European Journal of Applied Physiology (2019) 119:103–111
https://doi.org/10.1007/s00421-018-4004-y

ORIGINAL ARTICLE

Spontaneous cardiac baroreflex sensitivity is enhanced during post-exercise ischemia in men but not in women

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Received: 9 March 2018 / Accepted: 1 October 2018 / Published online: 6 October 2018

Abstract

Purpose To investigate the effect of isolated muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity (cBRS), and to characterize the potential sex-related differences in this interaction in young healthy subjects.

Methods 40 volunteers (20 men and 20 women, age: 22 ± 0.4 year) were recruited. After 5-min rest period, the subjects performed 90 s of isometric handgrip exercise at 40% of maximal voluntary contraction followed by 3 min of post-exercise ischemia (PEI). Beat-to-beat heart rate and arterial blood pressure were continuously measured by finger photopletysmography. Spontaneous cBRS was assessed using the sequence technique and heart rate variability was measured in time (RMSSD—standard deviation of the RR intervals) and frequency domains (LF—low and HF—high frequency power).

Results Resting cBRS was similar between men and women. During PEI, cBRS was increased in men (Δ3.0 ± 1.1 ms mmHg^{-1}, $P = 0.03$) but was unchanged in women (Δ-0.04 ± 1.0 ms mmHg^{-1}, $P = 0.97$). In addition, RMSSD and HF power of heart rate variability increased in women (Δ7.4 ± 2.6 ms, $P = 0.02$; Δ373.4 ± 197.3 ms^2; $P = 0.04$, respectively) and further increased in men (Δ26.4 ± 7.1 ms, $P < 0.01$; Δ1874.9 ± 756.2 ms^2; $P = 0.02$, respectively). Arterial blood pressure increased from rest during handgrip exercise and remained elevated during PEI in both groups, however, these responses were attenuated in women.

Conclusions These findings allow us to suggest a sex-related difference in spontaneous cBRS elicited by isolated muscle metaboreflex activation in healthy humans.

Keyword Exercise pressor reflex · Autonomic nervous system · Heart rate · Blood pressure · Sex differences

Abbreviations

ANOVA	Analyses of variance
BMI	Body mass index
BSA	Body surface area
cBRS	Cardiac baroreflex sensitivity
CI	Cardiac index
CO	Cardiac output
DBP	Diastolic blood pressure
HF	High frequency
HR	Heart rate
HRV	Heart rate variability
ICC	Intraclass correlation coefficient
IHG	Isometric handgrip
LF	Low frequency
MBP	Mean blood pressure
MVC	Maximal voluntary contraction
NK1-R	Neurokinin-1 receptor
NTS	Nucleus tractus solitaries
PEI	Post-exercise ischemia
RMSSD	Root of the mean of the sum of successive differences
SBP	Systolic blood pressure
SV	Stroke volume
TVC	Total vascular conductance
TVCI	Vascular conductance index
VLF	Very low frequency

Communicated by Massimo Pagani.

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Introduction

Several neural mechanisms are responsible for regulating autonomic adjustments during exercise and through complex interactions control the cardiovascular and hemodynamic changes. These cardiovascular responses are mediated by

feedforward signals from high brain centers (i.e. central command) (Kaufman 2012; Raven et al. 2006), and feedback reflex mechanisms via afferent nerves from exercising skeletal muscle receptors (i.e. muscle mechanoreflex and metaboreflex) (Mitchell et al. 1983; Mitchell 1990; Rowell and O'Leary 1990; Vianna et al. 2018). In addition, there are arterial and cardiopulmonary baroreceptors (i.e. arterial and cardiopulmonary baroreflexes) (Rowell and O'Leary 1990; Potts et al. 1993; Fadel and Raven 2012; Fisher et al. 2015) contributing to these adjustments. Despite extensive knowledge of the central and peripheral mechanisms involved in the cardiovascular adjustments during exercise, their possible interactions are not fully understood. Specifically, the effect of the muscle metaboreflex activation on spontaneous cardiac baroreflex sensitivity (cBRS) remains particularly controversial.

