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Aspirin and Clopidogrel Alter Core Temperature and Skin Blood Flow during Heat Stress

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Abstract

Antithrombotic therapy with oral aspirin or clopidogrel (PlavixR) is associated with an attenuated skin vasodilator response and a greater rate of rise in core temperature in healthy, middle-aged individuals during passive heating in a water perfused suit.

Purpose—The present double-blind, crossover study examined the functional consequences of 7 days of low-dose aspirin (ASA, 81 mg/day) vs. clopidogrel (CLO, 75 mg/day) treatment in 14 healthy, middle-aged (50–65 yrs) men and women during passive heating in air (40 min at 30°C, 40% rh) followed by exercise (60% $\dot{V}O_{2peak}$).

Methods—Oral temperature (T_{or}) was measured in the antechamber ($23.0 \pm 0.1^\circ\text{C}$) before entering a warm environmental chamber. After 40 minutes of rest subjects cycled on a recumbent cycle ergometer for up to 120 minutes. Esophageal temperature (T_{es}) and laser Doppler flux were measured continuously, and the latter was normalized to maximal cutaneous vascular conductance (%CVC_{max}).

Results—Prior to entry into the environmental chamber there were no differences in T_{or} among treatments; however, after 40 minutes of rest in the heat, T_{es} was significantly higher for ASA and CLO vs. placebo ($37.2 \pm 0.1^\circ\text{C}$, $37.3 \pm 0.1^\circ\text{C}$, vs. $37.0 \pm 0.1^\circ\text{C}$, both $P < 0.001$), a difference that persisted throughout exercise ($P < 0.001$ vs. placebo). The mean body temperature thresholds for the onset of cutaneous vasodilation were shifted to the right for both ASA and CLO during exercise ($P < 0.05$).

Conclusion—ASA and CLO resulted in elevated core temperatures during passive heat stress and shifted the onset of peripheral thermoeffector mechanisms toward higher body temperatures during exercise heat stress.

Keywords

acetylsalicylic acid; PlavixR; esophageal temperature; laser Doppler flowmetry

INTRODUCTION

Primary human aging results in a diminished ability to vasodilate cutaneous arterioles in response to increasing ambient and/or body core temperatures (23, 25). Reflex cutaneous vasodilation is mediated in part by an unknown cholinergic cotransmitter (22) and is

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dependent on nitric oxide (NO) synthase (21, 36) and cyclooxygenase (COX)–dependent signaling (27) for full expression. Age-related impairments in reflex cutaneous vasodilation result from reduced nitric oxide (NO) and cotransmitter-mediated vasodilation and a shift from local vascular cyclooxygenase (COX)-derived vasodilators to vasoconstrictors (16, 17).

Aging is also associated with an increase in cardiovascular disease and in the use of cardiovascular medications, including systemic platelet inhibitors for both primary and secondary prevention of thromboembolic disease (7, 34). Aspirin (ASA) is the most common over the counter anti-platelet medication and is recommended for all men over 45 and women over 50 years of age with one or more cardiovascular disease risk factors (30). Clopidogrel bisulfate (CLO, PlavixR; 75 mg) is the most widely prescribed anti-platelet medication for the secondary prevention of thromboembolic events, prescribed to over 115 million individuals(1). While ASA and CLO inhibit platelets via different mechanisms (COX-1 and P₂Y₁₂ ADP receptors, respectively), both treatments independently attenuate reflex cutaneous vasodilation in middle-aged men and women during passive-whole body heat stress (18).

In the previous study (18), we noted that treatment with either of these orally-administered platelet inhibitors decreased the time required to raise oral temperature (T_{or}) by 1°C during passive heating in a water-perfused suit. However, the significant thermal strain imposed by a water-perfused suit, where skin temperature is clamped and the core-to-skin gradient is reversed, does not accurately replicate a natural environmental heat stress, e.g. resting or exercising in a warm environment. The functional thermoregulatory and cardiovascular implications of ASA versus CLO therapies during environmental heat stress and/or exercise in a warm environment have not been systematically explored.

The purpose of this study was to examine the effect of two commonly used antiplatelet medications, ASA and CLO, on core temperature and thermoeffector mechanisms during whole body heat stress at rest and during exercise in a warm environment. We performed a randomized double-blinded, crossover study design after 7 days of platelet COX-1 inhibition with systemic low-dose ASA (81 mg), 7 days of specific platelet P₂Y₁₂ ADP-receptor inhibition with CLO (75 mg), and placebo. We hypothesized that use of the ASA and CLO would result in a greater rise in body core temperature during heat stress in ambient warm air versus placebo. We further hypothesized that reflex cutaneous vasodilation would be attenuated with ASA and CLO, resulting in a rightward shift in the skin blood flow:core temperature relation during exercise in the heat.

