

Cardiovascular responses to forearm muscle metaboreflex activation during hypercapnia in humans

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Delliaux S, Ichinose M, Watanabe K, Fujii N, Nishiyasu T. Cardiovascular responses to forearm muscle metaboreflex activation during hypercapnia in humans. *Am J Physiol Regul Integr Comp Physiol* 309: R43–R50, 2015. First published April 22, 2015; doi:10.1152/ajpregu.00402.2014.—We characterized the cardiovascular responses to forearm muscle metaboreflex activation during hypercapnia. Ten healthy males participated under three experimental conditions: 1) hypercapnia (HCA, PET_{CO_2} : +10 mmHg, by inhalation of a CO_2 -enriched gas mixture); 2) muscle metaboreflex activation (MMA, by 5 min of local circulatory occlusion after 1 min of 50% maximum voluntary contraction isometric handgrip under normocapnia); and 3) HCA+MMA. We measured mean arterial pressure (MAP), heart rate (HR), and cardiac output (CO); calculated stroke volume (SV), and total peripheral resistance (TPR); and evaluated myocardial oxygen consumption ($\dot{M}V_{O_2}$) and cardiac work (CW) noninvasively. MAP increased in the three experimental conditions but HCA+MMA led to the highest MAP, CO, and HR. Moreover, HCA+MMA increased SV and was associated with the highest $\dot{M}V_{O_2}$ and CW. HCA and MMA exhibited inhibitory interactions with MAP, HR, TPR, $\dot{M}V_{O_2}$, and CW, increases of which were smaller during HCA+MMA than the sum of the increases during HCA and MMA alone (MAP: +28 ± 2 vs. +34 ± 2 mmHg, $P < 0.001$; HR: +15 ± 2 vs. +22 ± 3 bpm, $P < 0.01$; TPR: +1.1 ± 1.4 vs. +3.0 ± 1.5 mmHg·l·min⁻¹, $P < 0.05$; $\dot{M}V_{O_2}$: +50.25 ± 4.74 vs. +59.48 ± 5.37 mmHg·min⁻¹·10⁻², $P < 0.01$; CW: +59.10 ± 7.52 vs. +63.67 ± 7.71 ml mmHg·min⁻¹·10⁻⁴, $P < 0.05$). Oppositely, HCA and MMA interactions were linearly additive for CO (+2.3 ± 0.4 l/min) and SV (+13 ± 4 ml). We showed that muscle metaboreflex and hypercapnia interact in healthy humans, reducing vasoconstriction but enhancing SV.

hypercapnia; muscle metaboreflex; hypertension; stroke volume; myocardial oxygen consumption

REGULATION OF ARTERIAL BLOOD pressure involves the integration of humoral, hormonal, and neurogenic components. For example, whereas hypercapnia has direct humoral vasodilatory effects (19, 20), the neurally mediated central chemoreflex increases cardiovascular sympathetic nervous system activity, leading heart rate (HR), stroke volume (SV), cardiac output (CO), vascular resistance, and then blood pressure all to increase (31). During exercise, blood pressure is also regulated by muscle reflexes (2, 17). The muscle metaboreflex forms a second direct excitatory input for the

neural control of cardiovascular function (21, 29, 42) that also stimulates sympathetic pathways (29, 30, 33) leading to increases in HR, SV, CO, vascular resistance, and then blood pressure.

It is known that central chemoreflex and muscle metaboreflex signals converge to common areas in the brain (43) and exert similar excitatory effects through the cardiovascular autonomic nervous system. On the other hand, little is known about the hemodynamic effects of muscle metaboreflex activation during hypercapnia. Ponikowski (35) showed that muscle ergoreflex activation was an independent predictor of ventilation sensitivity to hypercapnia in chronic heart failure and argued for the existence of interactions between the muscle metaboreflex and hypercapnic chemoreflex. Despite the likelihood that these two reflexes interact during exercise, this topic received little direct attention before the pioneering work of Lykidis et al. (22, 23), who demonstrated the respiratory and cardiovascular responses to combined activation of the hypercapnic chemoreflex (hypercapnia) and muscle metaboreflex (postexercise local circulatory occlusion) in healthy humans. Their observation that minute ventilation (V_E) increased during postexercise local circulatory occlusion against a hypercapnic, but not normocapnic, background led them to suggest the possibility of central interactions between the hypercapnic chemoreflex and muscle metaboreflex. However, those investigators found no difference in HR, mean arterial pressure (MAP), or their respective increases during postexercise local circulatory occlusion between hypercapnic and normocapnic conditions. No data were reported concerning SV, CO, or vascular resistance.

The present human study focused on the cardiovascular effects of muscle metaboreflex activation during hypercapnia with the aim of characterizing the interactions between the two stimuli. We tested the hypothesis that muscle metaboreflex activation during hypercapnia and their interaction would increase SV and CO but would lower vascular resistance to limit the induced-hemodynamic response.

