

ORAL INGESTION OF ACETYLSALICYLIC ACID ON  
SKIN BLOOD FLOW AND LOCAL SWEAT RATE  
DURING HEAT STRESS

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## ABSTRACT

Acetylsalicylic acid (ASA) is an over-the-counter drug used for pain relief and fever suppression. As a nonselective cyclooxygenase-inhibitor, ASA has systemic effects that may influence hypothalamic-mediated temperature regulation. Three studies were performed to evaluate the effects of oral ASA ingestion on skin blood flow (SkBF) and sweating during passive and exercise-induced heat stress. Passive whole-body heating was achieved with the use of a water-perfused suit to raise body temperature. In a separate study, submaximal cycling was performed. Study one examined SkBF and local sweating responses during passive heating following acute ASA ingestion of different concentrations (325-mg and 81-mg). Study two examined the influence of four consecutive days of low-dose (81-mg) ASA ingestion on SkBF and local sweating responses during passive heating. Study three examined acute ASA administration of different concentrations (325-mg and 81-mg) on SkBF and local sweating during submaximal cycling at 50% heart rate reserve for 45 min and followed with 15 min of passive recovery (35 °C, 30-40% rH). Young, healthy male participants maintaining moderately active lifestyles volunteered for these studies. In a repeated measures, counter-balanced design, participants completed passive heating and submaximal cycling while SkBF and local sweat responses were continuously measured. As indicated by group mean data, results from study one suggested that acute ASA ingestion of a modest dose did not negatively influence thermoregulatory responses during whole-body passive heating. Results from study two suggested that low-dose ASA ingestion over four consecutive days did not alter

thermoregulatory responses during whole-body passive heating. Results from study three suggest that acute ASA ingestion of a modest dose did not alter thermoregulatory responses during exercise or passive recovery in the heat. The collective results from these investigations support the assertion that ASA use does not negatively affect temperature regulation in a cohort of young healthy male participants under conditions similar to those described.

## DEDICATION

I dedicate the entirety of this work to my wife and best friend, Kimberly Ann Carter. From the very beginning she has been a source of constant love and infinite support. Even from a young age, she has always believed in my abilities and what I can achieve. Despite the many challenges along this journey, Kimberly has remained faithful that “everything will work out.” Words fall short of expressing how much I love her and appreciate all her sacrifices. Together we will go far, as this triumph puts us one step closer to our dreams.

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## CHAPTER I

### INTRODUCTION

Acetylsalicylic acid (ASA) is a one of the most widely used over-the-counter medicines, largely due to its diverse therapeutic properties. ASA exerts its medicinal effect through the systemic inhibition of cyclooxygenase (COX) isoforms -1 and -2 (1). While many cells constitutively express COX-1, COX-2 has been shown to be inducible and quickly up-regulated at sites of trauma (7). Despite being considered a nonselective COX-inhibitor, ASA is 50- to 100-fold more effective at blocking COX-1 activity compared to COX-2 (3). Given the involvement of COX-1 in fever and inflammation, ASA is commonly taken for fever suppression and lowering of blood clotting potential (1). Due to its wide-ranging effects on central and peripheral targets (1), ASA may upset homeostatic function by interfering with normal cellular and enzymatic activity.

Invariably extended time in hot ambient conditions leads to a rise in core and skin temperature, and as such, the cutaneous circulation is tightly regulated (8, 11). Upon reaching a relative threshold, skin blood flow (SkBF) is increased via cutaneous vasodilation and eccrine sweating is stimulated to optimize heat dissipation (9). During heat stress, the release of acetylcholine acts to induce reflexive cutaneous vasodilation and initiate eccrine sweating by acting on muscarinic receptors (6). Accordingly, these thermo-effectors often exhibit a temporal relationship (9), however, in addition to being affected by SkBF, local sweat rate can also be independently affected by local skin temperature (12). Recently, it was determined that the onset and rise of sweating is dependent on increased SkBF during passive heat stress (10). Since short term ASA use (~7 days) has previously been shown to retard normal SkBF responses during passive heating (4, 5) and exercise (2), it is possible that ASA use may indirectly affect sweating

through an attenuation of SkBF. Importantly, a functional loss in either thermo-effector (e.g., SkBF and sweating) may hasten heat storage, threatening cellular homeostasis and physiologic function.

While previous work (2, 4, 5) has focused primarily on SkBF responses with ASA use, very little research has examined the influence of ASA on eccrine sweating. More specifically, it is unknown how different ASA doses (e.g., 325-mg and 81-mg) and administration (e.g., acute and short-term chronic) can influence SkBF and sweating responses during passive and exercise-induced heat stress. It is possible that the results from previous investigations (2, 4, 5) may be a consequence specific to platelet-inhibition in middle aged individuals.

Accordingly, the following series of studies aimed to elucidate the influence of different commonly used ASA doses (e.g., 325-mg and 81-mg) and compare acute *vs.* short-term chronic (e.g., 4 days) administration on thermoregulatory responses during passive whole-body heating and submaximal exercise in hot conditions. Certainly, it was of primary interest for these investigations to determine if oral ASA ingestion altered normal hypothalamic-mediated temperature regulation as evidenced by changes in SkBF and local sweating in a cohort of young, healthy males.

## References

1. Aronoff DM, Neilson EG. Antipyretics: mechanisms of action and clinical use in fever suppression. *Am J Med.* 2001; 111(4):304-15.
2. Bruning RS, Dahmus JD, Kenney WL, Alexander LM. Aspirin and clopidogrel alter core temperature and skin blood flow during heat stress. *Med Sci Sports Exerc.* 2013; 45(4):674-82.
3. Cipollone F, Patrignani P, Greco A, Panara MR, Padovano R, Cucurullo F, Patrono C, Rebuzzi AG, Liuzzo G, Quaranta G, Maseri A. Differential suppression of thromboxane biosynthesis by indobufen and aspirin in patients with unstable angina. *Circulation.* 1997; 96(4):1109-16.
4. Holowatz LA, Kenney WL. Chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985).* 2009; 106(2):500-5.
5. Holowatz LA, Jennings JD, Lang JA, Kenney WL. Systemic low-dose aspirin and clopidogrel independently attenuate reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985).* 2010; 108(6):1575-81.
6. Kellogg DL,Jr, Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossmann M, Johnson JM. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res.* 1995; 77(6):1222-8.
7. Mitchell JA, Warner TD. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. *Br J Pharmacol.* 1999; 128(6):1121-32.
8. Pergola PE, Kellogg DL,Jr, Johnson JM, Kosiba WA, Solomon DE. Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *Am J Physiol.* 1993; 265(3 Pt 2):H785-92.
9. Roddie IC, Shepherd JT, Whelan RF. The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *J Physiol.* 1957; 136(3):489-97.
10. Smith CJ, Kenney WL, Alexander LM. Regional relation between skin blood flow and sweating to passive heating and local administration of acetylcholine in young, healthy humans. *Am J Physiol Regul Integr Comp Physiol.* 2013; 304(7):R566-73.
11. Taylor WF, Johnson JM, O'Leary D, Park MK. Effect of high local temperature on reflex cutaneous vasodilation. *J Appl Physiol Respir Environ Exerc Physiol.* 1984; 57(1):191-6.
12. Wingo JE, Low DA, Keller DM, Brothers RM, Shibasaki M, Crandall CG. Skin blood flow and local temperature independently modify sweat rate during passive heat stress in humans. *J Appl Physiol (1985).* 2010; 109(5):1301-6.

## CHAPTER II

### INFLUENCE OF ACUTE ACETYLSALICYLIC ACID INGESTION ON SKIN BLOOD FLOW AND LOCAL SWEAT RATE DURING WHOLE-BODY PASSIVE HEAT STRESS

#### ABSTRACT

Acetylsalicylic acid (ASA) is one of the most commonly used over-the-counter medications. Due to its potent systemic effects as a cyclooxygenase inhibitor, ASA may interfere with normal hypothalamic-mediated temperature regulation. The purpose of this study was to determine if ASA would alter: 1) The onset and relative increase in cutaneous vascular conductance; and 2) The onset and slope (i.e., sensitivity) of local sweat rate during whole-body passive heat stress. Seven, healthy male participants (mean  $\pm$  SD;  $28 \pm 3$  yr) completed counter-balanced trials to compare ASA treatments (325-mg ASA and 81-mg ASA) to a control trial (no ASA). A 10-day washout period separated subsequent trials in order to ensure normal blood platelet restoration between ASA treatments. Skin blood flow was indexed by laser-Doppler flowmetry (LDF) placed on the dorsal aspect of the left forearm. A capacitance hygrometry capsule was used to measure local sweat rate. Participants' rested supine while hot water ( $\sim 49$  °C) was perfused through a tube-lined suit until mean body temperature ( $\bar{T}_b$ ) was raised 1 °C. Neither dose of ASA affected  $\bar{T}_b$  at baseline (control,  $36.5 \pm 0.1$  °C; 325-mg,  $36.4 \pm 0.2$  °C; 81-mg,  $36.5 \pm 0.2$  °C;  $p = 0.40$ ); the onset of cutaneous vasodilation (control,  $36.7 \pm 0.2$  °C; 325-mg,  $36.7 \pm 0.2$  °C; 81-mg,  $36.7 \pm 0.2$  °C;  $p = 0.87$ ) or relative increase in cutaneous vascular conductance from baseline (control,  $523 \pm 219\%$ ; 325-mg,  $714 \pm 372\%$ ; 81-mg,  $458 \pm 298\%$ ;  $p = 0.27$ ). Additionally, ASA did not alter the onset (control,  $36.9 \pm 0.1$  °C; 325-mg,  $36.8 \pm 0.1$  °C; 81-mg,  $36.8 \pm 0.2$  °C;  $p = 0.53$ ) or sensitivity of local sweat rate [ $(\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}) / \text{°C}$ ] (control,  $1.23 \pm 0.26$ ; 325-mg,  $1.25 \pm 0.34$ ; 81-mg,  $1.16 \pm 0.33$ ;  $p = 0.60$ ). Acute ASA ingestion did not

affect skin blood flow or local sweat responses during whole-body passive heat stress under the conditions of this study.

**KEY WORDS:** aspirin, sweating, thermoregulation, vasodilation

## INTRODUCTION

With respect to temperature homeostasis, sensory feedback concerning environmental cues and core body temperature is relayed to the hypothalamus through nerves originating from central and peripheral thermoreceptors (4). To mitigate a rise in core temperature, skin blood flow (SkBF) is increased and eccrine sweating responses are stimulated to optimize heat dissipation (20). Since normal physiologic function necessitates a narrow thermo-balance ( $37 \pm 1$  °C) (16), any environmental or pharmacologic challenges that threaten such balance may pose a health risk. One such challenge may be triggered by acetylsalicylic acid (ASA), a commonly used over-the-counter medication for treating fever and pain.

The primary mechanism of action for ASA is through the inhibition of the rate-limiting enzyme cyclooxygenase (COX). Two primary isoforms of the COX enzyme have been identified with a third (COX-3) considered a covariant of COX-1 and similarly involved in fever and pain signaling (5, 28). Importantly, this enzyme is known to contribute to the development and maintenance of fever by catalyzing the conversion of arachidonic acid to prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) (2). Increased concentrations of PGE<sub>2</sub> in the preoptic hypothalamus result in an elevated core temperature by raising the ‘normal’ thermoregulatory set-point (25). Since ASA is a nonselective COX inhibitor, it follows that ASA likely acts as a fever suppressant by interfering with PGE<sub>2</sub> synthesis. Previous research has shown that another COX-inhibitor, acetaminophen, can lower core body temperature by  $\sim 0.22$  °C in afebrile stroke patients (12). Due to its systemic effect as a COX-inhibitor, it is believed that ASA may alter normal thermoregulatory responses. Additionally, it is unknown how acute oral ingestion of ASA affects healthy individuals exposed to a direct exogenous heat stress.

COX-mediated pathways are essential during reflex cutaneous vasodilation (17) and, interestingly, habitual low-dose (81-mg) ASA use in middle-aged participants has been shown to attenuate SkBF during passive heat stress (9). Researchers from that study concluded that the diminished SkBF responses may have been a consequence specific to platelet inhibition which may have impaired vascular wall signaling (9). However, it is unclear if an acute ASA dose would also affect SkBF and/or local sweat responses during passive heating.

Cutaneous vasodilation and eccrine sweating often exhibit a temporal relationship (24), possibly do to the release of acetylcholine acting on muscarinic receptors to stimulate eccrine gland sweating (14). Recent work by Smith and colleagues (26), determined that the onset and rise of sweating is dependent on increased SkBF during passive heat stress. Accordingly, it is possible that ASA may indirectly exert its influence over sweating responses through a modification in SkBF during whole-body heating. Certainly, a functional impairment in either SkBF or sweating responses may accelerate heat storage thereby potentially threatening hypothalamic-mediated temperature homeostasis.

The purpose of this study was to determine if an acute ASA dose of different concentrations would alter SkBF and local sweat rate (SR) during whole-body passive heat stress. Since high acute doses of ASA are not advised due to the potential for gastrointestinal discomfort (25), standard U.S. full-dose (325-mg) and low-dose (81-mg) were used. It was hypothesized that ASA would affect SkBF responses as evidenced by a reduced increase in SkBF relative to baseline. This effect was thought to be more pronounced during the 325-mg trial compared to 81-mg. Additionally, it was hypothesized that the onset, slope (i.e., sensitivity) and peak local SR would be reduced by ASA ingestion of 325-mg compared to 81-mg and control trials.

## **METHODS**

### **Ethical Approval**

This study was approved by the Institutional Review Board at The University of Alabama. All participants were fully informed of the risks and methods prior to participation in the investigation. Both verbal and written consent were obtained from each participant. All methods and procedures conformed to the guidelines set forth by the Declaration of Helsinki.

### **Participants**

Seven, healthy male participants were recruited to complete this study. A power analysis (21) revealed this sample size was adequate to detect a moderate effect size ( $d = 0.60$ ) (23) for peak local SR, assuming a power of  $\sim 0.85$  and correlation among repeated levels of each factor of  $\sim 0.90$ . All participants were free from cardiovascular and metabolic disease while maintaining moderately active lifestyles in accordance with guidelines established by the American College of Sports Medicine (1). Participants were self-reported non-smokers not taking any prescription medications or herbal supplements. During the 12 hours prior to each of the three experimental trials, participants refrained from caffeine and alcohol before reporting to the laboratory. All experimental trials were counterbalanced to reduce any ordering effect. Time of day for individual testing was kept consistent to minimize the influence of circadian rhythms.

