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RESEARCH ARTICLE

Caffeine alters thermoregulatory responses to exercise in the heat only in caffeine-habituated individuals: a double-blind placebo-controlled trial

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Abstract

To assess the impact of acute caffeine ingestion on thermoregulatory responses during steady-state exercise under moderate heat stress conditions in caffeine-habituated and nonhabituated individuals. Twenty-eight participants [14 habituated (HAB) (4 females) and 14 nonhabituated (NHAB) (6 females)] cycled at a fixed metabolic heat production (7 W·kg^{-1}) for 60 min on two separate occasions 1 h after ingesting *1*) 5 mg·kg^{-1} caffeine (CAF) or *2*) 5 mg·kg^{-1} placebo (PLA), in a double-blinded, randomized, and counterbalanced order. Environmental conditions were 30.6 ± 0.9°C, 31 ± 1% relative humidity (RH). The end-exercise rise in esophageal temperature (ΔT_{es}) from baseline was greater with CAF in the HAB group (CAF = 0.88 ± 0.29°C, PLA = 0.62 ± 0.34°C, $P < 0.001$), but not in the NHAB group (CAF = 1.00 ± 0.42°C, PLA = 1.00 ± 0.39°C, $P = 0.94$). For a given change in mean body temperature, rises in % of maximum skin blood flow were attenuated with CAF on the forearm ($P = 0.015$) and back ($P = 0.021$) in the HAB group, but not in the NHAB group ($P \geq 0.65$). Dry heat loss was similar in the HAB (CAF = 31 ± 5 W·m^{-2}, PLA = 33 ± 7 W·m^{-2}) and NHAB groups (CAF = 31 ± 3 W·m^{-2}, PLA 30 ± 4 W·m^{-2}) ($P \geq 0.37$). There were no differences in whole body sweat losses in both groups (HAB: CAF = 0.59 ± 0.15 kg, PLA = 0.56 ± 0.17 kg, NHAB:CAF = 0.53 ± 0.19 kg, PLA 0.52 ± 0.19 kg) ($P \geq 0.32$). As the potential for both dry and evaporative heat loss was uninhibited by caffeine, we suggest that the observed ΔT_{es} differences with CAF in the HAB group were due to alterations in internal heat distribution. Our findings support the common practice of participants abstaining from caffeine before participation in thermoregulatory research studies in compensable conditions.

NEW & NOTEWORTHY We provide empirical evidence that acute caffeine ingestion exerts a thermoregulatory effect during exercise in the heat in caffeine-habituated individuals but not in nonhabituated individuals. Specifically, caffeine habituation was associated with a greater rise in esophageal temperature with caffeine compared with placebo, which appears to be driven by a blunted skin blood flow response. In contrast, no thermoregulatory differences were observed with caffeine in nonhabituated individuals. Caffeine did not affect sweating responses during exercise in the heat.

body temperature; heat balance; skin blood flow; sweating; thermosensitivity

INTRODUCTION

Products containing caffeine are consumed extensively worldwide (1). Dietary caffeine is sourced from multiple foods and beverages (such as tea, coffee, energy drinks, and chocolate) with the most common source of high and regular doses of caffeine obtained through coffee consumption (1). In human thermoregulatory research studies, a widely adopted practice is to require participants to abstain from caffeine consumption ~12–48 h before their trial. However, the evidence supporting this standard procedure, particularly for steady-state exercise studies that seek to examine potential mediators of thermoregulatory impairment, appears inconclusive.

Caffeine is an adenosine receptor antagonist (2), where adenosine potentiates both endothelium-independent and -dependent vasodilation (3) and thus has the potential to affect the internal transfer of heat to the skin surface via the circulatory system. However, it remains unclear whether systematic differences in the rise in core temperature, or if any alterations in sweating and/or skin blood flow responses, occur with caffeine ingestion during steady-state exercise in the heat. Some studies suggest that at rest and during the early stages of exercise, caffeine attenuates the rise in skin blood flow and vascular conductance in temperate (4, 5), but not warm conditions (6). A higher absolute mean body temperature with a high dose of caffeine (9 mg·kg^{-1}) has been reported during fixed relative intensity cycling (50% $\dot{V}O_{2peak}$) in a 40°C, 25% RH environment (7). However, these differences in body temperature appear to be simply a product of differences in baseline values and therefore do not indicate an alteration in the physiological modification of heat transfer once exercise begins. Notably, other existing studies do not indicate an effect of caffeine on core temperature (8, 9).

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There is limited evidence indicating that caffeine ingestion can modulate the human sweating response to exercise and/or heat stress. However, a lower esophageal temperature onset threshold and/or a greater sweat rate for a given internal body temperature via increased circulating catecholamines could be possible (10). No study to the best of our knowledge has examined whether caffeine alters vasomotor and sudomotor control, whereby thermoeffector responses (i.e., increases in sweat rate and skin blood flow) are evaluated relative to increases in mean body temperature (T_b) using esophageal temperature (T_{es}).

Requiring participants to abruptly abstain from regular caffeine consumption could result in negative psychological and physiological effects such as headaches, drowsiness, lethargy, and irritability within 12 h of cessation (11), possibly unnecessarily. There is evidence that some physiological responses (e.g., blood pressure, heart rate, plasma renin activity, epinephrine, and norepinephrine) to caffeine ingestion at rest are dependent on caffeine habituation status (12). However, the impact of such habituation from a thermoregulatory perspective remains unknown.

In this double-blind, placebo-controlled study, our aims were twofold: *1*) to compare the independent effect of an acute dose of caffeine (5 mg·kg^{-1}) on changes in core temperature, sweating, and skin blood flow during steady-state exercise in a compensable heat stress environment; and *2*) to determine whether any interaction exists between the effects of caffeine ingestion and caffeine-habituation status on these thermoregulatory responses to exercise. We hypothesized that *1*) acute caffeine ingestion would lead to a greater rise in core temperature during exercise compared with the ingestion of a placebo and *2*) this effect of caffeine would be different between the caffeine habituated and nonhabituated individuals.