METHODS

This study was approved by the Institutional Review Board at The Pennsylvania State University and conformed by the guidelines set forth by the Declaration of Helsinki. Verbal and written consent were voluntarily obtained from each subject before participation. Participants aged 50–65 years were studied because systemic platelet inhibitor therapy is most commonly prescribed for this age cohort.

All subjects underwent a complete medical screening which included a resting electrocardiogram (ECG), physical examination, blood chemistry and lipid profile (Quest Diagnostics Nichol Institute, Chantilly, VA), and coagulation study (prothrombin time and international normalization ratio (INR); iSTAT[®]1 Analyzer, Abbott, Abbott Park, IL). A $\dot{V}O_{2peak}$ test (ParvoMedics, Salt Lake City, UT) with a 12-lead ECG was performed to ensure that subjects were free of any potential underlying cardiovascular disease. For the subsequent exercise studies, a $\dot{V}O_{2peak}$ test was performed on an electromagnetically braked

recumbent cycle ergometer (Lode Corival, Groningen, Netherlands) and an exercise workload (watts) that elicited 60% $\dot{V}O_{2peak}$ was determined. No subject was taking ASA or CLO therapy or any other medication prior to the study, including any anti-inflammatory medications, hormone replacement therapy, oral contraceptives, vitamins, or nutritional supplements. All subjects were normally active, non-diabetic, and non-smokers. Five of the women studied were postmenopausal and the remaining two were studied in the early follicular phase of the menstrual cycle. Participants were asked to refrain from drinking alcohol for at least 24 hours and to refrain from consuming caffeine-containing products for at least 12 hours before the experiment.

Blinded drug treatments

Non-identifiable capsules were compounded by a registered pharmacist (Boalsburg Apothecary) and were given to subjects to take once daily over 7–10 days. The study design was a randomized, double-blind, crossover study with 81 mg of ASA (BayerR), 75 mg of clopidogrel bisulphate (PlavixR, Bristol-Myers Squibb), and placebo (sucrose) treatments. The duration and dose of ASA and CLO used in the present study was chosen because ASA has shown to be efficacious for full platelet inhibition within 4–6 days (32), whereas CLO reaches a dose- and time-dependent inhibition of platelet aggregation (40–60%) after 3–5 days (33). In addition, the doses of ASA and CLO used in the present study are commonly used for primary and secondary prevention of thromboembolic events, respectively. Participants were instructed to take the experimental medications each morning with breakfast and their final pill was taken the morning of the experiment. The average circulation time of a platelet is 10 days (26); therefore, a minimum of a 3 week washout period separated each experimental trial to allow for full platelet recovery and removal of the experimental medications (32).

Subjects

14 middle-aged men and women (55 ± 1 years; 7 men and 7 women) underwent seated passive heat stress in a warm environmental chamber followed by cycle exercise in the same environmental conditions. Because 1 man did not finish all 3 trials, data were analyzed for 13 subjects.

Experimental Protocol

Urine samples were obtained and urine specific gravity (Refractometer, Atago A300CL) and osmolality were measured upon arrival to the laboratory to ensure euhydration. Subjects then entered a thermoneutral antechamber ($23.0 \pm 0.1^\circ\text{C}$) where they had their oral temperature (T_{or}) measured (WelchAllyn, Sure Temp Plus, Navan, Ireland) and a 20 gauge intravenous (IV) catheter placed in the antecubital vein for periodic blood sampling during the experiment. Participants were weighed prior to baseline measurements and post exercise to determine whole body sweat losses and calculate sweating rates.

Subjects then entered the environmental chamber ($T_{db} = 30^\circ\text{C}$; $T_{wb} = 22^\circ\text{C}$, 40% relative humidity) where they were instrumented (see instrumentation) and remained quietly seated on the recumbent cycle ergometer for 40 minutes. After 40 minutes of passive heat stress, subjects began the exercise portion of the protocol in the same environmental conditions. The subjects exercised at 60% of their $\dot{V}O_{2peak}$ for 2 hours or until they (1) requested to stop, (2) reached an esophageal temperature (T_{es}) of 39°C , or (3) reached 90% of their heart rate max. After cessation of exercise, subjects remained seated on the recumbent cycle ergometer for 30 additional minutes while a local heating protocol was performed to elicit maximum cutaneous vasodilation for normalization of laser-Doppler flowmetry data. During this time the chamber dry bulb temperature was decreased to $T_{db} = 23^\circ\text{C}$.