MATERIALS AND METHODS

Subjects

Ten young healthy males participated in the study. Their mean (± SE) age, height, and weight were 24 ± 1 years, 173 ± 1 cm, and 64 ± 1 kg, respectively. None of the subjects smoked or took any medication. The subjects were instructed to refrain from exercising or

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drinking alcohol, coffee, or tea for at least 24 h before their participation. The study was approved by the Ethical Committee of the University of Tsukuba and was conducted in accordance with the Declaration of Helsinki. Each subject provided written consent, and all experiments were conducted in the presence of a medical doctor.

Protocols and Interventions

Maximal voluntary contraction. Subjects were asked to empty their bladder and then to assume a supine position. A room temperature of 25°C was maintained. The subjects gripped a hand dynamometer (digital dynamometer; TKK 5101; Takei, Niigata, Japan) and performed three maximum voluntary contraction (MVC) maneuvers using their dominant arm. We then considered only the highest value.

Local circulatory occlusion. A rapidly inflatable cuff was placed on the upper part of the dominant arm to provide local circulatory occlusion. The cuff pressure rose to a supersystolic pressure of 240 mmHg in less than 1 s. The local circulatory arrest was started before beginning the handgrip exercise and/or inhaling a CO₂-enriched gas mixture to prevent 1) local metabolic conditions within the contracting muscles from being affected by the inhaled gas composition (thus, the arterial CO₂ partial pressure) and 2) the release of metabolites produced by contraction to the systemic circulation, which could stimulate systems outside the contracting muscles. Local circulatory occlusion is used to trap metabolites that are produced by contraction and is known to stimulate the metaboreflex. As result, the muscle metaboreflex continues to be activated after contraction stops, with no associated mechanoreflex or central command (21, 45, 46).

Voluntary hyperventilation challenge. On the basis of our pilot studies and in accordance with a recent work (44), we asked the subjects to perform voluntary hyperventilation with a 30 l/min target V_E . This corresponded to the spontaneous ventilatory level obtained in response to a +10 mmHg increase in CO₂ end-tidal partial pressure (PETCO₂) when inhaling a CO₂-enriched gas mixture. Because hemodynamics are conditioned by V_E and one's breathing pattern, we asked all of the subjects to perform calibrated voluntary hyperventilation, even when such hyperventilation was not physiologically necessary [muscle metaboreflex activation (MMA)]. Breathing frequency (BF) was kept at 20 breaths per min (electronic metronome) and a 1,500 ml tidal volume (V_t) was targeted (visual feedback of the instantaneous V_t). N₂ and CO₂ supplies were adjusted to ensure normoxia and normocapnia or hypercapnia.

Protocols. In one unique experimental day, subjects performed three protocols in random order. Each protocol entailed a sequence of three periods: Control, reflex activation, and recovery. Control was designed to provide 5 min of resting baseline data with normoxia, normocapnia, and spontaneous breathing. Reflex activation was characterized by a 6-min experimental stimulus: 1) hypercapnia (HCA), which consisted of a 6-min rest with +10 mmHg PETCO₂; 2) MMA, which consisted of a 1-min isometric handgrip exercise at 50% MVC followed by a 5-min rest, all during normocapnia; and 3) muscle metaboreflex activation during hypercapnia (HCA+MMA). In all three protocols, dominant upper-arm ischemia induced by local circulatory occlusion and voluntary hyperventilation were maintained throughout the 6 min of reflex activation. Recovery consisted of a 5-min rest period (no exercise, no ischemia) under free air-breathing conditions. Finally, at least 30 min of rest interspaced the protocols.

Measurements

Subjects wore a nose clip that ensured exclusive mouth breathing through a mouthpiece connected in sequence to a pneumotachograph and a T-shaped nonrebreathing Rudolph valve. N₂ and CO₂ tanks with manual flow meters were connected to the inspiratory path, which enabled us to control the N₂ and CO₂ supplies. The expiratory path was opened to the room air. Inspiratory and expiratory gases were sampled at the mouthpiece. Sampled gases and the respiratory flow signal were fed into a mass spectrometer (Arco

2000; Arco System, Chiba, Japan). Analog signals for the respiratory variables, electrocardiogram (three-lead; Nihon Kohden, Tokyo, Japan), and arterial pressure waveform (finger photoplethysmography; Finometer; Finapres Medical System, Amsterdam, The Netherlands) were digitized at a sampling frequency of 1,000 Hz (PowerLab 16/30 and LabChart Pro; ADInstruments Japan, Nagoya, Japan) and fed into a personal computer.

We evaluated SV using Doppler ultrasound (27). A Doppler ultrasound system (Echo Doppler; HDI 3500; At/Philips Ultrasound, Bothwell, WA), equipped with a hand-held transducer probe (operating frequency: 2 MHz), was used to measure ascending aortic blood velocity. Our system collects aortic blood velocity at 100 Hz together with the analog electrocardiogram and blood pressure waveform signals. Aortic diameter was measured in a separate resting session. SV was calculated as the product of the aortic blood velocity (cm/s) and aortic cross-sectional area (cm²).

Ratings of perceived exertion (RPE; based on the Borg scale of 6 to 20) were obtained at the end of the 1-min handgrip exercise.

