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Hand cooling induces changes in the kinetics of oxygen uptake

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The objective of this investigation was to assess the impact of elevated catecholamine concentrations, induced through cold-water hand immersion, on the oxygen consumption ($\dot{V}O_2$) kinetics during intense exercise, and to contrast this effect with that of the priming effect. Ten active participants underwent three 8-minute constant work rate exercises (CWR) at Δ25%, with one CWR preceded by hand cooling (2 min at 0 °C, HC) and two consecutive CWR to induced priming effect on the second bout (SB). Pulmonary gas exchange and blood samples were analyzed to measure levels of epinephrine (E) and norepinephrine (NE). Results demonstrated a significant increase in the primary phase amplitude of $\dot{V}O_2$ kinetics in response to both hand HC (33.9 mL.min^{-1}.kg^{-1}; CI [32.2;35.7], $p < 0.001$) and SB (34.6 mL.min^{-1}.kg^{-1}; CI [33.0;36.3], $p < 0.001$) relative to the control (32.7 mL.min^{-1}.kg^{-1}; CI [31.5;35.1]). Additionally, the amplitude of the $\dot{V}O_2$ slow component was reduced for both HC (3.2 mL.min^{-1}.kg^{-1}; CI [2.2;4.1], $p = 0.018$) and SB (2.9 mL.min^{-1}.kg^{-1}; CI [1.8;4.2], $p = 0.009$) in comparison to control (3.9 mL.min^{-1}.kg^{-1}; CI [2.9;4.2]). These findings suggest that the increase in E and NE induced by hand cooling prior to exercise modifies $\dot{V}O_2$ kinetics in a manner akin to the priming effect. This research underscores the potential role of catecholamines in facilitating the priming effect and its subsequent impact on $\dot{V}O_2$ kinetics. However, further studies are necessary to clearly establish this link.

Keywords Oxygen consumption, VO2 kinetic, Hand cooling, Catecholamines

Oxygen uptake (VO_2) kinetics during constant work rate (CWR) exercise under the gas exchange threshold (GET) intensity has been described by two distinct phases[1,2]. First, a cardio dynamic phase (Phase I) is associated with a rapid increase in VO_2 and can be characterized by a mono exponential function. Then, a primary phase (Phase II), which also follows a mono-exponential rise of an amplitude (Ap) and a time constant (τp) reaches a steady state within 2–3 min during CWR exercise.[3] Above GET intensity, a third slow exponential increase of VO2 (Phase III) appears and has been identified as the slow component of VO_2 (VO_{2sc})[4].

VO_2 kinetics can be influenced by different factors such as ageing[5], endurance training[6] or the effect of a previous physical exercise[7]. The prior supra GET intensity exercise effect, also known as "priming effect", is referring to a modification of the overall VO_2 kinetics during the second bout of a two-bout supra GET intensity exercise[8,9], with an increase in the amplitude of the primary component and a decrease in the amplitude of the VO_{2sc} [7,10]. The VO_2 kinetics could also be accelerated by a faster τp but this remains a matter of debate as mixed results have been found across studies[11]. Recently, a review of the last 25-year literature suggested that the priming effect might be experimentally explained by an increase in intracellular oxygen utilization and/or delivery, as well as by a modification in the pattern of motor unit recruitment [11].

Catecholamines such as epinephrine (E) and norepinephrine (NE) are secreted in response to exercise[12]. On one hand, it has been observed that E infusion altered oxygen utilization by increasing metabolic rate up to 34% in animals at rest[13] as well as by increasing the quantity of available substrates (e.g. stimulation of glycogenolysis, utilization of glucose and/or fatty acids)[12,14]. On the other hand, NE has been shown to increase the excitability of motoneurons in animal studies[15], while drugs enhancing NE activity modify the size of the H and stretch reflexes in humans[16,17]. Moreover, catecholamines impact the muscle O_2 delivery because of their effects on the cardiovascular system (i.e. increased HR, alteration of vascular adaptation), visceral smooth muscles and respiratory system[12]. Thus, given that catecholamines can influence the same processes as those proposed to mediate the priming effect, it can be hypothesized that they represent key drivers of the priming effect.

So far, a few studies have explored the effect of E infusion on VO_2. One study demonstrated that continuous E infusion (0.04 μg.kg^{-1}.min^{-1}) during a 45-min exercise on a cycle ergometer at an average intensity of 54% of maximal oxygen consumption (VO_{2max}) induced an average 7% increase in O_2 utilization[18]. Another study

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showed that E (100 ng.kg^{-1}.min^{-1}) infused 10 min after initiation of a cycling exercise did not affect VO_{2sc} during the following 20 min of exercise performed at an average intensity of 65% of VO_{2max}[19]. Yet, there are currently no data available regarding the effect of elevated E concentration on the primary phase.

In addition to physical exercise, E and NE secretion can be increased by several factors such as hypoglycemia[20], hypoxia[21], acidemia[21], caffeine[22], and cold exposure[23–25]. Indeed, hand immersion in ice-cold water (i.e. 0–5 °C) is well known to stimulate the sympathetic nervous system[23–25].