## Instrumentation and Measurements

Upon reporting to the laboratory, participants consented to the study and completed a 24-hour history questionnaire to verify adherence to pretest instruction. Next, participants submitted a sample of urine to assess hydration status from urine specific gravity (USG) via refractometry (SUR-NE, Atago Inc., Higham, MA). Values  $< 1.020$  were considered adequately hydrated (7). Participants whose values were  $\geq 1.020$  were provided fluids to consume over a 30-min period and then were re-evaluated. Once hydration was verified, participants were given a small snack (e.g., breakfast bar) and either control (no ASA) or ASA treatment (U.S. standard full-dose, 325 mg; or U.S. standard low-dose, 81 mg) in accordance with their testing schedule. Following ASA ingestion, a period of 30 to 45-min was allowed to permit ASA to reach peak plasma concentration (22). During this period, participants had their standing height and nude body mass measured (Tanita Corporation, Tokyo, Japan). Additionally, body fat percentage was estimated using calipers (Lange, Beta Technology Inc., Cambridge, MD) from the sum of three skin-folds (11). Participants then self-inserted a rectal thermocouple (model RET-1, Physitemp, Clifton, NJ) 8-10 cm beyond the anal sphincter (18) to measure rectal temperature ( $T_{re}$ ). Next, participants were instrumented with a heart rate monitor (Polar, Stamford, CT) and 6 skin thermocouples located on the upper back, chest, lower back, abdomen, anterior thigh, and calf to measure weighted mean whole-body skin temperature ( $\bar{T}_{sk}$ ) (27).

To obtain an index of SkBF, cutaneous red blood cell flux was measured with a laser-Doppler flowmetry (LDF) probe (MoorVMS-LDF VP12, Moor Instruments, Devon, UK) held in position with a local heater placed on the dorsal aspect of the forearm. Cutaneous vascular conductance (CVC) was calculated from arbitrary LDF units divided by mean arterial pressure.

Mean arterial pressure was calculated as  $[(1/3 \cdot \text{systolic blood pressure}) + (2/3 \cdot \text{diastolic blood pressure})]$ .

Additionally, local SR was measured continuously using the ventilated-capsule method with compressed nitrogen at a rate of 300 ml/min. The humidity of the effluent air was measured via capacitance hygrometry. Total body sweating was estimated from a change in nude body mass from pre-heat stress to post-heat stress. To facilitate consistency between trials, measurements were taken from anatomical landmarks of the forearm to correctly position the LDF probe and ventilated capsule for each trial. Body temperature was controlled with a water-perfused suit that covered the entire body apart from the hands, feet, face, and instrumented forearm. To enhance heat storage and minimize evaporative heat loss, an impermeable garment (i.e., plastic suit) was worn over the water-perfused suit.

## **Experimental Protocol**

Experimental trials with ASA were separated by a minimum of 10 days to permit normal platelet re-synthesis (22). To ensure adequate recovery, control trials which took place before or between ASA trials were given at least 72 hours before another experimental session was conducted. Following instrumentation, participants wearing the perfusion suit and impermeable garment entered an environmentally controlled chamber held to thermoneutral conditions (22 °C, 30-35% rH) and aided into the supine position on a gurney. All instruments were connected and values verified. Thermoneutral water (~31.3-31.5 °C) was perfused through the suit to clamp  $\bar{T}_{sk}$  ~34 °C (thermoneutral). Next, a 10-min period was used to stabilize  $\bar{T}_{sk}$ . Data collected during the final min of this period was used as baseline. Hot water (~49 °C) was then perfused through the suit to raise mean body temperature ( $\bar{T}_b$ ) by 1.0 °C above baseline. Once the target  $\bar{T}_b$  had

been reached, peak data values for SkBF and local SR were obtained during the final min of heat stress.  $\bar{T}_b$  was calculated using the weighted measures of  $T_{re}$  and  $\bar{T}_{sk}$  from the following equation:  $\bar{T}_b = [(0.87 \cdot T_{re}) + (0.13 \cdot \bar{T}_{sk})]$  (3). Heart rate was continuously monitored throughout the protocol. Additionally, blood pressure was measured via automated oscillometry every 5 min. Once the appropriate  $\bar{T}_b$  was reached, cool water was perfused through the suit to return body temperature toward initial baseline. The experimental protocol ended once  $\bar{T}_b$  reached within  $\sim 0.4$  °C of baseline, after which participants were aided off the gurney to remove instrumentation. After towel drying, participants reported their nude body mass and were given fluids to drink *ad libitum*. All experimental trials were conducted in this manner.

## Statistical Analyses

Data were continuously sampled at a rate of 50 Hz using a data acquisition system (Biopac, MP150, Goleta, CA) and stored for offline analysis. CVC responses during the heat stress were expressed as a percent change from baseline (13). Local SR and thermal data (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) were averaged every 30 s during the heat stress (30). Changes in temperature variables before heating were compared with peak heat stress values and analyzed using a repeated-measures analysis of variance (ANOVA). The onsets of cutaneous vasodilation and sweating were determined by an experienced investigator, blinded to the treatment, by visually examining LDF units and local SR graphed as a function of time. The  $\bar{T}_b$  at the indicated time was then used to identify the onset of cutaneous vasodilation and onset of sweating. The  $\bar{T}_b$  at the plateau of local sweating or at the final  $\bar{T}_b$  if a plateau did not occur was used for calculation of slope (30). The slope of the local SR: $\bar{T}_b$  relationship was calculated using a linear regression of all data points between the onset of sweating and peak of heat stress (or plateau if appropriate). One-way

repeated measures ANOVA were used to compare the slope and peak local SR among control and ASA treatments. In instances where the assumption of sphericity was violated, subsequent df values for within-subject effects were adjusted using the Greenhouse-Geisser correction. Data were analyzed using SPSS v. 19 (IBM, Inc., New York, NY). Significance was accepted at  $p$  values  $\leq 0.05$ . All data are presented as means  $\pm$  SD.

## RESULTS

Participant characteristics are presented in Table 2.1. Prior to the heating protocol,  $\bar{T}_b$  at baseline was not affected by ASA treatments (control,  $36.5 \pm 0.1$  °C; 325-mg,  $36.4 \pm 0.2$  °C; 81-mg,  $36.5 \pm 0.2$  °C;  $p = 0.40$ ). Cardiovascular and thermal responses during heat stress are presented in Table 2.2. Acute ASA ingestion did not elicit a change in any dependent variable among trials. Following the completion of the heating protocol, total body water losses (mL) were not different among trials ( $p = 0.38$ ) (Table 2.2).

### Skin Blood Flow Responses

At rest, before the heating protocol, baseline measures of CVC were similar among trials ( $p = 0.24$ ). As body temperature increased, the onset of cutaneous vasodilation was unaffected by ASA use ( $p = 0.87$ ) (Figure 2.1). Following the onset of cutaneous vasodilation, the magnitude of change from baseline to the end of heat stress was similar among trials ( $p = 0.27$ ) (Figure 2.2).

### Local Sweating Responses

Acute ASA ingestion did not alter the onset of local SR among trials ( $p = 0.53$ ) (Table 2.3). The slope of local SR was measured as a function of  $\bar{T}_b$  during heat stress, and as such, provided an index of sensitivity regarding this thermo-effector response. Differences in sensitivity were not observed among trials ( $p = 0.60$ ) (Table 2.3). In addition, peak local SR was not affected by ASA use (Table 2.3).

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Table 2.1. Participant characteristics ( $n = 7$ ).

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Age (yr)	$28 \pm 3$
Body mass (kg)	$84.7 \pm 15.1$
Height (m)	$1.77 \pm 0.04$
Body fat (%)	$19.9 \pm 7.8$
Systolic BP (mm Hg)	$109 \pm 6$
Diastolic BP (mm Hg)	$70 \pm 7$
MAP (mm Hg)	$83 \pm 6$
Resting HR (beats·min <sup>-1</sup> )	$59 \pm 4$

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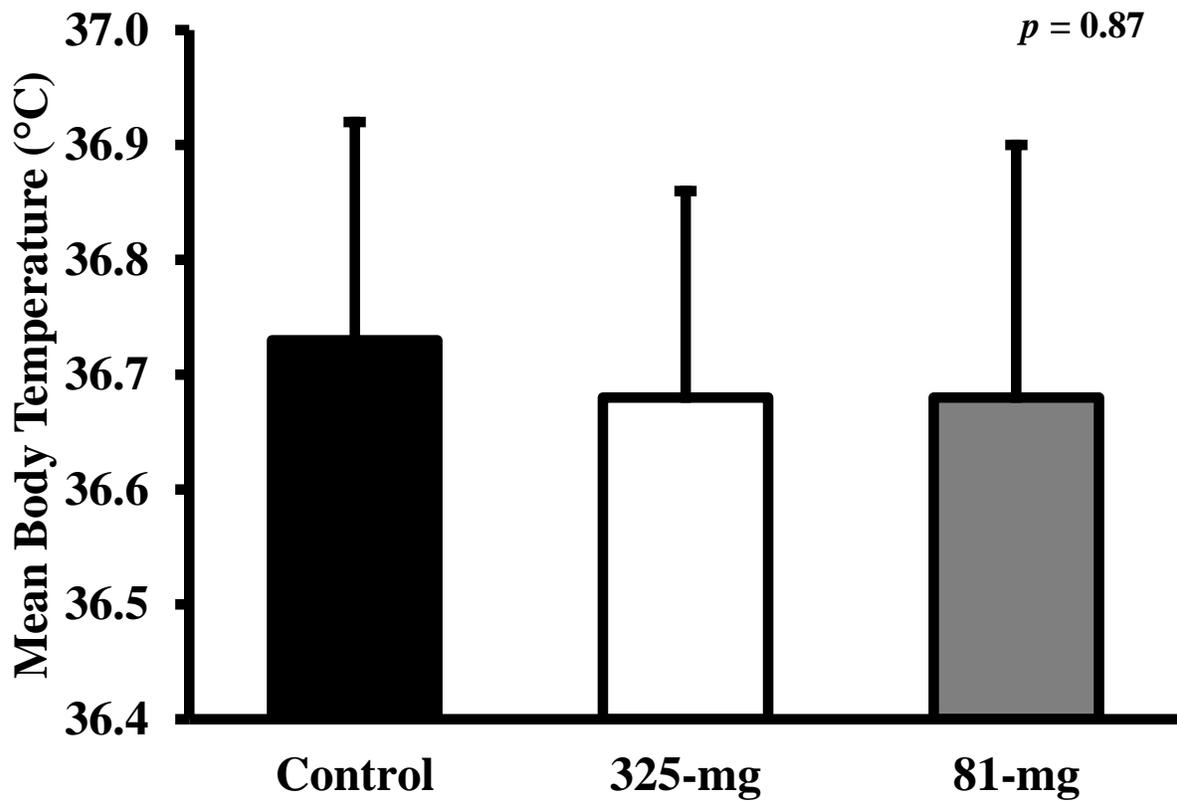
Values are means  $\pm$  SD; BP, blood pressure; MAP, mean arterial pressure; HR, heart rate.

Table 2.2. Cardiovascular and thermal responses to 1 °C increase in mean body temperature, ( $n = 7$ ).

	Control	325-mg	81-mg	<i>p</i> -value
Rectal temperature (°C)	+0.6 ± 0.1	+0.6 ± 0.1	+0.6 ± 0.3	0.35
Mean skin temperature (°C)	+5.1 ± 1.0	+4.7 ± 0.8	+5.0 ± 0.8	0.19
HR (beats·min <sup>-1</sup> )	+38 ± 4	+37 ± 8	+39 ± 6	0.56
Systolic BP (mm Hg)	+15 ± 10	+7 ± 10	+9 ± 6	0.12
Diastolic BP (mm Hg)	-1 ± 6	-4 ± 9	-4 ± 5	0.41
MAP (mm Hg)	+4 ± 5	0 ± 8	0 ± 5	0.24
Total body sweat loss (mL)	740 ± 220	830 ± 110	740 ± 200	0.38

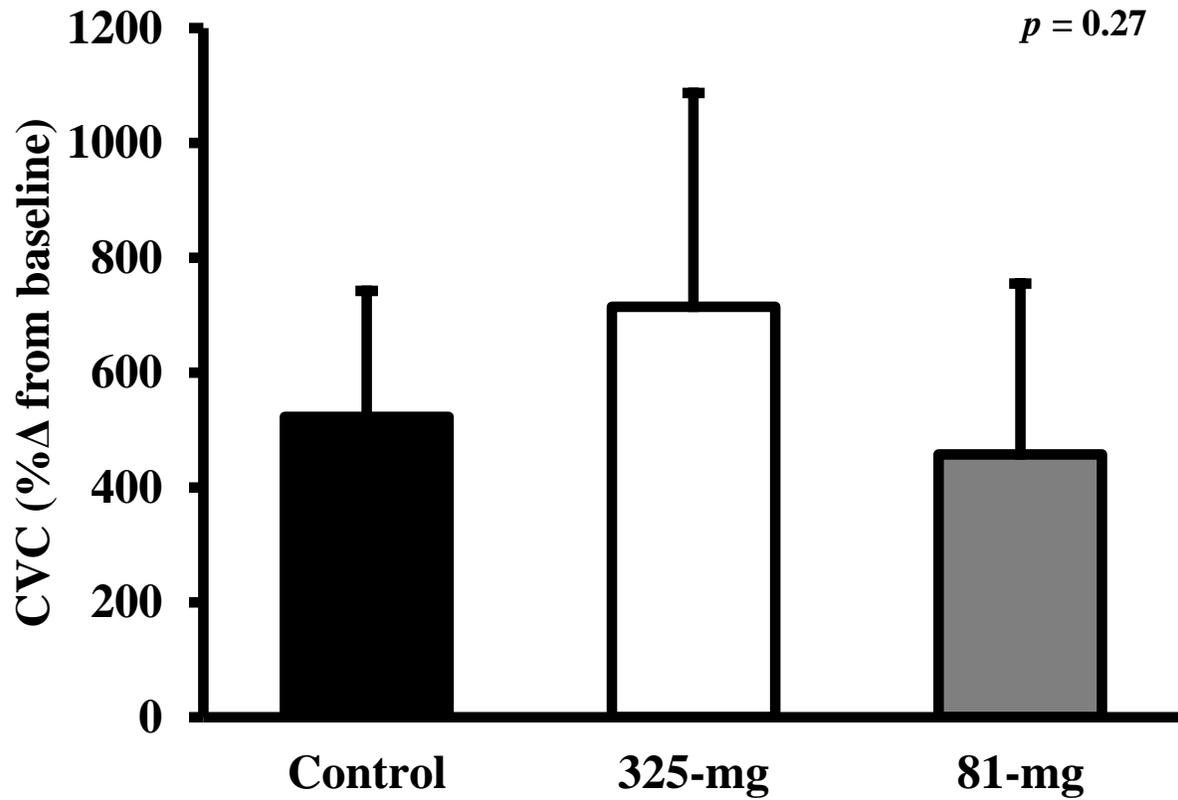
Values are presented as mean changes ± SD; HR, heart rate; BP, blood pressure; MAP, mean arterial pressure. *P*-values indicate omnibus ANOVA.