METHODS

Participants

A total of 28 participants [14 caffeine habituated (HAB, 4 females) and 14 nonhabituated (NHAB, 6 females)] were recruited (Table 1). Written informed consent was obtained for all individuals prior to participating in the study. All participants completed trials in a climate-controlled chamber at the Thermal Ergonomics Laboratory at The University of Sydney. Approval for the study was obtained from the Human Research Ethics Committee (File No. 2017/771). Written informed consent was obtained for all individuals prior to participating in the study. All participants met the predetermined criteria: 18–35 yr of age (inclusive), consumed either $\leq$ 35 mg·d^{-1} of caffeine (NHAB), or $\geq$ 100 mg·d^{-1} of caffeine (HAB) (assessed over a 1-wk period), had a minimum oxygen consumption ($\dot{V}O_{2max}$) $\geq$ 28 mL·kg^{-1}· min^{-1}, and had not recently undertaken a heat acclimation protocol. The threshold for inclusion in the HAB group was set at 100 mg·d^{-1} based on caffeine dependence being present after 9 days of repeated caffeine (>100 mg) ingestion (13). Testing of HAB and NHAB participants was distributed evenly throughout the course of data collection. For each participant, all three trials were conducted within a minimum of 7 days and a maximum of 21 days between testing days. A minimum fitness threshold was included to ensure that participants exercised at <80% of their $\dot{V}O_{2max}$ at the target rate of metabolic heat production (7 W·kg^{-1}) and would therefore be able to complete the experimental protocol. All participants had no self-reported adverse reaction to caffeine, never used tobacco products, and were free from respiratory, metabolic, and cardiovascular disease. Female participants were tested while in the follicular phase (i.e., between 2 to 8 days) of their menstrual cycle to avoid natural variation in body temperature (14). Based on previously reported differences in skin blood flow data (15), the number of participants for within-group comparisons for each group (HAB: n = 14; NHAB: n = 14) was determined by performing a power calculation (G*Power 3 software, Heinrich-Heine-Universität Düsseldorf, Germany) assuming a α of 0.05, a β of 0.2, and an effect size of 0.82.

Experimental Design

The study employed a double-blind, placebo-controlled design. The order in which participants completed caffeine and placebo trials was randomized (www.randomization.com) to a condition using a counterbalanced design. The randomization sequence was generated by an investigator not involved with the study to ensure concealment of each condition until the completion of data collection and data analysis. Placebo and caffeine pills were developed and dispensed by the same investigator not involved with data collection to ensure blinding. The caffeine and placebo pills were labeled as pill A or pill B in accordance with the randomization sequence and were encapsulated in identical white colored capsules designed to be indistinguishable by both the participant and the primary researcher conducting the data collection. All testing was conducted at the same time of day for each participant to avoid potential effects of circadian rhythm. Environmental conditions were set at 30°C, 30% RH, with a wind velocity of 0.3 m·s^{-1}. This combination of environmental conditions coupled with the metabolic heat production elicited during exercise was compensable [i.e., amount of evaporative heat loss required to attain heat balance (E_{req}) was less than the capacity for evaporative heat loss (E_{max})] (16).

All participants completed one preliminary trial, followed by two experimental exercise trials after ingesting either *1*) 5 mg·kg^{-1} caffeine (CAF) or *2*) 5 mg·kg^{-1} maltodextrin placebo (PLA), in a double-blinded randomized and counterbalanced order. The dose of caffeine was selected based on

Table 1. *Mean physical characteristics of participants in the habituated and nonhabituated groups*

Group	Age, yr	$\dot{V}O_{2max}$, mL·kg^{-1}·min^{-1}	Body Mass, kg	Resting MAP, mmHg	Resting HR, beats/min	Body Surface Area, m^2
Habituated	27 ± 5	45 ± 9	79.0 ± 11.2	88 ± 9	62 ± 9	2.0 ± 0.2
Nonhabituated	23 ± 3	40 ± 7	78.7 ± 45.6	93 ± 7	70 ± 10	1.9 ± 0.2
P value	0.054	0.127	0.950	0.152	0.027*	0.808

Values presented are means ± SD. HR, heart rate; MAP, mean arterial pressure; $\dot{V}O_{2max}$, maximal oxygen uptake. *Significant difference ($P < 0.05$) between groups.

previous work that has observed physiological effects from caffeine consumption in responses to exercise (17). During the experimental trials, participants cycled for 60 min on an upright bike at a fixed metabolic heat production (7 W·kg^{-1}) (see *Protocol* for further details). All participants were instructed to abstain from consuming caffeine in any form (liquid or solid), or alcohol, and from engaging in strenuous physical activity for 24 h before participating. Participants were also asked to avoid flavonoid-containing foods (e.g., apples, citrus fruits, and onions) and cruciferous vegetables (e.g., cabbage, cauliflower, broccoli) for 3 days before participating as they may influence caffeine metabolism (18).

Instrumentation

Body temperature.

Esophageal temperature (T_{es}) was measured using a general-purpose thermistor (TM400, Covidien, Massachusetts), inserted by the researcher through the nostril to a depth of 40 cm. T_{es} data were recorded every 5 s (NI cDAQ-91722 module, National Instruments, Texas). Skin temperature was measured at four sites (chest, shoulder, thigh, and calf) on the left side of the body using wireless temperature sensors (iButtons DS1921H-F5, Embedded Data Systems, Lawrenceburg, KY) secured to the skin using surgical tape. Mean skin temperature (T_{sk}) was calculated using a mean-weighted average of the four skin temperature sites (19). To account for the relative contributions of core and skin surface temperatures on the initiation, and subsequent increases in sweating (20) and skin blood flow (21), change in mean body temperature (ΔT_b) from baseline was calculated using the following equation (22)

$$\Delta T_b = (0.9 \times \Delta T_{es}) + (0.1 \times \Delta T_{sk}) \quad (1)$$

Dry heat exchange and the evaporative requirements for heat balance (E_{req}) were estimated using partitional calorimetry (23). Body surface area (BSA) was determined using the DuBois & DuBois method (24).

Skin blood flow.

Skin blood flow (SkBF) was measured in arbitrary flux units using single fiber laser-Doppler flowmetry (Moor Instruments MS-LDF, Axminster, UK). One sensor was placed on the right side, ventral forearm ~5 cm distal to antecubital fossa. Another sensor was placed on the right side of the upper back, ~5 cm above the spine of the scapula. Care was taken to place the sensors away from any large superficial blood vessels, freckles, moles, and any variations in pigmentation (25). The location of the sensors was marked with a surgical marker at the first trial to ensure consistent placement in the subsequent trial. Maximum skin blood flow ($SkBF_{max}$) was determined by heating both sites (forearm and upper back) to 44°C for 45 min, where $SkBF_{max}$ was defined as the average of the final 5 min of the 45-min heating period.