Data Analysis

V_t , BF, and V_E were computed from respiratory flows, while PETCO₂ and end-tidal O₂ partial pressure (PETO₂) were obtained from the mass spectrometer. We characterized hemodynamic responses to the experimental stimuli using five variables: 1) MAP and its determinants, 2) CO, which is a function of 3) HR, 4) SV, and 5) total peripheral resistance (TPR). MAP, HR, and SV were assessed using digital photoplethysmography, ECG, and echo Doppler, respectively. CO and TPR were calculated as follows: CO = SV × HR and TPR = MAP/CO. To better understand the hemodynamic responses and to search for changes in cardiac inotropism, we calculated indirect indexes of myocardial oxygen consumption (MVO₂) and cardiac work (CW) using the double product (SAP × HR), also called the systolic pressure-rate product, and the triple product (SV × SAP × HR), respectively (26, 37). In the absence of a direct way to assess inotropism or external work independently, comparison of MVO₂ and CW can reveal the catecholamine-oxygen wasting phenomenon. Simply stated, catecholamine-induced changes in inotropism alter myocardial oxygen consumption more than is predicted by indexes, such as the systolic pressure-rate product. In contrast to the double product, the triple product, which assesses CW, taking SV into consideration, is sensitive to catecholamine oxygen wasting (37). For each subject, data from the last minute of the resting period and the first 2 min of the reflex activation period were excluded to limit the hemodynamic effects of mental anticipation of the task to come and the effects of voluntary and involuntary muscle contraction, respectively. Variables were then averaged over the first 4 min of the 5-min control period and the last 4 min of the 6-min reflex activation period.

Statistics

Data are presented as means ± SE. Statistical analyses were performed using two-way repeated-measures ANOVA. The two factors for ANOVA were the period (control and reflex activation) and the experimental stimulus during reflex activation (HCA, MMA, and HCA+MMA). When main effects were observed, post hoc procedures were employed to compare pairs of means. When appropriate, paired two-tailed *t*-tests were also used. Values of *P* < 0.05 were considered significant. Because of the poor quality of the echo Doppler signal during some periods of the voluntary hyperventilation challenge in one subject, some of his hemodynamic data were excluded.

RESULTS

There was no difference in any respiratory variable during the control period across the three protocols (Table 1). Figure 1 shows the individual responses of a representative subject.

Table 1. Ventilatory parameters and respiratory gas partial pressure during control (free breathing) and reflex activation (voluntary hyperventilation)

	Control	Reflex Activation
V_t , liters		
HCA	0.7 ± 0.0	$1.6 \pm 0.0^{***}$
MMA	0.7 ± 0.0	$1.5 \pm 0.0^{***,\$}$
HCA+MMA	0.7 ± 0.1	$1.6 \pm 0.0^{***}$
BF, breath/min		
HCA	12 ± 1	$20 \pm 0^{***}$
MMA	13 ± 1	$20 \pm 0^{***}$
HCA+MMA	12 ± 1	$20 \pm 0^{***}$
V_e , l/min		
HCA	8.1 ± 0.4	$31.4 \pm 0.5^{***}$
MMA	8.5 ± 0.4	$29.5 \pm 0.5^{***,\$}$
HCA+MMA	8.2 ± 0.3	$31.9 \pm 0.4^{***}$
T_i/T_e		
HCA	62 ± 3	$90 \pm 2^{***}$
MMA	68 ± 4	$97 \pm 3^{***,\$}$
HCA+MMA	71 ± 5	$85 \pm 3^{***}$
PET_{CO_2} , mmHg		
HCA	45.9 ± 0.6	$56.8 \pm 0.5^{***}$
MMA	46.1 ± 0.7	$46.7 \pm 0.8^{***,\$}$
HCA+MMA	46.0 ± 0.7	$56.8 \pm 0.7^{***}$
PET_{O_2} , mmHg		
HCA	100 ± 1	102 ± 1
MMA	101 ± 1	102 ± 1
HCA+MMA	101 ± 1	101 ± 1

Values are expressed as means \pm SE. Control denotes resting period; reflex activation denotes the period defined by the experimental conditions (voluntary hyperventilation, local circulatory occlusion in the dominant arm, and hypercapnia (HCA); muscle metaboreflex activation (MMA); or hypercapnia plus muscle metaboreflex activation). V_t , tidal volume; BF, breathing frequency; V_e , minute ventilation calculated from the raw data and then rounded to the nearest significant digit; T_i/T_e , inspiratory time/expiratory time; PET_{CO_2} , end-tidal partial pressure of CO_2 ; PET_{O_2} , end-tidal partial pressure of O_2 . $^{***}P < 0.001$ vs. Control. $^{\$}$ Significant difference vs. HCA and HCA+MMA, $P < 0.05$. *** Significant difference vs. HCA and HCA+MMA, $P < 0.01$. **** Significant difference vs. HCA and HCA+MMA, and $P < 0.001$.

As seen in Fig. 2A, PET_{CO_2} quickly reached the targeted steady state during reflex activation in both the HCA and HCA+MMA conditions, reflecting the identically driven ventilation and gas management (Table 1). PET_{O_2} levels were similar and constant throughout all three protocols.

