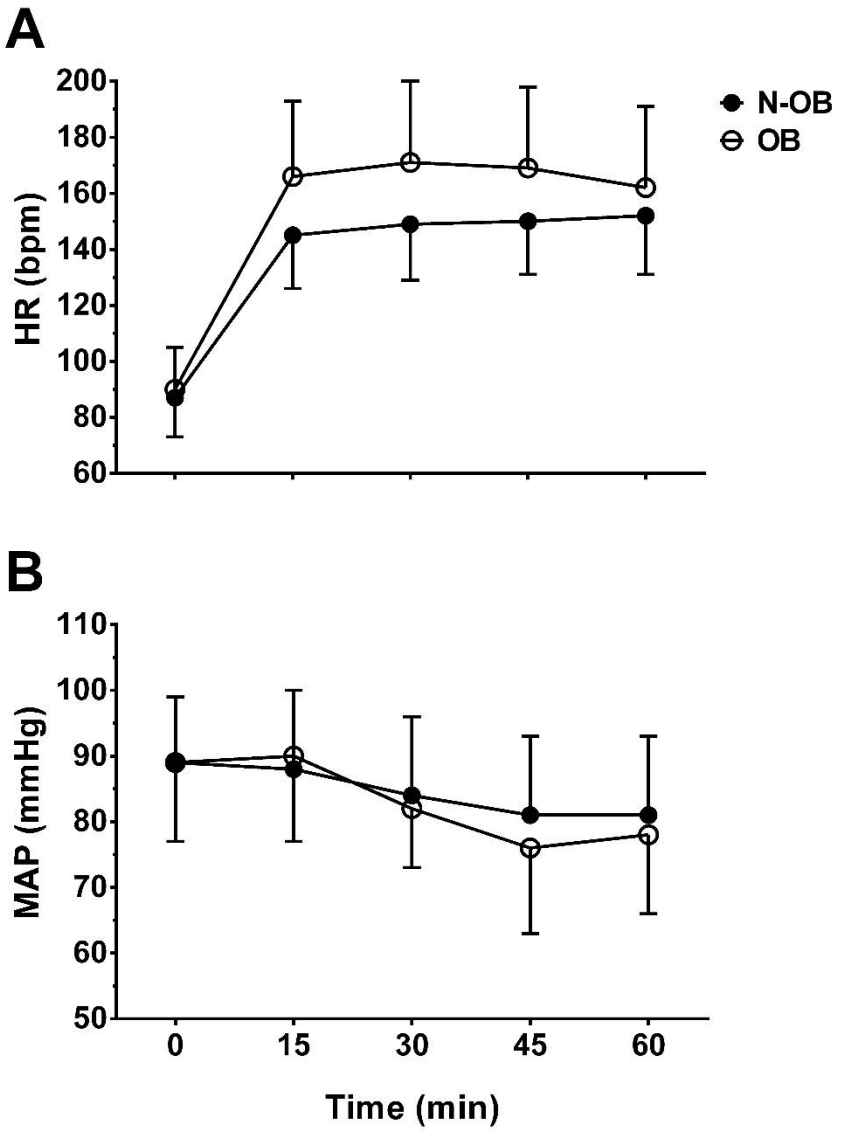
A
200
180
160
140
120
100
80
60
HR (bpm)
N-OB
OB
B
110
100
90
80
70
60
50
MAP (mmHg)
0
15
30
45
60
Time (min)

Figure 4.

Appendix C

UNIVERSITY OF ARKANSAS

Office of Research Compliance
Institutional Review Board

May 28, 2015

MEMORANDUM

TO:	Matthew Tucker	Stavros Kavouras
	Cory Butts	Nicole Moyen
	Cash Arcement	JD Adams
	Blake Robinson	Lemuel Brown
	Aleph Oliveria	Brendon McDermott
	Ethan Bagwell	Monty Matthews
	Matthew Ganio	
FROM:	Ro Windwalker IRB Coordinator	
RE:	New Protocol Approval	
IRB Protocol #:	15-05-726	
Protocol Title:	*Centrally and Peripherally Mediated Thermoregulatory Function in Lean and Obese Individuals*	
Review Type:	☐ EXEMPT ☐ EXPEDITED ☒ FULL IRB	
Approved Project Period:	Start Date: 05/28/2015 Expiration Date: 05/20/2016	

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 (https://vpred.uark.edu/units/rscp/index.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 40 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 109 MLKG Building, 5-2208, or irb@uark.edu.