Instrumentation and measurements

Upon entering the environmental chamber, the participants were instrumented and then rested for 40 minutes on the recumbent cycle ergometer in the warm environment. A copper-constantan thermocouple sealed in an infant feeding tube was inserted through the naris a distance of $\frac{1}{4}$ of the subject's standing height to measure T_{es} at the level of the left atrium. Skin temperatures were measured using copper-constantan thermocouples at six sites: calf, thigh, abdomen, chest, back and upper arm, and an unweighted mean of these sites were calculated ($\bar{T}_{sk}$) (38). Mean body temperature ($\bar{T}_b$) was calculated as $\bar{T}_b = 0.9T_{es} + 0.1 \bar{T}_{sk}$ (37) and the rate of rise in T_{es} was calculated during exercise as the slope of T_{es} versus time for each experiment ($\Delta T_{es} / \Delta \text{time}$).

An index of skin blood flow was continuously measured using laser-Doppler flowmetry at two sites on the ventral surface of the right forearm. Laser Doppler probes were held in place by local heaters, which were maintained at 34°C to locally clamp skin temperature to ensure changes in skin blood flow were of reflex origin (MoorLAB, Temperature Monitor SHO2, Moor Instruments, Devon, UK). Arterial blood pressure was measured by manual auscultation every 5 minutes. Cutaneous vascular conductance (CVC) was calculated as laser-Doppler flux divided by mean arterial pressure (MAP). In addition, beat-by-beat blood pressure and heart rate (HR) were measured continuously (FinapresR BP Monitor 2300, Ohmeda, Louisville, CO).

Forearm blood flow (FBF) was measured during seated rest on the cycle ergometer and every 5 minutes during exercise by venous occlusion plethysmography using a mercury-insilastic strain gauge (EC6 Plethysmograph, Hokanson, Bellevue, WA) while blood flow to the hand was occluded (40). Forearm vascular conductance was calculated as FBF/MAP.

Thermal sensation was obtained during seated rest on the cycle ergometer in the environmental chamber and every 5 minutes of exercise (1–8 scale) (41). Rating of perceived exertion (RPE) was obtained every 5 minutes during exercise (Borg RPE Scale, 6–20) (4).

Blood samples were drawn after 40 min of seated rest on the cycle ergometer, during the final minute of exercise, and following the recovery period. Samples were immediately analyzed for hematocrit (microhematocrit centrifugation) and hemoglobin concentration (Hemacue Hb 201+) and the percent change in plasma volume (ΔPV) was calculated from hematocrit and hemoglobin using the Dill and Costill method (10). All blood and urine samples were analyzed in triplicate.

Experimental timeline

The study ran over the course of 18 months with subjects entering at random times to minimize seasonal effects on thermoregulatory effector mechanisms. Time of day was standardized for each subject to prevent diurnal variations in body core temperature (2).

Data acquisition and analysis

Data were acquired using Windaq software and Dataq data-acquisition systems (Akron, OH). The data were collected at 40 Hz, digitized, recorded and stored on a personal computer for future analysis. T_{es} data were averaged over 1 min intervals every 5 min of exercise. CVC data were averaged over 3 min intervals every 5 min during seated rest and exercise. Absolute maximal CVC was calculated as the average of a 5 minute stable plateau in laser-Doppler flux after locally heating the skin to 43°C (~30–40 minutes of heating) and CVC was calculated and represented as a percentage of maximum ($\%CVC_{max}$). Slopes of the FBF data were calculated using the first derivative of the volume changes during each

venous occlusion period using Windaq advance codas analysis software. Forearm vascular conductance is reported in units of milliliters per 100 milliliters of forearm per minute per 100 mmHg ($\text{mL} \cdot 100 \text{ mL}^{-1} \cdot \text{min}^{-1} \cdot 100 \text{ mmHg}^{-1}$).

Statistical analyses

Separate two-way mixed model analysis of variance (ANOVA) with repeated measures were conducted to determine differences between trials for (1) the $\% \text{CVC}_{\text{max}}$, FVC, MAP, HR, $\bar{T}_{\text{sk}}$, T_{es} , and change in T_{es} responses versus time and (2) for the hematologic variables over the discrete sampling periods. Specific planned comparisons with Bonferroni corrections were performed when appropriate. The level of significance was set at $\alpha = 0.05$ and data are presented as mean $\pm$ SE.

