ΑΝΑΣΚΟΠΗΣΗ ΜΕΤΑΔΙΔΑΚΤΟΡΙΚΗΣ ΕΡΕΥΝΑΣ

Thermoregulatory and cardiovascular effects of capsaicin application on the skin at rest, during exercise and post-exercise state to temperate and warm conditions

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Introduction

The heat-sensitive TRPV1 channels are stimulated at the heat-pain temperature of 43°C [1,2] or by capsaicin, a constituent of red chilli peppers.[3] Classic studies have shown that the activation of TRPV1 through administration of capsaicin leads in considerable rectal temperature decline in rodents exposed in thermoneutral environment. [4,5] In support of this notion, more recent studies conducted in humans have shown that TRPV1 channels substantially contribute to thermal hyperaemia[6]accompanied by cutaneous active vasodilation.[7] In addition, capsaicin application on human skin has been shown to increase cutaneous vasodilation in resting participants exposed to different skin temperatures ranging from warm to hot[8]and to evoke warm or burning sensation.[9] In this direction, experiments in animals have shown that capsaicin reduces the synthesis of prostaglandins,[10] a group of chemicals with potent vasoconstrictive effect.[11]Moreover, also in animals, TRPV1 activation has been shown to stimulate the release of calcitonin gene-related peptide (CGRP), a potent endogenous vasodilator,[17] which in turn enhances Na^+ excretion and nitric oxide (NO) release in endothelial cells.[18] However, to date, the effects of capsaicin on human temperature homeostasis in different environmental temperatures (from temperate to warm) have not been determined. Moreover, it remains unknown whether the proven vasodilatory and heat-dissipating effects of capsaicin can be extrapolated during exercise, where cutaneous vasoconstrictive tone and sweating are activated, as well as at post-exercise state, where an abrupt, centrally mediated suppression in the heat loss responses occurs as the rate of metabolic heat production returns to baseline levels. Therefore our purpose was to investigate thermoregulatory and cardiovascular responses at rest, during exercise as well as at post-exercise state in a temperate (20ºC) and in a warm (30ºC) environment without and with the application of capsaicin on the skin.

Methods

In the first study, the effects of capsaicin were tested on ten participants for 30 minutes at rest. In the second study, eight participants cycled at 55% of their maximal aerobic power so long as to reach 38.2ºC in rectal temperature (Tre) to 20ºC or to 30°C: without (NCA) and with (CA) application of capsaicin patches on the skin. In the third study, capsaicin application was tested on seven participants who cycled so

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long as to reach 38.2ºC in Tre and then remained rested for 35 minutes at the same environmental conditions. In all trials, capsaicin patches (12X18 cm, 4.8.mg) were applied on different parts of the skin. In particular, one patch was applied on pectoralis major muscle, one was applied on trapezius muscle and the other two patches were applied on the two vastus lateralis muscles. Thermoregulatory (Tre, proximal-distal skin temperature gradient) and cardiovascular variables (mean arterial pressure, systolic and diastolic pressure, heart rate, stroke volume, cardiac output and total peripheral resistance) as well as oxygen uptake were continuously measured.

In the first study, before using parametric tests for the analysis of the data, the assumption of normality was verified using the Shapiro–Wilk test. Two variables (rectal temperature and thermal sensation) were found to violate this assumption, and as such, the area under the curve analysis was employed. Accordingly, these data were compared using the Friedman's nonparametric analysis of variance (ANOVA) followed by Wilcoxon tests. For all other variables with normal distribution, a three-way ANOVA for repeated measures on three factors (environmental temperature X capsaicin application X time) was used to define the overall differences in each variable. A Tukey test was employed to allocate post-hoc specific differences. Significance level was set at 0.05.

In the second study, before using parametric tests for the analysis of the data, the assumption of normality was verified using the Shapiro-Wilk test. All variables were found with normal distribution and, consequently, a 3-way analysis of variance (ANOVA) for repeated measures on three factors (environmental temperature X capsaicin application X time) was used to define the overall differences in each variable. A Tukey test was employed to allocate post-hoc specific differences. In addition, deviation from linearity using the least squares linear regression method was used to determine the rectal threshold for the initiation of sweating. Significance level was set at 0.05.

In the third study, before using parametric tests for the analysis of the data, the assumption of normality was verified using the Shapiro-Wilk test. All variables were found with normal distribution and, consequently, a 3-way analysis of variance (ANOVA) for repeated measures on three factors (environmental temperature X capsaicin application X time) was used to define the overall differences in each

variable. A Tukey test was employed to allocate post-hoc specific differences. Significance level was set at 0.05.

Results

In the first study, the area under the curve for Tre decline at 20°C was smaller in CA (-2.1±1.3 a.u.) than in NCA (-0.6±1.1 a.u., $p<0.01$). Likewise, at 30°C it was smaller in CA (-2.2±2.1 a.u.) compared to NCA (-0.8±2.0 a.u., $p=0.02$). Local vasomotor tone and oxygen uptake, were significantly lower by 36.7±94.2% and 12.3±12.3%, respectively, with capsaicin compared to NCA ($p=0.05$ and $p<0.01$, respectively). Additionally, in 30°C CA mean arterial pressure was lower by 10.7±5.9%, 8.9±5.9% and 10.6±7.0% compared to 30°C NCA, 20°C NCA and 20°C CA, respectively ($p<0.01$, $p=0.02$ and $p<0.01$, respectively).

In the second study, we observed that regardless of ambient temperature, during the first 14 min of exercise, the local vasoconstrictive tone was lower in CA than in NCA ($p<0.05$). Accordingly, sweating rate was higher and occurred at a lower Tre increase in CA compared to NCA ($p=0.03$). As a result, the time to reach Tre of 38.2ºC was longer by 5.4±6.1 min in CA compared to NCA ($p=0.03$). Moreover, oxygen consumption was higher in capsaicin than in non-capsaicin condition ($p<0.001$). Mean arterial pressure was lower during cycling in warm compared to temperate environment but was unaffected by capsaicin. In the third study, local vasoconstrictive tone was lower in 20 CA compared to the other trials ($p<0.01$) and sweating rate was higher in CA than NCA condition ($p=0.02$). As a result, Tre decline was faster in 20 CA compared to 20 NCA, 30 NCA and 30 CA ($p<0.01$). All cardiovascular variables remained unaffected by capsaicin application ($p>0.05$).

Discussion and Conclusions

We investigated the effects of capsaicin application on the skin (5% of the skin surface area) at rest, during dynamic whole-body exercise and at post-exercise state on thermoregulatory and cardiovascular responses at temperate and warm conditions. The principal findings of the first study are: (a) regardless of environmental temperature, capsaicin application on the skin reduced heat conservation due to lower vasomotor tone in conjunction with lower heat production and (b) in warm condition,

application of capsaicin patches were accompanied by arterial hypotension accredited to the lower total peripheral resistance compared to the temperate condition.

The principal findings of the second study were: a) regardless of ambient temperature, capsaicin application before the initiation of exercise reduced heat gain during exercise due to a greater heat loss indicated by a lower estimated vasoconstrictive tone of the skin as well as to an earlier onset and higher rate of sweating; in turn, both these responses ended in a slower rise of rectal temperature during exercise, and b) the higher heat dissipation observed after application of capsaicin was not accompanied by lessened blood pressure increase during exercise, as it was anticipated based on previous data at rest.

In parallel with findings at rest (first study) and during exercise (second study), the third study showed that capsaicin application on the human skin immediately after the termination of exercise induced faster rectal temperature decline in 20ºC compared to the other trials. The faster rectal temperature decline in 20ºC was likely the result of lower estimated vasocontrictive tone and higher sweating rate. Nonetheless, cardiovascular variables remained unaltered.

