Local arginase inhibition and low-dose sodium nitroprusside administration do not modulate heat loss responses in young and older men during exercise.

Robert D. Meade[1], Naoto Fujii[1,2], Gregory W. McGarr[1], Lacy M. Alexander[3], Pierre Boulay[4], Ronald J. Sigal[1,5,6], Glen P. Kenny[1,6]

[1]Human and Environmental Physiology Research Unit, School of Human Kinetics, University of Ottawa, Ottawa, Ontario, Canada, [2]Faculty of Health and Sport Sciences, University of Tsukuba, Tsukuba City, Japan; [3]Department of Kinesiology, Noll Laboratory, Pennsylvania State University, University Park, Pennsylvania, USA; [4]Faculty of Physical Activity Sciences, University of Sherbrooke, Sherbrooke, Canada; [5]Departments of Medicine, Cardiac Sciences and Community Health Sciences, Faculties of Medicine and Kinesiology, University of Calgary, Calgary, Alberta, Canada, and [6]Clinical Epidemiology Program, Ottawa Hospital Research Institute, Ottawa, Ontario, Canada.

Running head: Arginase, nitric oxide and heat loss, exercise, passive heating

Author Contributions:

R.D.M., N.F. L.M.A. and G.P.K. conceived and designed experiments. R.D.M., N.F., and G.W.M. contributed to data collection and analysis. All authors interpreted the experimental results. R.D.M. drafted the manuscript. All authors edited and revised the manuscript. All authors approved the final version of the manuscript. Experiments took place at the Human and Environmental Physiology Research Unit located at the University of Ottawa.

Dr. Glen P. Kenny
University of Ottawa,
School of Human Kinetics,
125 University Private, Room 367
Montpetit Hall
Ottawa, Ontario, Canada
K1N 6N5
(613) 562-5800 ext. 4282
(613) 562-5149 (fax)
Email: gkenny@uottawa.ca

ABSTRACT

Age-related impairments in cutaneous vasodilation and sweating may result from increased arginase activity (which can attenuate endogenous NO production), and/or altered NO sensitivity of the thermoregulatory end-organs (i.e., cutaneous vasculature and sweat gland). We evaluated whether local arginase inhibition or low-dose sodium nitroprusside (SNP; NO donor) modulate cutaneous vascular conductance (CVC) and sweat rate (SR) in young (n=9, 23±3 years) and older (n=9, 66±6 years) men during an exercise-heat stress. We also assessed the influence of these treatments during whole-body passive heating in older men (n=7, 64±7 years). During two 30-min bouts of moderate-intensity cycling (Ex1 and Ex2) in the heat (35˚C), CVC and SR were measured at forearm skin sites perfused with: 1) lactated Ringer's (Control); 2) 5mM N^{ω}-hydroxy-nor-Arginine + 5mM S-(2-boronoethyl)-L-cysteine (Nor-NOHA+BEC, arginase-inhibited); or 3) 1μM SNP. The influence of these treatments on CVC and SR was also evaluated at passively-induced elevations in esophageal temperature (ΔT_{es}) equal to those in Ex1 (0.6°C) and Ex2 (0.8°C). In both age-groups, CVC and SR were similar to Control at Nor-NOHA+BEC and SNP during exercise (all $P \geq 0.10$). During passive heating however, CVC was augmented compared to Control by Nor-NOHA+BEC at ΔT_{es} of 0.6°C (23±8%CVC_{max}; $P=0.04$) and 0.8°C (20±7%CVC_{max}; $P=0.04$). By contrast, no effect of SNP on CVC was seen (both $P \geq 0.66$) and SR was not influenced by any treatment during passive heating ($P=0.89$). Thus, neither arginase inhibition nor low-dose SNP modulate CVC or SR during moderate exercise in the heat, but arginase attenuates CVC in older men during whole-body passive heat stress.

Key words: Aging, nitric oxide, heat stress, skin blood flow, sweating

NEW AND NOTEWORTHY

In the current study we demonstrate that neither local arginase inhibition nor low-dose sodium nitroprusside administration influence the forearm cutaneous vasodilatory and sweating responses in young or older men during an exercise-heat stress. Consistent with previous findings, however, we observed augmented cutaneous blood flow with arginase inhibition during whole-body passive heat stress. Thus, arginase differentially affects cutaneous vasodilation depending on the mode of heat stress but does not influence sweating during exercise or passive heating.

INTRODUCTION

Aging is associated with a decline in cutaneous vasodilatory (17-20, 23, 44, 45) and sweating (21, 22, 27-30, 44) responses to both exercise- and passively-induced heat stress, with attenuated whole-body heat dissipation during exercise evident in adults as young as 40 years of age (27). Contributing to these age-related impairments are alterations in factors affecting heat loss at the level of the end-organ (i.e., cutaneous vasculature and eccrine sweat gland). For instance, nitric oxide (NO) is required for full expression of cutaneous vasodilation and sweating during heat stress (exercise or passive heating) in the young (8, 9, 17-20, 34-38, 45, 49). In older adults however, its contribution to these heat loss responses is diminished (9, 11, 17-20, 28, 30, 45, 46).

Potentially underpinning the attenuated NO-dependent cutaneous vasodilation and sweating responses in older adults is elevated arginase activity in the skin. In comparison to NO synthase, arginase preferentially binds L-arginine, the common precursor for both enzymes. Thus, an age-related increase in arginase activity may reciprocally inhibit endogenous NO production due to substrate depletion (4, 50). During whole-body passive heating in older adults, local arginase inhibition has been shown to augment cutaneous vasodilation to a level comparable to their younger counterparts (19, 20, 45). Additionally, studies of eccrine sweat urea nitrogen (a primary end-product of arginase) suggest that arginase is also localized in the sweat gland (15) and increased urea in the sweat of older vs. younger adults (2) indicates up-regulated sweat gland arginase activity with aging. That said, the role of arginase in mediating cutaneous vasodilation and sweating during exercise has not been directly evaluated,

and it is known that end-organ modulators of heat loss during whole-body passive heating are not always consistent with those during exercise (8, 33).

In addition to up-regulated arginase activity, changes in the sensitivity of the thermoregulatory end-organs to NO may play a role in the age-related reductions in NO-dependent heat loss. In response to a NO donor, attenuated cutaneous vasodilation has been observed in older relative to younger adults (18); however, others have found no age-related differences in cutaneous vasodilation during exogenous NO administration (10). Regardless, it is unknown whether the sensitivity of the cutaneous vasculature and eccrine sweat gland to NO is altered in older adults during exercise.