Cardiovascular Effects of Hypercapnia and/or Muscle Metaboreflex Activation

MAP was higher during reflex activation than control in all three protocols, with the largest increase in the HCA+MMA condition and the smallest in the HCA condition (Fig. 2B). MAP required around 1 min to stabilize after the exercise ended and was highest at the end of reflex activation (Fig. 2B). Reflex activation effects on TPR were not statistically significant (Fig. 3), as MMA increased ($P < 0.001$), while HCA tended to decrease ($P < 0.1$) TPR, variations of which were directionally opposite ($P < 0.05$) (Table 2). CO was higher during reflex activation than control in all three conditions, with the largest increase during HCA+MMA. Smaller but similar increases were observed in the HCA and MMA conditions (Fig. 3). HR increased during reflex activation in all three conditions, with the largest increase occurring in the HCA+MMA condition and the smallest in the MMA condition. SV increased significantly only during the HCA+MMA condition (Fig. 3). Therefore, although $M\dot{V}O_2$ and CW were higher

during reflex activation than control in all three conditions, they were the highest during HCA+MMA (Fig. 4, A and B).

RPE at the end of the handgrip exercise was similar between MMA and HCA+MMA (16 ± 0.75).

Reflex Interactions

Two types of interaction can exist between integrated physiological reflex systems: gain interaction and temporal interaction. Temporal interaction defines the ability of two interactive reflex systems to modify their outputs when their respective stimuli are delivered simultaneously. Comparing the sum of the output responses to each stimulation with the output response to simultaneous stimulations, the interaction can be characterized as facilitatory, linearly additive, or inhibitory, depending upon whether the response to the simultaneous stimulation is greater than, equal to, or less than the sum of the individual responses (38). We examined the effects of temporal interactions between hypercapnia and muscle metaboreflex on the tested hemodynamic variables (Table 2). We found that the blood pressure increase induced by HCA+MMA was smaller than the sum of the blood pressure increases induced by HCA and MMA separately. This inhibitory interaction of hypercapnia and the muscle metaboreflex was a consequence of effects on both TPR (vessels) and CO (heart). Effects on TPR were inhibitory, while effects on CO reflected changes in both HR and SV: the interaction was inhibitory for HR but linearly additive for SV, such that SV was significantly increased in the HCA+MMA condition. Finally, the interaction was also inhibitory for $M\dot{V}O_2$ and CW.

DISCUSSION

We studied the hemodynamic response to muscle metaboreflex activation during hypercapnia and the effects of their interactions. HCA+MMA led to the strongest response, but hypercapnia and the muscle metaboreflex exhibited inhibitory interactions affecting HR, TPR, and MAP. On the contrary, the interaction was linearly additive with respect to CO and SV, which increased.

Experimental Model

We chose to use hypercapnia instead of hypoxia because hypercapnia modifies MAP more efficiently through a smaller vasodilatory effect (44). Hypercapnia is also a powerful ventilatory stimulus, so we imposed the same voluntary hyperventilation challenge in all three conditions, even when such hyperventilation was not required (MMA). In addition, we tried to make comparable the muscle metaboreflex stimuli during MMA and HCA+MMA. Because inhaled CO_2 during HCA+MMA can modify muscle metaboreflex inputs at the muscle level (muscle metabolism during contraction, muscle nerve afferent activity), we started local circulatory occlusion just before the hypercapnia, hyperventilation, and muscle contraction onsets in all three protocols. Because CO_2 could not reach the contracting muscles, we concluded that the observed interactions took place outside the working muscles.

Cardiovascular Responses to Muscle Metaboreflex Activation During Hypercapnia

Previous human studies tested hypercapnia and the muscle metaboreflex and showed that both increase MAP, HR, and