In accordance with previous studies in animals[4,5], our findings suggest that TRPV1 activation through capsaicin application on the skin (5% of the skin surface area) induces heat loss responses at rest. Moreover, capsaicin reduces heat gains during whole-body exercise and accelerates heat loss responses at post-exercise state. Although the results of the first study showed that heat loss responses were accompanied by arterial hypotension, this was not evident during exercise and post-exercise state. This finding is in contrast to previous studies conducted both in animals[12,13] and humans[10] who received epidermal capsaicin administration. Nonetheless, our results are in accordance with the respective ones of Vianna et al.[14], who observed similar increase of blood pressure during handgrip exercise after the application of capsaicin on the skin compared to control condition. Several reasons may explain this interesting finding. First of all, different methodological approaches concerning the dosage of capsaicin have been applied. We presently used commercially available patches (12X18 cm; 5% of the skin surface area) popular for their analgesic properties containing 4.8 mg of capsaicin, whereas previous studies in humans[10] showing hypotensive effects had used greater dosage of capsaicin.

Additionally, it is known that the hypotensive effect of capsaicin is related to the capsaicin-induced desensitization of group III and IV muscle afferent fibers.[10,13] However, this was not the case in the present study, as the course of arterial blood pressure was similar between capsaicin and non-capsaicin conditions. Therefore, it could be alleged that a possible activation of group III and IV muscle afferent fibers as a result of a metabolically demanding exercise counteracts the possible role of capsaicin on the same fibers. In fact, the present exercise protocol was different from previous ones [10,14], wherein static exercise was applied.

The current investigation demonstrates that regardless of environmental condition (temperate or warm) TRPV1 activation by capsaicin application on the human skin (5% of the skin surface area) induces heat loss responses at rest and reduces heat gains during dynamic exercise thus resulting in a slower increase of rectal temperature. Accordingly, capsaicin appears to induce heat loss during post-exercise state, More research is needed to explore the effects of capsaicin at different dosages as well as during dynamic exercise in a different exercise intensity spectrum.

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HOMA-IR, and HOMA-IS were lower in LEX than SED (P<0.05). HIIT did not alter glucose, insulin, HOMA-IR, or HOMA-IS in LEX (P>0.05). HIIT reduced SED glucose compared to baseline (P=0.013, Cohen's *d*=0.36) and compared to post-preconditioning (P=0.009, Cohen's *d*=0.35). HIIT reduced HOMA-IR in SED compared to baseline (P=0.027, Cohen's *d*=0.25), and compared to after preconditioning (P=0.069, Cohen's *d*=0.36). Results of this study indicate that HIIT preceded by preconditioning without dietary intervention improve glucose regulation in SED, as evidenced by reduced fasting glucose and HOMA-IR.

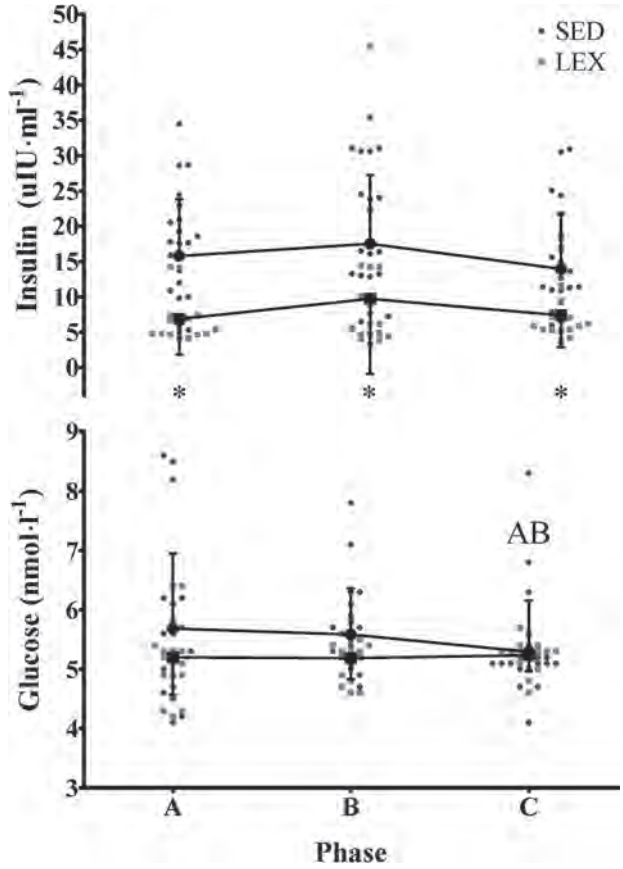

Figure 1: (upper panel) Insulin, and (lower panel) glucose, in a group of sedentary (SED) and lifelong exercising (LEX) older males. Data are presented as mean ± SD, plus individual data points. *Denotes significant differences between groups at this experimental phase (P<0.05). A = significantly different from phase A in SED (P<0.05). B = significantly different from phase B in SED (P<0.05).

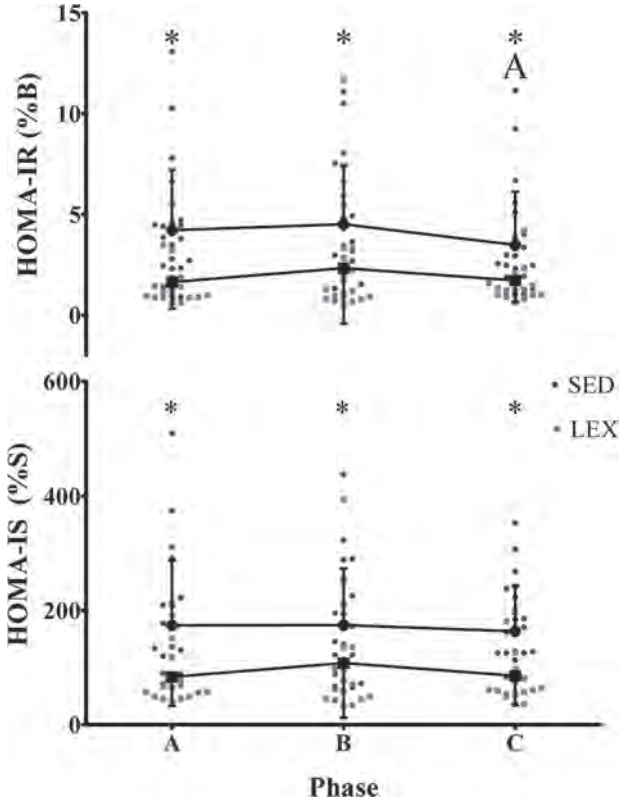

Figure 2: (upper panel) HOMA-IR, and (lower panel) HOMA-IS, in a group of sedentary (SED) and lifelong exercising (LEX) older males. Data are presented as mean ± SD, plus individual data points. *Denotes significant differences between groups at this experimental phase (P<0.05). A = significantly different from phase A in SED (P<0.05).

Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PCA163

Cardiovascular effects of capsaicin application on human skin at rest in temperate and warm conditions

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The heat-sensitive TRP vanilloid 1 (TRPV1) channels are stimulated at the heat-pain temperature of 43°C (1) or by capsaicin (2). It has been shown that capsaicin reduces the synthesis of prostaglandins, which stimulate the release of calcitonin gene-related peptide, a potent endogenous vasodilator (3), which, in turn, enhances Na^+ excretion and nitric oxide release in endothelial cells (4). Thus, TRPV1 activation through capsaicin may have important potential for promoting cardiovascular health (5). To date, no study has elucidated whether TRPV1 stimulation has any effect on humans' blood pressure regulation. Therefore, we explored cardiovascular responses at rest in temperate (20°C) and warm (30°C) environment without and with application of capsaicin on the skin of human subjects. Ten healthy males were exposed, while seated, for 30 min to 20°C or 30°C on two occasions: without (NCA) and with (CA) skin application of capsaicin patches (12 x 18 cm), each one impregnated with of 4.0g capsaicin. In CA, one patch was applied on right

pectoralis major muscle, another one on right trapezius and two more patches on the two vastus lateralis muscles. Mean arterial (MAP), systolic (SYS) and diastolic pressure (DIA), cardiac output (CO) and total peripheral resistance (TPR) were continuously measured via a photoplethysmometer (Finometer 2003, FMS, The Netherlands). Data were analyzed by 2-way ANOVA and the level of significance was set at $p<0.05$. Values are presented as mean±SD. MAP was lower ($p\leq0.01$) when subjects were exposed at 30°C (98.6±7.7 mmHg) compared to 20°C (103.1±5.7 mmHg). Moreover, it was lower ($p<0.01$) in CA (98.7±7.9 mmHg) than in NCA condition (103.0±5.4 mmHg). Both SYS and DIA were lower ($p\leq0.01$) in 30°C (131.0±9.8 and 79.0±7.7 mmHg, respectively) than in 20°C (140.3±9.2 and 81.5±4.6 mmHg, respectively) and in CA (133.3±12.4 and 78.1±6.8 mmHg, respectively) than in NCA condition (138.0±7.8 and 82.4±5.3 mmHg, respectively). No differences were detected in SYS and DIA among 20°C NCA, 20°C CA and 30°C NCA. Nonetheless, the mean values of SYS and DIA were lower ($p<0.05$) on average by 13.9 and 9.2 mmHg, respectively, in 30°C CA compared with the other three conditions. Regardless of capsaicin application, TPR was similar between 20°C and 30°C; it was lower ($p=0.02$), however, in CA than in NCA condition, with significant differences being evident only in 30°C. In addition, no differences were observed in CO among the experimental trials. At 30°C capsaicin induced arterial hypotension associated with vasodilation as indicated by the lower TPR. The absence of differences in blood pressure in CA at 20°C indicates a possible role of other heat-sensitive receptors on vascular relaxation. We conclude that the hypotensive effect of capsaicin is temperature-dependent.

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Where applicable, the authors confirm that the experiments described here conform with the ethical requirements.

PCA164

Hypoxia compounds exercise-induced free radical formation in humans; partitioning contributions from the cerebral and femoral circulation

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Historically considered as toxic, mutagenic "accidents" of in-vivo chemistry constrained to cellular oxidative damage and pathophysiology, it has become increasingly clear that at physiological, albeit undefined concentrations, free radicals and associated reactive oxygen species formation during hypoxia and exercise can equally serve as important signal transductants that collectively serve to defend cellular oxygen (O_2) homeostasis (Bailey *et al*, 2017). We designed the first human study to simultaneously measure free radical exchange across the cerebral and femoral circulation to evaluate the dynamic interplay taking place as a function of altered O_2 demand at rest and during exercise-induced responses to hypoxia. This was considered an ideal model system characterized by physiological extremes of O_2 flux (low in brain, high in muscle) facilitating experimental manipulation of local O_2 tension (PO_2) independently of flux (O_2). Healthy participants (5♂, 5♀) were randomly assigned single-blinded to normoxic (21% O_2) and hypoxic (10% O_2) trials with measurements taken at rest and 30 min after cycling at 70% of maximal power output in hypoxia and equivalent relative and absolute intensities in normoxia. Blood was sampled from the brachial artery (a), internal jugular and femoral veins (v) for non-enzymatic antioxidants (HPLC), ascorbate radical ($A^{\bullet-}$, electron paramagnetic resonance spectroscopy), lipid hydroperoxides (LOOH) and low density lipoprotein (LDL) oxidation (spectrophotometry). Cerebral and femoral venous blood flow was evaluated by transcranial Doppler ultrasound (CBF) and constant infusion thermodilution (FBF). With 3 participants lost to follow up (final n = 4♂, 3♀), hypoxia increased CBF and FBF ($P = 0.041$ *vs*. normoxia) with further elevations in FBF during exercise ($P = 0.002$ *vs*. rest). Cerebral and femoral ascorbate and α-tocopherol consumption (v < a) was accompanied by $A^{\bullet-}$/LOOH formation (v > a) and increased LDL oxidation during hypoxia ($P < 0.043$ to 0.049 *vs*. normoxia) implying free radical-mediated lipid peroxidation subsequent to inadequate antioxidant defense. This was pronounced during exercise across the femoral circulation in proportion to the increase in local O_2 uptake (r = -0.397 to -0.459, P = 0.037 to 0.045) but unrelated to any reduction in PO_2. These findings highlight considerable regional heterogeneity in the oxidative stress response to hypoxia that may be more attributable to local differences in O_2 flux than to O_2 tension. Since arterial hypoxaemia is a hallmark feature of circulatory disease, mapping the dynamic transvascular interplay of free radicals as a function of changing O_2 demand may help define sensitive biomarkers of vascular health and monitor the success of interventions designed to optimise tissue oxygenation in the critically ill.

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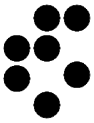

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Effects of capsaicin application on the skin during exercise at temperate and warm conditions

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Introduction

Heat-sensitive TRP vanilloid 1 (TRPV1) channels in the skin are stimulated at heat-pain temperature of 43°C or by capsaicin. In resting humans, the activation of TRPV1 channels induces cutaneous vasodilation. It is unknown whether skin surface capsaicin application facilitates heat dissipation during exercise, where cutaneous vasocontrictive tone and sweating are activated. Therefore, we explored humans' thermoregulatory responses during exercise in a temperate and in a warm environment without and with application of capsaicin on the skin. We hypothesized that regardless of environmental temperature, capsaicin application would stimulate heat loss mechanisms which, in turn, would diminuate exercise-induced rectal temperature (Tre) increase.

Methods

Eight healthy males cycled at 55% of aerobic power as long as to reach 38.2ºC in Tre at 20ºC and 30ºC without (NCA) and with (CA) application of capsaicin (4.0 mg) patches on the skin. Before the initiation of exercise in CA, one patch of capsaicin was applied on pectoralis major and trapezius muscles and the other two patches were applied on the two vastus lateralis muscles. Tre, the proximal-distal skin temperature gradient (Tsk_{f-f}) and sweating rate (SwR) were measured continuously.

Results

Regardless of ambient temperature, during the first 14 min of exercise, Tsk_{f-f} was lower in CA (0.88±1.89ºC) than in NCA (2.45±3.41ºC) ($p<0.05$). Accordingly, Tre threshold for SwR occurred at lower Tre in CA (0.10±0.15ºC), compared to NCA (0.24±0.13ºC) ($p<0.05$). As a result, the rate of rise in Tre was slower in CA (0.03±0.01ºC/min) than in NCA (0.04±0.02ºC/min) ($p<0.05$) and the time to reach Tre of 38.2ºC was longer by 6.0±6.2 min in CA compared to NCA ($p<0.05$).

Conclusion

Activation of TRPV1 by capsaicin immediately before exercise results in a slower Tre rise, which is mediated through greater skin vasodilation and earlier onset of sweating.