While the factors influencing VO_2 kinetics are relatively well established in the literature, the mechanisms governing the priming response remain elusive. As a result, warm-up strategies cannot currently be fully optimized to take full advantage of this priming effect. Although it has been suggested that catecholamines may play a role in driving the priming effect, their influence on the VO_2 kinetics is incompletely understood. This study therefore aimed to test the hypothesis that an indirect stimulation of the adrenergic system induced by hand cooling modifies VO_2 kinetics in a similar manner as a previous bout of exercise (i.e. priming effect). To test this hypothesis, the present study (i) evaluated the effects of blood increases in E and NE induced by cold-water hand immersion on VO_2 kinetics during a heavy intensity exercise bout, and (ii) determined the extent to which increases in E and NE induced by cold-water hand immersion can replicate the priming effect observed in response to a previous bout of heavy exercise. It was hypothesized that after a first bout of heavy-intensity exercise blood, catecholamine concentrations would remain elevated for several minutes leading to higher catecholamine concentrations present in the blood at the start of a second bout of exercise leading to an increase in A_p. VO_{2sc} should remain unaffected by the natural elevation of catecholamines induced by exercise stress, which is expected to raise their concentration levels above those induced solely by hand cooling.

Methods

Participants

A sample size calculation was performed using G*power and indicated that a sample size of $n = 10$ was needed to ensure that a repeated measures analysis of variance could detect an effect size of F = 0.3 with 80% power at a significance level of 5%.Ten healthy males (mean ± standard deviation (SD), age 23 ± 5 years, body mass 71 ± 6 kg, height 182 ± 6 cm) volunteered to participate to this study. Participants were at a recreational exercise level according to the World Health Organization definition and were accustomed to the laboratory procedures. The University of Auckland Human Participants Ethics Committee approved this study (2012–8209), and all procedures complied with the latest version of the declaration of Helsinki. All participants provided written informed consent prior to participation in the study. Inclusion criteria were (i) aged 18 to 40 years old, (ii) no chronic illness and (iii) complete positively the Physical Activity Readiness questionnaire. Participants were asked to avoid strenuous physical activity, alcohol, tobacco, and caffeine, in the 24 h prior to the testing session. Furthermore, participants were asked to remain hydrated and not to consume any food in the 2 h preceding a test.

Experimental design

Participants were asked to visit the laboratory three times over a period of two weeks. There was a minimum 48-hour period between two testing days. During the first visit, an incremental ramp test was performed on an electrically-braked cycle ergometer (Velotron, RacerMate, USA) to determine the intensity of the CWR exercises performed during the following sessions. During the second visit, two sets of CWR exercises separated by one hour of passive recovery were performed by the participants. One set consisted of 3 min of passive rest followed by 2 min of hand cooling in 0 °C-water, immediately followed by a CWR bout referred to as HC. The protocol is based on the foundational work of[26] in which hand cooling was performed at 4 °C for 1 to 2 min. To ensure a robust activation of the sympathetic nervous system, and target both norepinephrine and epinephrine release, a colder temperature of 0 °C for 2 min was chosen. This adjustment was made to intensify the physiological response and ensure consistent maximum sympathetic output, aligning with experimental design from studies using lower temperatures[27].The second set consisted of a 3-min rest followed by a first CWR bout (FB), which was followed by a second CWR bout (SB) realized after 9 min of passive recovery. The order in which the two sets were performed was randomized. The third visit was identical to the second one, but the order of sets was reversed in order to obtained two repetitions of each modality. Pulmonary gas exchange and heart rate (HR) were collected prior, during and after exercises and hand cooling, while blood samples were harvested before and after hand cooling and before and after each exercise bout.

Incremental ramp test

Participants completed a ramp incremental exercise test to determine $\dot{V}O_2peak$ and GET. Participants performed 3 min of cycling at 100 W. The work rate then gradually increased by 1 W every two seconds (30 W/min) until exhaustion. Breath-by-breath pulmonary gas exchanges were monitored throughout the test, and the $\dot{V}O_2peak$ was calculated as the mean value of the last 30-s attained before exhaustion. GET was identified through a series of determinations, comprising: (1) the initial discernible rise in CO_2 output (VCO_2) as observed from individual VCO_2*versus* VO_2 plots, (2) a rise in the ratio of expired ventilation (VE) to VO_2 without a corresponding rise in VE/VCO_2, and (3) an elevation in end-tidal oxygen partial pressure ($PetO_2$) without a decrease in end-tidal carbon dioxide partial pressure ($PetCO_2$).

CWR

CWR consisted of an 8-min heavy exercise at a power level eliciting a $\dot{V}O_2$ equal to Δ25 (GET plus 25% of the difference between the work rate at the GET and $\dot{V}O_2$peak) at 85 revolutions per minute.

Measurement

Pulmonary gas exchange and heart rate

Breath-by-breath pulmonary gas exchanges (O_2 and CO_2) and ventilation (VE) were measured continuously throughout all the exercise sessions using a MetaMax 3B computerized system (Cortex, Cologne, Germany). VO_2 and VCO_2 data were calculated and displayed breath-by-breath using Metasoft software (Cortex, Cologne, Germany). Immediately prior to each exercise, the gas analyzers and flowmeter turbine were calibrated with known gas concentrations (O_2 = 14.01% and CO_2 = 6.03%) and with a 3-l Rudolph syringe (Hans Rudolph, Kansas City, MO), respectively. A polar HR monitor (A3, Polar, Finland) was used to monitor HR.