Figure 2.1. Mean body temperature (means  $\pm$  SD) at the onset of cutaneous vasodilation during passive, whole-body heat stress among control and acetylsalicylic acid trials (325-mg; 81-mg), ( $n = 7$ ).



*P*-value indicates omnibus ANOVA.

Figure 2.2. The percent change (% $\Delta$ ) of cutaneous vascular conductance (CVC) (means  $\pm$  SD) from baseline to the end of passive, whole-body heat stress among control and acetylsalicylic acid trials (325-mg; 81-mg), ( $n = 7$ ).



*P*-value indicates omnibus ANOVA.

Table 2.3. Sweat responses during whole-body, passive heat stress among control and acetylsalicylic acid trials (325-mg; 81-mg), ( $n = 7$ ).

	Control	325-mg	81-mg	$p$ -value
$\bar{T}_b$ at onset of sweating ( $^{\circ}\text{C}$ )	$36.9 \pm 0.1$	$36.8 \pm 0.1$	$36.8 \pm 0.2$	0.53
SR sensitivity [ $(\text{mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1})/^{\circ}\text{C}$ ]	$1.23 \pm 0.26$	$1.25 \pm 0.34$	$1.16 \pm 0.33$	0.60
Peak SR ( $\text{mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ )	$0.89 \pm 0.24$	$0.88 \pm 0.25$	$0.92 \pm 0.22$	0.29

Values are presented as means  $\pm$  SD;  $\bar{T}_b$ , mean body temperature; SR, sweat rate; SR sensitivity, expressed as a function of  $\bar{T}_b$ .  $P$ -values indicate omnibus ANOVA.

## DISCUSSION

The purpose of this study was to determine if an acute ASA dose of different concentrations (325-mg; 81-mg) would alter SkBF and local sweat rate (SR) during whole-body passive heating. The principal findings were that ASA did not affect SkBF or local SR in this cohort of young, healthy participants. These findings suggest that during whole-body passive heating, modest doses of ASA taken acutely do not interfere with normal hypothalamic-mediated thermal effectors (SkBF and local SR) in young healthy men.

Under the conditions of the current study (i.e., passive whole-body heating), cardiovascular and thermal data (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) were similar among control and ASA trials at baseline and through the conclusion of heat stress (Table 2.2). However, these findings are in contrast to a recent investigation that demonstrated acute acetaminophen ingestion, also a COX-inhibitor, significantly reduced  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$  and extended the time to exhaustion during cycling in the heat (15). Since this effect was achieved in afebrile participants, researchers from that study speculated that exercise combined with the influence of hot ambient conditions may increase levels of prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in the central control centers of the brain (15).

The discrepant results between the present study and the one mentioned above may be related to the notion that cytokine release during exercise differs from that which is produced during infection or fever (19). For example, exercise stimulates the release of interleukin-6 (a pro-inflammatory cytokine) (19) and is often associated with PGE<sub>2</sub> production. Methodological differences between the present study and the aforementioned study with acetaminophen may explain the different findings. First, acetaminophen preferentially inhibits COX-3, which is most abundantly expressed in the cerebral cortex (5). Similar to ibuprofen, ASA acts on COX-1 and -2 enzymes, and thus exerts both central and peripheral effects. It is possible that the modest doses

used in the present study were not large enough to elicit a meaningful systemic effect. Moreover, Mauger and colleagues (15) used considerably high concentrations of acetaminophen ( $\sim 20$  mg·kg lean body mass<sup>-1</sup>). The standard full-dose (325-mg) and low-dose (81-mg) ASA concentrations were selected in this study to examine the practical relevance that acute ingestion may pose during a passive heating situation. Lastly, exercise-induced hyperthermia (endogenous heating) is fundamentally different from the exogenous, whole-body passive heating achieved through the water-perfused suit. Therefore, it is reasonable to speculate that the lack of an effect among trials in the present study may be a product of these combined differences. Apparently, under conditions of passive heat stress, conventional doses of ASA do not effect measures of body temperature (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ).

Although we did not observe any differences in SkBF, previous work (9, 10) has shown that chronic use of low-dose (81-mg) ASA significantly attenuates reflexive cutaneous vasodilation during whole-body passive heat stress in healthy, middle-aged humans. Whereas baseline core temperatures were similar between their groups, a higher core body temperature was required to stimulate the onset of cutaneous vasodilation in ASA users. Researchers hypothesized that since low-dose (ASA) COX-inhibition remains isolated to blood platelets (22), a smaller shear stress stimulus along the endothelial wall during heat stress may have diminished cutaneous vasodilation (9). Though baseline body temperatures were similar among participants in the present study, the onset of cutaneous vasodilation was unaffected by acute ASA ingestion (Figure 2.1). Previous research has highlighted a role for COX-dependent vasodilator pathways during reflexive cutaneous vasodilation in young, healthy participants (17). However, it is likely that the highest concentration used in the present study was insufficient to acutely alter

endothelial-COX (8). Indeed, the relative increases in CVC from baseline were similar among trials in this study (Figure 2.2).

While much research has investigated SkBF responses and ASA use, few studies have examined the influence of ASA on local SR during passive heat stress. It is well understood that increased SkBF and sweating share a temporal relationship; however, recent work has demonstrated that decreased SkBF and decreased local temperature independently lower SR sensitivity (30). Recent work has shown that the onset and increase in eccrine sweating is dependent on increased SkBF during passive heat stress (26). Accordingly, it was believed that ASA may influence local sweat rate indirectly through a modification in SkBF responses. Stated differently, acute ASA use was thought to alter the COX-mediated pathways needed for complete vasodilator expression (17) and in so doing indirectly affecting sweat release. However, no such differences were detected in the present study. This observation is in agreement with previous work examining the effect of acute ibuprofen ingestion (800-mg) on local sweating responses in female participants at high and low hormone phases of their menstrual cycle (6). While ibuprofen is also a COX-inhibitor, it exhibits a much greater degree of COX-2 selectivity compared to ASA (29). Still, no differences in local SR were found, suggesting that the control of eccrine sweating is not sensitive to COX-inhibition.

To investigate the sensitivity of the sweating response, the slope of local SR was expressed as a function of mean body temperature ( $\bar{T}_b$ ). Consequently, this approach permitted a continuous examination of local sweat rate during the +1 °C heating protocol. However, ASA treatment did not alter SR sensitivity or peak SR during heat stress (Table 2.3). Given that no discernable differences in thermal data (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) were observed in the present study, it follows that local SR would be similar across all conditions.

## **Limitations**

Several limitations arose in this study. Despite counterbalancing the treatment order, it is possible that the participants developed some degree of acclimation by the third visit. Given the small number of participants, any differences that may have been present could have been masked by repeated exposure to a potent thermal stress. Still, the protocol used was tightly controlled, and as shown, the physiological responses among trials were highly reproducible. Irrespective of ASA treatment, inter-individual differences in body mass and aerobic fitness may have impacted individual responses to the heating protocol. Finally, whether ASA affects modulators of temperature regulation like pro-inflammatory cytokines (e.g., interleukin-1 $\beta$ , tumor necrosis factor- $\alpha$ ) or hormones (e.g., prostaglandins) under these conditions was not measured and remains to be investigated.

## **Conclusions**

This study demonstrated that acute ASA ingestion did not produce any differences in SkBF and local SR, and therefore  $\bar{T}_b$ ,  $T_{re}$ , and  $\bar{T}_{sk}$  were also unaffected. It is possible that such observations were due in part to the relatively modest dose of ASA used. Future work should seek to examine the effect of different COX-inhibitors on thermo-effectors in order to delineate the influence of acetaminophen (COX-3) from ASA and ibuprofen (COX-1, -2). We conclude that acute ingestion of ASA in 325-mg and/or 81-mg doses do not adversely impact human heat tolerance under conditions of passive heat stress such as those imposed in this study.

## References

1. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. 8th ed. Baltimore: Lippincott, Williams, and Wilkins; 2010. 400 p.
2. Aronoff DM, Neilson EG. Antipyretics: mechanisms of action and clinical use in fever suppression. *Am J Med*. 2001; 111(4):304-15.
3. Baum E, Bruck K, Schwennicke HP. Adaptive modifications in the thermoregulatory system of long-distance runners. *J Appl Physiol*. 1976; 40(3):404-10.
4. Boulant JA. Neuronal basis of Hammel's model for set-point thermoregulation. *J Appl Physiol (1985)*. 2006; 100(4):1347-54.
5. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, Simmons DL. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci U S A*. 2002; 99(21):13926-31.
6. Charkoudian N, Johnson JM. Altered reflex control of cutaneous circulation by female sex steroids is independent of prostaglandins. *Am J Physiol*. 1999; 276(5 Pt 2):H1634-40.
7. Chevront S SM. Hydration assessment of athletes. *Sports Sci Exchange*. 2005; 18:1-6.
8. Heavey DJ, Barrow SE, Hickling NE, Ritter JM. Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man. *Nature*. 1985; 318(6042):186-8.
9. Holowatz LA, Kenney WL. Chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985)*. 2009; 106(2):500-5.
10. Holowatz LA, Jennings JD, Lang JA, Kenney WL. Systemic low-dose aspirin and clopidogrel independently attenuate reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985)*. 2010; 108(6):1575-81.
11. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. *Br J Nutr*. 1978; 40(3):497-504.
12. Kasner SE, Wein T, Piriyaawat P, Villar-Cordova CE, Chalela JA, Krieger DW, Morgenstern LB, Kimmel SE, Grotta JC. Acetaminophen for altering body temperature in acute stroke: a randomized clinical trial. *Stroke*. 2002; 33(1):130-4.
13. Kellogg DL, Jr, Johnson JM, Kosiba WA. Control of internal temperature threshold for active cutaneous vasodilation by dynamic exercise. *J Appl Physiol (1985)*. 1991; 71(6):2476-82.
14. Kellogg DL, Jr, Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossmann M, Johnson JM. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res*. 1995; 77(6):1222-8.

15. Mauger AR, Taylor L, Harding C, Wright B, Foster J, Castle PC. Acute acetaminophen (paracetamol) ingestion improves time to exhaustion during exercise in the heat. *Exp Physiol*. 2014; 99(1):164-71.
16. Maughan RJ, Shirreffs SM, Watson P. Exercise, heat, hydration and the brain. *J Am Coll Nutr*. 2007; 26(5 Suppl):604S-12S.
17. McCord GR, Cracowski JL, Minson CT. Prostanoids contribute to cutaneous active vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291(3):R596-602.
18. Mead J, Bonmarito CL. Reliability of rectal temperatures as an index of internal body temperature. *J Appl Physiol*. 1949; 2(2):97-109.
19. Moldoveanu AI, Shephard RJ, Shek PN. Exercise elevates plasma levels but not gene expression of IL-1beta, IL-6, and TNF-alpha in blood mononuclear cells. *J Appl Physiol (1985)*. 2000; 89(4):1499-504.
20. Nagashima K. Central mechanisms for thermoregulation in a hot environment. *Ind Health*. 2006; 44(3):359-67.
21. Park I SR. "Quick and easy" formulae for approximating statistical power in repeated measures ANOVA. *Meas Phys Educ Exerc Sci*. 1999; 3:249-70.
22. Patrono C, Collier B, Dalen JE, FitzGerald GA, Fuster V, Gent M, Hirsh J, Roth G. Platelet-active drugs : the relationships among dose, effectiveness, and side effects. *Chest*. 2001; 119(1 Suppl):39S-63S.
23. Potvin PJ SR. Statistical power for the two-factor repeated measures ANOVA. *Behav Res Methods Instrum Compt*. 2000; 32(2):347-56.
24. Roddie IC, Shepherd JT, Whelan RF. The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *J Physiol*. 1957; 136(3):489-97.
25. Rosenthal TC, Silverstein DA. Fever. What to do and what not to do. *Postgrad Med*. 1988; 83(8):75-84.
26. Smith CJ, Kenney WL, Alexander LM. Regional relation between skin blood flow and sweating to passive heating and local administration of acetylcholine in young, healthy humans. *Am J Physiol Regul Integr Comp Physiol*. 2013; 304(7):R566-73.
27. Taylor WF, Johnson JM, O'Leary D, Park MK. Effect of high local temperature on reflex cutaneous vasodilation. *J Appl Physiol Respir Environ Exerc Physiol*. 1984; 57(1):191-6.
28. Warner TD, Mitchell JA. Cyclooxygenase-3 (COX-3): filling in the gaps toward a COX continuum? *Proc Natl Acad Sci U S A*. 2002; 99(21):13371-3.

29. Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclo-oxygenase-1 rather than cyclo-oxygenase-2 are associated with human gastrointestinal toxicity: a full in vitro analysis. *Proc Natl Acad Sci U S A*. 1999; 96(13):7563-8.
30. Wingo JE, Low DA, Keller DM, Brothers RM, Shibasaki M, Crandall CG. Skin blood flow and local temperature independently modify sweat rate during passive heat stress in humans. *J Appl Physiol (1985)*. 2010; 109(5):1301-6.

## CHAPTER III

### EFFECTS OF SHORT TERM ACETYLSALICYLIC ACID USE ON SKIN BLOOD FLOW AND LOCAL SWEAT RATE DURING WHOLE-BODY PASSIVE HEAT STRESS

#### ABSTRACT

Daily use of low-dose (81-mg) acetylsalicylic acid (ASA) is often recommended for its antithrombotic properties. Previous work has revealed attenuated skin blood flow (SkBF) responses during heat stress in older participants habitually using ASA. Accordingly, the purpose of this study was to investigate the effect of short-term (4 days) ASA treatment on measures of SkBF and local sweat rate (SR) during whole-body, passive heat stress in college-aged participants. Seven, male participants completed counter-balanced trials to compare ASA treatment (i.e., 4 days of low-dose ASA) to control trials (no ASA). Laser-Doppler flowmetry was used to provide an index of SkBF. Local sweat rate was measured using a ventilated-capsule via capacitance hygrometry. Participant's mean body temperature ( $\bar{T}_b$ ) was increased by 1 °C above baseline using a water-perfused suit. ASA did not affect ( $\bar{T}_b$ ) at baseline (control,  $36.5 \pm 0.1$  °C; ASA,  $36.5 \pm 0.2$  °C;  $p = 0.93$ ). Additionally, ASA treatment did not affect the onset of cutaneous vasodilation (control,  $36.7 \pm 0.2$  °C; ASA,  $36.7 \pm 0.3$  °C;  $p = 0.86$ ), or the increase of cutaneous vascular conductance during heat stress (control,  $523 \pm 219\%$ ; ASA,  $545 \pm 173\%$ ;  $p = 0.81$ ). Measures of local SR were similar between treatments at the onset (control,  $36.9 \pm 0.1$  °C; ASA,  $36.8 \pm 0.2$  °C;  $p = 0.61$ ) and SR sensitivity [ $(\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1})/\text{°C}$ ] (control,  $1.23 \pm 0.26$ ; ASA,  $1.20 \pm 0.36$ ;  $p = 0.85$ ). Short-term low-dose ASA does not appear to influence measures of SkBF or local SR during whole-body, passive heat stress under these conditions.