Restoring endogenous NO synthesis through arginase inhibition or providing exogenous NO might alter thermoregulatory responses during exercise heat stress in older adults. Therefore, the purpose of this study was to assess the independent influences of local arginase inhibition and exogenous NO administration on cutaneous vasodilation and sweating in older men during exercise in the heat (35˚C). It was hypothesized that local arginase inhibition to improve endogenous NO production via NO-synthase or exogenous NO administration through low-dose sodium nitroprusside (SNP; a NO donor) would augment cutaneous vasodilation and sweating in older but not younger men. Furthermore, given that it has been repeatedly demonstrated that the mechanisms regulating these heat loss responses can differ between exercise- and passively-induced heat stress, we also evaluated the influence of arginase inhibition and low-dose sodium nitroprusside administration on cutaneous vasodilation and sweating in older men during whole-body passive hyperthermia.

MATERIALS AND METHODS

Ethical approval

This study was in accordance with the *Declaration of Helsinki* and was approved by the University of Ottawa Health Sciences and Science Research Ethics Board. Prior to their participation in the study, informed consent was obtained from all volunteers.

Participants

A total of nine young and sixteen older men participated in the study (see results section for physical characteristics). Of these individuals, all of the young and nine of the older men completed an exercise-heat stress test and the remaining seven older men participated in the whole-body passive heating protocol. All participants were healthy (i.e., no history of cardiovascular, metabolic or respiratory disease and not currently taking medication related to these conditions) and habitually active (i.e., performing ≥30-min of structured physical activity a minimum of 2 times per week) as determined by a standardized questionnaire (26). Men were chosen given the potential modulation in the mechanisms underpinning heat loss associated with fluctuations in female sex hormone levels (i.e., estrogen and progesterone) (5, 6). Furthermore, women exhibit an altered capacity to dissipate heat (for a given level of heat stress) independent of sex-related differences in body morphology and aerobic capacity in comparison to males (12-14).

146 **Experimental Design**

147 Each participant completed one preliminary and one experimental session
148 (exercise- or passively-induced heat stress). Prior to the start of each session,
149 participants were instructed to refrain from alcohol, caffeine and/or strenuous physical
150 activity for a minimum of 24 hours. Furthermore, participants were instructed to drink
151 500 mL of water the night before as well as ~2 hours prior to each session to ensure
152 adequate hydration. During the preliminary session, body height, mass and density
153 were measured. For those participating in the exercise-heat stress protocol, peak rate of
154 oxygen uptake (VO_{2peak}) was also determined. Body height and mass were measured
155 with a stadiometer (Detecto, model 2391, Webb City, MO, USA), and a digital high-
156 performance weighing terminal (model CBU150X, Mettler Toledo Inc., Mississauga, ON,
157 CAN), respectively. Body surface area was calculated from the measurements of body
158 height and mass (7). Body density was measured using the hydrostatic weighing
159 technique and used to calculate body fat % (43). VO_{2peak} was assessed using an
160 automated indirect calorimetry system (MCD Medgraphics Ultima Series; MGC
161 Diagnostics, MN, USA) during a progressive incremental cycling protocol on a semi-
162 recumbent cycle ergometer (Corival, Lode B.V., Groningen, the Netherlands). The
163 protocol began with 1 min of cycling at a starting workload of 100 W for the younger
164 adults and 60 W for the older adults. Following the first minute, the workload was
165 increased by 20 $W \cdot min^{-1}$ until volitional fatigue and/or the participant could no longer
166 maintain a pedaling cadence >50 $revolutions \cdot min^{-1}$.

167 As mentioned above, the experimental session consisted of either an exercise-
168 induced heat stress or whole-body heating at rest. In both protocols participants

provided a urine sample for the measurement of urine specific gravity (Reichert TS 400 total solids refractometer, Reichert Inc., Depew, NY, USA) upon arrival to the laboratory, prior to a baseline measurement of body mass. Thereafter, four microdialysis fibers (Bioanalytical Systems Inc., West Lafayette, IN, USA) were inserted in the dermal layer of the dorsal forearm skin while participants rested in an upright-seated position in a thermoneutral (~24°C) experimental room. Fibers were inserted under aseptic conditions by advancing a 25-gauge needle 20-25 mm. The needle was withdrawn after the fiber passed through the lumen, leaving a 10 mm dialysis membrane within the dermal layer. Each fiber was secured to the skin using surgical tape and separated by ~2-4 cm. All fibers were then perfused with lactated Ringer's solution for ~30 min at a rate of 4 $\mu L \cdot min^{-1}$ via a micro-perfusion pump (model 400, CMA, Microdialysis, Solna, Sweden). Each skin site was instrumented for the measurement of local cutaneous blood flow and sweat rate.

Exercise-heat stress

In the exercise-heat stress protocol, participants entered a thermally controlled chamber regulated to 35˚C and 20% relative humidity following microdialysis fiber insertion. For ~60 min, participants were seated on a semi-recumbent cycle ergometer while the four microdialysis fibers were perfused at a rate of $4 \mu L \cdot min^{-1}$ via a microinfusion pump (Model 400, CMA Microdialysis, Solna, Sweden) with either 1) lactated Ringer's (Control), 2) 10 mM N^G-nitro-L-arginine methyl ester (Sigma-Aldrich, St Louis, MO, USA), to inhibit NO synthase activity (L-NAME), 3) 5 mM N^{ω}-hydroxy-nor-Arginine (Nor-NOHA; Sigma-Aldrich) + 5 mM S-(2-boronoethyl)-L-cysteine (BEC;

Sigma-Aldrich) arginase inhibitors, to enhance endogenous NO synthesis (Nor-NOHA + BEC), or 4) 1 μM SNP (Sigma-Aldrich), an exogenous NO donor (low-dose SNP). The concentration of each drug was based on previous work (8, 9, 11, 17-20, 37, 38, 46).