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Effects of capsaicin application on the skin during resting exposure to temperate and warm conditions

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Funding information
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Abstract

We investigated thermoregulatory and cardiovascular responses at rest in a temperate (20°C) and in a warm (30°C) environment (40% RH) without and with the application of capsaicin on the skin. We hypothesized that regardless of environmental temperature, capsaicin application would stimulate heat loss and concomitantly deactivate heat conservation mechanisms, thus resulting in rectal temperature (Tre) and mean blood pressure decline due to excitation of heat-sensitive TRPV1. Ten male subjects were exposed, while seated, for 30 minutes to 20.8 ± 1.0°C or to 30.6 ± 1.1°C: without (NCA) and with (CA) application of capsaicin patches on the skin. Thermoregulatory (Tre, proximal-distal skin temperature gradient) and cardiovascular variables (modelflow technique) as well as oxygen uptake were continuously measured. The area under the curve for Tre decline at 20°C was smaller in CA (−2.1 ± 1.3 a.u.) than in NCA (−0.6 ± 1.1 a.u., $P < 0.01$, $r = 0.8$). Likewise, at 30°C it was smaller in CA (−2.2 ± 2.1 a.u.) compared to NCA (−0.8 ± 2.0 a.u., $P = 0.02$, $r = 0.7$). Local vasomotor tone and oxygen uptake, were significantly lower by 36.7% ± 94.2% and 12.3% ± 12.3%, respectively, with capsaicin compared to NCA ($P = 0.05$ and $P < 0.01$, respectively). Additionally, in 30°C CA mean arterial pressure was lower by 10.7% ± 5.9%, 8.9% ± 5.9%, and 10.6% ± 7.0% compared to 30°C NCA, 20°C NCA, and 20°C CA, respectively ($P < 0.01$, $P = 0.02$, and $P < 0.01$, respectively, d = 1.4-1.8). In conclusion, capsaicin application on the skin induced vasodilation and Tre decline. At 30°C CA, thermal responses were accompanied by arterial hypotension most likely due to the interactive effects of both stressors (warm environment and capsaicin) on cutaneous vascular regulation.

KEYWORDS

core temperature, skin warm sensors, temperature and vascular regulation

1 | INTRODUCTION

The role of transient receptor potential (TRP) family ion channels on thermal and vascular regulation has attracted ample attention from the research community. In this context, earlier and more recent studies have suggested the application of a chemical agonist on cold-sensitive (TRPM8) channels (eg, menthol) or on heat-sensitiveTRP vanilloid 1 (TRPV1) channels (eg, capsaicin) as a novel approach for studying the integrative mechanisms affecting temperature[1-4] and vascular regulation,[5-8] in mammals.

In particular, the heat-sensitive TRPV1 channels are stimulated at the heat-pain temperature of 43°C[9,10] or by capsaicin, a constituent of red chili peppers.[11] Classic studies have shown that the activation of TRPV1 through the administration of

capsaicin leads in considerable rectal temperature decline in rodents exposed in thermoneutral environment.[1,12] In support of this notion, more recent studies conducted in humans have shown that TRPV1 channels substantially contribute to thermal hyperaemia[7] accompanied by cutaneous active vasodilation.[8] In addition, capsaicin application on human skin has been shown to increase cutaneous vasodilation in resting participants exposed to different skin temperatures ranging from warm to hot[6] and to evoke warm or burning sensation.[5] The effect of capsaicin on rectal temperature decline may not be exclusively caused by an ensuing vasodilation, but also by an attenuation of oxygen uptake, according to the reciprocal cross-inhibition theory originally introduced by Sherrington and further elaborated by Bligh.[13] To date, the effects of capsaicin on human temperature homeostasis in different environmental temperatures (from temperate to warm) have not been determined.

Alongside with its thermoregulatory properties, TRPV1 activation through capsaicin may be promising for relieving local pain[14] and promoting cardiovascular health.[15] Experiments in animals have shown that capsaicin reduces the synthesis of prostaglandins,[16] a group of chemicals with potent vasoconstrictive effect.[17]

Moreover, also in animals, TRPV1 activation has been shown to stimulate the release of calcitonin gene-related peptide (CGRP), a potent endogenous vasodilator,[18] which in turn enhances Na^+ excretion and nitric oxide (NO) release in endothelial cells.[19] Thus, it appears that in animals capsaicin possesses vasodilatory[18] and anti-vasoconstrictive action.[19] Despite the possible cardioprotective effects of capsaicin and its popularity as analgesic, yet to date no study has elucidated whether TRPV1 stimulation has any effect on humans' blood pressure regulation.

Therefore, the purpose of the current study was to explore thermoregulatory and cardiovascular responses at rest in a temperate (20°C) and in a warm (30°C) environment without and with the application of capsaicin on the skin of human subjects. Based on the long-standing cross-inhibition theory,[13,20] we hypothesized that, regardless of environmental temperature, capsaicin application would stimulate heat loss (cutaneous vasodilation) and concomitantly deactivate heat conservation mechanisms (oxygen uptake), thus resulting in rectal temperature and mean blood pressure decline; this response would be probably due to TRPV1 excitation. It was further assumed that at 30°C the capsaicin effect would be greater due to the interactive effects of both stressors (warm environment and capsaicin) on cutaneous vascular regulation, which might, in turn, affect blood pressure homeostasis.

2 | MATERIALS AND METHODS

2.1 | Subjects

Ten healthy Caucasian male subjects (age: 20.3 ± 2.1 years, body mass: 79.6 ± 6.6 kg, stature: 179.2 ± 6.5 cm, body fat: 11.7% ± 4.4%) volunteered to participate in this study. All participants were non-smokers, physically active (2-5 d/wk), without being acclimatized to hot environment nor diagnosed with any cardiovascular, respiratory or metabolic disease at the time of the study. All participants provided informed consent to participate in this study that was approved by the faculty review board and conformed to the Declaration of Helsinki.

2.2 | Study outline

During the experiments, all subjects were dressed in shorts and a t-shirt. Subjects were initially familiarized with the experimental protocol and equipment and were tested for a possible undesirable reaction to capsaicin by applying a patch containing the substance on the skin area of the forearm. In four subsequent visits, separated by a 2-5 days interval, subjects remained seated and relaxed for 30 minutes, without (NCA) and with (CA) skin surface capsaicin application. A pilot study showed that this time period was adequate to elicit physiological response. The first reaction to capsaicin patches (burning or hot sensation) is manifested within 2 minute. In CA condition, four commercially available patches (12 × 18 cm), each one impregnated with of 4.8 mg capsaicin ($C_{18}H_{27}NO_3$), were applied on four different parts of the body, which were previously shaved. In particular, one patch was applied on pectoralis major muscle, one was applied on trapezius muscle and the other two patches were applied on the two vastus lateralis muscles. Before initiation of each experimental trial, there was a 5-minute initial pre-experimental period of collecting reference data at the respective environmental temperature (20 or 30°C) without capsaicin. In CA condition, there was no time interval between this pre-experimental resting phase and application of capsaicin. Experimental data collection started immediately upon patches application. The environmental temperature was either warm (30.6 ± 1.1°C, 40.8% ± 1.1% relative humidity) or temperate (20.8 ± 1.0°C, 40.6% ± 0.9% relative humidity). The testing order was counterbalanced in terms of capsaicin application and environmental temperature. All experiments were conducted during the spring season (between March and June) or autumn season (between October and November), when temperature in Athens varied around 25°C, and at the same time of the day.

2.3 | Procedure

For 2 days prior to their first experimental trial, subjects were asked to record their diet and physical activity and were instructed to replicate these habits before each experiment, to ensure similar levels of body energy sources and hydration. In addition, subjects had to abstain from strenuous exercise,

caffeine and alcohol consumption during the day before the experiments. On the experimental day, after having emptied their bladder, subjects' body mass was measured (Bilance Salus, Milano, Italy) and a rectal thermistor was inserted 13-15 cm beyond the anal sphincters.