Blood samples collection and analyses

During the second and third visits, a cannula was inserted into the brachial vein of the participant preferred arm 30 min before the start of exercise. During both of these sessions, 5 mL of venous blood were collected in standard EDTA vacutainer tubes before and after each cycling bout, as well as before hand cooling (i.e. 7 blood samples drawn during each visit, Fig. 1). Immediately upon collection, blood was centrifuged at 2000 g for 10 min and plasma was collected prior to being stored at − 80 °C. NE and E concentrations were subsequently measured by a professional and independent laboratory (Cardinal bioresearch, AUS) on all plasma samples using High-Performance Liquid Chromatography (HPLC) coupled with Electrochemical Detection (ED) .

Data Analysis

Oxygen consumption analysis

The breath-by-breath VO_2 data from each test was initially examined to exclude errant breaths that may have arisen from swallowing, sighing, or coughing. Residue values lying more than three standard deviations from local average were also removed. The breath-by-breath data was subsequently linearly interpolated to produce second-by-second values, and the two identical repetitions of each modality were time aligned to the start of exercise and the ensemble averaged. The first 20 s of data after the onset of exercise (i.e. cardio dynamic phase) were removed[1,2,4]. A bi-exponential model was used to characterize the VO_2 responses:

$$\dot{V}O_2(t) = \dot{V}O_{2rest} + A_P(1 - e^{-(t\text{-}TD_p)/\tau p}) + A_s(1 - e^{-(t\text{-}TD_s)/\tau s}) \quad (1)$$

Where $VO_{2(t)}$ represents the oxygen consumption at a given time t; VO_{2rest} the VO_2 at rest; A_p, TD_p, and τ_p represent the amplitude, time delay, and time constant, respectively describing the primary phase; and A_s, TD_s, and τs represent the amplitude, time delay, and time constant describing the VO_{2sc}. A nonlinear least squares algorithm was used to minimize the error between model and data. Since the asymptotic values (A_s) of the exponential terms describing the VO_{2sc} may represent a higher value than the one reached, the amplitude of VO_{2sc} were defined as A_s' and calculated as follows:

$$A_s' = A_s(1 - e^{-(tend\text{-}TD_s)/\tau s}) \quad (2)$$

were t_{end} is the time at the end of exercise.

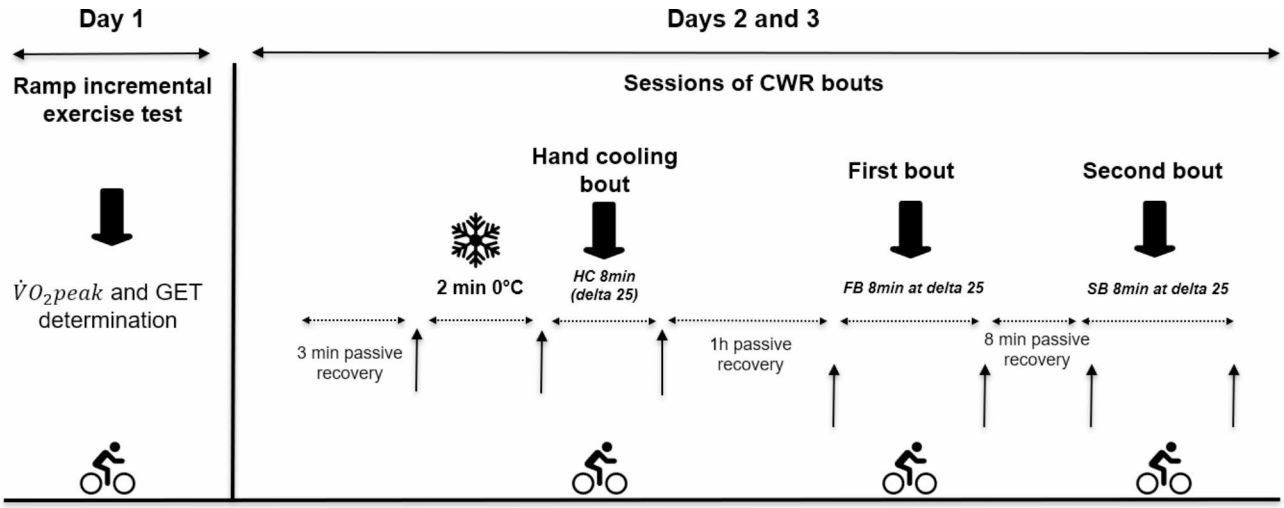

Fig. 1. Sequence of the experimental protocol. Participants were randomized into three conditions for a counter-balanced crossover study. During the first visit, participants were asked to perform a ramp incremental exercise test. Then, on two occasions, participants performed the same constant work rate (CWR) exercise on ergocycle (FB), preceded or not by hand cooling (HC) and followed by a second bout of the same exercise (SB). One hour-break was conceded between HC and FB, and 9 min between FB and SB. Arrows indicate times at which blood samples were collected.