RESULTS

Subject characteristics are presented in Table 1. All subjects were healthy and moderately physically active.

The effects of ASA vs. CLO relative to placebo on T_{es} after 40 minutes of passive heat stress in the environmental chamber are shown for each individual in Figure 1. Both ASA (1A) and CLO (1B) treatment resulted in a significantly higher T_{es} after seated passive heat exposure compared to the placebo trial (both $P < 0.05$). Prior to this heat exposure, before entering the environmental chamber, T_{or} obtained in the thermoneutral antechamber was not different between the groups ($P = 0.156$ main effect).

Figure 2 shows the mean core temperatures (T_{c}) responses during the time course of the experiment. Twenty five minutes before entering the environmental chamber T_{or} was measured in a thermoneutral antechamber. Upon entering the environmental chamber (time = 0), T_{es} responses were recorded for 35 minutes during seated rest (time = 5–40 minutes) and subsequent exercise in the heat (time = 40–95 minutes). For clarity, data are presented until 25% of subjects dropped out of exercise ($n = 4$), while all subjects are included in the recovery data (time = 100, 110, and 120 minutes). The elevation in T_{es} that occurred during passive heating with both drugs compared to placebo persisted throughout exercise heat stress ($P < 0.001$). There was no difference in the rate of rise in T_{es} during exercise between trials (Placebo: $0.05 \pm 0.01^{\circ}\text{C}$, ASA: $0.05 \pm 0.02^{\circ}\text{C}$, CLO: $0.05 \pm 0.01^{\circ}\text{C}$; $P = 0.883$). During recovery from exercise, T_{es} remained significantly elevated with CLO treatment ($P < 0.001$).

Figure 3 shows the mean $\% \text{CVC}_{\text{max}}$ (Figure 3A) and FVC responses (Figure 3B) after 35–40 minutes of seated rest followed by exercise in the heat (time 40–95 minutes). There were no differences between CLO treatment and placebo in either $\% \text{CVC}_{\text{max}}$ or FVC during passive heat exposure as a function of time ($P > 0.05$). In contrast, ASA treatment significantly attenuated these responses compared to both the CLO and placebo trials for both $\% \text{CVC}_{\text{max}}$ and FVC responses. Both ASA and CLO treatments resulted in a rightward shift of $\% \text{CVC}_{\text{max}}$: mean body temperature relation (Figure 4), such that skin blood flow was lower for a given mean body temperature. Mean body temperature thresholds for reflex vasodilation were shifted for both ASA and CLO treatments (both $37.3 \pm 0.1^{\circ}\text{C}$) compared to placebo trials ($37.1 \pm 0.1^{\circ}\text{C}$; $P < 0.05$) during exercise. Finally, there were no differences in absolute CVC_{max} (flux/MAP) among treatments (ASA: 1.8 ± 0.2 , CLO: 2.1 ± 0.3 , and placebo: 1.8 ± 0.3 flux/mmHg; $P > 0.05$).

Table 2 shows the cardiovascular and performance variables during passive and exercise heat stress. There were no differences in heart rate, T_{sk} , or subjective thermal sensation during passive warm air exposure prior to the start of exercise. MAP prior to exercise was slightly higher with ASA treatment vs. placebo ($P < 0.05$). There were no differences in the

reason for terminating exercise, exercise duration, the final exercise T_{es} , heart rate, MAP, or T_{sk} . Likewise, the change in plasma volume, whole body sweating rates, and thermal sensation ratings were similar among the trials.

DISCUSSION

The principal findings from the present study were that ASA and CLO treatment did not affect thermoneutral core temperature as measured orally, but resulted in an elevated core temperature when the subjects sat in warm ambient conditions for 40 minutes. After the passive heat exposure, the increase in core temperature with ASA and CLO persisted throughout exercise. Treatment with ASA, but not CLO, attenuated the skin blood flow response during exercise heat stress compared to placebo as a function of time. Finally, ASA and CLO treatment shifted the thresholds for reflex vasodilation during exercise after a passive heat exposure toward higher mean body (and core) temperatures, such that skin blood flow was lower for a given mean body temperature.

