Am J Physiol Heart Circ Physiol 314: H452–H463, 2018.
First published November 10, 2017; doi:10.1152/ajpheart.00436.2017.

RESEARCH ARTICLE | *Integrative Cardiovascular Physiology and Pathophysiology*

Metaboreflex-mediated hemodynamic abnormalities in individuals with coronary artery disease without overt signs or symptoms of heart failure

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Submitted 14 July 2017; accepted in final form 6 November 2017

Magnani S, Roberto S, Sainas G, Milia R, Palazzolo G, Cugusi L, Pinna V, Doneddu A, Kakhak SA, Tocco F, Mercuro G, Crisafulli A. Metaboreflex-mediated hemodynamic abnormalities in individuals with coronary artery disease without overt signs or symptoms of heart failure. *Am J Physiol Heart Circ Physiol* 314: H452–H463, 2018. First published November 10, 2017; doi:10.1152/ajpheart.00436.2017.—This study was devised to investigate the effect of coronary artery disease (CAD) without overt signs of heart failure on the cardiovascular responses to muscle metaboreflex activation. We hypothesized that any CAD-induced preclinical systolic and/or diastolic dysfunction could impair hemodynamic response to the metaboreflex test. Twelve men diagnosed with CAD without any sign or symptoms of heart failure and 11 age-matched healthy control (CTL) subjects participated in the study. Subjects performed a postexercise muscle ischemia (PEMI) test to activate the metaboreflex. They also performed a control exercise recovery test to compare data from the PEMI test. The main results were that the CAD group reached a similar mean arterial blood pressure response as the CTL group during PEMI. However, the mechanism by which this response was achieved was different between groups. In particular, CAD achieved the target mean arterial blood pressure by increasing systemic vascular resistance ($+383.8 \pm 256.6$ vs. $+91.2 \pm 293.5$ dyn·s^{-1}·cm^{-5} for the CAD and CTL groups, respectively), the CTL group by increasing cardiac preload (-0.92 ± 8.53 vs. 5.34 ± 4.29 ml in end-diastolic volume for the CAD and CTL groups, respectively), which led to an enhanced stroke volume and cardiac output. Furthermore, the ventricular filling rate response was higher in the CTL group than in the CAD group during PEMI ($P < 0.05$ for all comparisons). This study confirms that diastolic function is pivotal for normal hemodynamics during the metaboreflex. Moreover, it provides evidence that early signs of diastolic impairment attributable to CAD can be detected by the metaboreflex test.

NEW & NOTEWORTHY Individuals suffering from coronary artery disease without overt signs of heart failure may show early signs of diastolic dysfunction, which can be detected by the metaboreflex test. During the metaboreflex, these subjects show impaired preload and stroke volume responses and exaggerated vasoconstriction compared with controls.

cardiac afterload; cardiac preload; blood pressure; myocardial contractility; stroke volume

INTRODUCTION

During exercise, the accumulation of metabolic byproducts produced by muscle contraction activates the sympathetic nervous system by triggering a cardiovascular reflex commonly known as the muscle metaboreflex (21, 37, 39, 51). The postexercise muscle ischemia (PEMI) method is often used to study the metaboreflex because it allows for the metaboreflex to be isolated from the other two mechanisms that increase the sympathetic discharge during exercise, i.e., the central command and mechanoreflex (15, 51).

Because in the PEMI setting the recruitment of the heart rate (HR) reserve is absent (15–17, 26, 38, 62), this method appears particularly useful to study the hemodynamic responses to metaboreflex activation when impairments in cardiac preload, inotropism, and afterload are present. These cardiac reserves are in fact all recruited in healthy individuals during PEMI (1, 13, 14, 25, 31, 32, 36, 37, 41, 49, 50, 53, 59, 60).

By using the PEMI method, hemodynamic dysregulation has been demonstrated in different cardiovascular and metabolic and nervous diseases. In particular, it has been observed that, whenever the preload and inotropic reserves cannot be recruited, the hemodynamic regulation relies more on the afterload reserve and arteriolar vasoconstriction (14, 16, 31, 33, 50). This appears to be the consequence of the incapacity to enhance stroke volume (SV), which normally is stable or increases during PEMI in healthy individuals (13, 17). Indeed, in healthy individuals, the SV response maintains or increases cardiac output (CO) during PEMI, whereas the HR reserve is usually not involved in the phenomenon (13, 15, 17, 38, 39, 40, 42). The impossibility to increase SV in turn leads to exaggerated systemic vascular resistance (SVR) increments to achieve the target blood pressure (16, 44, 47, 48).

In short, it seems that in healthiness the preferred cardiovascular adjustment to PEMI is a flow-mediated mechanism obtained by recruiting the inotropic and preload reserve, whereas, when these reserves cannot be used any longer, SVR increments become pivotal.

Coronary artery disease (CAD) is the most prevalent form of cardiovascular disease. Because CAD in its severe form affects both inotropism and heart diastolic function, it is possible to hypothesize that hemodynamics would be dysregulated in these patients. Indeed, one of the most deleterious hemodynamic consequences of CAD is overt heart failure, with impairments in both systolic and diastolic function. The conse-

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quences of CAD-induced overt heart failure during the metaboreflex have been already studied. It has been previously reported that an exaggerated SVR increment in response to the PEMI test is attributable to the impaired systolic and diastolic function (16, 50). However, although the consequences of overt CAD-induced systolic and diastolic impairment during PEMI have been already investigated (16, 50), to the best of our knowledge, nothing is known about the hemodynamic consequences of CAD with no overt signs of heart failure. A study of this kind would help to establish whether the PEMI test was able to detect preclinical signs of myocardial dysfunction. This would provide a possible diagnostic tool.