**KEY WORDS:** aspirin, sweating, thermoregulation, vasodilation

## INTRODUCTION

During prolonged exposure to very hot ambient conditions, core temperature invariably rises. Upon reaching a relative ‘threshold,’ increased skin blood flow (SkBF) and eccrine sweating are optimized to defend against an unsafe rise in body temperature (29). Accordingly, hypothalamic-mediated temperature regulation relies on central and peripheral signaling to preserve cellular and enzymatic activity (23). However, any pharmaceuticals that may interfere with such signaling could pose a health risk by upsetting temperature regulation.

Acetylsalicylic acid (ASA), a popular over-the-counter pain and fever suppressant, is one such drug that may threaten intrinsic thermo-balance. Through the inhibition of cyclooxygenase (COX), ASA can elicit powerful systemic effects believed to suppress central and peripheral signaling (2). While the efficacy of ASA to treat fever is widely recognized, more recently a daily regimen of low-dose (81-mg) ASA has been prescribed for its antithrombotic properties (27).

With regard to temperature regulation, current understanding suggests nitric oxide synthase- (20, 30) and cyclooxygenase- (COX-) (21) mediated pathways are obligatory for complete reflexive cutaneous vasodilator expression. However, functional losses in SkBF responses during whole-body, passive heat stress have been observed in middle-aged participants habitually using low-dose ASA (12, 14). Researchers postulated that diminished SkBF effects may have resulted from altered vascular wall signaling (12). Similarly, acute ASA ingestion has been shown to inhibit current-induced cutaneous vasodilation (i.e. a model for neurogenic inflammation) (32). Remarkably, researchers noted a long lasting effect (> 5-days), suggesting that COX inactivation of central origin was not responsible for the observed outcome (9, 32).

Instead, modifications in platelet activity were believed to be responsible, and as such, highlight the importance of peripheral feedback for proper homeostatic function.

However, little is known about the effects of short-term (4 days) low-dose ASA use on local sweat rate (SR) in young, healthy participants. In light of the previous work showing ASA use significantly blunts SkBF responses during heat stress (12, 14), it is possible that ASA may also affect sweating. During heat stress, the release of acetylcholine initiates reflexive cutaneous vasodilation, and additionally, stimulates sweating through muscarinic receptor activation (19). Thus, SkBF and sweating often occur sequentially as body temperature rises, though, work has demonstrated that decreased SkBF and decreased local temperature independently alter SR sensitivity (34). More recently Smith and colleagues (31) determined that the onset and rise of sweating is dependent on increased SkBF during passive heat stress. Due to the importance of COX-mediated pathways in cutaneous vasodilation (21), ASA induced modifications in SkBF may indirectly affect local sweating. Indeed, any impairment in heat dissipation via SkBF and/or sweating may increase heat storage, possibly threatening thermo-balance.

The purpose of this study is to determine if short-term (4 days), low-dose (81-mg) ASA use would alter SkBF and/or local SR during whole-body, passive heat stress. The duration and dose of ASA chosen have previously been shown to be sufficient for complete blood platelet inhibition (27). Similar to previous work (12, 14) it was hypothesized that SkBF responses would be attenuated, and additionally, eccrine sweating responses would be weakened as evidenced by lowered SR sensitivity and lowered peak SR.

## **METHODS**

### **Ethical Approval**

Prior to inclusion, all participants were informed of the methods and risks associated with this study. Verbal and written consent were acquired from each participant during each visit. The consent and experimental procedures were approved by the Institutional Review Board at The University of Alabama. All procedures conformed to the standards set by the Declaration of Helsinki.

### **Participants**

Seven healthy, male participants (mean  $\pm$  SD;  $28 \pm 3$  yr) were recruited to participate in this study. A power analysis (25) revealed this sample size was adequate to detect a moderate effect size ( $d = 0.60$ ) (28) for peak local SR, assuming a power of  $\sim 0.85$  and correlation among repeated levels of each factor at  $\sim 0.90$ . All participants were normotensive and self-reported nonsmokers, maintaining moderately active lifestyles in accordance with American College of Sports Medicine guidelines (1). Participants were not taking any medications, including herbal supplements, and did not report an allergy to acetylsalicylic acid (ASA). During the 12 hours prior to each experimental session, participants refrained from caffeine, alcohol, and exhaustive physical activity. A counterbalanced, crossover design (control vs. ASA) was used to reduce any ordering effects. Experimental trials were conducted at a similar time of day to minimize the influence of circadian rhythms on body temperature.

## Instrumentation and Measurements

Following arrival at the laboratory, participants provided their consent and completed a 24-hr health history questionnaire to confirm guideline adherence. Hydration status was assessed from urine specific gravity (USG) through refractometry (SUR-NE, Atago Inc., Higham, MA). Adequate hydration was defined as values  $< 1.020$  (6). Once hydration status was confirmed, participants had their standing height and nude body mass measured (Tanita Corporation, Tokyo, Japan). Calipers (Lange, Beta Technology Inc., Cambridge, MD) were used to estimate body fat percentage from the sum of three skinfolds (15). To measure rectal temperature ( $T_{re}$ ), participants self-inserted a rectal thermocouple (model RET-1, Physitemp, Clifton, NJ) 8-10 cm beyond the anal sphincter (22). Mean skin temperature ( $\bar{T}_{sk}$ ) was assessed by weighted measures of six thermocouples placed on the upper back, upper chest, lower back, abdomen, anterior thigh, and calf. Mean body temperature ( $\bar{T}_b$ ) was calculated from weighted measures of  $T_{re}$  and  $\bar{T}_{sk}$  from the following equation (3):  $[\bar{T}_b = [(0.87 \cdot T_{re}) + (0.13 \cdot \bar{T}_{sk})]$ . Participants were then instrumented with heart rate monitors (Polar, Stamford, CT).

An index of SkBF was obtained via laser-Doppler flowmetry (LDF) (MoorVMS-LDF2, Moor Instruments, Devon, UK) with a probe positioned on the dorsal aspect of the forearm. Cutaneous vascular conductance (CVC) was calculated from arbitrary LDF units divided by mean arterial pressure. Mean arterial blood pressure was determined from the following equation:  $[(1/3) \cdot \text{systolic blood pressure}] + [(2/3) \cdot \text{diastolic blood pressure}]$ . Local SR was continuously measured from a ventilated-capsule placed near the LDF probe. Humidity from the effluent air was measured via capacitance hygrometry. Measurements from anatomical landmarks of the forearm were taken to facilitate consistency between trials. Body temperature

was manipulated with a water-perfused suit covering the whole-body apart from the face, hands feet, and instrumented forearm. An impermeable suit was worn over the perfusion suit to maximize heat storage and encourage sweating. Total body sweating was estimated from the difference in nude body mass before and after the heating protocol.

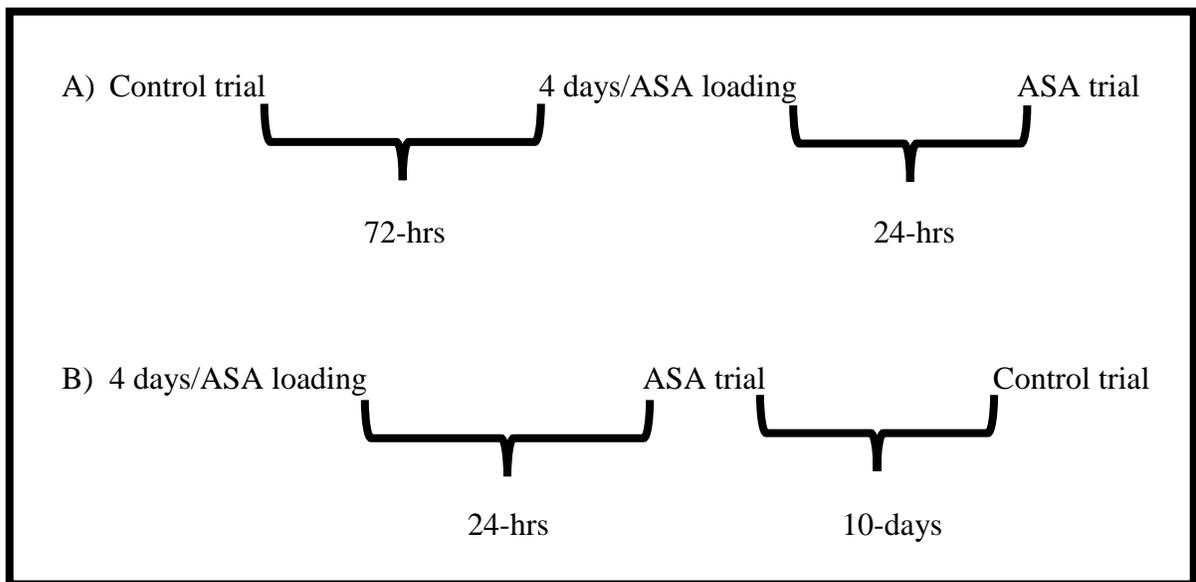
## **Experimental Protocol**

Standard U.S., low-dose (81-mg) ASA was purchased over-the-counter and was selected for its antithrombotic properties (27). A 4 day loading period has been shown to be a sufficient time-course to completely inhibit platelet activity (27). Previous work has demonstrated that endothelial-COX can recover activity within 24 hours (11), and as such, ASA was not taken the same day of experimental trials to avoid acute effects. Figure 3.1 describes experimental sequences. Due to counter-balancing, participants began the study with either a control trial (A) or 4 days of ASA loading (B). Following control trials, a 72-hr period was taken to ensure recovery prior to ASA loading. A 10 day washout period was enforced to permit normal blood platelet re-synthesis (27). During the loading period, no experimental procedures were conducted and participants were free to leave upon receiving ASA treatment.

During experimental trials, instrumented participants entered an environmentally controlled chamber set to temperate conditions (22 °C, 30-35% rH). Participants were aided onto a gurney and remained in the supine position throughout the duration of the experiment. All instruments were connected and values verified. To clamp  $\bar{T}_{sk}$  at ~34 °C (thermoneutral), water (~31.3-31.5 °C) was perfused through the suit. A 10-min period was permitted to allow  $\bar{T}_{sk}$  to stabilize. During the final minute of this period, baseline data were collected. Next, hot water (~49 °C) was circulated through the suit to increase  $\bar{T}_b$  by 1.0 °C above baseline temperature.

Upon reaching the target  $\bar{T}_b$  and during the final minute of heat stress, peak data values were collected. Cool water was perfused through the suit to lower  $\bar{T}_b$  within  $\sim 0.4$  °C of baseline. Throughout the heating protocol, heart rate was continuously monitored, while blood pressure was measured every 5 min via automated oscillometry. Participants were aided off the gurney to remove instrumentation. After towel drying, participants reported their nude body mass and were given fluids to consume *ad libitum*. All experimental trials were conducted in this manner.

Figure 3.1. Experimental sequence.



ASA, acetylsalicylic acid (81 mg).

### Statistical Analyses

Data were continuously sampled at 50 Hz using a data-acquisition system (Biopac, MP150, Goleta, CA) and stored for offline analysis. Thermal data (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) and local SR were averaged every 30 s during heat stress (34). Changes in temperature variables before heating were compared with peak heat stress values and analyzed using a two-tailed, paired Student's *t*-test. Averaged CVC values during the final min of exercise (peak) were expressed as

a percent change from baseline  $[(\text{peak CVC} - \text{baseline CVC}) / \text{baseline CVC}] \cdot 100$  (18). The onset of cutaneous vasodilation and sweating were determined by an experienced investigator, blinded to the treatment, by visually examining arbitrary LDF units and local SR graphed as a function of time. The  $\bar{T}_b$  at the indicated time was then used to identify the onset of cutaneous vasodilation and sweating. The  $\bar{T}_b$  at the plateau of local sweating or at the final  $T_b$  if a plateau did not occur was used for calculation (34). A linear regression was used to calculate the slope of the local SR: $\bar{T}_b$  relationship from all data points between the onset of sweating and peak of heat stress (or plateau if appropriate). Two-tailed, paired Student's *t*-tests were used to compare the slope and peak local SR between control and ASA trials. Data were analyzed using SPSS v. 19 (IBM, Inc., New York, NY). Significance was accepted at *p* values  $\leq 0.05$ . All data are presented as means  $\pm$  SD.

## RESULTS

Participant characteristics are shown in Table 3.1. At baseline  $\bar{T}_b$  did not differ between trials (control,  $36.5 \pm 0.1$  °C; ASA,  $36.5 \pm 0.2$  °C;  $p = 0.93$ ). Cardiovascular and thermal responses during the final minute of heat stress are presented in Table 3.2. Four days of low-dose ASA treatment did not produce any change in dependent variables. Additionally, total body water losses (mL) were not different between trials ( $p = 0.13$ ) (Table 3.2).

### Skin Blood Flow Responses

Prior to the heating protocol, at rest, baseline measures of CVC were similar among ASA and control trials ( $p = 0.55$ ). At the onset of cutaneous vasodilation, mean body temperature was similar between trials (control,  $36.7 \pm 0.2$  °C; ASA,  $36.7 \pm 0.3$  °C;  $p = 0.86$ ) (Figure 3.2). During the final minute of heat stress, the magnitude of change from baseline (CVC) was not different between trials (control,  $523 \pm 219\%$ ; ASA,  $545 \pm 173\%$ ;  $p = 0.81$ ) (Figure 3.3).