Following the ~60 min perfusion period, 10 min of baseline data collection ensued followed by two successive 30 min bouts of semi-recumbent cycling at a metabolic heat production of 400 W. Exercise was performed at a fixed rate of heat production to ensure similar drives for whole-body heat loss between groups (24, 39). Each exercise bout was followed by 20 min of recovery. An intermittent exercise protocol was employed given the potential influence of intermittent exercise bouts on the mechanisms underpinning the heat loss responses. Specifically, recovery from exercise is associated with an attenuation of cutaneous vasodilation and sweating to near baseline levels, followed by a rapid increase in these responses upon initiation of a subsequent exercise bout (19). Following the second recovery, each microdialysis fiber was perfused with 50 mM SNP at a rate of 6 $\mu L \cdot min^{-1}$ to evaluate maximum cutaneous blood flow. A measurement of blood pressure was taken once a stable plateau in cutaneous blood flow was achieved (~20-30 min) for the determination of maximal cutaneous vascular conductance (CVC_{max}). The remaining instrumentation was then removed and a final nude body mass was obtained.

Passive heating protocol

Following completion of the exercise-heat stress study, a whole-body passive heating sub-study was performed for the purpose of comparing the effects of arginase inhibition and low-dose SNP administration on heat loss responses between active

(exercise) and passively-induced heat stress. Importantly, this protocol also allowed us to compare our findings to previous work, in which augmentation of CVC in response to arginase inhibition was observed in older adults during whole-body passive heating (19, 20). In this protocol, participants remained in the thermoneutral room (~24°C) in the semi-recumbent position following fiber insertion while wearing a tube-lined water-perfusion suit. The microdialysis fibers were perfused for ~60 min with the same agents as in the exercise-heat stress protocol (i.e., Ringer, L-NAME, Nor-NOHA+BEC and low-dose SNP), while suit temperature was maintained at 34°C. The temperature of water perfusing the suit was then increased to 49.5°C to initiate whole-body heating. Once esophageal temperature had increased by 0.8°C (similar to the peak increase in esophageal temperature during exercise-heat stress), the suit water temperature was reduced to 40°C and CVC_{max} was determined as described in the preceding section.

Measurements

Esophageal temperature was measured in both protocols with a pediatric thermocouple probe ~2 mm in diameter (Mon-a-therm, Mallinckrodt Medical, St. Louis, MO) inserted through the nose and advanced 40 cm. In one older participant (exercise-heat stress), tympanic temperature was taken due to intolerance of the esophageal probe. Mean skin temperature was calculated using 4 skin sites weighted to the regional proportions: 30% chest, 30% bicep, 20% quad and 20% calf (42), measured during the exercise protocol. Due to technical difficulties, mean skin temperature was not reported for one young participant. Temperature data were collected with a data acquisition

module at a 15 s sampling rate and displayed and recorded with LabVIEW software (version 7.0; National Instruments, TX, USA).

During both exercise-heat stress and whole-body passive heating systolic and diastolic pressures were measured by manual auscultation every 5 min using a validated mercury column sphygmomanometer (Baumanometer Standby Model, WA Baum Co, Copiague, NY, USA). Mean arterial pressure was calculated as diastolic pressure plus one-third the difference between systolic and diastolic pressure (i.e., pulse pressure). Additionally, heart rate data were recorded and stored every 5 s during exercise using a Polar coded WearLink and transmitter, Polar RS400 interface, and Polar Trainer 5 software (Polar Electro, Oy, Kempele, Finland).

Cutaneous red blood cell flux, an index of cutaneous blood flow expressed in perfusion units, was locally measured at a sampling rate of 32 Hz via laser-Doppler flowmetry (PeriFlux system 5000, Perimed, Stockholm, Sweden) using integrated laser-Doppler flowmetry probes with a 7-laser array (Model 413, Perimed). Each probe was placed over the center of each microdialysis fiber and housed within a specialized ventilated sweat capsule (see below). Cutaneous vascular conductance (CVC) was calculated as cutaneous red blood cell flux divided by mean arterial pressure (perfusion units·mm Hg^{-1}) and presented as a percentage of CVC_{max}.

Specialized ventilated capsules, designed to cover the entire area of skin perfused by the microdialysis fiber (40), were used for the measurement of local sweat rate. Each capsule was placed directly over the center of each fiber membrane and affixed to the skin using double-sided adhesive and topical skin glue (Collodion HV, Mavidon Medical products, Lake Worth, FL, USA). Dry compressed air was passed

through each capsule at a constant flow rate (0.4 $L \cdot min^{-1}$) while capacitance hygrometry (Model HMT333, Vaisala, Helsinki, Finland) was used to measure the water content of the effluent air. Connections between the gas tanks and sweat capsules, and between the sweat capsules and hygrometers, comprised long vinyl tubes to allow for gas temperature to equilibrate with ambient temperature. Local sweat rate was calculated every 5 s using the water content of the effluent air times the flow rate and normalized to capsule surface area ($mg \cdot min^{-1} \cdot cm^{-2}$).

During exercise, endogenous heat production was then estimated as metabolic rate minus external work (24). The oxygen and carbon dioxide concentrations of expired air were analyzed using electrochemical gas analyzers (AMETEK model S-3A/1 and CD3A, Applied electrochemistry, Pittsburg, PA, USA), which were calibrated using a gas mixture of known concentrations. A 3 L syringe was used to calibrate the turbine ventilometer. Participants wore a full-face mask (Model 7600 V2, Hans-Rudolf, Kansas City, MO, USA) attached to a 2-way T-shape non-rebreathing valve (Model 2700, Hans-Rudolf). Oxygen uptake and respiratory exchange ratio were obtained as 30 s averages and subsequently used to calculate metabolic rate (41).

Urine specific gravity (an index of body fluid status) was evaluated using a handheld total solids refractometer (Model TS400, Reichter Inc., Depew, NY, USA) prior to each experimental protocol. Participants with a urine specific gravity of <1.020 were deemed adequately hydrated to begin the experimental session (3).