The primary prime amplitude (A_p') refers to the amplitude of the primary phase of $\dot{V}O_2$ at TDs, while the absolute prime amplitude of the primary phase (Absolute A_p') was defined as the sum of $\dot{V}O_{2baseline}$ and A_p'. The mean response time (MRT) correspond to the sum of the time delay and the time constant of the primary phase (i.e. $TD_p + \tau_p$). The baseline of $\dot{V}O_2$ ($\dot{V}O_{2baseline}$) was calculated as the mean of the last minute of rest before exercise.

Heart rate analysis

HR pre-exercise (HR_{pre}) was defined as the average HR recorded during the last minute preceding CWR exercises, and HR end-exercise (HR_{end}) was computed as the average HR recorded during the last minute of CWR exercises.

Statistics

Due to the non-normality of the distribution and the presence of non-sphericity within the data, a bootstrapping approach has been used for an analysis of variance on repeated measures. Thanks to this method, a global hypothesis test has been made to determine if the mean of the independent variable was the same in all conditions. The distribution of the decision variable (F of Fisher-Snedecor) specific to H_0 has been construct with bootstrap with sampling on centered measurements[28]. Then, it was possible to calculate the probability of the decision variable F* taking a value greater than or equal to the empirical value of the decision variable F. This probability corresponded to the *p*-value. A bootstrap multiple comparison was performed. Two approaches were used. The first is analogous to the hypothesis test explained above, while the second consisted in calculating a confidence interval for each condition.

Bootstrap consisted in setting several bootstraps resamples (1000). For each iteration, the following procedure was applied: (1) Randomly sample data with replacement; (2) Apply the ANOVA model to the bootstrap sample; (3) Record parameter estimates. Parameter estimation is done by aggregating parameter estimates from the 1000 iterations allowing for robust estimates. Then a construction of confidence intervals based on the bootstrap distribution was done. The bootstrap distribution was utilized to conduct hypothesis tests on model parameters as part of the model validation process. This involved evaluating the model fit by comparing it with observed data and analyzing residuals. Validation conditions were controlled and satisfactory.

Values are expressed as mean ± 95% CI [lower limit, upper limit]. Statistics significance was set at $p < 0.05$ with a Bonferroni correction for the multiple comparison and effect sizes (d) were calculated and interpreted as follow: trivial effect ($d < 0.10$), small effect ($d = 0.20$), medium effect ($d = 0.50$), and large effect ($d = 0.80$)[29].

Results

Incremental test and heavy square wave exercise

Participants $\dot{V}O_{2peak}$ was 47.9 $mL.min^{-1}.kg^{-1}$, 95% CI [39.8;53.5]. The oxygen uptake at GET was 28.2 $mL.min^{-1}.kg^{-1}$, 95% CI [22.9;36.5] representing 59% of $\dot{V}O_{2peak}$. The mean value of $\dot{V}O_2$ at delta 25% was 33.12 $mL.min^{-1}.kg^{-1}$, 95% CI [27.2;40;7] representing an estimated oxygen consumption of 69% of $\dot{V}O_{2peak}$. Typical $\dot{V}O_2$ of a participant as well as the mean bootstrapped $\dot{V}O_2$ of all participants are presented in Fig. 2.a et 2.b respectively.

Catecholamines concentrations

E concentrations before and after hand cooling were significantly different ($d = 4.04$, $p = 0.032$) increasing by 347% from 0.3 nM, 95% CI [0.2;0.4] prior to hand cooling to 1.09 nM, 95% CI [0.7;1.4] after hand cooling. Regarding E concentrations before exercise (Fig. 3b), significant differences were found between FB (0.37 nM, 95% CI [0.2;0.4]) and HC (1.09 nM, 95% CI [0.7;1.4], $d = 1.97$, $p < 0.001$), between FB (0.37 nM, 95% CI [0.2;0.4]) and SB (0.6 nM, 95% CI [0.4;0.7], $d = 3.80$, $p < 0.001$), and between HC (1.09 nM, 95% CI [0.7;1.4]) and SB 0.6 nM, 95% CI [0.4;0.7], $d = 4.77$, $p < 0.001$)

At the end of exercise (Fig. 3d), no significant differences were observed in E concentrations between FB (1.44 nM, 95% CI [0.8;2.0]) and HC (1.54 nM, 95% CI [0.9;2.4], $d = 0.65$, $p = 0.176$) while E concentration at the end of SB (2.35 nM, 95% CI [1.5;3.3]) was significantly higher than at the end of FB (1.44 nM, 95% CI [0.8;2.0], $d = 4.07$, $p < 0.001$) and HC (1.54 nM, 95% CI [0.9;2.4], $d = 4.49$, $p < 0.001$).

NE concentration significantly increased from 5.35 nM, 95% CI [4.5;6.1] to 6.98 nM, 95% CI [5.8;8.1] in response to hand cooling ($d = 4.53$, $p = 0.011$). NE concentrations from SB (6.90 nM, 95% CI [5.6;8.2]) and HC (6.98 nM, 95% CI [5.8;8.1]) were not significantly different ($d = 3.41$, $p = 1$; Fig. 3a) but were significantly higher than NE concentration measured prior to FB (5.56 nM, 95% CI [4.6;6.7], $d = 0.45$, $p = 0.001$ and $d = 2.00$, $p < 0.001$, for HC and SB respectively). At the end of exercise (Fig. 3c), lower NE concentrations were recorded following FB (18.16 nM, 95% CI [13.0;23.5]) compared to HC (20.15 nM, 95% CI [14.9;25.4] ; $d = 1.76$, $p < 0.001$) and SB (20.41 nM, 95% CI [15.1;26.1]; $d = 2.56$, $p = 0.001$).