2.4 | Analytical methods and equipment

Throughout the experiment, skin temperatures (forearm, fingertip, chest, and trapezius muscle) were recorded additionally to rectal temperature. Delta values were calculated by subtracting the average initial pre-experimental value of 5-minute rest, where no treatment was applied even in the capsaicin conditions. Chest and back (trapezius muscle) skin thermistors were applied on skin areas free of capsaicin and the mean skin temperature was calculated as the average of forearm, chest and back temperatures. All temperatures were recorded continuously with thermistors (SS7, BIOPAC Systems Inc, California, USA) connected to a mobile unit (TEL 100D, BIOPAC Systems Inc, California, USA). For each measurement, the signal was transferred from the mobile unit (TEL 100D, BIOPAC Systems Inc, California, USA) to a signal processing unit (MP 100A, BIOPAC System, Inc, California, USA) and then stored on a computer. The difference in temperature between forearm and fingertip (Tskf-f) was calculated as an index of skin vasomotor tone with higher difference suggesting lower local blood flow.[21]

Cardiovascular variables (blood pressure, total peripheral resistance, cardiac output, stroke volume, and heart rate) were continuously recorded non-invasively via a photoplethysmometer with the cuff attached on the right middle finger (Finometer 2003, FMS, The Netherlands). Finometer traces a finger pulse waveform to measure arterial pressure and reconstruct aortic flow and thus stroke volume is estimated via a three-element modelflow approach using characteristics of aortic impedance, arterial compliance, and systemic vascular resistance.[22] Before each experiment, Finometer device was automatically calibrated for pressure, distance of finger sensor from the heart level, and detection of sound derived from "return to arm flow", according to the manufacturer's standards. Moreover, oxygen uptake (VO_2), ventilation (VE), and respiratory rate (RR) were measured continuously with a metabolic cart (MedGraphics, CPX-D, Minnesota, USA), which was calibrated with two different gas mixtures and a syringe of known volume before each testing.

Thermal whole-body sensation was reported by the subjects every 2 minutes using a scale questionnaire (1, cold; 3, cool; 5, slightly cool; 7, neutral; 9, slightly warm; 11, warm; 13, hot)[23] and this refers to whole-body feeling. Body fat was assessed by taking measurements of several skinfolds (chest, thigh, triceps, subscapular, suprailiac, abdominal, and axillary) and using the equation of Jackson and Pollock[24] for the calculation of body fat percentage.

2.5 | Statistical analysis

Before using parametric tests for the analysis of the data, the assumption of normality was verified using the Shapiro–Wilk test. Two variables (rectal temperature and thermal sensation) were found to violate this assumption, and as such, the area under the curve analysis was employed. Accordingly, these data were compared using the Friedman's nonparametric analysis of variance (ANOVA) followed by Wilcoxon tests. For all other variables with normal distribution, a three-way ANOVA for repeated measures on three factors (environmental temperature X capsaicin application X time) was used to define the overall differences in each variable. A Tukey test was employed to allocate post-hoc specific differences. As a measure of effect size the Cohen's d was calculated dividing the difference between sample means by the standard deviation of difference scores. Values of 0.20, 0.50, and above 0.80 were considered as small, medium, and large, respectively. For nonparametric comparisons, the effect sizes (r) were also calculated by dividing the Z to the root square of the sample size (N = 10). Values of 0.1, 0.3, and 0.5 were considered as small, medium, and large, respectively.[25] Data are presented as mean ± standard deviation (SD), unless indicated otherwise. Significance level was set at 0.05.

3 | RESULTS

3.1 | Thermoregulatory responses

3.1.1 | Rectal temperature

Initial pre-experimental resting values for rectal temperature were similar among conditions (20°C NCA: 37.1 ± 0.3°C, 20°C CA: 37.1 ± 0.3°C, 30°C NCA: 37.2 ± 0.2°C, 30 CA: 37.1 ± 0.2°C $P > 0.05$). However, Friedman's ANOVA showed that the area under the rectal temperature curve (Figure 1) was different between experimental conditions (main effect: $P = 0.04$). Wilcoxon tests revealed that regardless of environmental temperature, the area under the rectal temperature curve was significantly smaller (ie, the rectal temperature was lower) with than without capsaicin application. Namely, the area under the curve at 20°C was smaller in CA (−2.1 ± 1.3 a.u.) than in NCA (−0.6 ± 1.1 a.u.; $P < 0.01$, $r = 0.8$). Likewise, at 30°C it was smaller in CA (−2.2 ± 2.2 a.u.) compared to NCA (−0.8 ± 2.0 a.u., $P = 0.02$, $r = 0.7$). Moreover, the area under the rectal temperature curve tended to be smaller in 30°C CA than in 20°C NCA ($P = 0.07$, $r = 0.6$). No differences were observed between 20°C and 30°C CA conditions ($P = 0.72$; Figure 1).

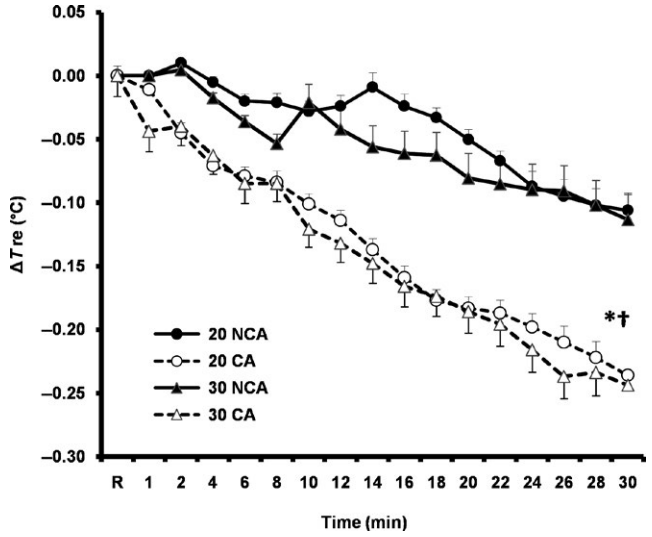

FIGURE 1 Relative changes (mean ± SE) from initial pre-experimental resting values (R) in rectal temperature (ΔTre) during 30-minutes resting exposure to temperate (20°C) and warm (30°C) environmental temperature with (CA) and without (NCA) capsaicin application. (N = 10). *Significant difference between 20°C CA and 20°C NCA ($P < 0.01$). †Significant difference between 30°C CA and 30°C NCA ($P < 0.05$)

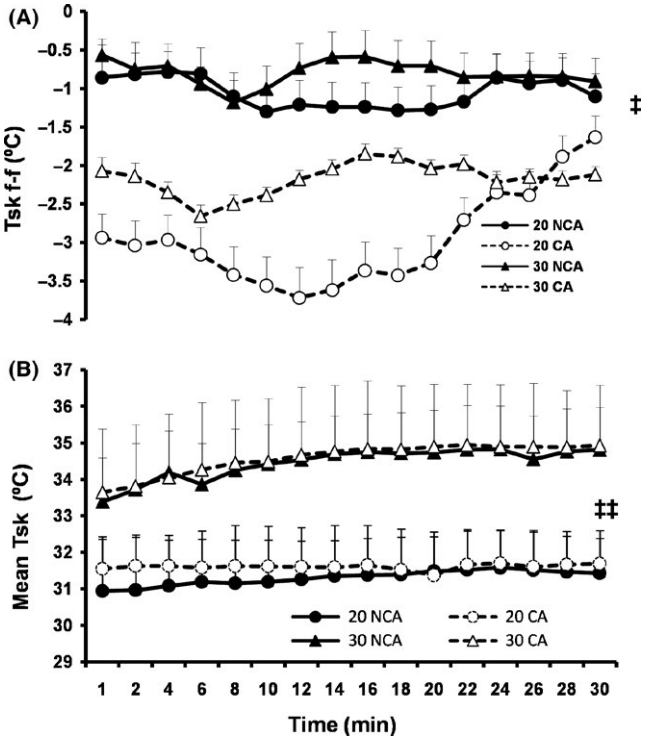

FIGURE 2 Mean (SD) values of temperature difference between forearm and fingertip ($\Delta T_{skf\text{-}f}$) (A) and mean skin temperature (Tsk) (forearm, chest and back, B) during 30-minutes resting exposure to temperate (20°C) and warm (30°C) environmental temperature with (CA) and without (NCA) capsaicin application on the skin (N = 10). ‡Significant difference between CA and NCA (main effect: $P = 0.05$, $d = 0.6$). ‡‡significant difference between 20°C and 30°C (main effect: $P < 0.01$, $d = 1.6$)