Local sweat rate.

Local sweat rate (LSR) was measured using 4.1-cm^2 ventilated sweat capsules taped to the skin using surgical tape (Transpore, 3M, Minnesota). One capsule was placed on right ventral forearm and the other was placed on the right upper back, above the spine of the scapula. The flow rate of anhydrous air at each capsule was set at 700 mL·min^{-1} and was measured in real time using a flow rate monitor (Omega FMA-A2307, Omega Engineering, Connecticut). The anhydrous air was passed over the skin and the difference between the temperature and humidity of the effluent and influent air was measured every 5 s using factory calibrated capacitance hygrometers (HMT333, Vaisala, Vantaa, Finland). This difference was then normalized for the skin surface area under the capsule to yield LSR expressed as mg·min^{-1}·cm^{-2}. Onset thresholds for local sweat rate were determined via segmented linear regression (26).

Heart rate and blood pressure.

Heart rate (HR) was measured continuously with a wireless 6-lead electrocardiography (ECG) system (Quark ECG stress system, Cosmed, Rome, Italy). Blood pressure (BP) was measured every 15 min during exercise and every 10 min postexercise using a Tango M2 device (Suntech Medical, Inc.). Mean arterial pressure (MAP) was then calculated using systolic (SBP) and diastolic (DBP) values

$$MAP = [SBP + 2(DBP)]/3 \quad (2)$$

Whole body sweat loss.

Fully instrumented body mass measurements were taken in triplicate immediately pre and post exercise using a platform scale (Mettler Toledo, Germany; ± 2 g), with participants toweled dry of sweat before the measurement after exercise. Whole body sweat loss (WBSL) was taken as the difference between the average of the three pre and three post measurements after adjusting for respiratory mass loss during exercise (27). Given the minimal, standardized clothing worn, and the practical limitations of having to remain seated in the climate chamber postexercise, to obtain maximum skin blood flow measures, any sweat trapped inside the clothing after exercise was not quantified.

Habitual Caffeine Consumption

Caffeine intake was assessed during the preliminary trial using a validated Caffeine Food Frequency Questionnaire (C-FFQ) (28). Participants were asked to recall and record all items that contained caffeine in the past 7 days and were then stratified into either the habituated (HAB) or the nonhabituated (NHAB) group based on the >100 mg·day^{-1} and <35 mg·day^{-1} cutoffs, respectively. As per the inclusion criteria, if an individual had an average daily caffeine intake between 35 and 100 mg·day^{-1} they were excluded from the study. Caffeine withdrawal symptoms (with 7 discrete categories–fatigue, low alertness, mood disturbances, low sociability, nausea, flue like feelings, headache) were recorded upon arrival to the laboratory, before pill ingestion, using a validated 23-item Caffeine Withdrawal Symptom Questionnaire (CWSQ) (29). Participants were required to rate from 0 (not at all) to 4 (extremely) how they were feeling in the morning relating to each item.

Protocol

Preliminary session.

During the preliminary trial, resting heart rate, resting blood pressure, height, and body mass were recorded. Participants then completed a maximal oxygen uptake test ($\dot{V}O_{2max}$) on an upright cycle ergometer (Corival Upright, Lode BV, Groningen, The Netherlands) in a 20°C 50% RH room. The

maximal exercise protocol began at 80 W and the workload was increased by 20 W every minute while participants maintained a cadence of 80 ± 5 rpm. The exercise test was concluded upon volitional fatigue, inability to maintain target cadence, and/or reaching an oxygen consumption plateau. Following 10 min of rest, $\dot{V}O_{2max}$ was verified using a supramaximal effort at 110% of the final workload in the previous test, as previously described by Poole and Jones (30). During the $\dot{V}O_{2max}$ test, heart rate (Polar, Sweden) and oxygen consumption (Quark CPET, Cosmed, Rome, Italy) were measured continuously, whereas rating of perceived exertion (RPE) (31) was recorded at the end of each stage.

Experimental session.

Participants were asked to consume a light meal and 500 mL of water ~2 h before arriving at the laboratory for the experimental trials. On arrival to the laboratory, participants were given a pill containing 5 mg·kg^{-1} of either caffeine or maltodextrin, with 250 mL of water in the CAF and PLA trials, respectively. This dosing (5 mg·kg^{-1}) would result in a 70 kg individual being given 350 mg of caffeine, equivalent to ~4 cups of coffee (32). Immediately after CAF or PLA ingestion, participants entered the climate-controlled chamber and were instrumented. All participants wore standardized running shoes, socks, and short cotton shorts, whereas females also donned a sports bra. Fully instrumented baseline body mass measurements were taken just before the commencement of the baseline period. Exercise commenced 60 min post CAF or PLA ingestion, which encompassed 45 min seated on a chair and 15 min of rest (baseline) while seated on the upright cycle ergometer, both while in a climate chamber. Ambient conditions were 30.6 ± 0.9°C, 31 ± 1% RH. Exercise began 60 min after pill ingestion to ensure that peak blood caffeine concentration had been achieved before beginning exercise and would remain at peak concentration for the duration of the trial (33). When compared with rest, steady-state exercise has been shown to increase caffeine clearance; however, this clearance effect of exercise does not occur until 250 min after caffeine ingestion (12).

Participants exercised for 60 min at a fixed metabolic heat production (H_{prod}) of 7 W·kg^{-1} to account for any differences in total body mass between HAB and NHAB on core temperature changes during exercise (34). After the exercise period, participants dismounted the bike and were toweled down to remove nonevaporated sweat from the skin. Subjects were then weighed again in triplicate. To determine maximum skin blood flow ($SkBF_{max}$), participants were seated in a chair, whereas both skin blood flow heater probes were set to 44°C for 45 min. The skin blood flow heater probes were only turned on to 44°C at the conclusion of exercise but remained switched off during baseline and throughout exercise. During this period, participants remained in the climate-controlled chamber, and no fluids were ingested.