Heart rate

Before exercise, HR was significantly higher prior to HC (95 bpm, 95% CI [87.6;103.2]) and SB (97 bpm, 95% CI [86.9;108.0]) compared to FB (78 bpm, 95% CI [69.6;86.5], $d = 1.55$, $p = 0.03$ and $d = 2.23$, $p = 0.003$), while at the end of exercise, HR was significantly higher during SB (163 bpm, 95% CI [153.4;172.7]) compared to during FB (155 bpm, 95% CI [145.8;165.4] bpm, $d = 1$, $p < 0.001$) and HC (156 bpm, 95% CI [147.3;166.2] bpm, $d = 1$, $p = 0.003$).

$\dot{V}O2$ kinetics

The different variables of $\dot{V}O_2$ kinetics are presented in Table 1. $\dot{V}O_{2baseline}$ was significantly lower prior to FB than SB ($d = 3.74$, $p < 0.001$) and HC ($d = 1.46$, $p = 0.006$). A_p and Absolute A_p' were significantly higher during SB than during FB ($d = 2.07$, $p < 0.001$ for A_p and $d = 1.05$, $p < 0.001$ for Absolute A_p') and HC ($d = 0.31$,

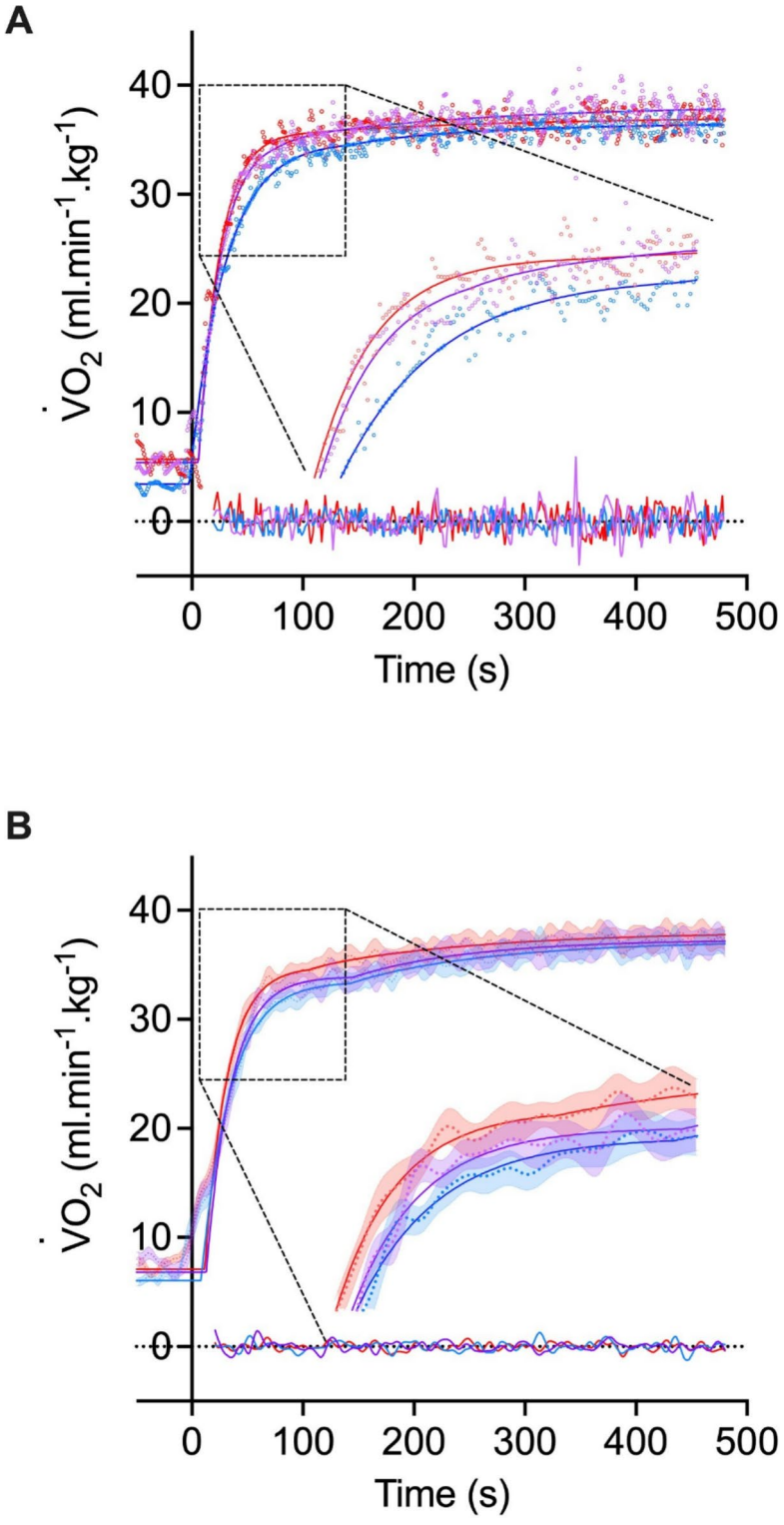

Fig. 2. Figure 2.a represent the raw VO_2 signal of one participant in each condition. Figure 2.b represent the mean bootstrapped VO_2 of all participants in each condition including the corresponding residual plots. Red represents the second bout (SB) condition, blue indicates the first bout (FB) condition, and purple corresponds to the hand cooling (HC) condition.