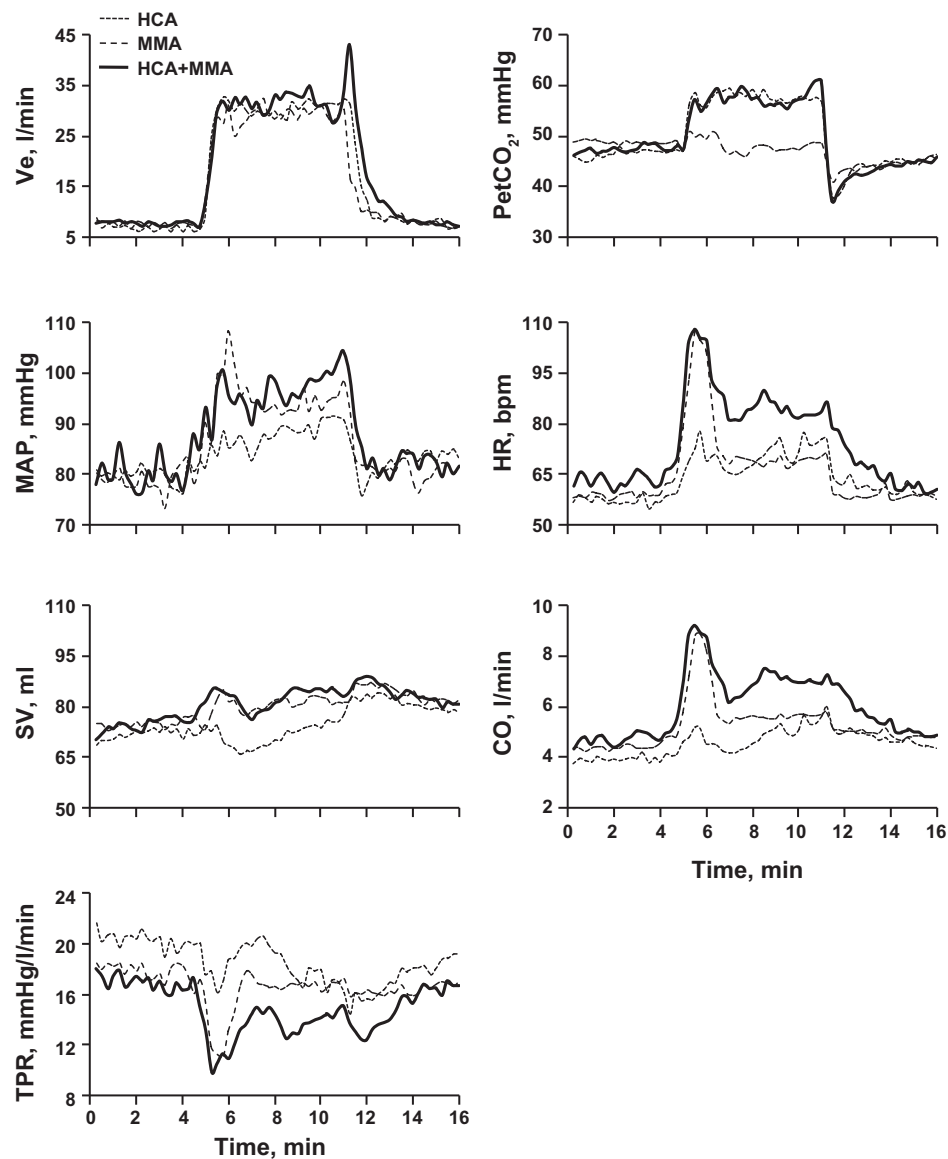


Fig. 1. Representative subject data. Kinetics of minute ventilation (V_e), CO_2 end-tidal partial pressure (PETCO_2), mean arterial pressure (MAP), heart rate (HR), stroke volume (SV), cardiac output (CO), and total peripheral resistance (TPR) for one representative subject during hypercapnia (HCA), muscle metaboreflex activation (MMA), and HCA+MMA protocols. Values were recorded beat-to-beat and then averaged over every 15 s.

CO, but only the muscle metaboreflex exerts vasoconstrictive and inotropic effects (36, 11, 18, 44, 4). Other investigators focused on coactivation of the muscle metaboreflex and hypoxic chemoreflex and reported no interaction and no specific effect on blood pressure response (39, 14). By contrast, we found that blood pressure was higher during HCA+MMA than HCA or MMA alone, but that the increase in MAP was smaller than the sum of the HCA and MMA responses. Because on the whole, we observed directionally opposite TPR responses to HCA and MMA (Table 2), we expected that TPR during HCA+MMA (Control TPR plus the algebraic summation of the TPR responses to HCA and MMA) would inevitably be within the range of cardiovascular system responsiveness. In addition, because on the one hand CO and SV during HCA+MMA did not differ from their expected values, and on the other hand the expected MAP and HR reached values frequently seen in humans, 114 (80+12+22) mmHg and 83 (60+9+22) bpm, we discarded the idea that the observed responses were limited by the range of cardiovascular system responsiveness.

In addition, our finding that MAP was highest during HCA+MMA prompted us to question whether increased baroreflex buffering could explain our results. Physiologically, when MAP increases, baroreflex-induced response combines reduction in CO (due to decreases in HR and SV) with arterial vasodilation (decrease in TPR). Although we observed reductions in vasoconstrictive (TPR) and chronotropic (HR) effects, we also observed a linearly additive effect on inotropism (SV and CO), which is not explainable solely on the basis of baroreflex physiology. Our results show that the reduction in the response was more a modification of its qualitative nature during HCA+MMA than merely a reduction in amplitude, i.e., the response was less vasoconstrictive and chronotropic but more inotropic. However, our data do not exclude the possibility of a modification of baroreflex function through HCA and MMA interactions.