Statistical Analyses

All data are expressed as means ± SD unless otherwise noted. Participant characteristics were compared (HAB vs. NHAB) using a two-tailed independent sample *t* test. Mean trial H_{prod} and E_{req} were compared within HAB and NHAB groups between CAF and PLA trials using separate two-tailed paired sample Student's *t* tests. A three-way mixed analysis of variance (ANOVA) utilizing the repeated factors of "time" (five levels: 0, 15, 30, 45, 60 min) and "condition" (two levels: CAF, PLA) and the nonrepeated factor of "group" (two levels: HAB, NHAB) was employed to assess the change in T_{es} from baseline, T_{sk}, HR, MAP, and RPE. Furthermore, a three-way mixed ANOVA with the repeated factors of "change in mean body temperature (ΔT_b)" (six levels: 0 to 0.5°C in 0.1°C increments) and "condition" and the nonrepeated factor of "group" was used to analyze $\%SkBF_{max}$ and LSR on the arm and back. A two-way mixed ANOVA using the repeated factor of "condition" and the nonrepeated factor of "group" was used to assess the LSR onset threshold (using change in mean body temperature) and whole body sweat losses. During one participant's trial, the esophageal sensor provided erroneous data and was omitted from this data set. Therefore, due to incomplete groupings of the participants, a mixed-effect analysis method was employed in place of an ANOVA. Significance was set at an α of 0.05, and when a significant main effect or interaction was found, between-group differences were assessed using Sidak's post hoc testing. All statistical analyses were performed with GraphPad Prism (v. 9.0.2, GraphPad Software, La Jolla).

RESULTS

Participant Characteristics

No differences were observed between HAB and NHAB for age, $\dot{V}O_{2max}$, BSA, and resting MAP (Table 1). As per the study design, free-living 7-day caffeine intake was higher in the HAB group than the NHAB group [HAB: 284 (130–429) mg·day^{-1}, NHAB: 2 (0–34) mg·day^{-1}; reported as median and range].

Metabolic Heat Production

Values for metabolic heat production (H_{prod}), the evaporative requirement to attain heat balance (E_{req}), and dry heat exchange are presented for both groups in Table 2. By design, no within-group differences were observed between CAF and PLA for H_{prod} in W·kg^{-1}, W, or W·m^{-2}, nor for E_{req}.

Esophageal Temperature

A main effect of CAF was observed for the rise in esophageal temperature from baseline (ΔT_{es}), with a greater end-exercise ΔT_{es} observed with CAF (0.94 ± 0.36°C) relative to PLA (0.81 ± 0.41°C) (P = 0.006). However, this effect of CAF on the rise in esophageal temperature with exercise time was different depending on caffeine habitation status (P = 0.001). Specifically, in the HAB group, there was a greater ΔT_{es} after 15-min and for the remainder of exercise in the CAF trial (Fig. 1*A*), but no differences were observed between CAF and PLA throughout exercise in the NHAB group (Fig. 1*B*). At the end of 60-min of exercise, ΔT_{es} was greater ($P < 0.001$) in CAF (0.88 ± 0.29°C) relative to PLA (0.62 ± 0.34°C) in the HAB group (Fig. 1*C*), but end-exercise ΔT_{es} values were not different (CAF = 1.00 ± 0.42°C, PLA = 1.00 ± 0.39°C, P = 0.94) in the NHAB group (Fig. 1*D*). Baseline esophageal temperature (T_{es}) was not different with CAF relative to PLA in either the HAB (CAF = 36.79 ± 0.25°C, PLA = 36.89 ± 0.36°C) or the NHAB group (CAF = 36.87 ± 0.22°C, PLA = 36.90 ± 0.24°C) ($P \geq 0.08$).

Skin Temperature

There was no main effect of CAF on mean skin temperature (P = 0.75). However, the change in T_{sk} with exercise time

Table 2. *Average metabolic heat production and required amount of evaporation to attain heat balance for habituated and nonhabituated groups in the caffeine vs. placebo trials*

Group	Metabolic Heat Production, H_{prod}			E_{req}, W	Dry Heat Exchange
	W	W·kg^{-1}	W·m^{-2}		W·m^{-2}
Habituated					
Caffeine	553 ± 103	7.0 ± 0.5	280 ± 33	437 ± 80	31 ± 5
Placebo	531 ± 85	6.7 ± 0.6	270 ± 28	414 ± 77	33 ± 7
P value	0.15	0.21	0.18	0.07	0.42
Nonhabituated					
Caffeine	554 ± 133	7.0 ± 0.7	282 ± 39	429 ± 90	31 ± 3
Placebo	543 ± 116	6.9 ± 0.7	277 ± 33	432 ± 97	30 ± 4
P value	0.48	0.62	0.54	0.79	0.37

Values given are means ± SD for the duration of the exercise (60 min) in watts (W), watts/unit mass (W·kg^{-1}), and watts/unit surface area (W·m^{-2}). E_{req}, evaporative requirement to attain heat balance; H_{prod}, metabolic heat production.

was altered by caffeine depending on habituation status (P = 0.013) with a lower T_{sk} in the HAB group observed at rest and for the initial 15-min of exercise in the CAF condition (P < 0.001). Mean skin temperature with exercise was the same in both the CAF and PLA conditions (P = 0.53) for the NHAB group (Fig. 2*B*).

Dry Heat Exchange

Irrespective of caffeine-habituation status, there was no effect of CAF on dry heat exchange (P = 0.72) (Fig. 2, *C* and *D*).

Local Sweat Rate

No main effect of CAF was observed for the onset threshold (using change in mean body temperature) of sweating on the arm (HAB: CAF = 0.24 ± 0.12°C, PLA = 0.23 ± 0.07°C; NHAB: CAF = 0.35 ± 0.17°C, PLA = 0.36 ± 0.15°C, P = 0.97) or the back (HAB: CAF = 0.22 ± 0.12°C, PLA = 0.23 ± 0.05°C; NHAB: CAF = 0.31 ± 0.17°C, PLA = 0.30 ± 0.15°C, P = 0.75).

Local sweat rate on both the arm and back increased with increasing ΔT_b (P < 0.001); however, this increase was unaffected by habituation status (arm: P = 0.31; back: P = 0.47) (Fig. 3, *A*–*D*).

Skin Blood Flow

The effect of CAF on the $\%SkBF_{max}$ on the arm for a given ΔT_b during exercise was altered by caffeine habituation status (P = 0.045). A lower $\%SkBF_{max}$ was observed with CAF compared with PLA in the HAB group at all levels of ΔT_b (P = 0.015) (Fig. 4*A*), whereas similar $\%SkBF_{max}$ values on the arm were observed between CAF and PLA at all levels of ΔT_b in the NHAB group (P = 0.57) (Fig. 4*B*).