$p < 0.001$ and d = 0.63, $p = 0.003$, respectively). Absolute A_p' recorded during HC was also significantly higher than Absolute A_p' recorded during FB (d = 2.11, $p < 0.001$). TD_p was significantly higher during HC than during FB (d = 1.46, $p < 0.001$) and SB (d = 1.21, $p < 0.001$), while no significant difference was found for τp between HC and FB (d = 0.53, $p = 0.306$), FB and SB (d = 1.01, $p = 0.063$) and SB and HC (d = 0.79, $p = 0.072$). With

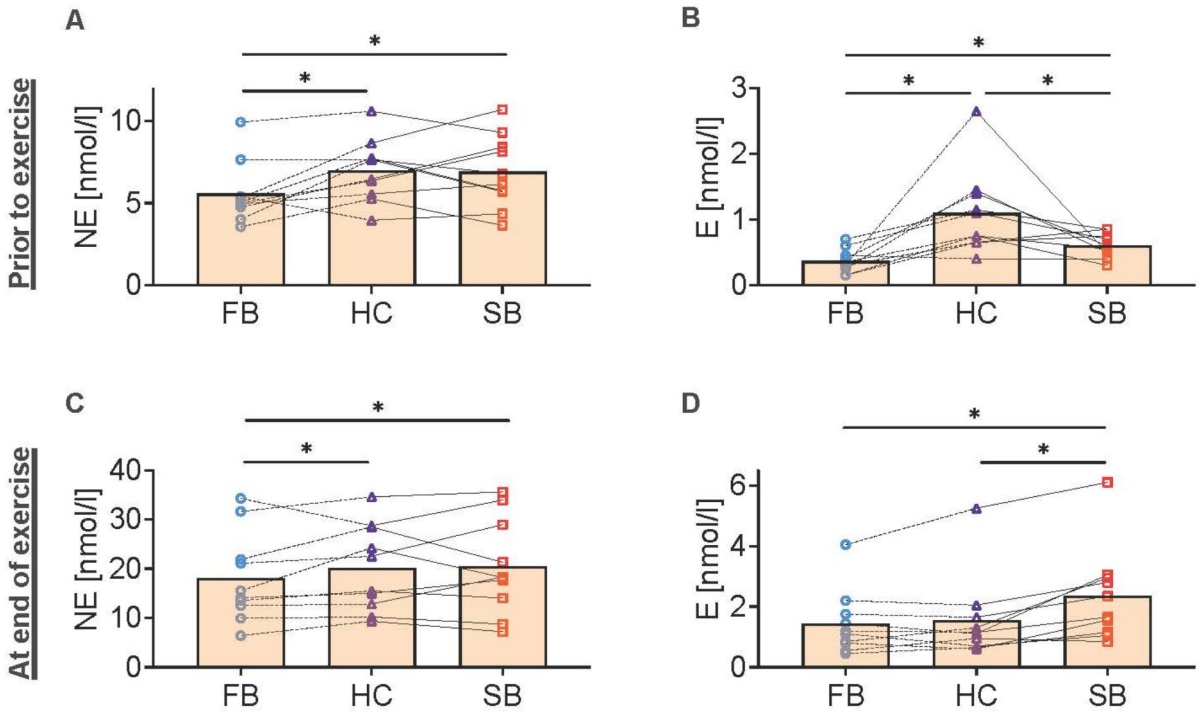

Fig. 3. Epinephrine and Norepinephrine plasma concentration during each condition. HC, exercise after hand cooling; FB control bout; SB second exercise bout." * " represents the significant difference between conditions. Figure 2.a represents the Norepinephrine plasma concentration [nmol/l] before the onset of exercise. Figure 2.b represents the Epinephrine plasma concentration [nmol/l] before the onset of exercise. Figure 2.c represents the Norepinephrine plasma concentration [nmol/l] at the end of exercise while Fig. 2.d represents the Epinephrine plasma concentration [nmol/l] at the end of exercise.

respect to the slow component, A_s' was significantly lower during FB than during HC (d = 1.19, $p = 0.018$) and SB (d = 1.25, $p = 0.009$), while TD_s and τ_s were only significantly different between FB and HC (d = 0.99, $p = 0.042$ and d = 0.53, $p = 0.021$). MRT was significantly lower during SB compared to during HC (d = 2.11, $p < 0.001$) and FB (d = 1.05 $p = 0.015$). Lastly, VO_{2end} was different between SB and FB (d = 2.08, $p < 0.001$) and no differences were found compared to HC (d = 0.88, $p = 0.090$ and d = 0.58, $p = 0.192$).