### Local Sweating Responses

Four-days of low-dose ASA treatment did not influence any measures of local sweating responses (Table 3.3). Changes in local SR were measured as a function of  $\bar{T}_b$ , and thus, provided insight into the sensitivity of this thermo-effector response. Consequently, differences in local SR sensitivity [ $(\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}) / \text{°C}$ ] were not seen between trials (control,  $1.23 \pm 0.26$ ; ASA,  $1.20 \pm 0.36$ ;  $p = 0.85$ ). Furthermore, peak local SR was similar between trials and did not appear to be influenced by 4 days of low-dose ASA treatment ( $p = 0.90$ ) (Table 3.3).

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Table 3.1. Participant characteristics ( $n = 7$ ).

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Age (yr)	$28 \pm 3$
Body mass (kg)	$84.7 \pm 15.1$
Height (m)	$1.77 \pm 0.04$
Body fat (%)	$19.9 \pm 7.8$
Systolic BP (mm Hg)	$109 \pm 6$
Diastolic BP (mm Hg)	$70 \pm 7$
MAP (mm Hg)	$83 \pm 6$
Resting HR (beats·min <sup>-1</sup> )	$59 \pm 4$

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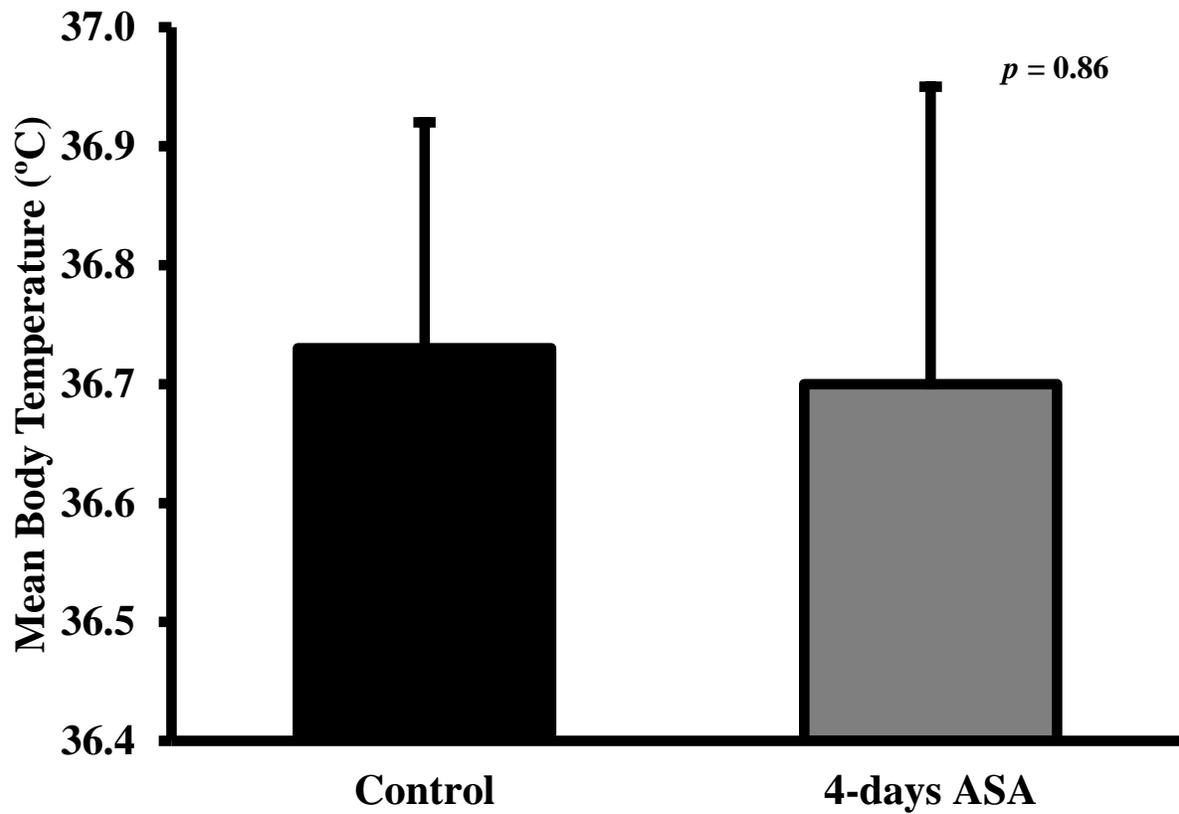
Values as means  $\pm$  SD; BP, blood pressure; MAP, mean arterial pressure; HR, heart rate.

Table 3.2. Cardiovascular and thermal responses to 1 °C increase in mean body temperature, ( $n = 7$ ).

	Control	4 days ASA	<i>p</i> -value
Rectal temperature (°C)	+0.6 ± 0.1	+0.6 ± 0.1	0.29
Mean skin temperature (°C)	+5.1 ± 1.0	+4.6 ± 0.6	0.24
HR (beats·min <sup>-1</sup> )	+38 ± 4	+36 ± 5	0.29
Systolic BP (mm Hg)	+15 ± 10	+8 ± 6	0.08
Diastolic BP (mm Hg)	-1 ± 6	0 ± 7	0.91
MAP (mm Hg)	+4 ± 5	+2 ± 5	0.46
Total body sweat loss (mL)	740 ± 220	940 ± 390	0.13

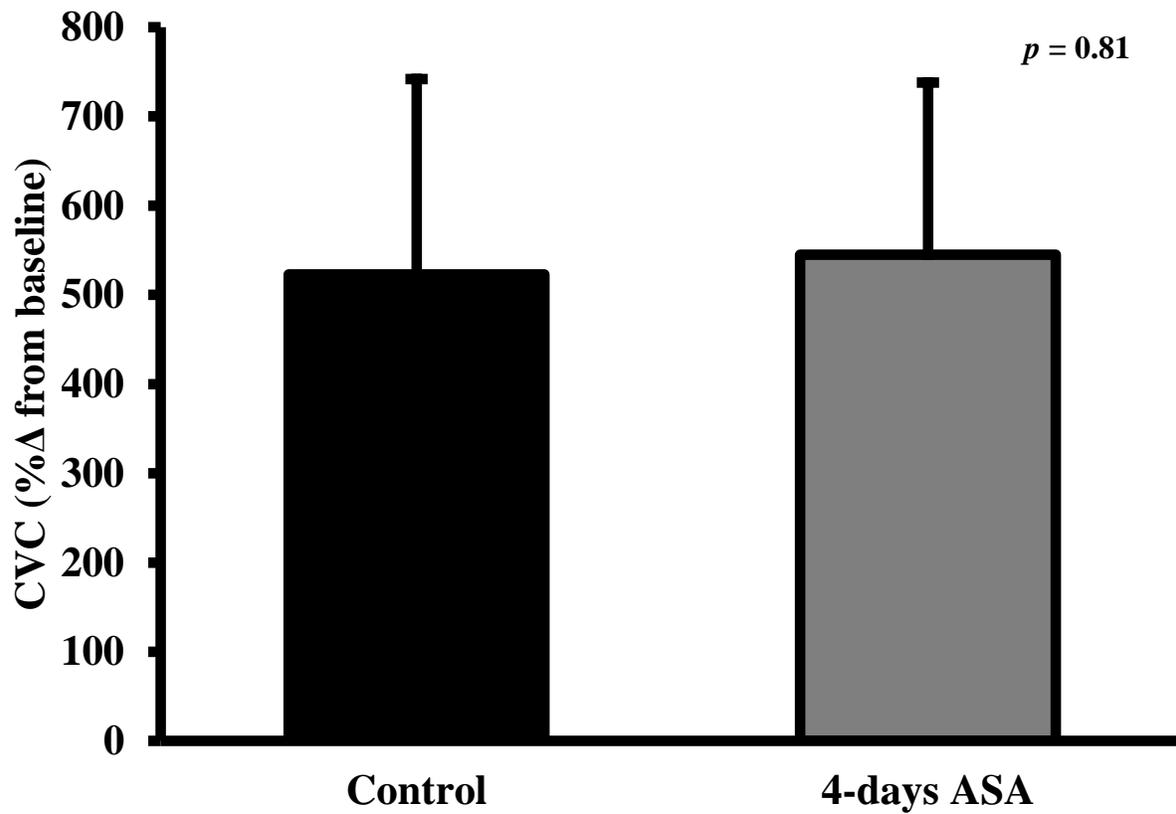
Values are presented as means ± SD; HR, heart rate; BP, blood pressure; MAP, mean arterial pressure; ASA, acetylsalicylic acid. *P*-values indicate two-tailed, paired Student's *t*-test.

Figure 3.2. Mean body temperature (means  $\pm$  SD) thresholds for the onset of cutaneous vasodilation during passive, whole-body heat stress among control and 4-days of acetylsalicylic acid (ASA) trials, ( $n = 7$ ).



*P*-value indicates two-tailed, paired Student's *t*-test.

Figure 3.3. The percent change ( $\Delta\%$ ) of cutaneous vascular conductance (CVC) (means  $\pm$  SD) from baseline to the end of passive, whole-body heat stress among control and 4-days of acetylsalicylic acid (ASA) trials, ( $n = 7$ ).



*P*-value indicates two-tailed, paired Student's *t*-test.

Table 3.3. Local sweating responses during whole-body, passive heat stress among control and 4-days low-dose (81-mg) acetylsalicylic acid (ASA) trials, ( $n = 7$ ).

	Control	4 days ASA	$p$ -value
$\bar{T}_b$ at onset of sweating ( $^{\circ}\text{C}$ )	$36.9 \pm 0.1$	$36.8 \pm 0.2$	0.61
SR sensitivity [ $(\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}) / ^{\circ}\text{C}$ ]	$1.23 \pm 0.26$	$1.20 \pm 0.36$	0.85
Peak SR ( $\text{mg} \cdot \text{cm}^{-2} \cdot \text{min}^{-1}$ )	$0.89 \pm 0.24$	$0.88 \pm 0.17$	0.90

Values are presented as means  $\pm$  SD;  $\bar{T}_b$ , mean body temperature; SR, sweat rate; SR sensitivity, expressed as a function of  $\bar{T}_b$ .  $P$ -values indicate two-tailed, paired Student's  $t$ -test.

## DISCUSSION

Previous work has demonstrated that daily ingestion of low-dose acetylsalicylic acid (ASA) significantly attenuates SkBF responses during passive heat stress (12, 14). Accordingly, the purpose of the present study was to investigate the effect of short-term (4 days) low-dose ASA on SkBF and local SR in healthy, young male participants. Similar to previous work (12, 14), it was hypothesized that SkBF responses would be diminished with short term ASA use, and thus indirectly blunting local sweating responses. Contrary to the initial hypotheses, the principal findings of this investigation were that 4 days of low-dose ASA treatment did not effect SkBF or local SR responses during passive, whole-body heat stress in these participants.

Given that ASA readily permeates the blood-brain barrier and irreversibly inhibits blood platelet activity, it exerts both central and peripheral effects. Yet, in the current study baseline measures of mean body temperature and cardiovascular responses were not different between ASA and control trials (Table 3.2). However, Bruning et al. (4) demonstrated that 40 min exposure to warm ambient air (30 °C) resulted in higher esophageal temperatures in ASA groups compared to controls despite having similar oral temperatures prior to heating. Researchers from that study reported significantly attenuated SkBF responses with ASA during heat exposure. Since, increased SkBF is one of the primary strategies to prevent a rise in body temperature, diminished cutaneous vasodilator responses were believed to contribute to the elevated core temperatures.

While speculative, it is possible that the changes in SkBF were unlikely a direct result of hypothalamic COX-inhibition, as much higher doses are needed to influence central temperature control in afebrile participants (17). Indeed, low-dose ASA treatment may affect thermal sensitivity through a modification in afferent signaling. Blunted peripheral vasodilator activity

has been repeatedly observed during heat stress (4, 12, 14) and may be consequence-specific to blood platelet inhibition (via ASA) which raises two possibilities: 1) altered vascular wall signaling and/or 2) reduced platelet-derived vasodilator factors (e.g., ADP, serotonin) (10, 24). Accordingly, a lowered blood viscosity may retard the mechanical stress along the endothelial wall resulting in significantly weakened nitric oxide-dependent cutaneous vasodilation (12). During prolonged heat stress, the resultant hyperthermic body temperature may neurogenically activate platelets via sensory nerves or vessel-wall interactions thereby stimulating the release of adenosine diphosphate or serotonin to induce cutaneous vasodilation (4, 10, 14, 33). While SkBF responses were similar between ASA and control trials in the present study, our results may have been attributed to the inherent redundancy of the thermoregulatory effectors (5) and relatively young age of participants. Previous research has demonstrated that during healthy aging the cutaneous tissue becomes less responsive (13), and as such, the healthy young participants in the present study did not seem to be affected by short-term ASA use. Still, the results from previous investigations (4, 12, 14) provide reasonable evidence to suggest that the diminished SkBF responses to low-dose ASA treatment during heat stress may be of peripheral origin though further research is warranted.

As previously stated, SkBF responses in the present study were hypothesized to be attenuated during passive heat stress. Consequently, eccrine sweating was thought to be indirectly influenced, however, no such differences were seen in the present study suggesting that local SR is unaffected by 4 days of low-dose ASA treatment (Table 3.3). Admittedly, few studies have examined the effects of ASA on body temperature regulation and sweating responses. Given the absence of thermal differences and SkBF responses, it follows that local SR would be unaffected. Nevertheless, previous work has demonstrated that systemic exposure to

very high doses (7.8 g) of sodium salicylate increased SR during walking exercise in hot conditions (16). Since, ingestion of sodium salicylate has been reported to markedly increase oxygen uptake at rest (7), it was believed that exercise in the heat combined with ingestion of sodium salicylate created a ‘hyper-metabolic state’ resulting in amplified sweating responses (16). Yet, due to the low-concentrations (81-mg) of ASA used in the current study, direct action of salicylate (ASA’s active metabolite) on central temperature regulation is likely negligible. Furthermore, exercise-induced hyperthermia (endogenous heating) is profoundly different from exogenous heating in the present study thereby limiting inferences.

### **Limitations**

Daily ingestion of low-dose (81-mg) ASA is often prescribed as a preventative for acute cardiac events due to its antithrombotic properties (27). Accordingly, this dose and administration schedule was used in the present study to further out understanding of daily low-dose ASA on temperature regulation during passive heat stress. While 4 days has been shown to be an effective time-course to full inhibit platelet activity (27) , it is possible that such a result was not achieved. In addition, ASA treatment was not titrated to body mass or blood volume. However, ASA doses as low as 30-mg/day have been shown to be just as efficacious on platelet activity as 75-mg (26). Finally, only young healthy, male participants were tested in this study. Consequently, extrapolation of these data to other demographics must be done cautiously.