Similarly, the effect of CAF on the $\%SkBF_{max}$ on the back for a given ΔT_b during exercise was also altered by caffeine

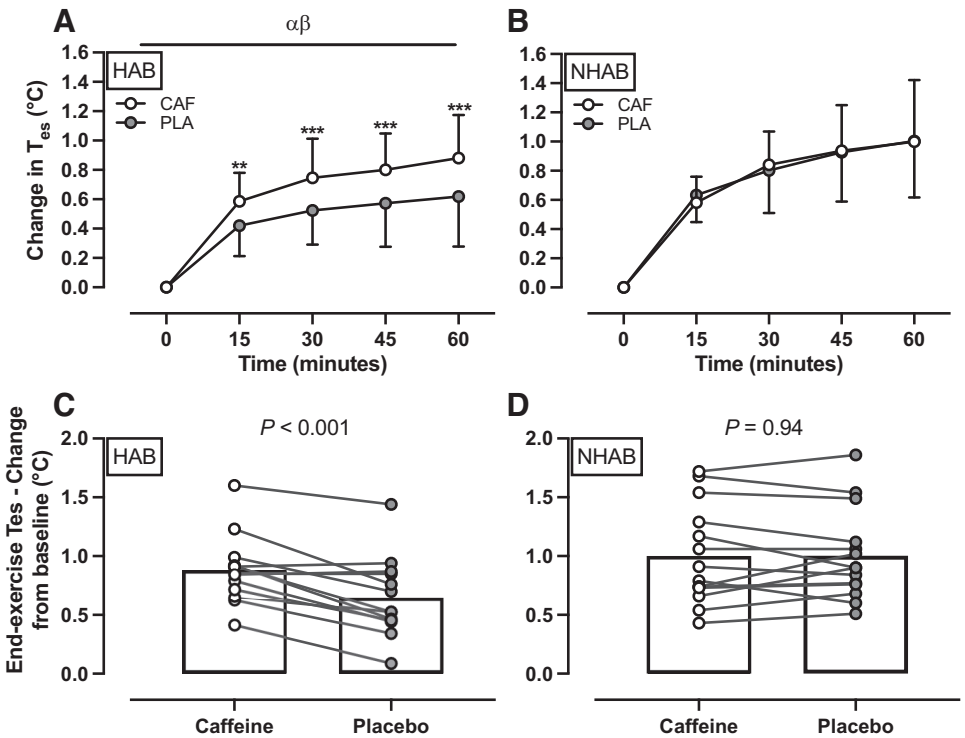

Figure 1. Changes in esophageal temperature are plotted across time in the caffeine and placebo conditions for the habituated (n = 14, *A*) and nonhabituated groups (CAF n = 13 vs. PLA n = 14, *B*). End-exercise change in esophageal temperature from baseline is plotted between caffeine and placebo conditions for the habituated (*C*) and nonhabituated (*D*) groups. **P < 0.01, ***P < 0.001 relative to PLA. α denotes main effect of caffeine (P < 0.01), β denotes interaction (P < 0.001). CAF, caffeine; HAB, habituated; NHAB, nonhabituated; PLA, placebo; T_{es}, esophageal temperature. Mixed-effects analysis with Sidak post hoc test (*A* and *B*); effect of treatment (CAF vs. PLA). Students *t*-test (*C* and *D*); effect of treatment (CAF vs. PLA).

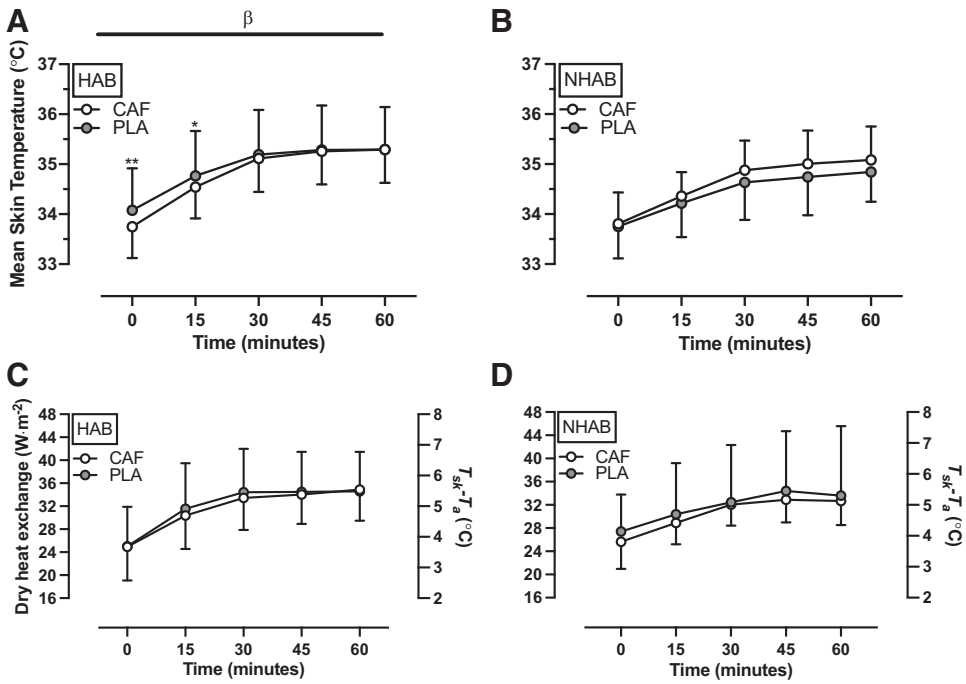

Figure 2. Changes in mean skin temperature, calculated dry heat exchange, the difference between skin temperature and air temperature are plotted with time for habituated (CAF vs. PLA, *A* and *C*) and nonhabituated groups (CAF vs. PLA, *B* and *D*) (n = 14). *P < 0.05, **P < 0.01. β denotes interaction (P < 0.001). CAF, caffeine; HAB, habituated; NHAB, nonhabituated; PLA, placebo; T_a, air temperature; T_{sk}, mean skin temperature. Three-way repeated-measures ANOVA with Sidak post hoc test (*A-D*); effect of treatment (CAF vs. PLA).

habituation status (P = 0.021). During the initial lower levels of ΔT_b in the HAB group, lower $\%SkBF_{max}$ values were observed in the CAF condition (P = 0.02) (Fig. 4*C*), but similar $\%SkBF_{max}$ values were observed in the CAF and PLA conditions at all levels of ΔT_b in the NHAB group (Fig. 4*D*).