Discussion

The results of this study showed that hand cooling increased Absolute A_p' and decreased the amplitude of the VO_{2sc}, indicating that hand cooling can alter the VO_2 kinetics in a similar manner but with less intensity to the priming effect induced by a prior bout of heavy exercise suggesting that other mechanisms are involved in the priming effect.

The present results showed that hand cooling increased plasma E and NE concentrations before the beginning of exercise compared to FB. In turn, these elevated E and NE concentrations increased HR, VO_2 and ventilation to values comparable to those observed before the SB exercise. This is consistent with the established effects of the activation of the beta-adrenergic receptors in response to elevated E (and to a lesser extent NE), including heightened HR and cardiac contractility, enhanced muscle blood flow, and increased ventilation. These physiological responses should ultimately contribute to greater muscle perfusion [12]. The increase in HR and ventilation suggests that a higher work is being performed by the myocardium and ventilatory muscles which may explain the increased $VO_{2baseline}$ observed in the present study following hand cooling and a first bout of exercise. Yet, it cannot be discarded that the increased $VO_{2baseline}$ could be the result of the effects of E and/or NE on cellular metabolism. Indeed, E has been shown to increase fat mobilization and triacylglycerol breakdown, glycogenolysis and gluconeogenesis[30]. For instance,[31] reported that an E infusion resulting in an increase in E concentration similar to the one observed in the present study led to an 18% increase in carbohydrate oxidation in healthy individuals. These authors further showed that this metabolism shift could be explained by increased muscle glycogenolysis along with an increased pyruvate dehydrogenase (PDH) activity (which represents the rate limiting step for the conversion of pyruvate to acetyl-CoA). Further supporting the ability of E to shift the energy metabolism, an increase oxidative phosphorylation was observed in mitochondria isolated from skeletal muscle following an E infusion[32], while a study using a rodent model demonstrated that E and NE could increase succinate dehydrogenase (SDH) and cytochrome c oxidase (COX) activity[33].Considering these previous studies, it can be hypothesized that the increased VO_2 recorded prior to exercise in the present study might be explained

	FB	HC	SB
Baseline			
$VO_{2baseline}$ ($mL.min^{-1}.kg^{-1}$)	6.1 [5.70; 6.51]	6.7 [5.45; 8.01]^{FB}	6.8 [6.36; 7.33]^{FB}
VO_2primary component			
Ap ($mL.min^{-1}.kg^{-1}$)	27.0 [25.90; 28.11]	27.2 [25.54; 28.98]	28.0 [26.46; 29.62]$^{HC, FB}$
TDp (s)	9.2 [5.57; 11.73]	12.8 [10.16; 15.19]^{FB}	11.1 [8.38; 13.81]^{HC}
τp (s)	24.5 [19.46; 29.98]	22.3 [19.54; 25.66]	20.3 [16.41; 24.22]
Absolute A_p' ($mL.min^{-1}.kg^{-1}$)	32.7 [31.51; 35.06]	33.9 [32.21; 35.73]^{FB}	34.6 [33.03; 36.27] $^{HC, FB}$
VO_2slow component			
A_s' ($mL.min^{-1}.kg^{-1}$)	3.9 [2.86; 4.92]	3.2 [2.21; 4.16]^{FB}	2.9 [1.76; 4.2]^{FB}
TD_s (s)	122.8 [91.99; 165.44]	145.1 [134.99; 163.20]^{FB}	115.9 [83.99; 180.00]
τ_s (s)	150.5 [105.19; 206.67]	112.4 [62.10; 174.03] ^{FB}	145.8 [78.13; 248.39]
Overall response			
MRT (s)	33.7 [29.96; 37.71]	35.1 [33.47; 36.71]	31.5 [28.96; 34.71]$^{HC, FB}$
VO_{2end} ($mL.min^{-1}.kg^{-1}$)	36.9 [35.05; 38.95]	37.2 [34.92; 39.62]	37.7 [35.76; 39.79] ^{FB}

Table 1. Oxygen uptake kinetics responses during each condition. Values are expressed as mean [lower and upper bound of 95% CI]. FB control exercise bout, HC, exercise bout after hand cooling, SB second exercise bout, $VO_{2baseline}$oxygen uptake during 1 min before the start of exercise, Ap amplitude of the primary component of oxygen uptake, TD_ptime delay of the primary component of oxygen uptake, τp time constant of the primary component of oxygen uptake; A_s' amplitude of the slow component of oxygen uptake, TD_stime delay of the slow component of oxygen uptake, $\tau_s$time constant of the slow component of oxygen uptake, VO_2end oxygen uptake of the total amplitude ($VO_{2baseline}$ + Ap + A_s'), MRT mean response time. HC, significantly different from hand cooling ($p < 0.05$), FB, significantly different from the first exercise bout ($p < 0.05$).

by a catecholamine-mediated increase in oxidative metabolism. Yet, future studies are required to test this hypothesis.