During isolated muscle metaboreflex activation, via post-exercise ischemia (PEI), there is a loss of the powerful inhibitory input from central command and muscle mechanoreflex to cardiac parasympathetic, and muscle metaboreflex activation remains elevated (i.e. sustaining the rise in blood pressure). This maintains the elevated baroreflex-mediated excitation input of the cardiac vagal motoneurones. Functionally, activation of the muscle metaboreflex at some time after the onset of exercise may act to counter muscle mechanoreceptor inhibition of baroreflex driven, vagally mediated, events and thereby protect blood pressure from abnormal oscillations (Carrington and White 2001). Previous studies showed that cBRS is unchanged during isolated muscle metaboreflex activation via PEI after IHG exercise (Spaak et al. 1998; Iellamo et al. 1999b, a; Cui et al. 2001; Ichinose et al. 2002; Fisher et al. 2008), or single leg extensor exercise (Iellamo et al. 1999b). In contrast, activation of metabolically sensitive skeletal muscle afferents leads to a decrease in spontaneous cBRS in exercising dogs (Sala-Mercado et al. 2007), and this findings was extended to healthy humans during leg cycling exercise (Hartwich et al. 2011). The reason behind these discrepancies could be attributable to variation in the methods used to assess cBRS function, the exercise modality, intensity and size of exercising muscle mass, and, less discussed, but also important, the possible sex-related differences. Indeed, the majority of these studies included both men and women, and was not designed to investigate the potential existence of sex-related differences in spontaneous cBRS during isolated muscle metaboreflex activation.

Whilst studies investigating sex differences in muscle metaboreflex activation and cBRS are few, there is a growing body of evidence to suggest that here are important distinctions. For example, there are a plethora of studies indicating sex-differences in blood pressure control (Hart and Charkoudian 2014; Joyner et al. 2015; Teixeira et al. 2018c). In addition, Abdel-Rahman et al. (1994) first reported in humans that females had lower cBRS than males when blood pressure was acutely elevated by a bolus of intravenous injection of phenylephrine. In summary, these finding suggest that the difference in cBRS between males and females relates, at least in part, to the pattern of blood pressure increase, and this was confirmed later by others (Beske et al. 2001; Huikuri et al. 1996; Convertino 1998). In addition, several studies have shown attenuated muscle metaboreflex activation in women compared with men (Ettinger et al. 1996; Jarvis et al. 2011; Smith et al. 2016). Taken together, to date, it is unknown whether there are sex-related differences in spontaneous cBRS during isolated muscle metaboreflex activation following IHG exercise. Given this background, the purpose of the present study was to investigate the effect of isolated muscle metaboreflex activation on cBRS, and to characterize the potential sex-related differences in this interaction in healthy young subjects.

Methods

Subjects

40 volunteers (20 men and 20 women, age: 22 ± 0.4 year) were studied. All subjects were healthy, normotensive, non-smoker and physically active (self-report for at least 6 months and 3 days/week). No subjects were using any controlled medications and had no history or symptoms of cardiopulmonary, metabolic or neurological diseases. To avoid potential influence of female sex hormones on blood pressure control, all women were non-users of oral contraceptive pills for at least six consecutive months and were studied during the early follicular phase of their menstrual cycle (i.e. first 5 days after menstruation onset). All subjects gave written informed consent to participate in this study, which was approved by the Ethic Committee research of University of Brasília (CAAE 76504017.1.0000.0030) in accordance with Declaration of Helsinki. All subjects were asked to refrain from consuming caffeine/alcohol and from engaging in physical exercise for 6 and 24 h, respectively, prior to the tests. Subjects were 2-h postprandial upon arrival to the laboratory. To avoid potential diurnal variations, subjects were always tested at the same time of day for each subject and in the same quiet, temperature-controlled room (22–24 °C).

Experimental Protocols

Initially, all subjects were familiarized with all the study equipment and procedures. Weight and height were determined via standard methods, and body mass index (BMI) calculated. Subjects performed three maximal efforts each

separated by at least 1 min, and the higher value was considered the maximal voluntary contraction (MVC).

In the experimental protocol, after instrumentation, the subjects were asked to be seated (90° of hip and knee flexion) and rested for 10 min to stabilization of cardiovascular variables. Resting baseline hemodynamic measures were recorded for the last 5 min. Heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure were continuously measured on a beat-to-beat basis by photoplethysmography using a Finometer device (Human NIBP Controller, AD instruments, NSW, Australia), placed at the middle finger of the non-dominant hand, with the hand positioned at heart level. Brachial arterial blood pressure was also measured with an automated digital sphygmomanometer (Dixtal, DX2022, Brazil) for absolute measures of blood pressure to confirm finger measurement accuracy. Respiratory movements were monitored using a pneumatic belt placed around the subjects's abdomen (MLT 1132 Piezo Respiratory Belt Transducer-ADInstruments) to ensure that the subjects did not perform Valsalva maneuver during the protocol.