### **Conclusion**

This study determined that 4 days of low-dose ASA did not influence measures of SkBF or local SR during whole-body passive heat stress. It is possible that the similarities in  $\bar{T}_b$ ,  $T_{re}$ ,

and  $\bar{T}_{sk}$  between ASA and control trials may have contributed to such findings. Given that previous work has shown that ASA treatment blunts SkBF responses in middle-aged participants (4, 12, 14), it is plausible that age may be linked to the dissimilarity in results from previous studies (8). Accordingly, future work should seek to understand if low-dose ASA treatment affects eccrine sweating other groups and special populations.

## References

1. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. 8th ed. Baltimore: Lippincott, Williams, and Wilkins; 2010. 400 p.
2. Aronoff DM, Neilson EG. Antipyretics: mechanisms of action and clinical use in fever suppression. *Am J Med*. 2001; 111(4):304-15.
3. Baum E, Bruck K, Schwennicke HP. Adaptive modifications in the thermoregulatory system of long-distance runners. *J Appl Physiol*. 1976; 40(3):404-10.
4. Bruning RS, Dahmus JD, Kenney WL, Alexander LM. Aspirin and clopidogrel alter core temperature and skin blood flow during heat stress. *Med Sci Sports Exerc*. 2013; 45(4):674-82.
5. Charkoudian N. Skin blood flow in adult human thermoregulation: how it works, when it does not, and why. *Mayo Clin Proc*. 2003; 78(5):603-12.
6. Cheuvront S SM. Hydration assessment of athletes. *Sports Sci Exchange*. 2005; 18:1-6.
7. Cochran JB. Further observations on the metabolic stimulating effect of salicylate. *Br Med J*. 1954; 1(4864):733-4.
8. Dufour A, Candas V. Ageing and thermal responses during passive heat exposure: sweating and sensory aspects. *Eur J Appl Physiol*. 2007; 100(1):19-26.
9. Durand S, Fromy B, Tartas M, Jardel A, Saumet JL, Abraham P. Prolonged aspirin inhibition of anodal vasodilation is not due to the trafficking delay of neural mediators. *Am J Physiol Regul Integr Comp Physiol*. 2003; 285(1):R155-61.
10. Forstermann U, Mugge A, Bode SM, Frolich JC. Response of human coronary arteries to aggregating platelets: importance of endothelium-derived relaxing factor and prostanoids. *Circ Res*. 1988; 63(2):306-12.
11. Heavey DJ, Barrow SE, Hickling NE, Ritter JM. Aspirin causes short-lived inhibition of bradykinin-stimulated prostacyclin production in man. *Nature*. 1985; 318(6042):186-8.
12. Holowatz LA, Kenney WL. Chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985)*. 2009; 106(2):500-5.
13. Holowatz LA, Houghton BL, Wong BJ, Wilkins BW, Harding AW, Kenney WL, Minson CT. Nitric oxide and attenuated reflex cutaneous vasodilation in aged skin. *Am J Physiol Heart Circ Physiol*. 2003; 284(5):H1662-7.
14. Holowatz LA, Jennings JD, Lang JA, Kenney WL. Systemic low-dose aspirin and clopidogrel independently attenuate reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985)*. 2010; 108(6):1575-81.

15. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. *Br J Nutr.* 1978; 40(3):497-504.
16. Jacobson ED, Bass DE. Effects of Sodium Salicylate on Physiological Responses to Work in Heat. *J Appl Physiol.* 1964; 19:33-6.
17. Kasner SE, Wein T, Piriyaawat P, Villar-Cordova CE, Chalela JA, Krieger DW, Morgenstern LB, Kimmel SE, Grotta JC. Acetaminophen for altering body temperature in acute stroke: a randomized clinical trial. *Stroke.* 2002; 33(1):130-4.
18. Kellogg DL,Jr, Johnson JM, Kosiba WA. Control of internal temperature threshold for active cutaneous vasodilation by dynamic exercise. *J Appl Physiol (1985).* 1991; 71(6):2476-82.
19. Kellogg DL,Jr, Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossmann M, Johnson JM. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res.* 1995; 77(6):1222-8.
20. Kellogg DL,Jr, Crandall CG, Liu Y, Charkoudian N, Johnson JM. Nitric oxide and cutaneous active vasodilation during heat stress in humans. *J Appl Physiol (1985).* 1998; 85(3):824-9.
21. McCord GR, Cracowski JL, Minson CT. Prostanoids contribute to cutaneous active vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol.* 2006; 291(3):R596-602.
22. Mead J, Bonmarito CL. Reliability of rectal temperatures as an index of internal body temperature. *J Appl Physiol.* 1949; 2(2):97-109.
23. Nagashima K. Central mechanisms for thermoregulation in a hot environment. *Ind Health.* 2006; 44(3):359-67.
24. Oskarsson HJ, Hofmeyer TG. Platelets from patients with diabetes mellitus have impaired ability to mediate vasodilation. *J Am Coll Cardiol.* 1996; 27(6):1464-70.
25. Park I SR. "Quick and easy" formulae for approximating statistical power in repeated measures ANOVA. *Meas Phys Educ Exerc Sci.* 1999; 3:249-70.
26. Patrono C, Rocca B. Aspirin: promise and resistance in the new millennium. *Arterioscler Thromb Vasc Biol.* 2008; 28(3):s25-32.
27. Patrono C, Collier B, Dalen JE, FitzGerald GA, Fuster V, Gent M, Hirsh J, Roth G. Platelet-active drugs : the relationships among dose, effectiveness, and side effects. *Chest.* 2001; 119(1 Suppl):39S-63S.
28. Potvin PJ SR. Statistical power for the two-factor repeated measures ANOVA. *Behav Res Methods Instrum Compt.* 2000; 32(2):347-56.

29. Roddie IC, Shepherd JT, Whelan RF. The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *J Physiol*. 1957; 136(3):489-97.
30. Shastry S, Dietz NM, Halliwill JR, Reed AS, Joyner MJ. Effects of nitric oxide synthase inhibition on cutaneous vasodilation during body heating in humans. *J Appl Physiol (1985)*. 1998; 85(3):830-4.
31. Smith CJ, Kenney WL, Alexander LM. Regional relation between skin blood flow and sweating to passive heating and local administration of acetylcholine in young, healthy humans. *Am J Physiol Regul Integr Comp Physiol*. 2013; 304(7):R566-73.
32. Tartas M, Bouye P, Koitka A, Jaquinandi V, Tan L, Saumet JL, Abraham P. Cathodal current-induced vasodilation to single application and the amplified response to repeated application in humans rely on aspirin-sensitive mechanisms. *J Appl Physiol (1985)*. 2005; 99(4):1538-44.
33. Vanhoutte PM, Houston DS. Platelets, endothelium, and vasospasm. *Circulation*. 1985; 72(4):728-34.
34. Wingo JE, Low DA, Keller DM, Brothers RM, Shibasaki M, Crandall CG. Skin blood flow and local temperature independently modify sweat rate during passive heat stress in humans. *J Appl Physiol (1985)*. 2010; 109(5):1301-6.

## CHAPTER IV

### EFFECTS OF ACETYLSALICYLIC ACID INGESTION ON SKIN BLOOD FLOW AND LOCAL SWEAT RATE DURING SUBMAXIMAL EXERCISE AND PASSIVE RECOVERY IN THE HEAT

#### ABSTRACT

Acetylsalicylic acid (ASA) has been shown to attenuate cutaneous vasodilation during passive heat stress, but effects on local sweat rate (SR) during exercise in a hot environment have not been tested. The purpose of this study was to investigate if, during exercise in the heat, acute ingestion of either standard U.S. full-dose (325-mg) or low-dose (81-mg) ASA would alter the following during exercise in a hot environment: 1) Increase in cutaneous vascular conductance (CVC) from baseline; 2) Onset and slope (i.e., sensitivity) of local SR; and 3) CVC and local SR responses during a period of passive recovery. Seven, healthy male participants (mean  $\pm$  SD; 27  $\pm$  5 yr) completed counter-balanced trials to compare ASA treatments (325-mg ASA and 81-mg ASA) to a control trial (no ASA). A 10-day washout period was used to ensure normal blood platelet restoration between ASA treatments. Participants performed recumbent cycling in the heat (35 °C; 30-40% rH) for 45-min at an intensity corresponding to 50% heart rate reserve, followed by a 15-min passive recovery. ASA did not affect the increase in CVC from baseline (control, 342  $\pm$  172%; 325-mg, 319  $\pm$  122%; 81-mg, 335  $\pm$  219%;  $p = 0.92$ ). Similarly, ASA did not alter the onset (control, 36.8  $\pm$  0.4 °C; 325-mg, 36.8  $\pm$  0.3 °C; 81-mg, 36.8  $\pm$  0.3 °C;  $p = 0.82$ ) or local SR sensitivity [control, 0.69  $\pm$  0.28 (mg·cm<sup>-2</sup>·min<sup>-1</sup>)/°C; 325-mg, 0.69  $\pm$  0.20 (mg·cm<sup>-2</sup>·min<sup>-1</sup>)/°C; 81-mg, 0.67  $\pm$  0.22 (mg·cm<sup>-2</sup>·min<sup>-1</sup>)/°C;  $p = 0.94$ ]. Additionally, CVC change from the end of exercise (control, -28  $\pm$  20%; 325-mg, -29  $\pm$  16%; 81-mg, -30  $\pm$  18%;  $p = 0.93$ ) and local SR change from the end of exercise (control, -48  $\pm$  26%; 325-mg, -45  $\pm$  21%; 81-mg, -53  $\pm$  19%;  $p = 0.70$ ) during passive recovery were unchanged among control and ASA

treatments. Acute ASA ingestion did not affect thermoregulatory responses during submaximal exercise or passive recovery under the conditions of this investigation.

**KEYWORDS:** aspirin, sweating, thermoregulation, vasodilation

## INTRODUCTION

Exercise performed in hot ambient conditions increases heat storage, leading to a rise in core and skin temperatures (36). Appropriate thermo-balance necessitates intact central and peripheral afferent signaling to the hypothalamus (4). Humans rely on increased skin blood flow (SkBF) and sweating to enhance heat dissipation (27). To maintain thermal homeostasis, interacting neural and local factors ensure tight control of the cutaneous circulation (31, 39). Once a relative core temperature threshold has been reached, noradrenergic tone is withdrawn to increase SkBF (33). As core temperature continues to rise active vasodilation and sweating responses are stimulated in an effort to alleviate a given thermal load (33). Due to the importance of body temperature the cutaneous microvasculature exhibits a degree of redundancy where nitric oxide synthase (21, 37) and cyclooxygenase (COX) (23) are considered essential for complete vasodilator expression. Thus, any pharmaceuticals that may interfere with such pathways could threaten homeostatic function by upsetting typical thermoregulatory responses (i.e., SkBF or sweating).

An example of one such drug is acetylsalicylic acid (ASA), commonly taken for its diverse therapeutic qualities including pain and fever relief. Due to its systemic effects, ASA has been the subject of physiological research for many years (12). While many cells express COX-1 enzyme activity, COX-2 is inducible and quickly up-regulated at sites of inflammation (25). Consequently, ASA acts as a nonspecific COX (-1 and -2) isoform inhibitor (35), and as such, suppresses central and peripheral signaling (2).

During heat stress, reflexive cutaneous vasodilation is mediated through the release of acetylcholine which also acts on muscarinic receptors to stimulate eccrine sweat glands (20). Accordingly, SkBF and sweating often share a temporal relationship (34). In fact, SkBF has been

shown to independently affect local sweat rate (40). Recently, it was determined that the onset and rise of sweating is dependent on increased SkBF during passive heat stress (38). Since short term ASA use (~7 days) has previously been shown to negatively affect SkBF responses during exercise (6), it is possible that acute ASA use may affect sweating indirectly through a modification of SkBF. Importantly, attenuation of either thermo-effector (e.g., SkBF and sweating) may accelerate heat storage during exercise, thereby exacerbating heat strain and increasing risk of heat illness.

Interestingly, research by Downey and Darling (11) observed a cooling trend following exercise in ASA-dosed groups. While counterintuitive, authors from that investigation stated that ASA dosing may have up-regulated vasomotor (i.e., SkBF) and sudomotor (i.e., sweating) function to facilitate heat loss during recovery. Taken together, these studies illustrate that evidence regarding the influence of ASA on temperature regulation remains equivocal. Therefore, it is unclear if acute ASA ingestion influences normal physiologic responses (i.e., SkBF or sweating) in young healthy participants during exercise in the heat.

The aim of the present study was to investigate the influence of an acute ASA dose on SkBF and sweating responses during submaximal exercise and recovery in the heat. Standard U.S. full-dose (325-mg) and low-dose (81-mg) ASA concentrations were selected to provide an ecologically valid approach to understanding this issue, as ASA administration in very high doses is not advised (35). As a result, there was a three-fold hypothesis: 1) ingestion of 325-mg would blunt the increase in SkBF during exercise in the heat to a greater extent than control and 81-mg trials; 2) 325-mg would interrupt local sweating responses as evidenced by a diminished slope (i.e., sensitivity) and peak output during exercise compared to control and 81-mg trials; and

3) 325-mg would alter SkBF and local sweating responses compared to control and 81-mg ASA trials by exhibiting a greater magnitude of change during passive recovery.

## **METHODS**

### **Ethical Approval**

This study was approved by the local Institutional Review Board at The University of Alabama. All participants were informed of the methods and risks before the study. Verbal and written consent was obtained from participants during each visit to the laboratory. All procedures conformed to the guidelines set forth by the Declaration of Helsinki.

### **Study Participants**

Seven healthy male participants (mean  $\pm$  SD;  $27 \pm 5$  yr) were recruited to participate in this study. A power analysis (29) revealed this sample size was adequate to detect a moderate effect size ( $d = 0.60$ ) (32) for peak local SR, assuming a power of  $\sim 0.85$ . Participants were free from cardiovascular and metabolic disease while maintaining moderately active lifestyles (1). At least 12 hours before each experimental session, participants abstained from alcohol and caffeine. Treatments were administered in a counter-balanced fashion to reduce any ordering effects. Individual testing times of day were kept consistent among trials to avoid body temperature variance due to circadian rhythms.