ASA, CLO and Passive Thermal Stress

We previously demonstrated that reflex cutaneous vasodilation during whole body heating with a water-perfused suit was attenuated in subjects taking ASA and CLO versus no drug (18, 19). In the previous study examining the neurovascular signaling mechanisms mediating the reduction in skin blood flow with these drugs, we observed that the time it took subjects to increase their body core temperature by 1.0°C was significantly reduced when they were taking ASA or CLO. The aim of the current study was to determine the potential thermoregulatory and cardiovascular consequences of ASA and CLO therapy in natural warm air environment during both passive heat stress and exercise. Our present findings demonstrate that even mild heat exposure results in an increased resting core temperature when subjects are taking either ASA or CLO, as both of these treatments resulted in a higher body core temperature after 40 minutes of passive heat stress in a compensable warm air environment compared to placebo trials.

We previously reported that ASA and CLO consistently resulted in attenuated reflex cutaneous vasodilation during hyperthermia using the water-perfused suit versus no drug (18). In the present study, after 40 minutes of passive heating in warm air there were no differences in the $\%CVC_{max}$ or the FVC responses among treatments. Considering the core temperature increase after the 40 minutes of warm ambient air exposure in the present study, the threshold for reflex cutaneous vasodilation had not been reached. Therefore it is not surprising that we were unable to detect a difference in skin blood flow responses during rest with the mild thermal stress utilized in the present study. It would be necessary to increase the heating stimulus, like that achieved in a water perfused suit, to reach the threshold and observe any potential differences.

ASA, CLO and Exercise in the Heat

The ASA- and CLO-related elevation in core temperature that appeared by 40 min of passive heating persisted throughout exercise heat stress and into recovery. Further, there was a shift in the threshold for the onset of reflex cutaneous vasodilation toward higher body temperatures with both ASA and CLO. While there was no increase in the rate of rise in body core temperature during exercise, there was a rightward shift in the skin blood flow ($\%CVC_{max}$):mean body temperature relation. These data indicate that given the same environmental conditions, ASA and CLO alter thermoregulatory effector mechanisms such that skin blood flow is lower for a given mean body temperature. Plotted against time (Fig. 3), ASA treatment resulted in lower skin blood flow as demonstrated by both $\%CVC_{max}$ and FVC responses versus placebo, an effect not observed with CLO treatment. The $\%CVC_{max}$

and FVC data were similar in both direction and magnitude for each of the given treatments. Based on previous skin blood flow data using the water-perfused suit model, we originally hypothesized that reflex vasodilation during exercise would be attenuated with CLO treatment. However in the present study, because core temperatures were somewhat higher after passive heat stress with CLO, differences in skin blood flow as a function of time are not different from placebo.

While we observed differences in skin blood flow with ASA and CLO in the present and previous studies (18, 19), there appeared to be no differences in fluid balance and evaporative heat loss among trials, as evidenced by similar changes in plasma volume and absolute whole body sweating rates. Although it is unlikely that ASA or CLO treatments affected initial absolute plasma volume, we did not measure total plasma volume and are therefore relying on the relative change in plasma volume. Further studies using thermal modeling along with highly sensitive indirect calorimetric measurements are needed to more precisely measure changes in dry heat loss mechanisms while undergoing ASA and CLO therapy and the potential thermoregulatory consequences that may occur in more severe environmental temperatures like summer heat waves or saunas where the evaporation of sweat is limited.

Based on the present data, the mechanisms underlying the effect of these drugs on thermoregulation remain speculative. However, the consistency of findings across the present and previous studies (18) using different modes of passive heating demonstrate altered mechanisms of temperature regulation and/or thermal balance with ASA and CLO during passive heat stress. A similar pattern of altered thermoregulatory responses is observed during the luteal phase of the menstrual cycle or with synthetic progesterone administration (6, 8, 9). Because progesterone shifts the threshold for reflex vasodilation to higher mean body temperatures but does not alter the sensitivity of the response, this has been interpreted as an alteration in central thermoregulatory control, i.e. shifting the “set point” for activation of peripheral vasodilator skin sympathetic nerve activity (29). Our data suggest that ASA and CLO may alter central hypothalamic thermoregulatory control, despite evidence for minimal transfer of CLO across the blood brain barrier in tissue distribution studies of CLO in rat models (15). In addition to the direct drug actions of these antithrombotic drugs across the blood brain barrier, the central resetting for the onset of active vasodilation while undergoing ASA or CLO therapy could be mediated by altered neural afferent nerve signaling to the hypothalamus (5). However, little is known about the afferent control of skin blood flow and much research in this area remains to be done.