The present study further showed that hand cooling impacted VO_2 kinetics during a heavy/severe-intensity exercise in a similar manner as a previous bout of heavy/severe-intensity exercise. Specifically, an increased Absolute A_p' and decreased the amplitude of the VO_{2sc} was observed during the HC and SB exercise bouts. To date, several mechanisms have been proposed to explain how a prior bout of heavy/severe-intensity exercise affects VO_2 kinetics of a subsequent exercise, with several evidence supporting an altered motor unit recruitment and/or an enhanced intracellular O_2 utilization[34]. For instance, a study using ^{31}P magnetic resonance spectroscopy and electromyographic activity recordings supports these proposed mechanisms[35]. In this study, a greater electromyographic activity was recorded during the initial phase of an exercise bout when a prior heavy-intensity exercise was performed, suggesting an increase in motor unit recruitment during the early phase of the subsequent exercise. A greater motor unit recruitment occurred during the early phase of the subsequent exercise, a greater initial O_2 consumption (i.e. amplitude of the primary component) and reduced VO_{2sc} amplitude (due to less accumulation of by-products at a given power as a result of the anticipated lower force produced by each muscle fiber) might be expected.[35] indeed reported an increase in O_2 uptake during the early part of exercise along with a shift in the energetic system supporting ATP production from anaerobic to oxidative metabolism when a prior heavy-intensity exercise was performed (i.e., the priming phase[35]). As mentioned above, catecholamines, especially E, can enhance the oxidative metabolism providing a possible mechanistic explanation of how a prior bout of heavy-intensity exercise shifts ATP metabolism.The present results support such a mechanism, as an increase in E concentration in response to hand cooling increased absolute A_p' compared to FB.

It is to be noted that while hand cooling altered VO_2 kinetics in a similar manner as a prior bout of heavy/severe intensity exercise, it did not fully replicate the effects of such a prior exercise bout. For instance, A_p' was even more increased during SB compared to HC. While the present results do not allow to determine the exact mechanisms responsible for this difference, it can be speculated that this greater A_p' increase observed during SB compared to HC might be explained by a greater increase in E concentration during the initial phase of SB compared to HC. Indeed, E has been shown to increase fat mobilization and triacylglycerol breakdown, glycogenolysis and gluconeogenesis, and uncoupling of the oxidative phosphorylation (see[30] and [36] for reviews). Yet, it was not possible to measure E (and NE) at the end of the primary component due to the nature of its determination (i.e. determined after exercise completion). However, a more significant elevation in E levels was noted at the end of SB compared to HC, highlighting that that a prior session of heavy-intensity exercise appears to enhance E secretion during subsequent physical activity to a greater extent than hand cooling. A possible explanation for this might involve the prolonged elevation of cortisol concentrations in response endurance exercise performed at intensities above GET[37, 38]), and in turn the cortisol-mediated conversion of NE to E[39]. It might thus be hypothesized that FB led to an increase in cortisol concentration prior to SB that contributed to further increase E production during SB explaining the greater increased E secretion observed during SB. In contrast, hand cooling either did not increase cortisol or led to a lower increase than FB. While the present

results would align with this mechanism, they cannot confirm it as cortisol concentrations were not measured, and thus cannot discard other mechanisms to explain the differences observed between hand cooling and a prior bout of heavy/severe intensity exercise.

Taken together the present results established that hand cooling alters the VO_2 kinetics in a similar fashion as the priming effect and that catecholamines are likely to play a role in this response. Yet, the exact cellular and molecular mechanisms mediating this response remain to be fully determined. For instance, it appears clear that a deeper understanding of the influence of catecholamines during exercise is currently needed, particularly concerning oxygen consumption. While the role of catecholamines in the priming effect is currently not a well-established hypothesis, the results of this study suggest that the influence of catecholamines on the priming effect cannot be dismissed but might not be the only factor that influence the VO_2 kinetics as the effect of hand cooling didn't completely match the priming effect. Given the importance of the VO_2 kinetics for performance, further research is warranted to determine whether hand cooling and/or other strategies triggering an increase in catecholamine release could benefit performance and training.

The present study has several limitations. First, it is unknown if the present results can be translated to women. While women were eligible to participate, only two women volunteered and ended-up by dropping out due to mental burden associated with blood sampling. Second, the measurement of electromyographic activity, lactate and cortisol would have helped supporting or discarding mechanisms which could potentially explain our results. Third, while the present results allow to formulate a number of hypotheses that could explain the mechanisms through which both hand cooling and prior heavy/severe-intensity exercise can mediate the priming effect, future mechanistic studies are required to definitely test those hypotheses and establish the molecular and cellular mechanisms driving the priming effect. Yet, it is important to note that current technologies are limiting the ability to assess molecular and cellular processes during exercise.

Conclusion

The present study demonstrates that hand cooling altered VO_2 kinetic, positing hand cooling as a potential valuable strategy to implement during warm-ups to enhance performance. Given the increase in catecholamine concentrations observed in response to both hand cooling and a prior bout of heavy/severe-intensity exercise, the present results suggest that circulating catecholamines might mediate the observed changes in VO_2 kinetic (i.e. increase in VO_2 primary component amplitude and reduction in VO_2 slow component amplitude) and underscore the potential key role of catecholamines in mediating the priming effect. Future studies are however required to obtain a greater mechanistic understanding of these phenomena.

Data availability

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

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Author contributions

FB Conceived and designed the experiments: FB Performed the experiments.LF Analyzed the data. LF Designed the Tables and Figures. LF and DN Wrote the paper: .JPA Contributed reagents/materials/analysis tools: AS and FB. Revision of the manuscript: All the authors read the manuscript and approved the final version.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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