### **Instrumentation and Measurements**

On arrival to the laboratory, participants consented and completed a 24-hr history questionnaire to ensure adherence to the pre-trial instructions. Participants' urine specific gravity (USG) was measured via refractometry (SUR-NE, Atago Inc., Higham, MA). Participants with USG values  $\geq 1.020$  were given fluids to consume for a 30-min period then re-evaluated. If USG

values remained elevated, participants were rescheduled for another visit and tested. One participant was rescheduled due to inadequate USG level (e.g., >1.020). USG values  $\leq 1.020$  were considered adequately hydrated (9). Following hydration verification, ASA (e.g., 325-mg; 81-mg) or control (no ASA) treatments were administered in counterbalanced order along with a small snack (i.e., breakfast bar) according to individual testing progression. Following ASA ingestion, a 30-45 min period was given to allow ASA to reach peak plasma concentration before beginning the experimental protocol (30). During this period, participants had their standing height (SECA-213, Los Angeles, CA, US) and nude body mass measured (Tanita Corporation, Tokyo, Japan). Body fat percentage was estimated using the sum of three skinfolds (16) (Lange calipers, Beta Technology Inc., Cambridge, MD).

Next, participants inserted a rectal thermocouple (model RET-1, Physitemp, Clifton, NJ) 8-10 cm beyond the anal sphincter (24) to measure rectal temperature ( $T_{re}$ ). Six skin thermocouples were positioned on the upper back, chest, lower back, abdomen, anterior thigh, and calf to measure weighted mean whole-body skin temperature ( $\bar{T}_{sk}$ ) (39). Participants were also instrumented with a heart rate monitor (Polar, Stamford, CT). Mean body temperature ( $\bar{T}_b$ ) was calculated using weighted measures of  $T_{re}$  and  $\bar{T}_{sk}$  from the following equation:  $\bar{T}_b = [(0.87 \cdot T_{re}) + (0.13 \cdot \bar{T}_{sk})]$  (3). To maintain consistency between trials, all participants wore the same compression shorts and cotton T-shirt during each experimental session.

Laser-Doppler flowmetry (LDF) (MoorVMS-LDF2, Moor Instruments, Devon, UK) provided an index of SkBF with the probe positioned on the dorsal aspect of the forearm. Cutaneous vascular conductance (CVC) was calculated from arbitrary LDF units divided by mean arterial pressure (MAP). MAP was calculated as  $[(1/3) \text{ pulse pressure} + \text{diastolic pressure}]$ . Local sweat rate (SR) was continuously measured via capacitance hygrometry using a ventilated

capsule (3.98 cm<sup>2</sup>) placed near the LDF probe. Compressed nitrogen perfused through the capsule at a fixed rate of 300 mL/min. Measurements were taken from the antecubital fossa of the forearm relative to the LDF probe and ventilated capsule to consistently position for each trial.

## **Experimental Protocol**

Participants made three separate visits to the laboratory: control, 325-mg, and 81-mg ASA. Approximately 5-7 days separated control and ASA trials to enable adequate recovery. ASA trials were separated by a minimum of 10 days to ensure normal blood platelet re-synthesis (30). Following instrumentation, participants entered an environmentally controlled chamber set to 35 °C, 30-40% rH (33 °C WBGT) and positioned themselves on a recumbent cycle ergometer. All instruments were connected and values verified (lasting 5-7 min). Two small tables were located on either side of the participant and elevated to heart level. This set-up allowed participants to steady their torso and facilitated SkBF and local SR measurements throughout the duration of the experiment.

Participants began cycling at an intensity initially equivalent to 50% heart rate reserve:  $[(220 - \text{age}) - \text{resting heart rate}] \cdot (0.50) + (\text{resting heart rate})$  (1). Exercise duration was 45 min and immediately followed with a 15-min passive recovery period where participants remained stationary on the cycle ergometer. Heart rate was continuously measured and recorded every 10 min. Blood pressure was taken via manual auscultation of the brachial artery every 5 min. Upon the conclusion of the experimental protocol (60 min), instrumentation was removed and participants were assisted out of the environmental chamber. After towel drying, participants

measured post-exercise nude body mass, which was used to estimate total body sweat loss (22). Water was then provided *ad libitum*. All experimental trials were conducted in this manner.

## Statistical Analyses

Data were continuously sampled at a rate of 50 Hz using a data acquisition system (Biopac, MP150, Goleta, CA) and stored for analysis. Averaged CVC values during the final min of exercise (peak) were expressed as a percent change from baseline [ $((\text{peak CVC} - \text{baseline CVC}) / \text{baseline CVC}) \cdot 100$ ] (19). Similarly, average CVC values during the final min of recovery were compared to the final min of exercise (peak) and expressed as a percent change: [ $((\text{end of recovery CVC} - \text{peak CVC}) / \text{peak CVC}) \cdot 100$ ]. A one-way repeated measures analysis of variance (ANOVA) was used to compare CVC changes among control and ASA treatments. Local SR and thermal data (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) were averaged every 30 s during the experimental protocol (40). The onset of sweating was determined by an experienced investigator, blinded to the treatment, by visually examining local SR graphed as a function of time. The  $\bar{T}_b$  at the indicated time was then used to identify the onset of sweating. The slope of the local SR: $\bar{T}_b$  relationship was calculated using a linear regression of all data points between the onset of sweating and final min of exercise. Peak local SR, taken during the final min of exercise, was compared with the final min of recovery and expressed as a percent change. One-way repeated measures ANOVAs were used to compare respective analyses of the onset, slope, peak, and percent changes during recovery of local SR among control and ASA treatments. In instances where the assumption of sphericity was violated, subsequent df values for within-subject effects were adjusted using the Greenhouse-Geisser correction. Data were analyzed using SPSS v. 19

(IBM, Inc., New York, NY). Significance was accepted at  $p$  values  $\leq 0.05$ . All data are presented as means  $\pm$  SD.

## RESULTS

Participant characteristics are presented in Table 4.1. All 7 participants completed the trials. Before exercise,  $\bar{T}_b$  was similar between treatments and not affected by ASA use ( $p = 0.93$ ). Heart rate responses and thermal data (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) were similar among control and ASA treatments during the final min of exercise (Table 4.2). ASA ingestion did not produce a significant change in any dependent variable during exercise or recovery among trials. Following completion of the experimental protocol, total body sweat losses were not different among trials (control,  $910 \pm 200$  mL; 325-mg,  $960 \pm 350$  mL; 81-mg,  $910 \pm 270$  mL;  $p = 0.86$ ).

### Skin Blood Flow Responses

Baseline measures of CVC were similar among treatments (control,  $0.259 \pm 0.056$ ; 325-mg,  $0.244 \pm 0.067$ ; 81-mg,  $0.238 \pm 0.103$ ;  $p = 0.81$ ). As expected, during exercise body temperature increased, but the magnitude of CVC change (%) between baseline and the final min of exercise did not differ among control and ASA treatments (control,  $342 \pm 172\%$ ; 325-mg,  $319 \pm 122\%$ ; 81-mg,  $335 \pm 219\%$ ;  $p = 0.92$ ) (Figure 4.1). During the rest period, CVC responses fell similarly among all treatments ( $p = 0.93$ ) (Table 4.3).

### Local Sweating Responses

As shown in Table 4.4, acute ASA ingestion did not modify the  $\bar{T}_b$  threshold for sweating ( $p = 0.82$ ). Likewise, differences in SR sensitivity were not observed among treatments. Additionally, local SR during passive recovery was unaffected by ASA use and exhibited similar patterns compared to controls ( $p = 0.70$ ) (Table 4.3).

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Table 4.1. Participant characteristics ( $n = 7$ ).

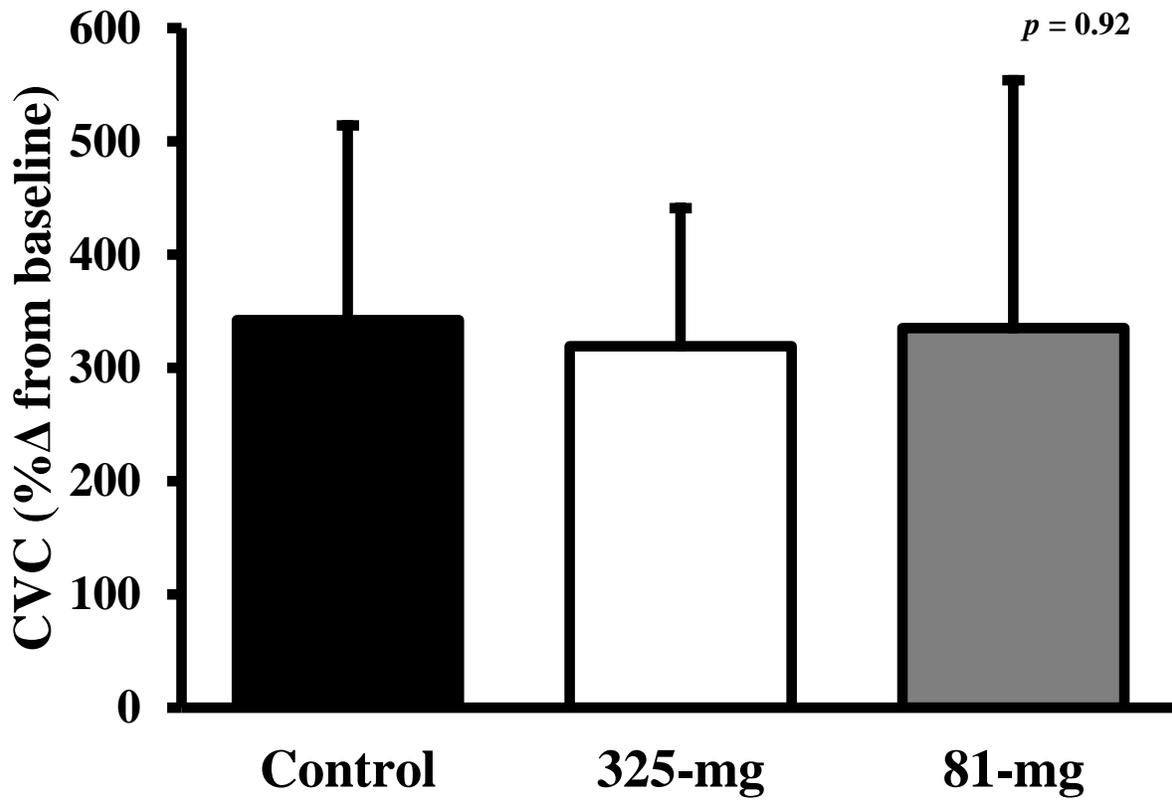
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Age (yr)	$27 \pm 5$
Body mass (kg)	$79.3 \pm 6.4$
Height (m)	$1.77 \pm 0.04$
Body fat (%)	$15.6 \pm 6.0$
Systolic BP (mm Hg)	$117 \pm 11$
Diastolic BP (mm Hg)	$78 \pm 5$
MAP (mm Hg)	$91 \pm 5$
Resting HR (beats·min <sup>-1</sup> )	$62 \pm 5$

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Values are presented as means  $\pm$  SD; BP, blood pressure; MAP, mean arterial pressure; HR, heart rate.

Figure 4.1. The percent change (% $\Delta$ ) of cutaneous vascular conductance (CVC) (means  $\pm$  SD) from baseline to the end of submaximal recumbent cycling in the heat during control and acetylsalicylic acid trials (325-mg; 81-mg), ( $n = 7$ ).



*P*-value indicates omnibus ANOVA.

Table 4.2. Heart rate and thermal responses to 45 min of submaximal recumbent cycling in the heat during control and acetylsalicylic acid trials (325-mg; 81-mg), ( $n = 7$ ).

	Control	325-mg	81-mg	<i>p</i> -value
Rectal temperature increase (°C)	+1.0 ± 0.4	+0.9 ± 0.3	+0.9 ± 0.1	0.42
Mean skin temperature increase (°C)	+1.0 ± 0.3	+0.9 ± 0.6	+1.1 ± 0.3	0.71
Mean body temperature increase (°C)	+1.0 ± 0.3	+0.9 ± 0.4	+0.9 ± 0.1	0.72
HR increase (beats·min <sup>-1</sup> )	+80 ± 13	+75 ± 12	+77 ± 9	0.42

Values are means ± SD; HR, heart rate. *P*-values indicate omnibus ANOVA.

Table 4.3. Percent (%) change of cutaneous vascular conductance (CVC) and local sweat rate (SR) during 15-min passive recovery after submaximal cycling in the heat among control and acetylsalicylic acid trials (325-mg; 81-mg), ( $n = 7$ ).

	Control	325-mg	81-mg	<i>p</i> -value
CVC (% $\Delta$ )	-28 $\pm$ 20	-29 $\pm$ 16	-30 $\pm$ 18	0.93
Local SR (% $\Delta$ )	-48 $\pm$ 26	-45 $\pm$ 21	-53 $\pm$ 19	0.70

Values are presented as means  $\pm$  SD; CVC, cutaneous vascular conductance; LDF, laser-Doppler flowmetry units; MAP, mean arterial pressure; local SR, local sweat rate. *P*-values indicate omnibus ANOVA.

Table 4.4. Local sweat responses during submaximal recumbent cycling in the heat among control and acetylsalicylic acid trials (325-mg; 81-mg), ( $n = 7$ ).

	Control	325-mg	81-mg	<i>p</i> -value
$\bar{T}_b$ at onset of sweating ( $^{\circ}\text{C}$ )	$36.8 \pm 0.4$	$36.8 \pm 0.3$	$36.8 \pm 0.3$	0.82
SR sensitivity [ $(\text{mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1})/^{\circ}\text{C}$ ]	$0.69 \pm 0.28$	$0.69 \pm 0.20$	$0.67 \pm 0.22$	0.94
Peak SR ( $\text{mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$ )	$0.95 \pm 0.24$	$0.89 \pm 0.18$	$0.93 \pm 0.26$	0.42

Values are presented as means  $\pm$  SD;  $T_b$ , mean body temperature; SR, sweat rate; SR sensitivity, expressed as a function of  $T_b$ . *P*-values indicate omnibus ANOVA.

## DISCUSSION

In the event that SkBF or sweating responses are weakened during heat stress, optimal physiologic function may become jeopardized. Therefore, the purpose of this investigation was to examine the influence of an acute ASA dose on thermoregulatory responses (i.e., SkBF and sweating) during submaximal exercise and recovery in the heat. Contrary to initial hypotheses and within the context of this investigation, the primary findings are that acute ASA ingestion does not alter thermo-effectors SkBF or local SR. These observations are likely due to similarities of thermal responses (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) during exercise and recovery in this cohort of young healthy participants.

In the present study, measures of  $\bar{T}_b$  were similar at rest ( $p = 0.93$ ) and remained similar at the end of exercise in all trials (Table 4.2). Despite discrepancies in administration and dose among previous investigations (6, 11, 14, 15), ASA ingestion does not appear to effect body temperature at rest in healthy participants (14, 15, 26). However, acetaminophen, also a COX-inhibitor has been shown to reduce core temperature by  $\sim 0.22$  °C in afebrile participants (18). It is possible that differences in mode of action between ASA and acetaminophen may account for such differences. Whereas ASA inhibits COX-1 and COX-2, acetaminophen preferentially inhibits COX-3 which is largely expressed in the cerebral cortex (8). As a result, this difference may help explain why acetaminophen exerts a potent influence over body temperature.