3.1.2 | Tskf-f

The index of skin vasomotor tone (Tskf-f) is depicted in Figure 2A. Initial pre-experimental resting values for Tskf-f were similar among conditions (20°C NCA: −0.9 ± 4.3°C, 20°C CA: −1.7 ± 3.5°C, 30°C NCA: −0.6.±2.1°C, 30°C CA: −0.1 ± 1.2°C, $P > 0.05$). Tskf-f was similar between 30°C and 20°C ($P > 0.05$), but lower in CA (−2.6 ± 2.3°C) than in NCA (−0.9 ± 2.7°C) condition (main effect: $P = 0.05$, $d = 0.6$). In addition, the rate of change in Tskf-f in the initial phase of the experimental procedure (ie, first 6 minute) was greater in capsaicin (−1.8 ± 1.8°C) than in non-capsaicin condition (−0.1 ± 0.7°C; $P < 0.01$, $d = 0.9$). No significant differences were detected among the other trials. The mean skin temperature (forearm, chest and back) was higher in 30°C than in 20°C, but similar between CA and NCA conditions (Figure 2B).

3.1.3 | Thermal sensation

Initial pre-experimental resting values for thermal sensation were higher when subjects were exposed to 30°C than to 20°C (20°C NCA: 5.7 ± 1.3 vs 30 NCA: 8.0 ± 0.6 a.u., main effect: $P < 0.01$). Moreover, values were similar in CA compared to NCA condition (main effect: $P > 0.05$; 20°C NCA: 4.9 ± 1.3 vs 20°C CA: 5.4 ± 1.3 a.u., $P > 0.05$, and 30°C NCA: 7.1 ± 0.6 vs 30°C CA: 7.2 ± 1.3 a.u.). The area under the thermal sensation curve was different among experimental conditions (main effect: $P < 0.01$). Wilcoxon tests showed that the area under the curve in 20°C NCA was smaller compared to the 20°C CA ($P < 0.01$, $r = 0.8$), as well as compared to 30°C NCA ($P = 0.01$, $r = 0.8$) and 30°C CA ($P < 0.01$, r = 0.9). Moreover, in 30°C the area under the thermal sensation curve was greater in CA than in NCA condition ($P = 0.03$, $r = 0.7$). Furthermore, it was greater in 30°C NCA than in 20°C CA ($P = 0.05$, $r = 0.6$; Figure 3).

3.2 | Cardiorespiratory responses

3.2.1 | Respiratory responses

Mean values for VO_2 were similar between the two environmental temperature conditions, but they were lower ($P < 0.01$, $d = 1.0$) with than without capsaicin patches on the skin (Figure 4, Table 1). In addition, subjects showed similar VE and RR responses across experimental trials ($P > 0.05$; Table 1).

3.2.2 | Mean arterial pressure

Mean arterial pressure was affected by ambient temperature and capsaicin application (main effect: $P < 0.01$, and

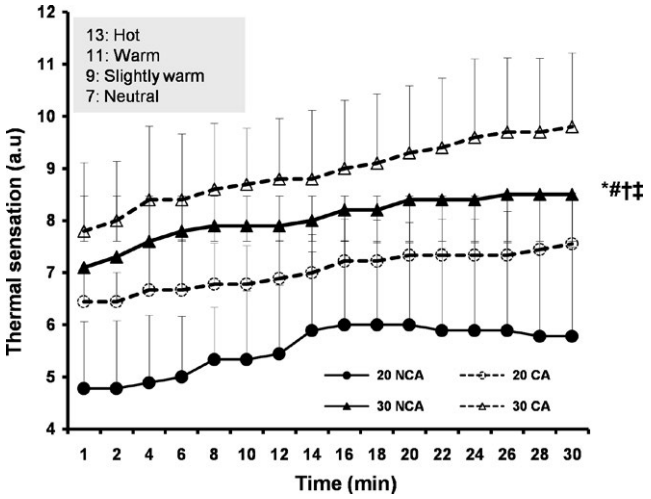

FIGURE 3 Mean (SD) values of thermal sensation during 30-minutes resting exposure to temperate (20°C) and warm (30°C) environmental temperature with (CA) and without (NCA) capsaicin application on the skin (N = 10). *Significant difference between 20°C NCA and 20°C CA, 20°C NCA and 30°C NCA, 20°C NCA and 30°C CA ($P \leq 0.01$). #Significant difference between 30°C NCA and 30°C CA ($P < 0.05$). †Significant difference between 30°C NCA and 20°C CA ($P \leq 0.05$)

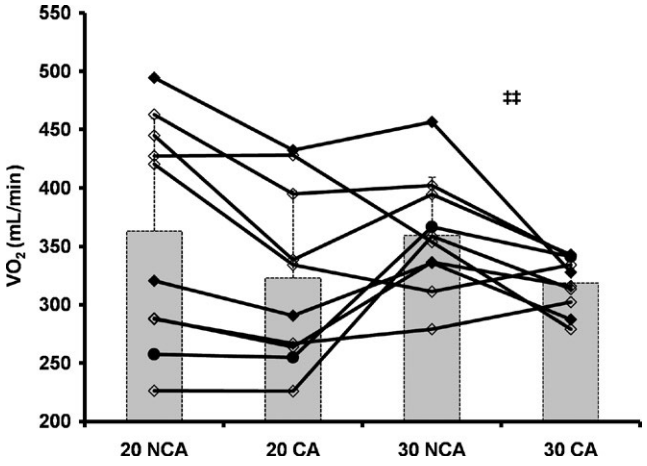

FIGURE 4 Individual and mean (SD) values (gray bars) of oxygen uptake (VO_2) during 30-minutes exposure to temperate (20°C) and warm (30°C) environmental temperature with (CA) and without (NCA) capsaicin application on the skin (N = 10). ‡‡Significant difference between CA and NCA (main effect: $P < 0.01$)

$P < 0.01$, respectively). Post-hoc analysis showed that the mean arterial pressure was lower at 30°C in CA compared to 30°C NCA ($P < 0.01$, $d = 1.8$), 20°C CA ($P < 0.01$, $d = 1.5$), and 20°C NCA ($P < 0.05$, $d = 1.4$; Figure 5A).

3.2.3 | Systolic and diastolic pressure

Significant main effects of ambient temperature and experimental condition were found on both systolic and diastolic pressure. Systolic pressure was lower at 30°C in CA compared to 30°C NCA ($P < 0.05$, $d = 1.2$), 20°C NCA ($P < 0.01$, $d = 1.3$), and 20°C CA ($P < 0.01$, $d = 1.3$; Figure 5B). Likewise, the diastolic pressure was lower in 30°C CA than in 30°C NCA ($P < 0.01$, $d = 1.7$), 20°C NCA ($P \leq 0.05$, $d = 1.4$), and 20°C CA ($P < 0.01$, $d = 1.5$; Figure 5C).