Hypercapnia exerts systemic humoral vasodilatory effects (36, 11), but TPR appears not to be affected by 30 min of hypercapnia at rest (18). We measured no difference in TPR between control and reflex activation during both HCA and

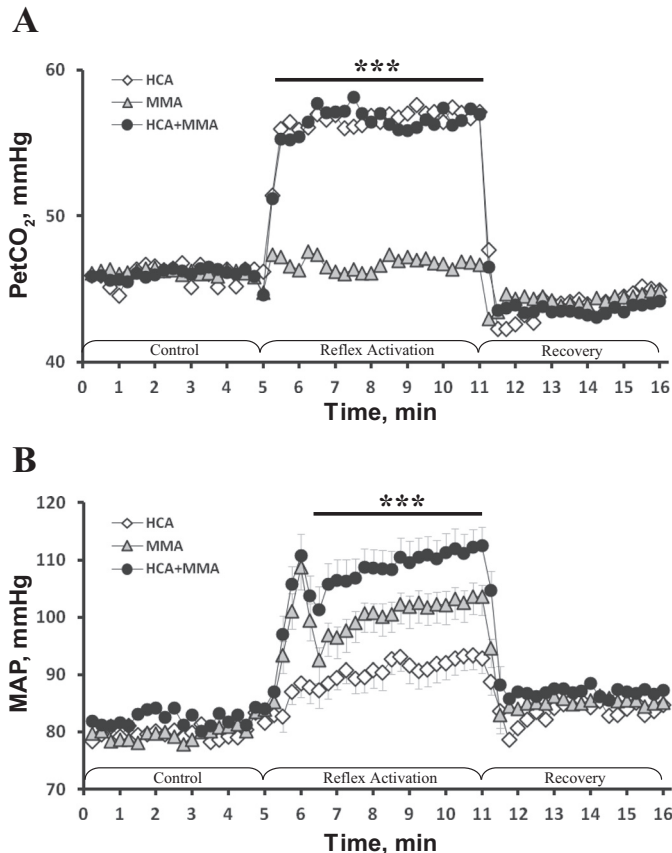


Fig. 2. Kinetics of the CO₂ PETCO₂ and MAP during the HCA, MMA, and HCA+MMA conditions. Mean PETCO₂ (A) values [SE are not shown (<1 mmHg)] and mean MAP (B) values (\pm SE) were recorded breath-to-breath and beat-to-beat, respectively, and then averaged over every 15 s throughout control, reflex activation, and recovery periods of the HCA, MMA, and MMA during concurrent hypercapnia (HCA+MMA) conditions, as indicated. A: *** P < 0.001 for reflex activation vs. control in the HCA and HCA+MMA conditions. B: *** P < 0.001 for the last 4 min of the reflex activation vs. control in all three experimental conditions.

HCA+MMA. We interpret this to be evidence of a modification of the muscle metaboreflex response, which would otherwise increase TPR (41, 15), as seen during MMA. Besides, the vasoactivity of hypercapnia is related to the prevailing sympathetic tone at the time of measurements (31). The blunted TPR response observed during HCA+MMA can also be interpreted as an enhanced vasodilatory effect of hypercapnia against a background of increased sympathetic activation, as MMA and HCA both increase cardiac and vascular sympathetic tone (33, 34).

HR and SV increased together only during HCA+MMA. As larger SVs are associated with longer systolic periods in the absence of a contractility change, our data suggest an increase in cardiac inotropism. Our energetics data (Fig. 4) strongly support this hypothesis. Both MV_O₂ and CW increased during the three experimental conditions, but HCA+MMA led to the highest values. While MV_O₂ peaked at the end of exercise, CW reached its maximum at the end of reflex activation, i.e., during interactions (Fig. 4). This discrepancy between the two different predictors of left ventricular oxygen consumption, with only CW taking SV into consideration, is suggestive of the