Beat-to-beat stroke volume (SV) was derived from arterial blood pressure waveform using the Modelflow method (Beatscope 1.1a; Finapres Medical Systems BV, Amsterdam, The Netherlands), which incorporates age, sex, weight, and height. Cardiac output (CO) was calculated from beat-to-beat HR and SV ($CO = HR \times SV$), and total vascular conductance (TVC) was calculated as the ratio between CO and mean blood pressure (MBP). To take body size into consideration, cardiac index (CI) was calculated from beat-to-beat CO divided by body surface area (BSA) [BSA (m^2) = $0.20247 \times$ height $(m)^{0.725} \times$ weight $(kg)^{0.425}$] (Du Bois and Du Bois 1989), and total vascular conductance index (TVCI) was calculated from beat-to-beat TVC divided by BSA. The blood pressure waveform were sampled at 1000 Hz (Powerlab, AD Instruments, Bella Vista, New South Wales, Australia), and beat-to-beat values of HR, SBP, DBP and MBP were stored for offline analysis (Chart version 5.2, ADInstruments).

The protocol consisted of 90s of IHG exercise at 40% of MVC followed by 3-min and 15-s of PEI to isolate muscle metaboreflex activation. The IHG exercise intensity was controlled and maintained by a visual feedback of researcher's computer screen. PEI was achieved by the rapid inflation of a cuff positioned around the exercising arm to suprasystolic pressure (240–250 mmHg) 5 s before the end of exercise. The initial 15-s of PEI was eliminated from analyses due to the confound influence of inherent nonstationarities in HR (Fisher et al. 2010; Teixeira et al. 2018a). Following completion of PEI, the cuff was released and recovery was monitored for 5-min.

Spontaneous cBRS

Spontaneous cBRS was assessed using the sequence technique as previously described (Antonino et al. 2017; Parati et al. 2000; Teixeira et al. 2018c, b, a; Sabino-Carvalho et al. 2018). Briefly, this approach is based on the identification of consecutives beats in witch progressive increases (or decreases) in SBP (input variable) are followed by a progressive lengthening (or shortening) in RR interval (output variable). Arterial baroreflex sequences were detected by software (CardioSeries v2.4, Brazil) only when variation in SBP and RR interval were ≥ 1 mmHg and ≥ 1.0 ms, respectively. A linear regression was applied to each individual sequence and only those sequences in which r was > 0.85 were accepted. cBRS was determined for all combined sequences ($Gain_{all}$), and also separately upward (increase in SBP−$Gain_{up}$) and downward (decrease in SBP−$Gain_{down}$).

HR variability

HR variability (HRV) was assessed following the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (1996). Only segments without interference were analyzed. All ectopic beats on the ECG trace were identified both automatically and manually before exclusion from the analysis. A fast Fourier transformation (512 points) was used for spectral analysis of HRV. HRV in linear methods in time domain was represented by the square root of the mean of the sum of successive differences in RR interval (RMSSD), which represent the parasympathetic activity of the heart, because they are drawn from adjacent RR intervals. In frequency domain, the spectral component was distributed in very low frequency power (VLF: < 0.04 Hz), high frequency (HF: 0.15–0.4 Hz), low frequency (LF: 0.4–0.15 Hz). Total power and normalized units of LF and HF were also calculated. Normalized units were calculated by dividing each spectral band by the total power minus the VLF power and were multiplied by 100. The LF/HF ratio was calculated to estimate the sympato-vagal balance of the heart (1996).