In addition to central alterations in the regulation of body temperature, ASA and CLO could be exerting their effects peripherally through neural or humoral signaling to the cutaneous microvasculature. One putative mechanism for the reduction in skin blood flow with ASA and CLO is through their ability to reduce platelet activation. ASA and CLO independently attenuate platelet activation for the life of the platelet (~10 days). Specifically, ASA acetylates COX-1 in the portal circulation thereby inhibiting COX-mediated PGH_2 and thromboxane synthesis (31) while CLO is metabolized in the liver and inhibits P_2Y_{12} ADP platelet surface receptors (32). During hyperthermia platelets may be activated neurogenically through sensory nerves or platelet vessel wall interactions causing the release of platelet derived vasodilators such as 5-hydroxytryptamine (5-HT) and adenosine diphosphate (ADP) from platelet dense core granules (18). The release of platelet-derived vasodilators may elicit endothelium-dependent vasodilations in a similar fashion as acetylcholine. Supporting peripheral neurogenic platelet vessel wall interactions, the axon reflex mediated neurogenic inflammation created by applying an anodal current to the skin is reduced with platelet COX-1 inhibition (35).

Other putative means of altering skin blood flow with ASA or CLO may be via altering direct cytokine and prostaglandin (ASA) signaling across the blood brain barrier or through attenuation of local production of the latter pyrogenic molecules. In the present study, 81 mg of ASA was chosen because it does not reach the vascular endothelium in significant concentrations or for an adequate period of time to fully inhibit vascular COX-1, like that of higher dose ASA regimens (600 mg), limiting COX-1 inhibition to the platelet (31). In a previous study, localized vascular nonspecific COX inhibition with ketorolac did not alter cutaneous vasodilation during whole body heating in a water perfused suit, suggesting that local vascular COX was not involved in the attenuated reflex vasodilation observed with ASA therapy (17).

ASA may alter central or peripheral thermoregulatory mechanisms by increasing systemic exposure to low concentrations of its active metabolite salicylate. Bass et al. demonstrated that oral treatment with much higher doses of sodium salicylate increased sweating rate in a compensable environment and tended to increase rectal temperature (T_{re}) in an uncompensable environment ($P < 0.07$) (20). Furthermore, salicylate attenuated the decrease in T_{re} with heat acclimation (3). However, due to the low concentration of salicylate in the dose of ASA used in the present study, the direct action of salicylate on thermoregulatory effector mechanisms is likely minimal.

There have been a few other studies that have examined the effects of higher doses of ASA on body temperature during exercise that have found no effect on body temperature in thermoneutral (11) and hot environments (13). In the present study body temperature was elevated after 40 minutes of passive heat exposure resulting in the maintenance of higher body temperatures throughout exercise. The aforementioned studies used up to 60 fold higher concentrations of ASA over a shorter duration which alters the mechanism of action of ASA through inhibition of both platelet and vascular endothelial COX. In addition, the environmental conditions, exercise modality, and means of assessing core temperature were different. In the present study, we measured esophageal temperature, which is considered to be the gold standard for assessing core temperature and has a high degree of sensitivity to measure small changes in the temperature of the blood that is perfusing the hypothalamic thermoregulatory control centers. These other studies used rectal (11) and tympanic temperatures (13), which are known to be influenced by lower body exercise, and have a significantly great lag time and variability (tympanic temperature).

Other anti-inflammatory drugs that have minimal antiplatelet effects, but alter prostaglandin production, have been used to study temperature regulation during exercise and whole body heat stress. Rofecoxib, a specific COX-2 inhibitor, *reduced* rectal and body temperature during treadmill exercise in a warm environment (5), whereas acute ibuprofen, a reversible non-selective COX 1 and 2 inhibitor (14) did not alter the onset of reflex vasodilation during whole body heat stress in a water-perfused suit (9). Similarly, these studies used different anti-inflammatory drugs that affect prostaglandin synthesis with a different mechanism of action, likely resulting in the disparate results. Further studies are needed to determine the effects of various dosages of anti-inflammatory medications on the control of body temperature.