281 **Data analysis**

282 For the exercise-heat stress protocol, local forearm CVC and sweat rate at each
283 treatment site as well as esophageal and skin temperatures (both absolute values and
284 elevations from baseline) and heart rate were presented as 5-min averages of data
285 recorded at the end of each of the following time periods: baseline, exercise 1 (25-30
286 min), recovery 1 (15-20 min), exercise 2 (25-30 min) and recovery 2 (15-20 min). Blood
287 pressure data were presented as an average of the two measurements taken over 10
288 min at the end of each time period. CVC_{max} was determined from averaged CVC data
289 over a minimum of 2 min once a plateau was attained during the maximal cutaneous
290 vasodilation procedure at the end of the protocol. In the passive heating protocol,
291 average CVC and sweat rate data were determined over the time-interval
292 corresponding to increases in esophageal temperature of 0.6°C (0.55-0.64°C) and
293 0.8°C (0.75-0.84°C) (similar to the increase in esophageal temperature noted in the
294 older adults during exercise 1 and 2 of the exercise-heat protocol, respectively).
295
296 **Statistical Analysis**

297 Local CVC and sweat rate were analyzed for the exercise-heat stress protocol
298 using a two-way repeated-measures analysis of variance (ANOVA) for both the young
299 and older men with the factors of time period (five levels: baseline, exercise 1, recovery
300 1, exercise 2 and recovery 2) and of treatment site (four levels: Control, L-NAME, Nor-
301 NOHA + BEC and low-dose SNP). A separate two-way mixed model ANOVA was
302 performed with the factors of time (five levels) and age (two levels: young and older) to
303 compare physiological responses between age-groups – namely, the heat loss

responses at the Control site (i.e., CVC and sweat rate) as well as body temperatures (i.e., esophageal and mean skin) and cardiovascular (i.e., mean arterial pressure and heart rate) variables. A two-way mixed-model ANOVA with the factors of treatment site (four levels) and age (two levels) was also used to evaluate local forearm CVC_{max} (expressed in perfusion units·mm Hg^{-1}) obtained during the maximal cutaneous vasodilation protocol. For the whole-body passive heating protocol, CVC and sweat rate were analyzed with a repeated-measures ANOVA with the factors of stage (two levels: 0.6°C and 0.8°C increase in esophageal temperature) and of treatment site (four levels). For the exercise-heat stress test, *post hoc* multiple comparisons were carried out using two-tailed Student's paired t-tests. Based on the previous observations of attenuated and augmented CVC with L-NAME and Nor-NOHA + BEC, respectively (19, 20), responses between treatment sites were compared using one-tailed t-tests in the passive heating protocol. CVC_{max} was evaluated with a one-way repeated measures ANOVA (factor of treatment site). The Holm-Bonferroni correction was employed to account for multiple comparisons. Differences in participant physical characteristics, urine specific gravity and the percent change in body mass between age-groups in the exercise-heat stress protocol were assessed with Student's independent sample t-tests (two-tailed). $P \leq 0.05$ was the level of significance. Unless otherwise mentioned, values were reported as mean ± 95 % confidence interval (standard error of the mean x 1.96).

RESULTS

Exercise-heat stress

Participant physical characteristics

Table 1 contains the mean ± standard deviation characteristics of the young and older participants. By design, age was greater in the older vs young men (P<0.01). No differences were noted for body height (*P=0.69*), mass (*P=0.30*) or surface area (*P=0.40*) but VO_{2peak} was lower (*P<0.01*) and body fat % was greater (*P<0.01*) in the older men in comparison to their younger counterparts.

Body fluid status

No differences in pre-session urine specific gravity were noted between the young (1.013 ± 0.005) and older (1.016 ± 0.006; *P=0.41*) men. Further, the change in body mass over the experimental protocol was similar between age-groups (young: -1.64 ± 0.28% vs older: -1.50 ± 0.28%; *P=0.49*).

Cutaneous vascular conductance

In the young men, local forearm CVC (main effect of time, *P<0.01*) was elevated during both exercise bouts at all treatment sites (all *P≤0.04*) except at L-NAME (*P=0.08*) during the first exercise bout. In contrast, CVC was similar to baseline values at each skin site during both recoveries (all *P≥0.10*) with the exception of an elevation at the Nor-NOHA + BEC site during the first recovery (*P<0.01*). Throughout the experimental protocol, CVC (main effect of treatment site, *P<0.01*; figure 1) was reduced relative to

346 Control at L-NAME (all *P<0.01*) but similar to Control at Nor-NOHA + BEC (all *P≥0.43*)
347 and low-dose SNP (all *P≥0.18*) in the young men.

348 In the older men, local forearm CVC (main effect of time, *P<0.01*) was elevated
349 from baseline at Control (both *P<0.01*), Nor-NOHA + BEC (both *P≤0.01*) and low-dose
350 SNP (both P≤0.02) during both exercise bouts. At L-NAME however, CVC was similar
351 to baseline throughout the experimental protocol (all *P≥0.12*). In comparison to Control,
352 CVC (main effect of treatment site, *P<0.01*; figure 1) was reduced at L-NAME during
353 baseline (*P<0.01*) and the first (*P=0.02*) but not second (*P=0.16*) exercise bout. Further,
354 CVC at L-NAME was attenuated relative to Control in both recoveries (both *P<0.01*). In
355 contrast, no differences in CVC between Control and both Nor-NOHA + BEC (all
356 P≥0.06) and low-dose SNP (all *P≥0.10*) were noted during the experimental protocol.
357 When a comparison between age groups was made, no differences in CVC at Control
358 were observed during the intermittent exercise protocol (main effect of age, *P=0.71*).
359 CVC_{max} was similar between and within the young and older adults at all treatment sites
360 (main effects of age and treatment site, both *P≥0.71*; table 2).
361
362 *Sweat rate*
363 Local forearm sweat rate (interaction of time and condition, *P=0.03*; figure 2) was
364 elevated relative to baseline values throughout the intermittent exercise protocol in the
365 young men at all treatment sites. While no between-site differences were observed at
366 baseline (all *P≥0.18*) sweat rate was attenuated relative to Control at the L-NAME (both
367 *P≤0.03*) but not Nor-NOHA + BEC (both *P≥0.57*) or low-dose SNP (both *P≥0.38*) skin
368 sites during both exercise bouts. In parallel to baseline, local sweat rate was not

different between treatment sites in the young men during both recovery periods (all P≥0.09). In the older men, local sweat rate (main effect of time, *P<0.01*) was elevated from baseline at all treatment sites during both exercise bouts and recovery periods (all *P≤0.01*); however, sweating responses were similar between treatment sites (main effect of treatment site, *P=0.28*). Finally, no differences in local sweat rate at Control were noted between age-groups (main effect of age, *P=0.76*; figure 2).