Previous work has also clarified a role for COX-2 specific inhibitors (e.g., Rofecoxib) with respect to temperature regulation during exercise (5). Notably, COX-2 inhibition contributed to a reduced core temperature and  $\bar{T}_b$  during 90-min of continuous aerobic exercise (5). Since exercise stimulates the release of prostaglandins and pro-inflammatory cytokines (e.g., interleukins) (28) it is possible that differences in thermal data (i.e.,  $\bar{T}_b$ ,  $T_{re}$ ,  $\bar{T}_{sk}$ ) from the present

study are a direct result of COX-2 inhibition. While ASA is a nonselective COX-inhibitor, it is 50- to 100-fold more potent at COX-1 inhibition compared to COX-2 (10). Given that COX-2 enzymes are inducible and quickly up-regulated at sites of inflammation (25), the reduced core and  $\bar{T}_b$  reported by Bradford et al. (5) may be due to exercise-induced inflammatory processes contributing to heat strain. However, no measures of cytokines were performed in the present study preventing evaluation of this hypothesis.

Recently, a 7 day loading period of low-dose (81-mg) ASA has been shown to result in a higher core temperature compared to control groups during exposure to hot ambient conditions, despite similar baseline core temperatures (6). Interestingly, core temperature remained elevated during a subsequent bout of exercise while researchers also noted a significant reduction in cutaneous vasodilation. It is possible that low-dose ASA use may have weakened the shear stress stimulus along the endothelial wall (6), and as such, disrupted normal cutaneous vasodilation. Accordingly, these results support the notion that a functional loss in SkBF may impede proper heat dissipation and accelerate heat storage.

In the present study, no differences in SkBF were noted at rest, exercise, or recovery which may be explained by two key elements. First, consecutive days (> 3 days) of low-dose administration has been shown to sufficiently inhibit systemic platelet activity thereby lowering blood viscosity and clotting potential (30). Whereas ASA irreversibly inhibits platelet COX activity, the modest acute doses used in the present study were unlikely to have produced a meaningful effect on platelet function (30). Secondly, previous investigations demonstrating a significant and consistent decrease in SkBF during heat stress have all been observed in healthy, middle-aged participants (6, 14, 15). Aging, in the absence of disease, has been shown to attenuate SkBF during heat stress due to an impairment in nitric oxide signaling (13). Since

complete vasodilator expression depends on nitric oxide synthase (21, 37) and COX-mediated (23) pathways, it is possible that the combined effects of aging and ASA use may contribute to the discrepancy in SkBF responses from the present study.

Given the similarities in SkBF and  $\bar{T}_b$  during each trial, it was unsurprising to note that no differences were apparent regarding local SR (Table 4.4). Initially, it was hypothesized that ASA use would influence local SR, as previous research has indicated a tight coupling of SkBF and eccrine sweating (34, 38). Peak SR was compared with SR at the end of recovery to provide an index of change in sudomotor function. However, local SR responses were similar among control and ASA trials (Table 4.3). Conversely, up-regulated sweating has been reported in groups taking high doses of sodium salicylate while exercising in hot ambient conditions (17). Researchers from that study postulated that the enhanced SR may have been due to the effect of exercise in the heat combined with very high doses (7.8 g) of sodium salicylate, creating a ‘hyper-metabolic state’ (17). Accordingly, within the framework of this study, eccrine sweating does not appear to be sensitive to ASA derived COX-inhibition.

## **Limitations**

Limitations arose in this study. Apart from ASA treatment, inter-individual differences in aerobic fitness may have impacted physiologic responses to the exercise protocol (7). While exercise intensity began at 50% heart rate reserve, the protocol used did not account for increased heart rate over time (i.e., cardiovascular drift). However, heart rate responses were similar among all conditions at the end of exercise (Table 4.2). Additionally, the identification of the onset of CVC was not possible due to artifact created during exercise. Despite efforts to restrict extraneous moment, normal oscillations during pedaling interfered with this calculation.

Despite similarities in onset of local SR (Table 4.4), it is possible that ASA may have delayed the onset of CVC. Moreover, the influence of ASA on exercise-induced pro-inflammatory cytokines (e.g., interleukins) and hormones (e.g., prostaglandins) which are known modulators of temperature regulation remains unclear.

## **Conclusions**

This study demonstrated that acute ASA ingestion, in contrast to acetaminophen and COX-2-specific inhibitors, did not produce any differences body temperature at rest or during exercise. Additionally, acute ASA ingestion did not affect SkBF and local SR during exercise or passive recovery. It is possible that such observations were due in part to the relatively modest dose of ASA used. Future work should seek to examine the effect of different COX-inhibitors on thermo-effectors in order to differentiate the influence of ASA (COX-1, -2) from acetaminophen (COX-3) and COX-2 specific inhibitors. Consequently, acute ASA doses of 325-mg and 81-mg do not appear to adversely affect temperature regulation during exercise or recovery in hot ambient conditions.

## References

1. American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. 8th ed. Baltimore: Lippincott, Williams, and Wilkins; 2010. 400 p.
2. Aronoff DM, Neilson EG. Antipyretics: mechanisms of action and clinical use in fever suppression. *Am J Med*. 2001; 111(4):304-15.
3. Baum E, Bruck K, Schwennicke HP. Adaptive modifications in the thermoregulatory system of long-distance runners. *J Appl Physiol*. 1976; 40(3):404-10.
4. Boulant JA. Neuronal basis of Hammel's model for set-point thermoregulation. *J Appl Physiol (1985)*. 2006; 100(4):1347-54.
5. Bradford CD, Cotter JD, Thorburn MS, Walker RJ, Gerrard DF. Exercise can be pyrogenic in humans. *Am J Physiol Regul Integr Comp Physiol*. 2007; 292(1):R143-9.
6. Bruning RS, Dahmus JD, Kenney WL, Alexander LM. Aspirin and clopidogrel alter core temperature and skin blood flow during heat stress. *Med Sci Sports Exerc*. 2013; 45(4):674-82.
7. Buono MJ, Sjolholm NT. Effect of physical training on peripheral sweat production. *J Appl Physiol (1985)*. 1988; 65(2):811-4.
8. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, Simmons DL. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. *Proc Natl Acad Sci U S A*. 2002; 99(21):13926-31.
9. Chevront S SM. Hydration assessment of athletes. *Sports Sci Exchange*. 2005; 18:1-6.
10. Cipollone F, Patrignani P, Greco A, Panara MR, Padovano R, Cuccurullo F, Patrono C, Rebuzzi AG, Liuzzo G, Quaranta G, Maseri A. Differential suppression of thromboxane biosynthesis by indobufen and aspirin in patients with unstable angina. *Circulation*. 1997; 96(4):1109-16.
11. Downey JA, Darling RC. Effect of salicylates on elevation of body temperature during exercise. *J Appl Physiol*. 1962; 17:323-5.
12. Fink GH. High Temperature after Labour and its Treatment with Aspirin. *Br Med J*. 1911; 1(2614):251-2.
13. Holowatz LA, Thompson-Torgerson CS, Kenney WL. Altered mechanisms of vasodilation in aged human skin. *Exerc Sport Sci Rev*. 2007; 35(3):119-25.
14. Holowatz LA, Kenney WL. Chronic low-dose aspirin therapy attenuates reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985)*. 2009; 106(2):500-5.

15. Holowatz LA, Jennings JD, Lang JA, Kenney WL. Systemic low-dose aspirin and clopidogrel independently attenuate reflex cutaneous vasodilation in middle-aged humans. *J Appl Physiol (1985)*. 2010; 108(6):1575-81.
16. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. *Br J Nutr*. 1978; 40(3):497-504.
17. Jacobson ED, Bass DE. Effects of Sodium Salicylate on Physiological Responses to Work in Heat. *J Appl Physiol*. 1964; 19:33-6.
18. Kasner SE, Wein T, Piriyaawat P, Villar-Cordova CE, Chalela JA, Krieger DW, Morgenstern LB, Kimmel SE, Grotta JC. Acetaminophen for altering body temperature in acute stroke: a randomized clinical trial. *Stroke*. 2002; 33(1):130-4.
19. Kellogg DL,Jr, Johnson JM, Kosiba WA. Control of internal temperature threshold for active cutaneous vasodilation by dynamic exercise. *J Appl Physiol (1985)*. 1991; 71(6):2476-82.
20. Kellogg DL,Jr, Pergola PE, Piest KL, Kosiba WA, Crandall CG, Grossmann M, Johnson JM. Cutaneous active vasodilation in humans is mediated by cholinergic nerve cotransmission. *Circ Res*. 1995; 77(6):1222-8.
21. Kellogg DL,Jr, Crandall CG, Liu Y, Charkoudian N, Johnson JM. Nitric oxide and cutaneous active vasodilation during heat stress in humans. *J Appl Physiol (1985)*. 1998; 85(3):824-9.
22. Maughan RJ, Shirreffs SM. Development of individual hydration strategies for athletes. *Int J Sport Nutr Exerc Metab*. 2008; 18(5):457-72.
23. McCord GR, Cracowski JL, Minson CT. Prostanoids contribute to cutaneous active vasodilation in humans. *Am J Physiol Regul Integr Comp Physiol*. 2006; 291(3):R596-602.
24. Mead J, Bonmarito CL. Reliability of rectal temperatures as an index of internal body temperature. *J Appl Physiol*. 1949; 2(2):97-109.
25. Mitchell JA, Warner TD. Cyclo-oxygenase-2: pharmacology, physiology, biochemistry and relevance to NSAID therapy. *Br J Pharmacol*. 1999; 128(6):1121-32.
26. Murray LK, Otterstetter R, Muller MD, Glickman EL. The effects of high- and low-dose aspirin on thermoregulation during and after acute cold exposure. *Wilderness Environ Med*. 2011; 22(4):321-5.
27. Nagashima K. Central mechanisms for thermoregulation in a hot environment. *Ind Health*. 2006; 44(3):359-67.
28. Nielsen AR, Pedersen BK. The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. *Appl Physiol Nutr Metab*. 2007; 32(5):833-9.

29. Park I SR. "Quick and easy" formulae for approximating statistical power in repeated measures ANOVA. *Meas Phys Educ Exerc Sci*. 1999; 3:249-70.
30. Patrono C, Collier B, Dalen JE, FitzGerald GA, Fuster V, Gent M, Hirsh J, Roth G. Platelet-active drugs : the relationships among dose, effectiveness, and side effects. *Chest*. 2001; 119(1 Suppl):39S-63S.
31. Pergola PE, Kellogg DL,Jr, Johnson JM, Kosiba WA, Solomon DE. Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. *Am J Physiol*. 1993; 265(3 Pt 2):H785-92.
32. Potvin PJ SR. Statistical power for the two-factor repeated measures ANOVA. *Behav Res Methods Instrum Compt*. 2000; 32(2):347-56.
33. Roddie IC, Shepherd JT, Whelan RF. The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *J Physiol*. 1957; 136(3):489-97.
34. Roddie IC, Shepherd JT, Whelan RF. The contribution of constrictor and dilator nerves to the skin vasodilatation during body heating. *J Physiol*. 1957; 136(3):489-97.
35. Rosenthal TC, Silverstein DA. Fever. What to do and what not to do. *Postgrad Med*. 1988; 83(8):75-84.
36. Rowell LB. Human cardiovascular adjustments to exercise and thermal stress. *Physiol Rev*. 1974; 54(1):75-159.
37. Shastry S, Dietz NM, Halliwill JR, Reed AS, Joyner MJ. Effects of nitric oxide synthase inhibition on cutaneous vasodilation during body heating in humans. *J Appl Physiol (1985)*. 1998; 85(3):830-4.
38. Smith CJ, Kenney WL, Alexander LM. Regional relation between skin blood flow and sweating to passive heating and local administration of acetylcholine in young, healthy humans. *Am J Physiol Regul Integr Comp Physiol*. 2013; 304(7):R566-73.
39. Taylor WF, Johnson JM, O'Leary D, Park MK. Effect of high local temperature on reflex cutaneous vasodilation. *J Appl Physiol Respir Environ Exerc Physiol*. 1984; 57(1):191-6.
40. Wingo JE, Low DA, Keller DM, Brothers RM, Shibasaki M, Crandall CG. Skin blood flow and local temperature independently modify sweat rate during passive heat stress in humans. *J Appl Physiol (1985)*. 2010; 109(5):1301-6.

## APPENDICES

Office for Research  
Institutional Review Board for the  
Protection of Human Subjects



May 1, 2013

Stephen Carter  
Department of Kinesiology  
College of Education  
The University of Alabama

Re: IRB Protocol # 13-007-ME  
"Influence of Aspirin on Skin Blood Flow and Local Sweat Rate  
During Passive Heat Stress"

Mr. Carter:

The University of Alabama IRB has received the revisions requested by the full board on 4/3/13. The board has reviewed the revisions and your protocol is now approved for a one-year period. Please be advised that your protocol will expire one year from the date of approval, 3/22/13.

If your research will continue beyond this date, complete the Renewal Application Form. If you need to modify the study, please submit the Modification of An Approved Protocol Form. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the Request for Study Closure Form.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Please use reproductions of the IRB approved stamped consent/assent forms to obtain consent from your participants.

Good luck with your research.



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Office for Research  
Institutional Review Board for the  
Protection of Human Subjects



June 27, 2013

Stephen Carter  
Department of Kinesiology  
College of Education  
The University of Alabama

Re: IRB Protocol # 13-016-ME  
"Influence of Aspirin on Skin Blood Flow and Local Sweat Rate  
During Submaximal Exercise"

Mr. Carter:

The University of Alabama IRB has received the revisions requested by the full board on 5/16/13. The board has reviewed the revisions and your protocol is now approved for a one-year period. Please be advised that your protocol will expire one year from the date of approval, 5/9/13.

If your research will continue beyond this date, complete the Renewal Application Form. If you need to modify the study, please submit the Modification of An Approved Protocol Form. Changes in this study cannot be initiated without IRB approval, except when necessary to eliminate apparent immediate hazards to participants. When the study closes, please complete the Request for Study Closure Form.

Should you need to submit any further correspondence regarding this proposal, please include the assigned IRB application number. Please use reproductions of the IRB approved stamped consent/assent forms to obtain consent from your participants.

Good luck with your research.



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