Data and statistical analysis

5 min data segments were used for the calculation of average cardiovascular variables at rest and recovery, while last 3-min segment was used for the PEI period. In addition, these same data segment were used to assess cBRS and HRV. Measurements obtained during IHG exercise were derived from the last 15-s. These indices (i.e., HRV and cBRS) were not assessed during IHG exercise due to the confounding influence of inherent non-stationarities in HR. The SBP range was calculated by maximum minus minimum from the rest and PEI. In addition, to determine

the duration of data collection needed during rest period to adequately and reliably estimate spontaneous cBRS we re-analyzed 18 subjects in which spontaneous cBRS measures were derived from beat-to-beat 3-min and 5-min periods and compared. The typical error of measurement and intraclass correlation coefficient (ICC) was calculated to describe the within participant variation between measurements (i.e. 3 min vs. 5 min—reliability) (Hopkins 2000). The typical error of measurement for $GAIN_{up}$ was 1.2 ms $mmHg^{-1}$, for $GAIN_{down}$ was 0.9 ms $mmHg^{-1}$ and $GAIN_{all}$ was 0.7 ms $mmHg^{-1}$. Furthermore, the ICC, another index of reliability, was quite elevated for $GAIN_{up}$ (ICC: 0.96, $P<0.01$), $GAIN_{down}$ (ICC: 0.95, $P<0.01$) and $GAIN_{all}$ (ICC: 0.96, $P<0.01$). Thus, 5 min or 3 min time series segments were considered a reliable duration to be compared in our experiments. The normality distribution of the data was verified by Shapiro–Wilk test. Subjects' characteristics were compared using independent sample *t* test. Comparisons of physiological variables were made using analyses of variance (ANOVA) with repeated measures in which sex (men and women) and moment (rest, IHG, PEI and recovery) were the main factors. Post-hoc analyses were employed using Fisher's test to investigate main effects and interaction. In non-parametric analyses, Friedman's ANOVA was used followed by Wilcoxon–Mann–Whitney tests. The relationship of the magnitude of the SBP and changes in cBRS during PEI was evaluated using a Pearson's correlation coefficients. Statistical significance was set at $P<0.05$ and values are presented as mean ± SE. All statistical analyses were conducted using IBM SPSS® Statistics software (version 20) for windows.

Results

The subject's characteristics are presented in Table 1. Men and women were matched for age, however, men had higher height, weight, BMI, BSA and MVC than women (all $P<0.05$).

Table 1 Subjects characteristics

	Men ($n=20$)	Women ($n=20$)	*P* value
Age (years)	21 ± 0.6	23 ± 0.6	0.08
Height (cm)	177 ± 0.01	163 ± 0.01	< 0.01
Weight (kg)	78.0 ± 2.2	61.4 ± 2.2	< 0.01
BMI (kg/m^2)	24.9 ± 0.6	23.0 ± 0.6	0.04
BSA (m^2)	1.95 ± 0.03	1.66 ± 0.03	< 0.01
MVC (N)	123.7 ± 5.9	80.5 ± 3.4	< 0.01

Values represents means ± SEM. *BMI* body mass index, *BSA* body surface area, *MVC* maximum voluntary contraction. *P* values are derived from independent sample *t* test

The hemodynamics responses are presented in Table 2. Resting SBP and MBP were lower in women than men ($P<0.01$), whereas DBP and HR were similar. SBP, DBP and MBP increased from rest during IHG exercise and remained elevated during PEI ($P<0.01$) in both men and women. However, the magnitude of increase in blood pressure response was greater in men. IHG exercise significantly increased HR in a similar manner in both men and women, restoring to baseline values during PEI. Importantly, no interaction was found for SBP range ($P=0.79$), but presented significant time effect ($P=0.03$) and was lower in women than men throughout the protocol ($P=0.003$) (Rest: 31 ± 2 vs. 28 ± 2 mmHg; PEI: 29 ± 2 vs. 24 ± 1 mmHg).

No interaction were found for SV, CO, CI, TVC and TVCI (All $P>0.05$) but all presented significant time effects ($P<0.01$). In addition, SV and CO was lower in women than in men throughout the protocol ($P<0.01$ for sex), however, when CO and TVC were corrected by BSA (i.e. CI and TVCI, respectively), the sex differences were abolished ($P>0.05$) (Table 2).

Time and frequency domains of HRV are presented in Table 3. Resting HF and LF components were lower in women compared to men, whereas RMSSD and LF/HF were similar. During PEI, RMSSD and the HF power of HRV, two index that represent a proxy of cardiac vagal activity, increased in women (Δ7.4 ± 2.6 ms, $P=0.02$; Δ373.4 ± 197.3 ms^2; $P=0.04$, respectively) and further increased in men (Δ26.4 ± 7.1 ms, $P<0.01$; Δ1874.9 ± 756.2 ms^2; $P=0.02$, respectively). In addition, these components remained elevated during recovery in both groups. On the other hand, LF power of HRV remained unchanged from rest in both men and women. After the normalization procedure, HF increased and LF decreased in men during PEI; and remained unchanged in women. In addition, during PEI, the ratio LF/HF decreased in men (Δ−0.5 ± 0.2; $P=0.003$) but not in women (Δ0.6 ± 0.6; $P=0.33$).