Body temperature and cardiovascular responses

Esophageal temperature (main effect of time, *P<0.01*; table 3) was elevated from baseline values in the young men during both exercise bouts (both *P<0.01*) as well as the first (*P<0.01*) but not second recovery (*P=0.08*). When expressed as both absolute values and elevations from baseline, esophageal temperature was greater in the second compared to first exercise bout in both age-groups (both *P=0.02*). In the older adults, esophageal temperature (absolute and relative responses) was elevated from baseline values during both exercise bouts (both *P<0.01*) and recovery periods (both *P<0.01*) and was greater in the second exercise relative to the first (both *P=0.01*). Esophageal temperature was not different between age-groups when expressed in absolute values (main effect of age, *P=0.61*) but elevations from baseline were greater in the older vs. younger men during both exercise bouts (both *P=0.01*).

Compared to baseline, mean skin temperature (main effect of time, *P<0.01*; table 3) was elevated during both exercise bouts (both *P≤0.03*) in the young men. In the older men, mean skin temperature was increased from baseline values throughout both exercise bouts (*P<0.01*) and during the second recovery period (*P=0.03*). No

differences in mean skin temperature were observed between age-groups whether expressed in absolute values or as a change from baseline (main effect of age, *P=0.61*).

Throughout the experimental protocol, mean arterial pressure (interaction of time and age, *P=0.03*; table 4) remained similar to baseline in the young men (all *P>0.01*). In the older men however, mean arterial pressure was elevated from baseline during both exercise bouts (both *P≤0.02*) but was attenuated during the second recovery (*P=0.02*). During the first exercise, mean arterial pressure was greater in the older relative to younger men (*P=0.03*). Heart rate (main effect of time, *P<0.01*, table 4) was elevated from baseline during both exercise bouts (both P<0.*01*) in the young and throughout the experimental protocol in the older men (all *P≤0.01*). Moreover, in the older men, heart rate increased from the first to second exercise bout (*P<0.01*) and recovery (*P<0.01*). Heart rate was not different between age-groups (main effect of age, *P=0.95*).

Whole-body passive heating

The mean ± standard deviation characteristics of the older men who participated in the whole-body passive heating protocol were: age, 64 ± 7 years; height, 1.75 ± 0.09 m; body mass, 77.8 ± 10.4 kg, surface area, 1.93 ± 0.13 m^2 and body fat, 22.3 ± 8.1%. Participants were adequately hydrated (urine specific gravity <1.020 (3)) prior to the heating protocol (urine specific gravity: 1.012 ± 0.009).

At all treatment sites, CVC (temperature-treatment interaction, *P=0.03*) was elevated when esophageal temperature was passively increased from 0.6°C to 0.8°C (all *P≤0.02*). In comparison to Control, CVC at the L-NAME skin site was similar when esophageal temperature was elevated 0.6°C (*P=0.09*) but reduced at an increase of

0.8°C (*P=0.04*). Arginase inhibition via Nor-NOHA + BEC augmented CVC relative to control at both levels of hyperthermia (both *P≤0.05*), whereas no influence of low-dose SNP was noted (both *P≥0.33*). CVC_{max} was 2.01 ± 0.50, 1.79 ± 0.48, 1.91 ± 0.55, and 1.82 ± 0.55 perfusion units·mm Hg^{-1} at Control, L-NAME, Nor-NOHA + BEC and Low-dose SNP, respectively, and was similar between treatments (main effect of treatment site, *P=0.89*). Finally, while sweat rate at each skin site increased when the change in esophageal temperature from baseline was elevated from 0.6°C to 0.8°C (all *P≤0.01*; main effect of temperature, *P<0.01*), responses were not affected by any treatment (main effect of treatment site, *P=0.83*).

DISCUSSION

In the present study the independent influences of age-related increases in arginase activity and changes in NO sensitivity of the thermoregulatory end-organs (i.e., cutaneous vasculature and the eccrine sweat gland) on cutaneous vasodilation and sweating during exercise in the heat (35˚C) were evaluated. We demonstrate that local arginase inhibition (to increase synthesis of endogenous NO via NO synthase) and low-dose SNP administration (to supply exogenous NO) do not independently modulate cutaneous vasodilation or sweating in either young or older men performing moderate-intensity intermittent exercise in the heat (35˚C). These results contrast with those observed during whole-body passive heating, wherein arginase inhibition augmented cutaneous vasodilation (but not sweating) at increases in esophageal temperature equivalent to those achieved in the exercise-heat stress test. Altogether our findings provide novel information regarding the control of cutaneous perfusion and sweating

438 during exercise while also highlighting the differential modulation of these responses
439 between exercise- and passively induced heat stress.
440
441 **Arginase**
442 Our hypothesis that age-related changes in the activity of the arginase enzyme
443 (4, 25, 31, 50) influence heat loss responses in older men during exercise was not
444 supported by the current findings. Specifically, increasing local synthesis of endogenous
445 NO via arginase inhibition did not augment the heat loss responses in either age-group,
446 despite reductions in NO-dependent cutaneous vasodilation (figure 1) and sweating
447 (figure 2) in the older men (evidenced by similar responses between the Control and L-
448 NAME skin sites). This contrasts our findings in the whole-body passive heating
449 protocol (figure 3), as well as those of Holowatz *et al*. (19, 20) in which augmented
450 cutaneous vasodilation was observed in older adults when arginase was locally
451 inhibited. While there is currently no explanation for the dissimilar roles for arginase in
452 cutaneous vasodilation between exercise- and passively-induced heat stress, it is
453 known that control of the heat loss responses is influenced by the mode of heating. For
454 example, Fujii et al (8) demonstrated that cyclooxygenase contributes to the cutaneous
455 vasodilatory response during exercise in the heat (in an interactive manner with NO)
456 while McCord et al (33) did not observe a role for this enzyme in whole-body passive
457 heating.
458 A potential contributor to the discrepancies in the influence of arginase on
459 cutaneous vasodilation may be differences in skin temperature between heating
460 modalities. Exposure to a hot environment (35˚C) in the current study resulted in