Whole Body Sweat Loss

There was no effect of CAF on whole body sweat losses irrespective of habituation status (P = 0.43). Whole body sweat losses were 0.59 ± 0.15 kg and 0.56 ± 0.17 kg for the CAF and PLA conditions respectively in the HAB group, and

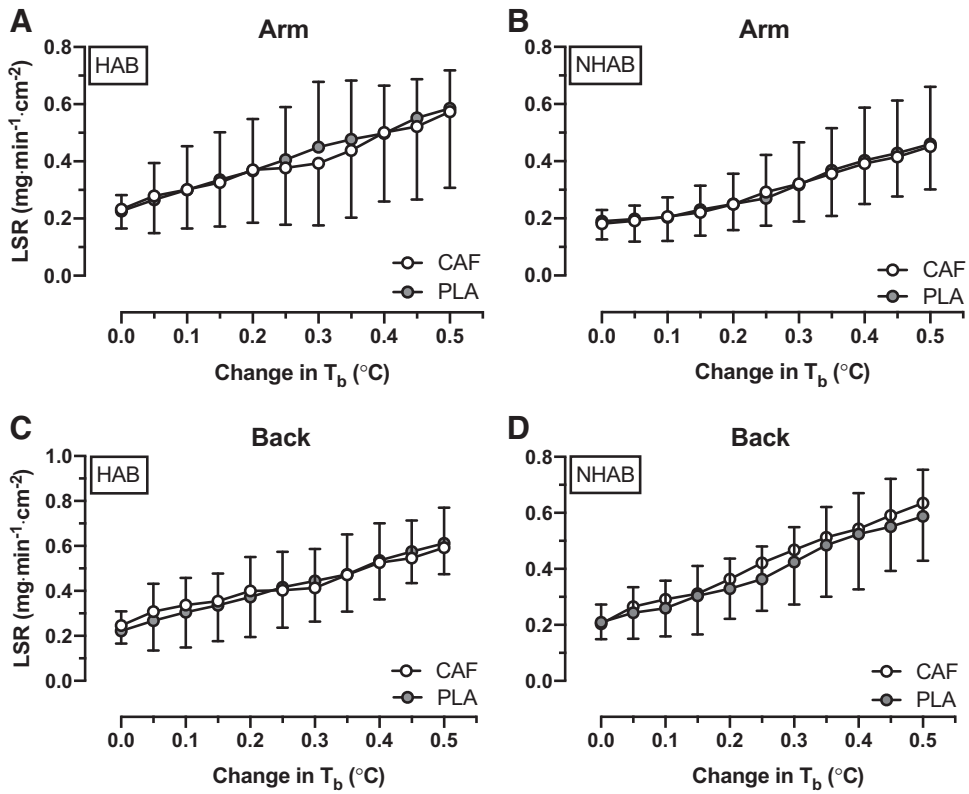

Figure 3. Local sweat rate values expressed as a change in mean body temperature from baseline are plotted for the arm and the back in the habituated and nonhabituated groups. Habituated (CAF, n = 14, n = 13 from 0.05°C onward vs. PLA, n = 14, n = 13 from 0.40°C onward, *A* and *C*) and nonhabituated (CAF, n = 14 vs. PLA, n = 14, *B* and *D*). CAF, caffeine; HAB, habituated; LSR, local sweat rate; NHAB, nonhabituated; PLA, placebo. Mixed-effects analysis with Sidak post hoc test (*A-D*); effect of treatment (CAF vs. PLA).

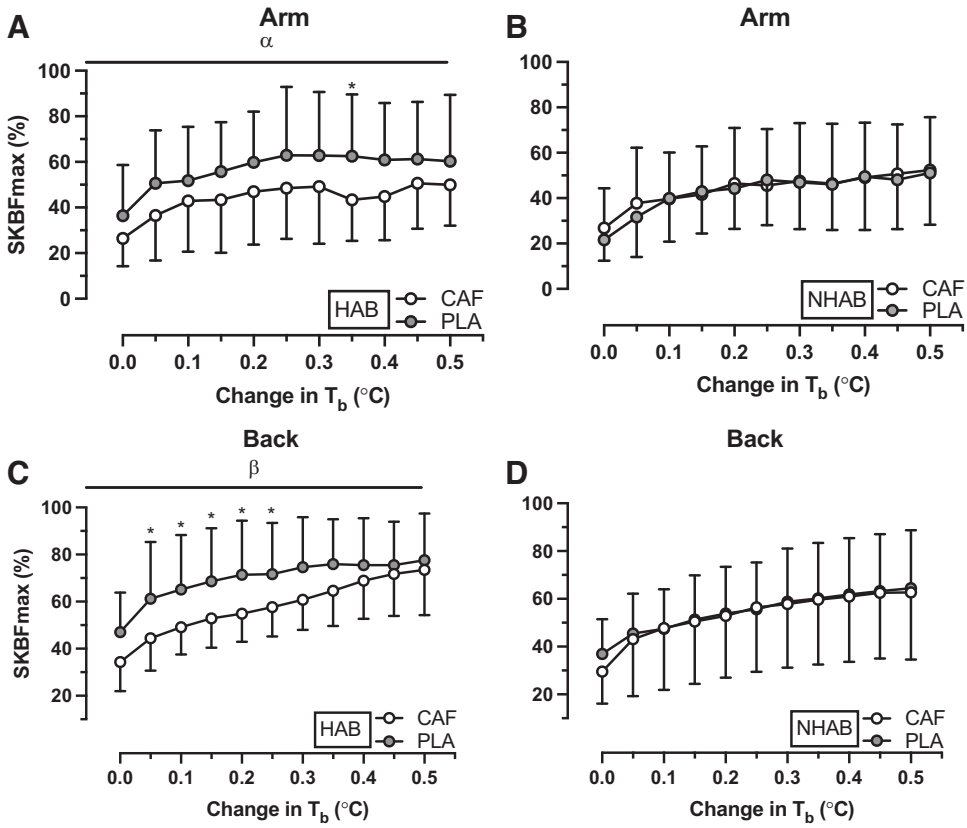

Figure 4. Percentage of maximum skin blood flow expressed as a change in mean body temperature from baseline (°C) on the arm (*A* and *B*) and the back (*C* and *D*). Habituated (CAF, n = 14, n = 13 from 0.05°C onward vs. PLA, n = 14, n = 13 from 0.40°C onward, *A* and *C*) and nonhabituated group (CAF, n = 14 vs. PLA, n = 14, *B* and *D*). *$P < 0.05$. α denotes main effect of CAF ($P < 0.01$) in *A*. β denotes interaction ($P < 0.005$) in *C*. CAF, caffeine; HAB, habituated; NHAB, nonhabituated; PLA, placebo; $SkBF_{max}$, maximum skin blood flow. Mixed-effects analysis with Sidak post hoc test (*A-D*); effect of treatment (CAF vs. PLA).