The spontaneous cBRS responses are presented in Fig. 1. Resting cBRS gain for up, down and all sequences were similar between men and women. cBRS increased from rest during PEI in men ($GAIN_{all}$: Δ3.0 ± 1.1 ms $mmHg^{-1}$, $P=0.03$; $GAIN_{up}$: Δ4.4 ± 2.1 ms $mmHg^{-1}$, $P=0.04$; $GAIN_{down}$: Δ3.3 ± 1.4 ms $mmHg^{-1}$, $P=0.03$) but not in women ($GAIN_{all}$: Δ− 0.04 ± 1.0 ms $mmHg^{-1}$, $P=0.97$; $GAIN_{up}$: Δ− 0.3 ± 0.9 ms $mmHg^{-1}$, $P=0.77$; $GAIN_{down}$: Δ0.2 ± 1.2 ms $mmHg^{-1}$, $P=0.87$). In addition, the cBRS in men remained elevated during recovery. Further, there was no interaction in the number of sequences ($P=0.31$), but presented significant time effect ($P<0.05$) and was not different between men and women throughout the protocol ($P=0.11$) (men: 41 ± 3 vs. women: 32 ± 3 at rest; Men: 20 ± 2 *vs*. Women: 17 ± 2 during PEI; men: 37 ± 3 vs. women: 28 ± 3 at recovery).

Table 2 Hemodynamics variables at rest, isometric handgrip exercise, post-exercise ischemia and recovery in men and women

	Rest	IHG	PEI	Recovery	*P* value		
					Sex	Time	Interaction
HR (beat min^{-1})							
Men	71 ± 2	96 ± 3	69 ± 2	65 ± 2	0.31	< 0.01	0.68
Women	74 ± 2	96 ± 5	73 ± 2	70 ± 2			
SBP (mmHg)							
Men	114 ± 2	168 ± 5*	153 ± 3*	124 ± 2*	< 0.01	< 0.01	< 0.01
Women	98 ± 1†	133 ± 4*†	122 ± 3*†	106 ± 2*†			
DBP (mmHg)							
Men	60 ± 1	92 ± 3*	82 ± 2*	62 ± 1	< 0.01	< 0.01	< 0.01
Women	56 ± 1	81 ± 2*†	71 ± 1*†	58 ± 1			
MBP (mmHg)							
Men	77 ± 1	118 ± 3*	108 ± 2*	83 ± 1*	< 0.01	< 0.01	< 0.01
Women	70 ± 1†	100 ± 3*†	90 ± 2*†	74 ± 1†			
SV (ml)							
Men	94 ± 3	96 ± 4	109 ± 4	106 ± 4	< 0.01	< 0.01	0.11
Women	70 ± 2	78 ± 3	85 ± 4	79 ± 3			
CO (l min^{-1})							
Men	6.6 ± 0.3	9.2 ± 0.5	7.4 ± 0.3	6.9 ± 0.2	< 0.01	< 0.01	0.85
Women	5.1 ± 0.2	7.5 ± 0.6	6.2 ± 0.2	5.5 ± 0.2			
CI (l $min\ m^{-2}$)							
Men	3.4 ± 0.1	4.7 ± 0.2	3.8 ± 0.1	3.5 ± 0.1	0.26	< 0.01	0.94
Women	3.1 ± 0.1	4.5 ± 0.3	3.7 ± 0.1	3.3 ± 0.1			
TVC (l min^{-1} $mmHg^{-1}$)							
Men	0.085 ± 0.004	0.080 ± 0.005	0.069 ± 0.003	0.083 ± 0.002	0.14	< 0.01	0.12
Women	0.073 ± 0.002	0.076 ± 0.006	0.069 ± 0.003	0.075 ± 0.003			
TVCI (l min^{-1} $mmHg^{-1}$ m^{-2})							
Men	0.044 ± 0.002	0.041 ± 0.003	0.036 ± 0.001	0.043 ± 0.001	0.13	< 0.01	0.20
Women	0.044 ± 0.001	0.046 ± 0.004	0.041 ± 0.001	0.045 ± 0.001			