461 unclamped mean skin temperatures of ~35˚C whereas the temperature of each
462 treatment site in the study by Holowatz *et al*. (19, 20) was maintained at ~33˚C. *In vitro,*
463 the activity of NO synthase has been shown to be temperature-sensitive and the
464 influence of temperature on the each isoform of the enzyme (i.e., endothelial, neuronal)
465 is inconsistent (48). In the context of the current work, this latter point may be especially
466 important given that endothelial NO synthase is the primary modulator of cutaneous
467 perfusion during exercise (9, 36) whereas the neuronal isoform is implicated during
468 passive heating (36). Additionally, exercise increases cutaneous vessel shear stress,
469 which can augment NO-mediated vasodilation (32), further complicating the comparison
470 of mechanisms between heating modes. Future research should be directed at
471 evaluating the factors associated with exercise (i.e., skin temperature, shear stress) that
472 not only influence NO-dependent heat loss, but also its interaction with the age-related
473 changes in arginase activity.
474
475 **End-organ sensitivity to exogenous NO**
476 Administration of low-dose SNP to provide exogenous NO did not alter
477 cutaneous vasodilation in the young men (figure 1). The relatively consistent
478 contribution of NO to cutaneous vasodilation (~26%CVC_{max} difference between Control
479 and L-NAME) in the young group throughout the experimental protocol suggests that it
480 is exposure to a hot environment and not exercise *per se* that modulates the
481 contribution of NO to cutaneous vasodilation. In other words, NO-dependent cutaneous
482 vasodilation appeared to reach its 'physiological peak' when skin temperature was
483 elevated from thermoneutral values of ~33˚C to ~35˚C during the initial resting heat

exposure period (~60 min at 35˚C ambient temperature) prior to baseline data collection. However, despite an attenuated contribution of NO to cutaneous vasodilation in the second exercise bout, exogenous NO (i.e., low-dose SNP) did not affect cutaneous vasodilation in the older men at any point during exercise heat stress (figure 1). Importantly, the lack of an effect of low-dose SNP was also observed during whole-body passive heating (figure 3), suggesting that providing exogenous NO does not modulate cutaneous vasodilation in older adults.

Consistent with the findings for cutaneous vasodilation, low-dose SNP did not influence sweat rate in the older men despite a lack of NO-dependent sweating during exercise (figure 2) or whole-body passive heating (figure 3). The age-related alteration in sweating (and potentially cutaneous vasodilation) may therefore stem from modulation of signaling pathways downstream of endogenous NO production. In support of this notion, we have demonstrated that cyclooxygenase contributes to the sweating response in young adults during moderate intensity exercise (heat production of 400 W) in a NO-interactive manner (8). Similarly, recent work suggests that in younger adults, purinergic receptor activation is also an important mediator of sweating during exercise, again via NO-dependent mechanisms (1). However, In parallel to age-related reductions in NO-dependent sweating (1, 11), neither cyclooxygenase (11) nor purinergic receptor (1) blockade modulates exercise-induced sweating in older adults. Combined with the lack of an effect of exogenous NO observed in the present study, those findings suggest that in men, age-related modulation of NO-dependent heat loss is secondary to alterations in pathways downstream of endogenous NO synthesis.

507 **Considerations**

508 Cutaneous vasodilation and sweating at the Control skin site were similar
509 between the young and older men throughout the exercise-heat stress protocol. While
510 age-related impairments in heat exchange are well established (24, 27, 28), they are not
511 always reflected in local measurements (28, 47). Differences in cutaneous vasodilation
512 between young and older adults can be influenced by the site of measurement (44) and
513 a more pronounced separation between groups may have been seen if measurements
514 were performed on the chest or back, skin sites which typically exhibit greater
515 sudomotor activity than the forearm (24). The forearm was chosen to reduce movement
516 artifacts during cycling and to facilitate comparison between previous studies, given that
517 the majority of work aimed at evaluating the mechanisms underpinning these heat loss
518 responses previously employed forearm skin sites (8, 9, 11, 16, 17, 19, 20, 37, 38, 40,
519 45, 46, 49). That said, we observed greater elevations in esophageal temperature
520 during exercise in the older vs. younger men (table 3), despite having both groups
521 exercise at the same absolute heat load (400 W) to elicit similar drives for whole-body
522 heat loss between age-groups (24, 39). Thus, whole-body heat loss was attenuated for
523 a given body core temperature in the older men. Our findings are therefore still
524 indicative of age-related modulation in the control of heat loss during exercise,
525 especially when considering the altered NO-dependent cutaneous vasodilation and
526 sweating (figures 1-3).
527

CONCLUSION

Our findings suggest that neither elevated arginase activity nor changes in the sensitivity of the thermoregulatory end-organs to exogenous NO affect cutaneous vasodilation and sweating in older men during moderate-intensity intermittent exercise in the heat. By contrast, cutaneous vasodilation was augmented by local arginase inhibition in older men during passive heating. In all, these findings highlight the differential mechanisms underpinning the heat loss responses to both exercise- and passively-induced heat stress. Future work is required to delineate the putative modulators of cutaneous perfusion and sweating with both heating modalities along with their implications for body temperature regulation.

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DISCLOSURES

None.

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FIGURE LEGENDS

Figure 1. Local forearm cutaneous vascular conductance (CVC; %CVC_{max}) during the two successive exercise bouts performed at a fixed rate of endogenous heat production (400 W) for the young (n=9; top panel) and older (n=9; bottom panel) men. Throughout the exercise-heat stress protocol, four skin sites were continuously perfused via intradermal microdialysis with 1) lactated Ringer's to act as a control; 2) 10 mM N^G-nitro-$_L$-arginine methyl ester (L-NAME,) to inhibit nitric oxide synthase activity; 3) 5 mM N^{ω}-hydroxy-nor-Arginine + 5 mM S-(2-boronoethyl)-L-cysteine (Nor-NOHA + BEC,), to inhibit arginase activity; or 4) 1 μM sodium nitroprusside (low-dose SNP,), a nitric oxide donor. Values are mean ± 95% confidence interval. Each data point is an average of the last 5 min of each time period. *, P≤0.05 vs. Control.

Figure 2. Local forearm sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$) during the two successive exercise bouts performed at a fixed rate of endogenous heat production (400 W) for the young (n=9; top panel) and older (n=9; bottom panel) men. Throughout exercise-heat stress protocol, four skin sites were continuously perfused via intradermal microdialysis with 1) lactated Ringer's to act as a control; 2) 10 mM N^G-nitro-$_L$-arginine methyl ester (L-NAME) to inhibit nitric oxide synthase activity; 3) 5 mM N^{ω}-hydroxy-nor-Arginine + 5 mM S-(2-boronoethyl)-L-cysteine (Nor-NOHA + BEC) to inhibit arginase activity; or 4) 1 μM sodium nitroprusside (low-dose SNP), a nitric oxide donor. Values are mean ± 95% confidence interval. Each data point is an average of the last 5 min of each time period.