3.2.4 | Total peripheral resistance and cardiac output

Total peripheral resistance (TPR) was similar between 20 and 30°C (main effect: $P = 0.77$), but it was lower in capsaicin than in non-capsaicin condition (main effect: $P = 0.02$, $d = 0.41$). In addition, a significant interaction (ambient temperature × capsaicin) was found ($P < 0.01$). The post-hoc analysis revealed that significant differences in TPR between CA and NCA were evident only at 30°C ($P \leq 0.01$, $d = 1.3$; Figure 5D). In addition, no differences were observed in cardiac output, stroke volume, and heart rate among the experimental trials. Namely, cardiac output was similar between 30 and 20°C as well as between capsaicin *vs.* non-capsaicin conditions. Regarding both stroke volume and heart rate,

TABLE 1 Selected cardiorespiratory responses during 30-minutes resting exposure to 20°C and 30°C without (NCA) and with (CA) capsaicin application on the skin (N = 10). All values are mean (SD)

	20°C		30°C		Effect of temperature	Effect of condition
	NCA	CA	NCA	CA	*P*	*P*
VO_2 (mL/min)	363 (97)	323 (75)	359 (50)	319 (23)	0.87	0.002
VE (L/min)	9.8 (2.7)	9.4 (1.6)	10 (1.1)	8.9 (1.0)	0.81	0.11
RR (breaths/min)	17.7 (4.4)	17.8 (4.0)	16.4 (3.1)	18.4 (5.1)	0.72	0.36
HR (beats/min)	68.6 (7.8)	66.2 (9.0)	71.4 (8.8)	71.8 (11.7)	0.06	0.54
SV (L/min)	103.2 (18.0)	101.7 (17.5)	92.1 (14.5)	97.6 (22.6)	0.09	0.31
CO (L/min)	6.9 (0.9)	6.6 (0.8)	6.6 (1.1)	6.9 (1.3)	0.84	0.64

CO, cardiac output; HR, heart rate; RR, respiratory rate; SV, stroke volume; VE, ventilation; VO_2, oxygen uptake.

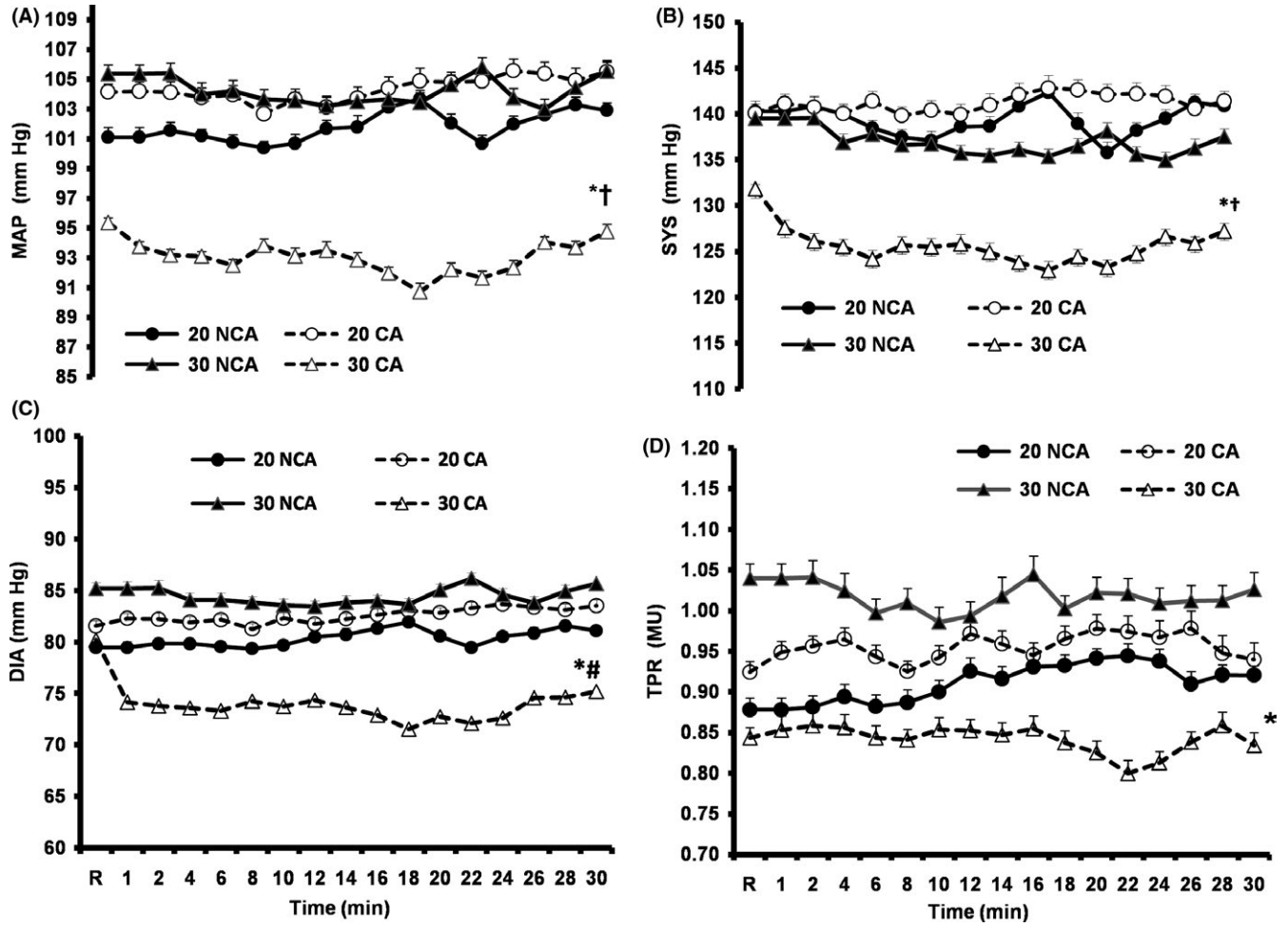

FIGURE 5 Mean (SE) arterial pressure (MAP, A), systolic pressure (SYS, B), diastolic pressure (DIA, C), and total peripheral resistance (TPR, D) during 30-minutes resting exposure to temperate (20°C) and warm (30°C) environmental temperature with (CA) and without (NCA) capsaicin application (N = 10). (A) *Significant difference between 30°C CA and 30°C NCA ($P < 0.01$, $d = 1.8$) and between 30°C CA and 20°C CA ($P < 0.01$, $d = 1.5$), †Significant difference between 30°C CA and 20°C NCA ($P < 0.05$, $d = 1.4$). (B) *Significant difference between 30°C CA and 20°C NCA ($P < 0.01$, $d = 1.29$) and between 30°C CA and 20°C CA ($P < 0.01$, $d = 1.3$), †Significant difference between 30°C CA and 30°C NCA ($P < 0.05$, $d = 1.2$). (C) *Significant difference between 30°C CA and 30°C NCA ($P < 0.01$, $d = 1.7$) and between 30°C CA and 20°C CA ($P < 0.01$, $d = 1.5$). #Significant difference between 30°C CA and 20°C NCA ($P \leq 0.05$, $d = 1.4$). (D) *Significant difference between 30°C CA and 30°C NCA ($P \leq 0.01$, $d = 1.3$)

the analysis showed a tendency for lower stroke volume and higher heart rate values in 30°C than in 20°C ($P = 0.09$ and $P = 0.06$, respectively) and no differences between capsaicin and non-capsaicin condition (Table 1).

4 | DISCUSSION

We investigated the effects of capsaicin application on the skin on resting thermoregulatory and cardiovascular responses during exposure to temperate and warm conditions. The principal findings of the present study are: (a) regardless of environmental temperature, capsaicin application on the skin reduced heat conservation due to lower vasomotor tone in conjunction with lower heat production and (b) in warm condition, application of capsaicin patches were accompanied by arterial hypotension accredited to the lower total peripheral resistance compared to the temperate condition.