Within our group of 13 participants, we detected greater variation in the CLO temperature data compared to ASA (Fig. 1). Both of these medications have large inter- and intra-individual variability on their effectiveness as platelet inhibitors (12, 28), and potential alterations in drug metabolism may have accounted for intra-individual variability. For example, CLO metabolism between individuals is variable due to genetic variations in the cytochrome P450 enzymes which metabolize the pro-drug clopidogrel to its active metabolite, and can also be affected by other nutritional factors that can either inhibit or

induce drug metabolism by cytochrome P450 enzymes (24, 39). In the present study subjects were instructed to maintain their normal diet and avoid the most common nutritional inhibitors of the cytochrome P450 enzymes including grapefruit juice. Within our subject group there were some individuals who exhibited more dramatic attenuation in %CVC_{max} and also had an increased rate of rise in T_{es} during exposure to mild thermal stress with CLO therapy, while others were relatively non-responsive. Based on our power calculations a sample size of 10 subjects was needed to observe a meaningful physiological difference in skin blood flow (~12% CVC_{max} difference). Due to the large number of patients using CLO, a larger scale study may result in a more marked effect of this drug on thermoregulatory outcomes during exercise in the heat.

Limitations

Before participants entered the heated environmental chamber T_{or} was carefully measured in a thermoneutral antechamber and was not significantly different among trials. Once the participant entered the environmental chamber they were instrumented with an esophageal probe and T_{es} was measured. During esophageal probe placement T_{es} was already raised above placebo (Fig. 2). We have corroborating data from our lab using the water-perfused suit model to induce passive heat stress that demonstrates that there was no significant difference in baseline T_{or} between platelet inhibitor treatments when measured continuously throughout heating (unpublished data).

We intentionally tested healthy subjects who have no underlying cardiovascular disease and who did not take ASA or CLO as a logical extension from our original studies. Because these drugs are intended for the primary and secondary prevention of thromboembolic disease our results may be significantly different had we tested the thermoregulatory effects of these drugs in populations with cardiovascular disease. However, following up on our previous observations our aim in the current study was to examine the functional thermoregulatory and cardiovascular consequences of the two most commonly used antithrombotic medications regimens during a hyperthermic stress in a more natural heat stress environment. Even though healthy subjects were tested these data are relevant because many healthy individuals engage in prophylactic ASA therapy.

In conclusion, oral administration of ASA and CLO treatment resulted in a higher core temperature during passive exposure to warm ambient temperatures. This elevation in core temperature persisted throughout exercise heat stress and into recovery. Furthermore, both antithrombotic drugs resulted in a threshold shift for the onset of reflex cutaneous vasodilation. While the mechanisms underlying the increase in core temperature remain speculative, the consistency of findings across two studies with differing modes of heating provides substantial evidence for the effects of ASA and CLO on human temperature regulation. From a functional perspective, these data highlight the need for future work examining the effects of these commonly used antithrombotic regimens on thermoregulatory effector mechanisms during passive exposure to high heat and humidity such as saunas and/or hot tubs.

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Figure 1.

Individual differences in esophageal temperatures (T_{es}) between placebo and systemic drug treatments after 40 minutes of mild passive heat stress (30°C, 40% rh) in an environmental chamber. Mean responses $\pm$ SE for low-dose aspirin (Graph A), clopidogrel (Graph B), compared to placebo trials. Both low-dose aspirin and clopidogrel significantly increased T_{es} versus placebo. * Significant difference versus placebo ($P < 0.05$).

Figure 2.

Temperature profile $\pm$ SEM during thermoneutral rest (23°C), passive warm ambient air exposure (30°C, 40%rh), exercise (60% $\dot{V}O_{2\text{peak}}$ on a recumbent cycle ergometer) in a warm environment, and the recovery period after exercise (23°C) during systemic low-dose aspirin (ASA), clopidogrel (CLO), and placebo trials. In thermoneutral conditions prior to exercise, there was no significant difference in oral temperature (T_{or}) with ASA or CLO therapy versus placebo. ASA and CLO treatment resulted in higher esophageal temperatures (T_{es}) during passive heat stress (time 0–40 minutes) which persisted throughout exercise (time 40–90 minutes) in a warm environment. CLO therapy resulted in higher T_{es} during the recovery period following exercise versus ASA and placebo. Data are shown during exercise until 25% of subjects terminated exercise (n 4). *Significant difference of drug treatment versus placebo ($P < 0.001$). # Significant difference versus CLO ($P < 0.05$).