Figure 3. Local forearm cutaneous vascular conductance (CVC; %CVC_{max}) and sweat rate ($mg \cdot min^{-1} \cdot cm^{-2}$) during whole-body passive heating to elevations in esophageal temperature (ΔT_{es}) from baseline of 0.6 and 0.8 in a group of older men (n=7). Throughout the passive heating protocol, four skin sites were continuously perfused via intradermal microdialysis with 1) lactated Ringer's to act as a Control; 2) 10 mM NG-nitro-L-arginine methyl ester (L-NAME) to inhibit nitric oxide synthase activity; 3) 5 mM Nω-hydroxy-nor-Arginine + 5 mM S-(2-boronoethyl)-L-cysteine (Nor-NOHA + BEC) to inhibit arginase activity; or 4) 1 μM sodium nitroprusside (low-dose SNP), a nitric oxide donor. Values are mean ± 95% confidence interval. *, P≤0.05 vs. Control.

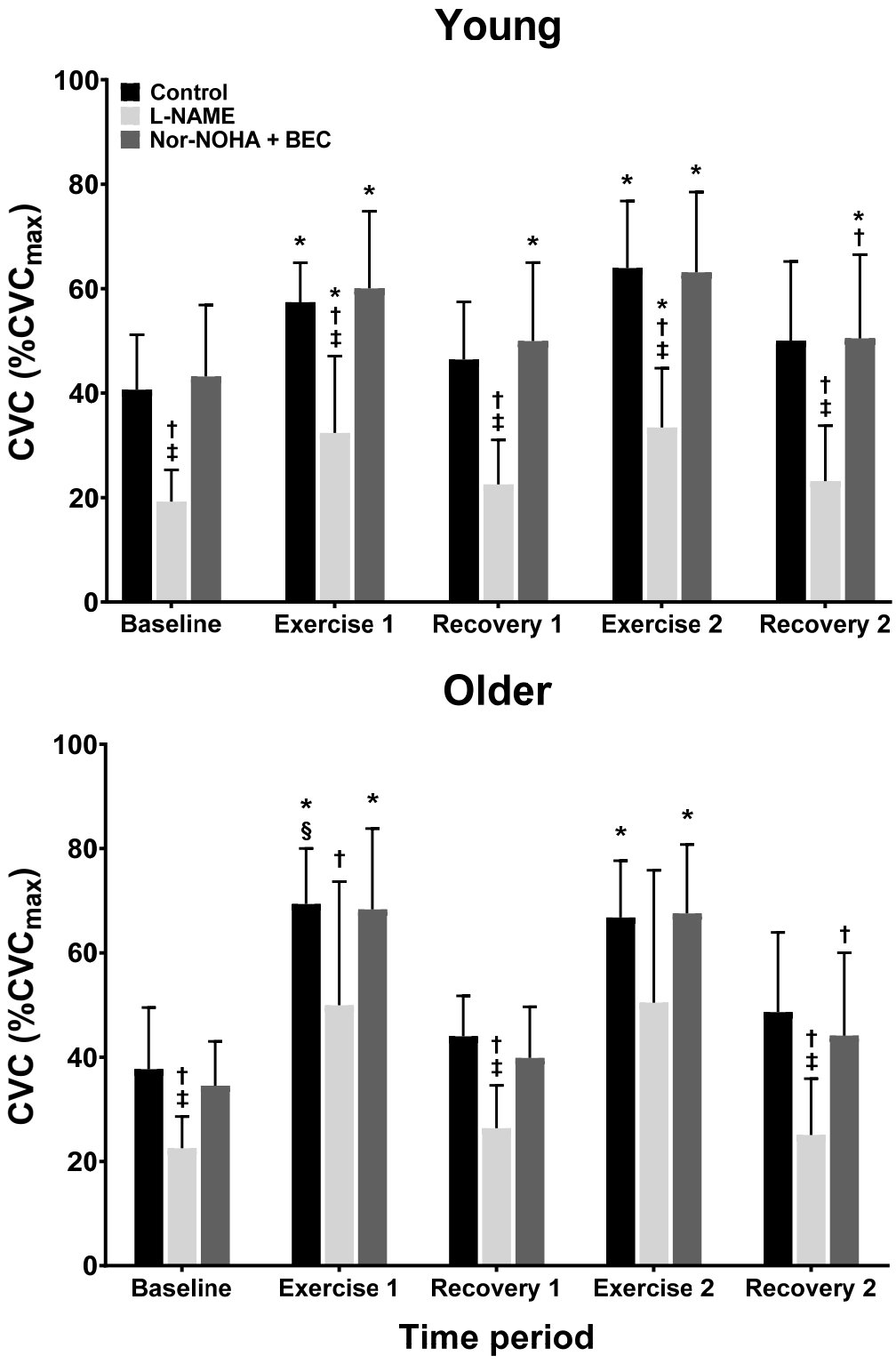
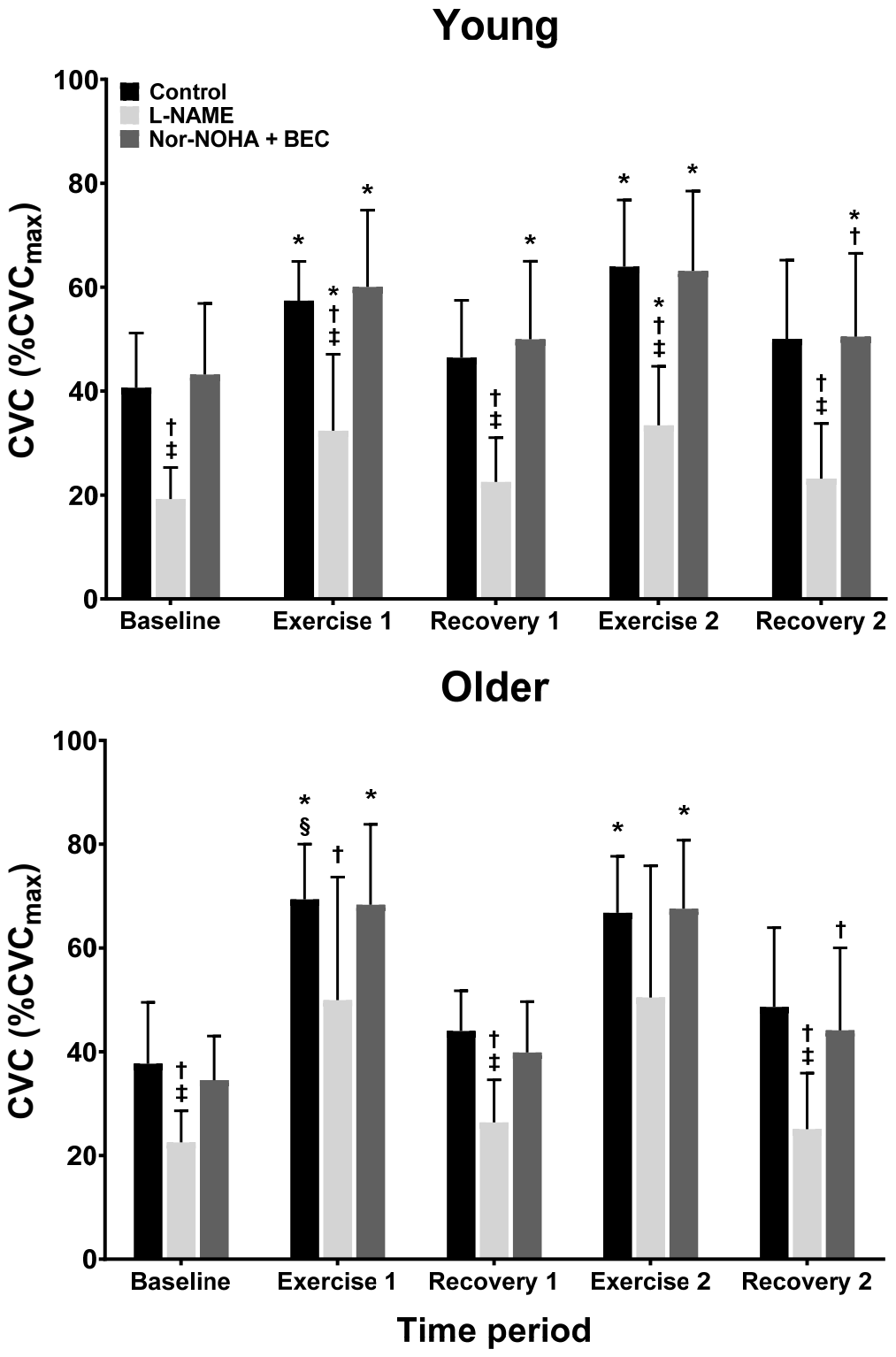
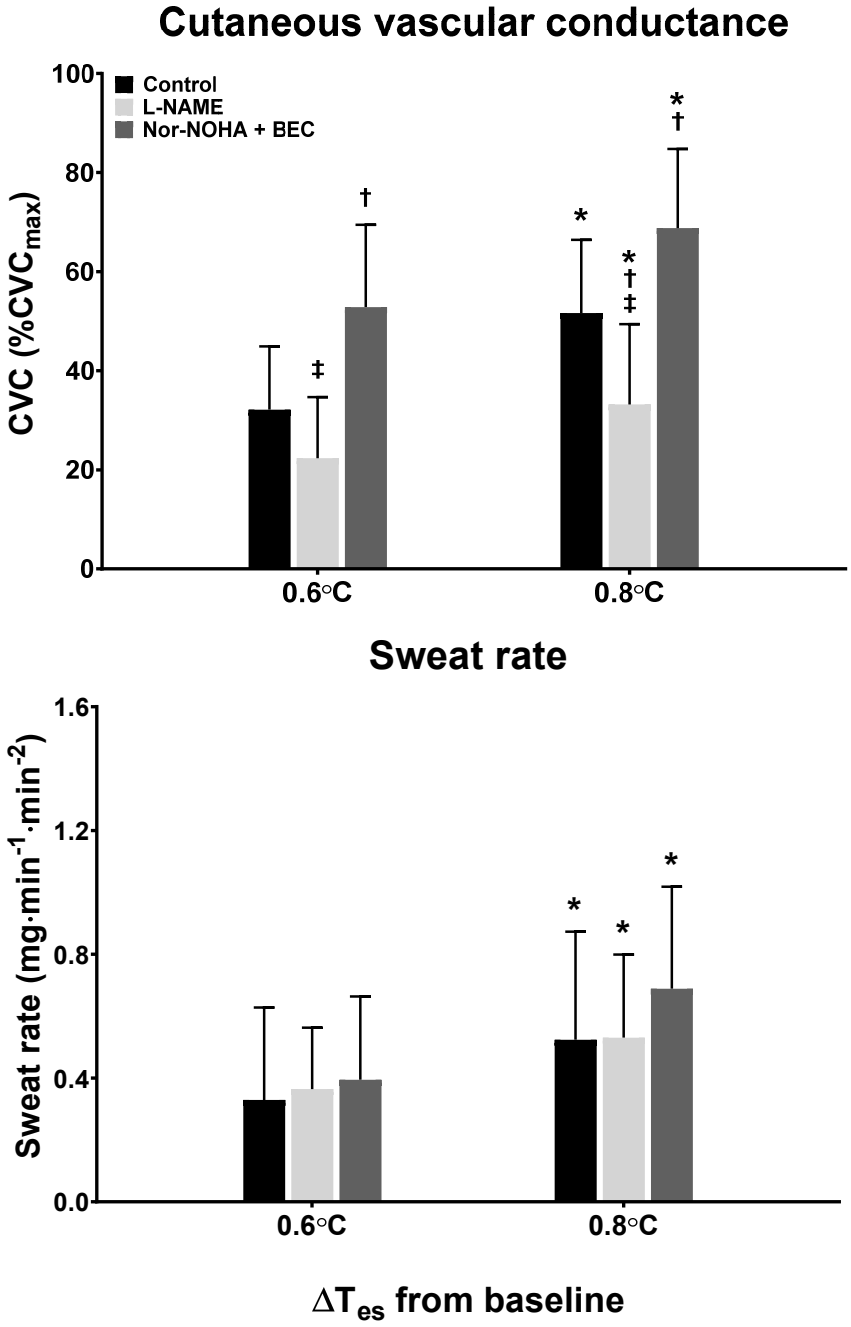

Young
Control
L-NAME
Nor-NOHA + BEC
CVC (%CVCmax)
100
80
60
40
20
0
Baseline
Exercise 1
Recovery 1
Exercise 2
Recovery 2
Older
CVC (%CVCmax)
Time period

FIGURE 1



735
736 **FIGURE 2**
737



738
739 **FIGURE 3**