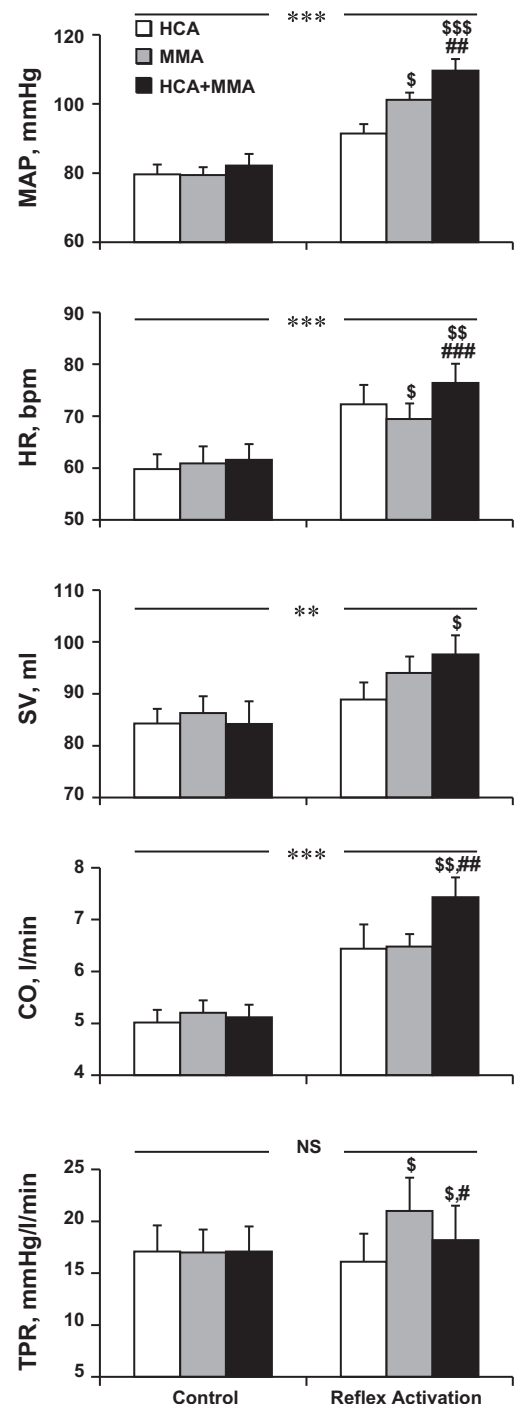


Fig. 3. MAP, HR, SV, CO, and TPR during control and reflex activation in the HCA, MMA, and HCA+MMA conditions. Values are expressed as means \pm SE recorded during control and reflex activation, as indicated. White, gray, and black bars depict values obtained in the HCA, MMA, and HCA+MMA conditions, respectively. NS, not significant. ** P < 0.01, *** P < 0.001 for reflex activation vs. control. \$Significant difference from HCA during reflex activation, P < 0.05. \$\$\$Significant difference from HCA during reflex activation, P < 0.001. #Significant difference from MMA during reflex activation, P < 0.05. ##Significant difference from MMA during reflex activation, P < 0.01. ###Significant difference from MMA during reflex activation, P < 0.001.

Table 2. Interactions between hypercapnia and muscle metaboreflex and their effects on the main cardiovascular variables

	HCA	MMA	HCA+MMA	Sum of HCA and MMA
Δ SAP	$+19 \pm 2$	$+30 \pm 3$	$+42 \pm 4$	$+49 \pm 5^{***}$
Δ MAP	$+12 \pm 1$	$+22 \pm 2$	$+28 \pm 2$	$+34 \pm 2^{***}$
Δ DAP	$+8 \pm 1$	$+18 \pm 2$	$+20 \pm 1$	$+26 \pm 2^{**}$
Δ CO	$+1.4 \pm 0.3$	$+1.3 \pm 0.2$	$+2.3 \pm 0.4$	$+2.7 \pm 0.4$
Δ HR	$+13 \pm 2$	$+9 \pm 2$	$+15 \pm 2$	$+22 \pm 3^{**}$
Δ SV	$+5 \pm 2$	8 ± 2	$+13 \pm 4$	13 ± 4
Δ TPR	-1.0 ± 0.6	$+4.0 \pm 1.2$	$+1.1 \pm 1.4$	$+3.0 \pm 1.5^*$
Δ MV \dot{O}_2	$+28.15 \pm 2.94$	$+31.34 \pm 2.87$	$+50.25 \pm 4.74$	$+59.48 \pm 5.37^{**}$
Δ CW	$+28.94 \pm 4.64$	$+34.73 \pm 3.56$	$+59.10 \pm 7.52$	$+63.67 \pm 7.71^*$

Values are expressed as means \pm SE rounded to the nearest significant digit. HCA, hypercapnia; MMA, muscle metaboreflex activation; HCA+MMA, muscle metaboreflex activation during concurrent hypercapnia; Δ , difference between beat-to-beat values recorded during reflex activation and averaged over the last 4 min and the corresponding beat-to-beat values recorded during control and averaged over the first 4 min in the indicated experimental conditions. SAP, systolic arterial pressure, mmHg; MAP, mean arterial pressure, mmHg; DAP, diastolic arterial pressure, mmHg; CO, cardiac output, l/min; HR, heart rate, bpm; SV, stroke volume, ml; TPR, total peripheral resistance ($\text{mmHg} \cdot \text{l} \cdot \text{min}^{-1}$); MV \dot{O}_2 , myocardial oxygen consumption ($\text{mmHg} \cdot \text{min}^{-1} \cdot 10^{-2}$); CW, cardiac work ($\text{ml} \cdot \text{mmHg}^{-1} \cdot \text{min} \cdot 10^{-4}$). $^*P < 0.05$ vs. HCA+MMA. $^{**}P < 0.01$ vs. HCA+MMA. $^{***}P < 0.001$ vs. HCA+MMA.

oxygen-wasting phenomenon usually associated with catecholamine-mediated increases in inotropism (37).

Limitations

First, we studied only temporal interactions. To study gain interactions, step-based activation is necessary to calculate the gain changes from the dose-response curve. Two experimental designs could enable us to achieve our aim in this study: 1) cumulative sequential activation of reflexes within a single trial, staggering the start times of the two stimuli in random order, or 2) three separate trials, one for each experimental condition. We performed the second, which we prefer because sequential activation can lead to time effects, as seen in Fig. 2B, or to the induction of adaptive mechanisms. The design that we used intrinsically overcomes this time effect.