Values represents means ± SE. *IHG* isometric handgrip exercise, *PEI* post-exercise ischemia, *HR* heart rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *MBP* mean blood pressure, *SV* stroke volume, *CO* cardiac output, *CI* cardiac index, *TVC* total vascular conductance, *TVCI* total vascular conductance index. *P* values are derived from ANOVA with repeated measures and Post-hoc analyses were employed using Fisher's test to verify the interaction. *$P < 0.05$ vs. rest; †$P < 0.05$ vs. men

Discussion

The main finding of this study is that spontaneous cBRS is enhanced during PEI following IHG exercise in men but not in women. In addition, arterial blood pressure increased during IHG exercise and remained elevated during PEI in both sexes; however, these responses were attenuated in women. Collectively, these findings suggest a sex-related difference in spontaneous cBRS elicited by isolated muscle metaboreflex activation in healthy young humans.

Previous studies in humans examining the influence of metabolically sensitive skeletal muscle afferents (i.e., muscle metaboreflex) on cBRS have found equivocal results. Some (Spaak et al. 1998; Iellamo et al. 1999b; Cui et al. 2001; Ichinose et al. 2002; Fisher et al. 2008) but not all (Sala-Mercado et al. 2007; Hartwich et al. 2011) reported that spontaneous cBRS was unchanged during muscle metaboreflex activation. Interestingly, Carrington and White (2001) verified an increase in spontaneous cBRS during PEI following electrically evoked plantar flexion in healthy men, which corroborates with our results (i.e., rise in spontaneous cBRS during PEI following IHG exercise in men). On the other hand, we found that spontaneous cBRS remained unchanged during PEI in women. Therefore, our study contributes to the literature indicating that muscle metaboreflex interact with cardiac baroreflex to the neural control of cardiovascular function in a sex-dependent manner.

The underlying mechanisms for sex differences in cBRS during muscle metaboreflex activation are unknown, but some physiological aspects should be considered. Neuroanatomical and electrophysiological evidences indicate that the arterial baroreflex and skeletal muscle afferents share common central neural pathways including the nucleus tractus solitaries (NTS) and rostral ventral lateral medulla

Table 3 Heart rate variability on time and frequency domain parameters at rest, post-exercise ischemia and recovery in men and women

	Rest	PEI	Recovery
RMSSD (ms)			
Men	53.8 ± 6.1	80.1 ± 12.0*	76.0 ± 10.9*
Women	43.2 ± 3.5	50.7 ± 2.8*†	50.6 ± 4.1*†
LF (ms^2)			
Men	1814.2 ± 312.5	1749.1 ± 271.4	2106.5 ± 433.8
Women	1322.9 ± 396.0†	1828.6 ± 562.2	1608.1 ± 709.9†
HF (ms^2)			
Men	2044.8 ± 662.2	3919.7 ± 1289.3*	3576.7 ± 1211.3*
Women	986.5 ± 184.8†	1359.8 ± 175.5*†	1238.7 ± 181.2*†
LF (nu)			
Men	49.1 ± 3.1	38.4 ± 3.6*	40.9 ± 3.0*
Women	48.6 ± 2.7	47.2 ± 4.2	42.7 ± 3.2
HF (nu)			
Men	50.7 ± 2.6	61.1 ± 3.0*	58.2 ± 2.6*
Women	52.0 ± 2.4	53.5 ± 3.8	57.6 ± 2.8
LF/HF			
Men	1.4 ± 0.2	0.9 ± 0.2*	1.0 ± 0.1*
Women	1.4 ± 0.2	2.0 ± 0.8	1.1 ± 0.2

Values represents means ± SE. *PEI* post-exercise ischemia, *RMSSD* square root of the mean of the sum of successive differences in R–R interval of HR variability, *LF* low frequency power spectral density, *HF* high frequency power spectral density. *P* values are derived from Friedman's ANOVA followed by Wilcoxon-Mann-Whitney test. * $P < 0.05$ *vs*. rest; † $P < 0.05$ vs. men