In accordance with our initial hypotheses, we observed that regardless of environmental temperature, the application of capsaicin on the skin reduced heat storage and resulted in a significant heat loss. Namely, the rectal temperature decline was greater in capsaicin than in non-capsaicin condition and this response was similar between temperate and warm conditions. Additionally, after capsaicin application both local vasomotor tone and VO_2 were lower by 36.7% and 12.3%, respectively, compared to the respective values observed in the non-capsaicin conditions. In this context, experiments in animals have demonstrated that capsaicin administration induces heat loss[1,12] due to the heat-sensitive TRPV1 channel

activation and that the blockade of TRPV1 induced hyperthermia by increasing skin vasoconstriction and thermogenesis.[26]

Although skin blood flow was not presently measured, the considerable reduction in the local vasomotor tone after capsaicin treatment implies higher skin blood flow,[27] which suggests increased heat loss. The present results are in agreement with earlier[6] and more recent observations[8] suggesting that the activation of TRPV1 plays a crucial role in cutaneous active vasodilation. In particular, Stephens et al[6] detected higher cutaneous vascular conductance after capsaicin treatment. Likewise, Wong and Fieber[12] indicated that the inhibition of TRPV1 attenuated the cutaneous active vasodilation, thus suggesting that TRPV1 nociceptors are important in the cutaneous vasodilator response. Accordingly, in the present study we observed that the rate of change in Tskf-f in the initial phase of the experimental procedure (ie, first 6 minute) was higher in capsaicin than in non-capsaicin condition, which was probably caused by an increased stimulation of heat-sensitive TRPV1, elicited by capsaicin application. It is also of note that we detected higher values of thermal sensation when subjects were treated with capsaicin. This finding supports previous observations in humans[5] showing warm or burning sensation after capsaicin administration. Moreover, the decline of rectal temperature in capsaicin conditions caused by TRPV1 stimulation was expected to increase resting oxygen consumption by 2% due to the Q_{10} effect.[28] In the present study, however, resting oxygen uptake was decreased by 12.3% despite the decline in rectal temperature. This was further substantiated by the fact that a significant correlation was detected between oxygen uptake and rectal temperature decline after capsaicin administration ($r = 0.69$, $P < 0.01$), whereas no such association was revealed ($r = -0.17$, $P > 0.05$) in non-capsaicin condition. This finding, for the first time in humans, supports the hypothesis that convergence of peripheral warm input at central neuroanatomical sites excites heat loss and concomitantly inhibits heat production. This cross-talk of the sensors input to opposite effectors pathways is known as reciprocal cross-inhibition in neuronal circuits.[13,20] It has been observed in animals where injection of a solution containing 5-hydroxytryptamine (5HT) in thermosensitive areas of the brain induced concurrent heat loss and a decrease in heat production.[29] Thus, capsaicin application on the skin mimics a thermal stimulus equivalent to a temperature of 43°C and appears to be a novice experimental approach for the study of cross-inhibition theory when combined with different ambient temperatures as in the present study.

An interesting finding of the present study was that our participants presented lower mean arterial as well as systolic and diastolic blood pressure at 30°C with capsaicin treatment compared to the other three conditions, wherein blood pressure remained unchanged. The reduction in human blood pressure observed in the current study is in line with studies conducted in rodents,[26,30] indicating a promising target for therapeutic intervention of hypertension.[31] Several mechanisms have been proposed to explain the underlying mechanisms behind the TRPV1 activation-induced hypotension. First, the activation of TRPV1 channel has been shown to produce vasodilation by increasing the phosphorylation of endothelial NO synthase.[31,32] Noteworthily, Yang et al[31] observed that TRPV1 activation by capsaicin enhanced endothelium-dependent vasorelaxation and that this effect was absent in TRPV1 deficient mice. Moreover, the activation of TRPV1 channels may induce vasodilation via increased release of calcitonin gene-related peptide (CGRP).[33] In support of this, recent findings indicate that the activation of TRPV1 channels through capsaicin ingestion modulate vascular function by opposing a-adrenergic receptor-mediated vasoconstriction and potentiating vasorelaxation.[34] Hence, the observed lower total peripheral resistance is possibly linked with the capsaicin-induced vasorelaxation or with NO increase and/or CGRP release. Additionally, experiments in animals have demonstrated that capsaicin reduces the synthesis of prostaglandins, which stimulate group III and IV muscle afferent fibers[35] and raise blood pressure through increased sympathetic release.[17] In this context, the administration of capsaicin both in animals[36] and humans[16] has been shown to attenuate the activation of group III and IV afferent fibers evoked by muscle contraction and to reduce peak blood pressure. Nonetheless, the present data imply that the hypotensive effect of capsaicin is temperature-dependent, as the blood pressure reduction was evident only in 30°C. This suggests that the decline in blood pressure occurs as a consequence of the interactive effects of warm temperature and capsaicin application on cutaneous vascular relaxation. It has been proposed that interactive effects could arise when combining two stressors, which are mechanistically similar (in our case 30°C and capsaicin application corresponding to 43°C), while additive effects may result from combining stressors subserving different mechanisms.[37] This argument is presently supported by the lower total peripheral resistance observed in warm compared to temperate environment. In interpreting this observation, it is worth considering recent observations on the role of other TRPV channels on vascular regulation.[38] In particular, Gifford et al[38] showed that during exposure to warm environment the stimulation of TRPV4 channels, which present a threshold of activation at around 30°C,[11] elicits sympatholysis-like inhibition of α1-adrenergic vasocontraction in human skeletal muscle feed arteries. Thus, apart from TRPV1, a possible role of TRPV4 channel on human vascular regulation during exposure to warm environment cannot be excluded. Conversely, any effect of warm environment and capsaicin administration on blood pressure was not apparent in temperate condition, in which the activation of cold-sensitive channels (TRPM8)[10,39] may counteract vasorelaxation.

The current study demonstrates that regardless of environmental condition (temperate or warm) TRPV1 activation by capsaicin application on the human skin increases heat loss. These effects are mediated through greater skin vasodilation and lower VO_2 response. These responses are in agreement with the Sherrington's theory of centrally mediated reciprocal cross-inhibition of stimuli, which assumes that peripheral effectors with opposite function cannot counteract each other during homeostatic maneuvers. Our study also suggests that there is an interactive role of skin surface capsaicin application and warm environment in the cardiovascular regulation, since only in the warm condition the capsaicin enhancing heat loss effects were also accompanied by arterial hypotension.

It is noteworthy that due to the burning effect of capsaicin, it was not possible to keep the experimental design blind. Thus, placebo effect cannot be excluded. Based on strong similarity of capsaicin physiological response between our data and data on animals,[1,12] it could be alleged that the placebo effect in our study must have been minimal and insignificant. Our data are interpreted mainly by several studies conducted on animals. It is acknowledged however, that thermoregulatory reactions might differ between animals and humans. Along this line, we also accept that the magnitude of the epidermal capsaicin application effect on thermal and cardiovascular responses may depend on the size of the animal. Moreover, we acknowledge that a large standard deviation is observed in some of the data, which is associated with the inter-day variation of physiological responses. The present study examined only male subjects and thus our results cannot be generalized to females.

5 | PERSPECTIVES

In the present study, we showed that, regardless of environmental condition, TRPV1 activation by capsaicin application on human skin reduces heat storage due to enhanced heat loss in resting participants. In addition, heat loss effects observed in 30°C after the application of capsaicin were accompanied by arterial hypotension accredited to the lower total peripheral resistance compared to the control condition. The absence of differences in blood pressure when subjects were exposed to 20°C indicates a possible role of other warm receptors on vascular relaxation in other tissues beyond skin. Therefore, further research is required to explore the contribution of skin warm receptors on tissue vascular relaxation. Moreover, in our study the capsaicin patches were applied on four different parts of the human body; the participants, however, anecdotally reported higher thermal or burning sensation on the back and chest sites compared to the quadriceps, which is most probably due to the higher quantity of warm receptors in these sites.[40] Therefore, it is still unknown whether capsaicin effect fluctuates among different areas of the human body and as such, the investigation of a more targeted application of capsaicin is needed.

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