Second, because we did not test the gains of the muscle metaboreflex and hypercapnic chemoreflex, we could not conclude whether hypercapnia acted through humoral or neural mechanisms; however, because we isolated the working muscles by starting the local circulatory occlusion before the onset of reflex activation, we ensured that the muscle metaboreflex inputs were similar during all three protocols and that interactions took place outside the contracting muscles. Consequently, V_e was lower during MMA than during HCA+MMA (Table 1). The respiratory muscle blood flow underlying this difference in V_e requires the muscle respiratory vascular beds to have greater conductance during HCA+MMA. This could explain the observed lower TPR. But with a V_e level near 30 l/min, the observed difference in V_e was less than $3 (\text{l/min of } V_e) \times 2 (\text{ml } \dot{V}O_2) = 6 \text{ ml/min of } \dot{V}O_2$ (1), which is clearly negligible. For these reasons, we think that hypercapnia acted through neural mechanisms; i.e., via the central chemoreflex.

Third, with respect to the limitations of the model used to evoke the metaboreflex, both static and dynamic contractions followed by local circulatory occlusion increase HR, SV, and

CO in humans (3, 24, 32) and in dogs (29), but the effects on the heart appear to be intensity-dependent (7, 8, 40). However, the local circulatory occlusion used to trap metabolites that activate metaboreflex prevents venous dilation, which is a major component of metaboreflex activation (10, 25). To avoid this obstacle, we chose to use a 1-min 50% MVC handgrip model, which is known to evoke the muscle metaboreflex without increasing HR, SV, or CO.

Perspectives and Significance

Our experiment on healthy young subjects provides new evidence that forearm muscle metaboreflex activation during hypercapnia induces substantial modification of cardiovascular function, leading to increases in blood pressure and SV. These data should also contribute to a better understanding of the pathophysiology of heart failure. Increasing evidence suggests the metaboreflex is exaggerated or tonically activated in heart failure (9, 13, 28) and that hypercapnia plays a key role (5, 6, 16, 12) in the disease complications. Furthermore, because heart failure is characterized by an inability to increase SV, hemodynamic effects related to the interactions between the muscle metaboreflex and hypercapnia, such as those observed in this study, could explain in part the heart failure-linked exercise intolerance. Increases in SV likely mediated by inotropic effects appears to be the key to the interaction between

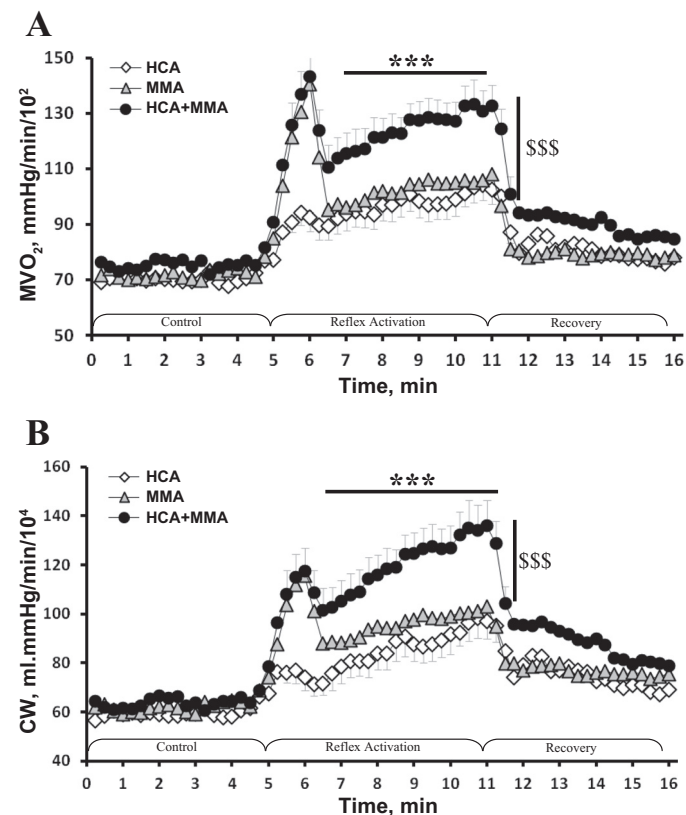


Fig. 4. Myocardial oxygen consumption (MV \dot{O}_2) and cardiac work (CW) during the HCA, MMA, and HCA+MMA conditions. Mean MV \dot{O}_2 (A) and CW (B) values (\pm SE) were recorded beat-to-beat and then averaged over every 15 s throughout the HCA, MMA, and HCA+MMA conditions, as indicated. $^{***}P < 0.001$ for reflex activation vs. control in all three experimental conditions. $SSSP < 0.001$ for HCA+MMA vs. HCA and MMA during reflex activation.

muscle metaboreflex and hypercapnia, but further study will be required to precisely determine the mechanisms in healthy subjects and heart failure patients.

In conclusion, we showed in healthy subjects that interactions between the muscle metaboreflex and hypercapnia lead to partially additive pressor effects, reflecting increases in SV, likely due to increased inotropism, combined with attenuation of the chronotropic and vasoconstrictive responses. These interactions take place outside the working muscles and appear to limit cardiac oxygen consumption and cardiac work.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: S.D. and T.N. conception and design of research; S.D., M.I., K.W., and N.F. performed experiments; S.D. and K.W. analyzed data; S.D. interpreted results of experiments; S.D. prepared figures; S.D. drafted manuscript; S.D., M.I., N.F., and T.N. edited and revised manuscript; S.D., M.I., and T.N. approved final version of manuscript.

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