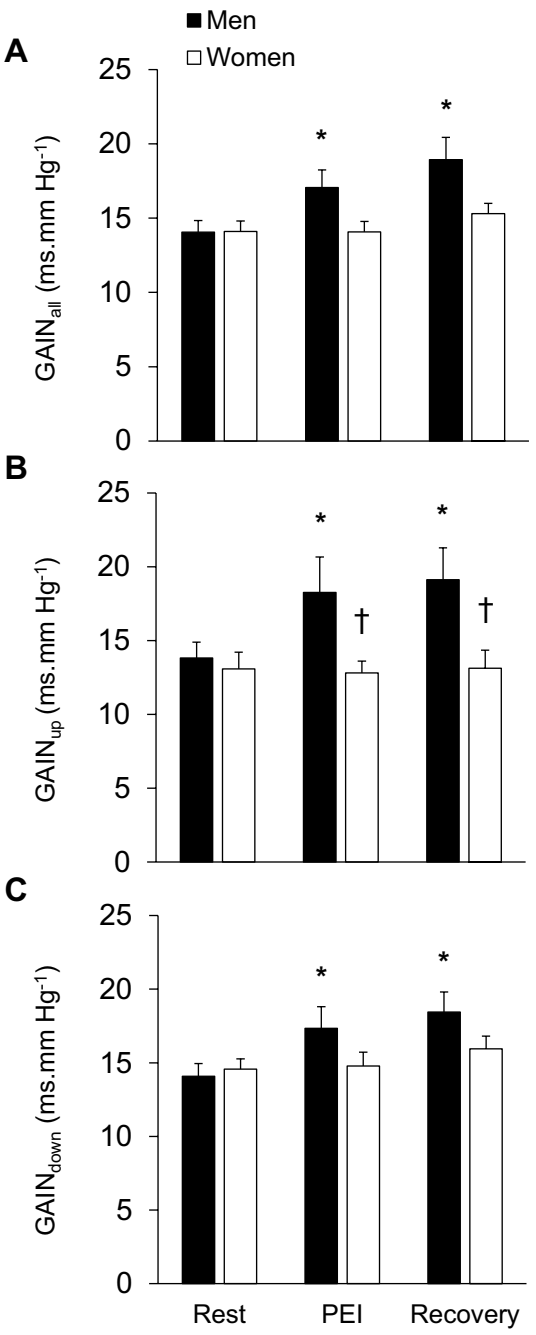

Fig. 1 Response of cardiac baroreflex sensitivity for all ($GAIN_{all}$—**a**), up ($GAIN_{up}$—**b**) and down ($GAIN_{down}$—**c**) sequences at rest, post-exercise ischemia and recovery in men (solid bars) and women (open bars). *PEI* post-exercise ischemia. * $P < 0.05$ vs. rest; † $P < 0.05$ vs. men

(Potts 2006). Chemically sensitive muscle metaboreceptors are mostly unmyelinated group IV neurons whose receptors are stimulated by metabolites produced by contracting skeletal muscle. When muscle metaboreceptor is activated via PEI, inputs to the NTS may diminish the tonic activation of the caudal ventrolateral medulla, thus attenuating the inhibition of rostral ventrolateral medulla, increasing sympathetic outflow (i.e., sympathoexcitatory reflex). The resultant effect is the maintenance of exercise-induced increases in arterial blood pressure (Fisher et al. 2008, 2010). In contrast, the arterial baroreflex are mechanoreceptor comprised of unencapsulated free nerve endings located at the medial-adventitial border of blood vessels in the carotid sinus bifurcation and aortic arch, respectively (Sheehan et al. 1941) and responds to beat-to-beat changes in blood pressure by reflexively, altering autonomic neural outflow to adjust CO and TVC. Specifically, when blood pressure is elevated, the baroreceptors are stretched and this deformation causes an increase in afferent neuronal firing which results in a reflex-mediated increase in parasympathetic nerve activity and decrease in sympathetic nerve activity (Fadel and Raven 2012). Previous studies (Potts and Mitchell 1998; Sheriff et al. 1990) demonstrated that input from muscle metaboreceptors interacts centrally with carotid baroreceptor inputs to alter baroreflex behavior. Based on this, as our finding demonstrated highest blood pressure response during PEI in men compared to women, we can speculate that these responses excites baroreflex afferents, and this provides an excitatory input to cardiac vagal motoneurons in the NTS (i.e., increasing parasympathetic nerve activity) and then