0.53 ± 0.19 kg and 0.52 ± 0.19 kg in the CAF and PLA conditions respectively in the NHAB group.

Heart Rate and Mean Arterial Pressure

Heart rate was not different between CAF and PLA throughout exercise irrespective of habituation status (P = 0.14) (Table 3). Similarly, mean arterial pressure was not different between CAF and PLA throughout exercise regardless of group (P = 0.52) (Table 3).

Rating of Perceived Exertion

There was no effect of CAF on rating of perceived exertion (RPE) during exercise in both groups (P = 0.68). RPE at the end of exercise was 11 ± 2 and 12 ± 2 for the CAF and PLA conditions respectively in the HAB group, and 13 ± 2 and 13 ± 2 in the CAF and PLA conditions respectively in the NHAB group.

Withdrawal Symptoms

Caffeine-habituated participants experienced greater levels of negative withdrawal symptoms associated with caffeine cessation (fatigue, low alertness, mood disturbances, low sociability, nausea, flue like feelings, headache) when compared with nonhabituated participants (HAB: 11 ± 7 AU, NHAB: 4 ± 4 AU, $P < 0.001$).

DISCUSSION

We conducted a double-blind, placebo-controlled study to examine the acute effects of a moderate dose of caffeine (equivalent to 4–5 cups of coffee), on thermoregulatory responses during steady-state exercise in the heat and sought to identify whether any potential effect of caffeine was modified by the caffeine-habituation status of the individual. Compared with placebo, caffeine ingestion resulted

Table 3. *Mean heart rate and mean arterial pressure throughout exercise for habituated and nonhabituated groups for both caffeine and placebo conditions*

Group	Mean Heart Rate, beats/min				Mean Arterial Pressure, mmHg			
	Habituated		Nonhabituated		Habituated		Nonhabituated	
Condition, min	CAF	PLA	CAF	PLA	CAF	PLA	CAF	PLA
0	73 ± 13	75 ± 10	73 ± 11	77 ± 9	95 ± 8	91 ± 11	98 ± 12	96 ± 7
15	124 ± 16	125 ± 16	137 ± 19	141 ± 19	99 ± 6	99 ± 12	105 ± 7	106 ± 11
30	128 ± 18	128 ± 15	145 ± 18	146 ± 14	98 ± 10	101 ± 14	98 ± 10	98 ± 11
45	132 ± 21	129 ± 16	145 ± 18	149 ± 14	97 ± 12	102 ± 13	97 ± 9	96 ± 11
60	136 ± 22	132 ± 17	145 ± 18	151 ± 14	97 ± 13	99 ± 13	95 ± 7	96 ± 12

Values given are means ± SD. CAF, caffeine; PLA, placebo.

in a greater (~0.3°C) increase in esophageal temperature after 60 min of steady-state exercise in the heat in caffeine-habituated individuals (Fig. 1, *A* and *C*), with no such difference observed in nonhabituated individuals (Fig. 1, *B* and *D*). This greater change in esophageal temperature with caffeine ingestion in the habituated group was paralleled by a lower skin blood flow response on both the arm and back (Fig. 4, *A* and *C*). No differences in sweat rate (Fig. 3) or in the onset of sweating were observed as a function of caffeine ingestion in either group. There were also no differences in calculated estimated dry heat loss between or within groups (Table 2 and Fig. 2, *C* and *D*).

In the habituated group, whole body sweat losses were the same between CAF and PLA conditions, however, the rise in esophageal temperature was greater in the CAF trial. We did not observe any differences in LSR thermosensitivity (Fig. 3) or onset of sweating. This follows previous steady-state exercise research where no differences in sweat rates were observed with low ($3\,mg{\cdot}kg^{-1}$) and moderate ($6\,mg{\cdot}kg^{-1}$) doses of caffeine (9). However, there is some evidence that acute caffeine ingestion could alter sweating control parameters (35). Performance research has observed higher sweat rates with caffeine ingestion (36); however, this likely reflects the higher work rate and attendant heat production with caffeine ingestion during self-paced exercise compared with placebo, rather than any caffeine-mediated sudomotor effects and concomitant impacts on evaporative heat losses. This evidence suggests that although the mechanism of action of caffeine (through an increased catecholamine response) indicates the potential for caffeine to modulate sudomotor responses to exercise and/or heat stress, our data show that there is no effect of caffeine on sweating during steady-state exercise in the heat.

Reductions in skin blood flow with CAF in the habituated group (Fig. 4, *A* and *C*) led to a ~0.3°C and ~0.2°C lower skin temperature in the habituated group at rest and after 15 min of exercise, respectively (Fig. 2*A*). However, no associated reductions in estimated dry heat transfer were observed between CAF and PLA (Table 2 and Fig. 2, *C* and *D*). Given that the greater rise in esophageal temperature in the CAF trial in the HAB group cannot be attributed to differences in either evaporative heat loss or dry heat transfer, it stands to reason that although the same internal heat content may have accumulated in the body throughout exercise in PLA and CAF conditions, the distribution of this heat energy among the tissues of the body may have been different. Specifically, a lower core-to-shell thermal conductance arising from a lower skin blood flow (37) in the CAF trial may have been responsible for the greater relative accumulation of heat closer to the body core and thus a greater rise in core temperature.

The influence of caffeine on vasomotor control (i.e., skin blood flow) has two components: as a locally mediated adenosine receptor antagonist, and as a central nervous system stimulant through catecholamine release (38, 39). Adenosine assists vasodilation through nitric oxide release (3) and acts as a synergistic vasodilator during exercise and environmental heat stress (40). Adenosine A_{2A} receptors are located throughout the cardiovascular system, in particular at the smooth muscle vasculature (41, 42) and it is the adenosine receptor that caffeine has the highest affinity for (43). Adenosine A_{2A} receptors stimulate endothelial nitric oxide synthase (eNOS) release when activated by adenosine (44), which is the mechanism responsible for ~30%–44% of vasodilation during heat stress (45, 46). The antagonism of adenosine A_{2A} receptors by caffeine may therefore explain the blunted vasodilatory response to exercise we observed in the HAB group (Fig. 4, *A* and *C*).