Figure 3. Indices of skin blood flow measured by (A) laser-Doppler flowmetry and (B) venous occlusion plethysmography after 40 minutes of resting warm air exposure (30°C, 40%rh) and exercise (60% $\dot{V}O_{2peak}$ on a recumbent cycle ergometer) in a warm environment. During resting warm air exposure there was no significant difference in cutaneous vascular conductance (%CVC_{max}) or forearm vascular conductance (FVC) with low dose aspirin (ASA) and clopidogrel (CLO) treatment versus placebo. After the initiation of exercise (40–90 minutes), ASA therapy resulted in lower %CVC_{max} and FVC responses versus CLO and placebo. Data are shown during exercise until 25% of subjects terminated exercise (n = 4). *Significantly different versus ASA ($P < 0.05$) (main effect for FVC data). †Significantly different versus placebo ($P < 0.05$).

Figure 4. Cutaneous vascular conductance $\pm$ SEM as a percentage of maximum ($\%CVC_{\text{max}}$) versus mean body temperature (T_b). Low dose aspirin (ASA) and clopidogrel (CLO) resulted in a rightward T_b threshold shift for the onset of reflex vasodilation ($P < 0.05$). Error bars were omitted for CLO trials for clarity.

Table 1**Subject Characteristics**

Sex (Men, Women)	7, 7
Age (years)	55 ± 1
Body Mass Index (kg·m ⁻²)	25.2 ± 0.7
Mean Arterial Pressure (mmHg)	90 ± 2
Total Cholesterol (mg·dl ⁻¹)	178 ± 9
High Density Lipoproteins (mg·dl ⁻¹)	60 ± 3
Low Density Lipoproteins (mg·dl ⁻¹)	101 ± 7
Fasting Blood Glucose, (mg·dl ⁻¹)	94 ± 2
Glycosylated Hemoglobin (HbA1c, %)	5.5 ± 0
$\dot{V}O_{2peak}$ (L · min ⁻¹)	2.32 ± 0.21
$\dot{V}O_{2peak}$ (L·kg ⁻¹ ·min ⁻¹)	29.5 ± 1.7

Values are mean ± SE.

Table 2

Cardiovascular and Performance. Variables in the Heat ($T_{db}=30^{\circ}\text{C}$; $T_{wb}=22^{\circ}\text{C}$, 40% relative humidity). End of exercise perception and performance variables.

After 40 Minutes of Passive Heating	Placebo	ASA	CLO
Heart Rate (beats $\cdot$ min $^{-1}$)	70 $\pm$ 2	69 $\pm$ 2	71 $\pm$ 2
Mean Arterial Pressure (mmHg)	85 $\pm$ 2	89 $\pm$ 1 *	86 $\pm$ 1
Mean Skin Temperature ($^{\circ}\text{C}$)	34.7 $\pm$ 0.1	34.5 $\pm$ 0.2	34.7 $\pm$ 0.2
Thermal Sensation (0–10 Scale)	4.2 $\pm$ 0.1	4.2 $\pm$ 0.1	4.5 $\pm$ 0.1
At the End of Exercise	Placebo	ASA	CLO
Reason for Terminating Exercise	7 VF, 4 HR limit, 2 T_{es} limit	7 VF, 4 HRlimit, 2 T_{es} limit	7 VF, 4HR limit, 1 T_{es} limit
Exercise Duration (minutes)	68 $\pm$ 6	71 $\pm$ 6	65 $\pm$ 7
Esophageal Temperature ($^{\circ}\text{C}$)	38.1 $\pm$ 0.1	38.3 $\pm$ 0.1	38.3 $\pm$ 0.1
Heart Rate (beats $\cdot$ min $^{-1}$)	143 $\pm$ 3	143 $\pm$ 4	144 $\pm$ 4
Mean Arterial Pressure (mmHg)	95 $\pm$ 3	97 $\pm$ 3	95 $\pm$ 2
Mean Skin Temperature ($^{\circ}\text{C}$)	35.0 $\pm$ 0.2	35.0 $\pm$ 0.2	35.1 $\pm$ 0.2
Change in Plasma Volume (%)	-10.5 $\pm$ 0.6	-11.4 $\pm$ 0.7	-10.4 $\pm$ 0.9
Sweating Rate (g $\cdot$ h $^{-1}$)	766 $\pm$ 157	767 $\pm$ 128	940 $\pm$ 202
Thermal Sensation (0–10 Scale)	6.9 $\pm$ 0.2	7.1 $\pm$ 0.2	7.1 $\pm$ 0.1
Borg Rating of Perceived Exertion	17 $\pm$ 1	17 $\pm$ 1	18 $\pm$ 1

Values are mean $\pm$ SE; VF, volitional fatigue; HR limit, heart rate limit (90% HR max); T_{es} limit, esophageal temperature limit (39°C);

* Significant difference versus placebo ($P<0.05$).