enhances the cBRS during PEI in men. Indeed, measures of parasympathetic indexes of HRV were robustly increased during PEI in men (i.e. RMSSD and HF power). In contrast, despite HF and RMSSD also increased during PEI in women, these increases were not enough to translate into increases in cBRS. The perspective of our findings is related to the fact that men present an enhancement in cBRS during PEI and, overall this can be part of a counteraction by the arterial baroreflex in response to the rise in blood pressure induced by the muscle metaboreflex, which would enhance the buffering effects of the arterial baroreflex. However, since there was no relationship between change in SBP and change in cBRS in men ($r = 0.07$, $P = 0.77$) and women ($r = 0.19$, $P = 0.42$), the magnitude of change in blood pressure could not be the reason of increased cBRS. In addition, it is important to consider the sex differences in absolute muscle strength. Recently, Notay et al. (2018) reported that the larger blood pressure responses to IHG exercise observed consistently in men compared with women are abolished after adjustment for handgrip MVC. Importantly, this study did not investigate the mechanism(s) responsible for these effects of handgrip MVC on blood pressure responses, thus future studies are required to investigate this concern. Moreover, there are some evidence of the existence of estrogen receptor within brainstem, such as ventrolateral medulla and NTS, providing a support to a possible mechanism through which circulating estrogen concentrations may influence the activity of this area (Simonian and Herbison 1997; Pelletier et al. 1988). However, Hartwich et al. (2013) reported that cBRS remained unchanged from rest during PEI in women independent of hormonal fluctuations across the menstrual cycle, indicating that the baroreflex and metaboreflex interaction is unlikely to be mediated by estrogen. Furthermore, one possible explanation should be that women demonstrated lower carotid artery distensibility compared to men (Hayward and Kelly 1997), and it is possible that this would result in a smaller mechanical transduction of arterial blood pressure into barosensory stretch, attenuating cardiovagal baroreflex response (Beske et al. 2001). However, further studies would be necessary to elucidate the possible effects of sex-differences in artery distensibility on the cBRS responses to PEI.

Of note, RMSSD and the HF power of HRV remained elevated during recovery following PEI in both men and women. Additionally, our finding showed maintenance of the increase in cBRS in men during recovery which could indicate a vagally mediated baroreflex mechanism. Interestingly, a previous study has demonstrated similar findings (Dipla et al. 2013), however, the underlying mechanism playing a role in this autonomic response during recovery following PEI is unknown. Nevertheless, in attempt to understand the mechanism for this autonomic phenotype observed during recovery, animal studies (Chen et al. 2009; Chen and Bonham 2010) suggest that exercise-induced neurokinin-1 receptor (NK1-R) internalization, which reduces GABA interneuron excitability after exercise, resulting in reduced GABA inhibition in the NTS. Overall, this disinhibition of the NTS neurons in the baroreflex pathway could translate to a higher excitatory output from NTS to different autonomic nuclei, which potentially translates to higher parasympathetic activity.

The limitations of the present study should be considered. We studied only young healthy and physically active subjects, limiting to extrapolate our results to other population such as older men, postmenopausal women and/or sedentary and diseased patients. In addition, cBRS was calculated based on spontaneous data which assesses a limited range of pressure for the stimulus–response baroreflex relationship. In this sense, further studies should investigate sex difference on cBRS using other perturbation methods, such as infusion of vasoactive drugs (i.e. modified Oxford) and whether menstrual cycle or age (i.e. postmenopausal women) influence on cBRS during isolated muscle metaboreflex activation.

In summary, our finding demonstrated that spontaneous cBRS is enhanced during PEI following IHG exercise in men but not in women. In addition, arterial blood pressure increased during IHG exercise and remained elevated during PEI; however, these responses were attenuated in women. Overall, these results allow us to suggest a sex-related difference in spontaneous cBRS elicited by isolated muscle metaboreflex activation in healthy humans.

Acknowledgements The time and effort expended by all the volunteer subjects is greatly appreciated.

Author contributions MS and LCV conceived and designed research. MS, ALT, JLSC and LCV performed experiments. MS, ALT and LCV analyzed data. MS, ALT and LCV interpreted results of experiments. MS prepared figures. MS, ALT and LCV drafted manuscript. All authors read and approved final version of manuscript.

Funding This study was supported by grants and scholarships from the Brazilian National Council of Scientific and Technological Development (CNPq), the Foundation for Research Support of Federal District (FAPDF), Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) and partially supported by an American Physiological Society Arthur C. Guyton Awards for Excellence in Integrative Physiology (to L.C. Vianna).

Compliance with ethical standards

Conflict of interest None of the authors declares a conflict of interest.

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