The caffeine-mediated responses of core temperature and skin blood flow outcomes in the present study were observed in caffeine-habituated participants, but not in the nonhabituated group. This observation may be partially explained via the processes implicated in caffeine habituation. Adenosine receptor affinity to caffeine increases with caffeine use (47, 48). Further, the volume of distribution (i.e., the apparent volume into which a drug disperses to produce the observed plasma concentration (49)) with caffeine ingestion increases with habitual caffeine use, when compared with occasional caffeine use (12). Therefore, acute caffeine ingestion in habituated participants could result in greater binding of caffeine to adenosine receptors, compared with nonhabituated individuals, thus blunting the vasodilatory effects of adenosine. The attenuated skin blood flow response in the habituated individuals (Fig. 4, *A* and *C*) may therefore be attributed to an increased affinity of caffeine to adenosine A_{2A} receptors when compared with nonhabituated individuals. However, this remains speculative and further research needs to be undertaken to examine the cause of diverging vasomotor and thermoregulatory responses to caffeine between habituated and nonhabituated individuals.

Our study indicates that a moderate, acute dose of caffeine impaired vasomotor thermoeffector responses led to a greater end-exercise change in core temperature in caffeine-habituated, but not in nonhabituated individuals. Previous research has reported that caffeine reduces vasomotor responses in temperate conditions (4) but not in hot conditions (6). Caffeine-mediated reductions in blood flow may be ameliorated in situations of higher thermal stress or longer exercise durations (Fig. 4*C*), due to increased internal drive for heat loss overriding the caffeine-mediated reductions in skin blood flow. Although a greater rise in esophageal temperature was observed in habituated individuals in this study, this was under compensable conditions, and given that maximum skin wettedness is the predominant determination of the limits of compensability (50), and sudomotor output was unimpaired in this study, it seems likely that no further effect of caffeine would be observed in uncompensable conditions. However, this notion must be experimentally addressed with future research.

Perspectives

The findings from this study support the practice of requiring all participants to abstain from caffeine before participation in thermoregulatory research. We caution the translation of our results to performance-based caffeine research and fixed intensity exercise thermoregulatory studies in uncompensable environmental conditions as our findings relate to steady-state exercise in compensable conditions only. The ingestion of caffeine (albeit a moderate dose) by caffeine-habituated individuals 1 h before an exercise heat stress challenge clearly results in a systematically greater change in core

temperature and a lower skin blood flow response than if caffeine is withheld before exercise. Caffeine withdrawal in this group did lead to some subjective discomfort, e.g., headache, lethargy; however, these effects were exclusively mild. For noncaffeine-habituated individuals, although caffeine ingestion is less common, our data indicate that any incidental ingestion of caffeine before an experimental trial should not be the cause for suspending that trial. By investigating whether participants should continue to be required to abstain from caffeine before participation in thermoregulatory research, this study complements previous work by our laboratory that has investigated similar modern exercise-based heat stress research study design questions [e.g., the influence of fitness (51, 52), heat production (34), body mass (34, 52), body surface area (53), relative exercise intensities (51–53), running economy (52), body fatness (54), and time of day (55)] on thermoregulatory research outcomes.

Many heat wave guidelines around the world include the recommendation that people should avoid consuming caffeine products. Previous research shows that acute caffeine ingestion increases in urine output in caffeine naïve individuals; however, tolerance to this effect rapidly occurs (56). Our data provide evidence that potentially supports any recommendations to avoid caffeine in heat waves, particularly in habituated individuals. However, our HAB group became hotter following caffeine ingestion apparently due to a blunted vasodilatory response to exercise heat stress, rather than any impact of caffeine on hydration status, albeit over a relatively short timeframe.

Limitations

Without continuous blood pressure measurements, we were unable to continuously monitor cutaneous vascular conductance, which would have provided deeper insight into vasodilatory control. However, we did capture a snapshot of this response with periodic blood pressure measures and observed no difference in mean arterial pressure between placebo and caffeine condition within both the HAB and NHAB groups throughout exercise. The measurement of baseline plasma caffeine concentrations both on arrival and during exercise could have strengthened our findings. Nevertheless, caffeine withdrawal symptoms reported in the blinded CAF trial indicate compliance. As we only employed one exercise intensity and one intervention, we are unable to draw conclusions across different caffeine dosages and alternate combinations of exercise intensity and climate.

Future Research

Whether the present findings hold true with different doses and forms of caffeine (i.e., small-to-large doses and through coffee, tea, and other beverages) remains to be investigated. As we provided participants with one moderate dose of caffeine rather than multiple smaller doses consumed throughout the day (through tea and coffee), the translatability of our findings to everyday more widely distributed dosages of caffeine may be limited. Insight into the nature of the dose-response influence of caffeine and a deeper understanding of how different forms of caffeine may influence thermoregulation in free-living settings are also unknown. For example, beverages such as coffee and tea contain other compounds that may affect vasomotor function through antioxidants and polyphenols. As previously suggested (4, 6), the diminished sensitivity of the vasomotor response to heat and exercise with caffeine ingestion in low-moderate heat stress conditions may be ameliorated in higher heat stress conditions; however, the heat stress "threshold" (combination of activity and climate) at which this occurs remains unknown.

Conclusion

In this double-blind, counterbalanced, and placebo-controlled study, caffeine ingestion by caffeine-habituated individuals attenuated the rise in skin blood flow on the arm and back, which was paralleled by a greater increase in esophageal temperature during exercise in a warm compensable environment. In contrast, caffeine did not alter the esophageal temperature or skin blood flow responses during exercise in the nonhabituated group. No sudomotor differences at the whole body or local level were observed in either group as a function of caffeine ingestion. Our findings support the practice of requiring participants to abstain from consuming caffeine before participation in thermoregulatory research.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.A.H., Y.M., and O.J. conceived and designed research; L.A.H. and L.H. performed experiments; L.A.H., Y.M., and O.J. analyzed data; L.A.H., L.H., J.W.S., Y.M., and O.J. interpreted results of experiments; L.A.H. prepared figures; L.A.H. drafted manuscript; L.A.H., L.H., J.W.S., Y.M., and O.J. edited and revised manuscript; L.A.H., L.H., J.W.S., Y.M., and O.J. approved final version of manuscript.

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