This study was devised to investigate the effect of CAD without clinical signs of heart failure on the cardiovascular adjustments during metaboreflex activation obtained by the PEMI method. We hypothesized that the presence of preclinical systolic and/or diastolic dysfunction reduced the possibility to achieve the target hemodynamic response by means of a SV-induced flow-mediated mechanism, thereby leading to an exaggerated SVR response in these patients.

Table 1. *Medication at the time of the study and echocardiographic data of the CTL and CAD groups*

	CTL (n = 11)	CAD (n = 12)
Aspirin		11
Angiotensin converting enzyme inhibitors		8
Angiotensin receptor blockers		3
β-Blockers		12
Ca^{2+} antagonists		1
Diuretics		2
Statins		11
Clopidogrel/ticagrelor		6
Carvasin		3
End-diastolic volume	137.20 ± 16.50	112.20 ± 18.30
End-systolic volume	70.20 ± 21.30	64.20 ± 17.80
Ejection fraction	52.50 ± 16.80	57.40 ± 12.50
E_{vel}	60.80 ± 10.70	52.80 ± 7.20
A_{vel}	38.00 ± 6.60	42.90 ± 9.00
E/A	1.56 ± 0.18	1.26 ± 0.21

Values are means ± SE; n, number of subjects. CTL, control; CAD, coronary artery disease; *E/A*, E wave velocity (E_{vel})-to-A wave velocity (A_{vel}) ratio.

METHODS

Study population. Two groups of subjects were studied. The first group was individuals with CAD. Inclusion criteria included patients with clinical and angiographic findings of severe CAD diagnosed at least 6 mo before enrollment, symptoms of stable CAD, and age between >30 and <70 yr. Exclusion criteria included unstable angina pectoris, significant valvular heart disease, the presence of pulmonary disease, and findings supportive of heart failure, such as reduced exercise capacity, early exertional fatigue, dyspnea at rest, and reduced left ventricular (LV) ejection fraction (EF) at rest (<45%) (58). Enrollment was restricted to male patients to eliminate any potential effect of hormonal changes during the menstrual cycle that might affect vascular responsiveness and interfere with the metaboreflex response. At the end of enrollment, 12 men (age: 45–67 yr, 57.3 ± 8.1 yr) were found eligible to take part in the present study. All recruited subjects received a diagnosis of non-ST elevation myocardial infarction at least 6 mo before the study (range: 6–46 mo, 19.8 ± 11.7 mo) and were successfully treated with coronary stent implantation during percutaneous coronary intervention. Seven subjects reported a diagnosis of hypertension before infarction, whereas six subjects were under treatment with statins for hypercholesterolemia. Their means ± SD of height and body mass were 171.7 ± 6.2 cm and 76.4 ± 5.8 kg, respectively. Medications and the main echocardiographic data at the time of the study are shown in Table 1. Echocardiography examination confirmed that none of them suffered from any valvular disease.

The second group was the healthy control (CTL) group, which constituted 11 men (aged between 32 and 69 yr) who were healthy and physically active, accumulating a total of at least 150 min/wk of moderate to vigorous exercise. Their means ± SD of age, height, and body mass were 56.2 ± 14.8 yr, 166.7 ± 5.7 cm, and 70.2 ± 8.5 kg, respectively. Subjects were free from any known cardiovascular, metabolic, or respiratory disease and were not taking any medication for chronic disease. Their echocardiographic data at the time of the study are shown in Table 1.

Written informed consent was obtained from all individuals, and the study was approved by the local Ethical Committee and conformed with principles of the Declaration of Helsinki.

Experimental design. Before taking part in the study, participants underwent a general medical examination with rest ECG. This examination also included an incremental exercise test with continuous ECG monitoring and gas exchange analysis (CPX) to assess subjects' physical capacity and to exclude exercise intolerance and the presence of detectable stress-induced myocardial ischemia during exercise. CPX was performed by a gas analyzer (ULTIMA CPX, MedGraphics, St. Paul, MN) while subjects pedaled on an electromagnetically braked cycle ergometer (CUSTO Med, Ottobrunn, Germany) to assess maximal O_2 uptake ($\dot{V}O_{2max}$). This test aimed to exclude the presence of reduced physical capacity on the basis of the Weber functional classification for heart failure (63). The incremental exercise test consisted of a linear increase of workload (10 or 20 W/min depending on the fitness level of the subject), starting at 10 W, at a pedaling frequency of 60 revolutions/min, until exhaustion, which was taken as the point at which the subject experienced fatigue (i.e., was unable to maintain a pedaling rate of at least 50 revolutions/min). Achievement of $\dot{V}O_{2max}$ was considered as the attainment of at least two of the following criteria: *1*) a plateau in O_2 uptake despite increasing workload (<80 ml/min), *2*) a respiratory exchange ratio above 1.10, and *3*) HR ± 10 beats/min of predicted maximum HR calculated as 220 − age of the subject (24).

After this preliminary medical examination (minimum 3-day interval, range: 3–7 days), subjects underwent our protocol to study the hemodynamic response during the activation of the muscle metaboreflex. One portion of the protocol was a PEMI session. Subjects rested for 3 min seated on a chair to have reference baseline. This period was followed by 3 min of exercise, consisting of a rhythmic (30 compressions/min) dynamic handgrip at 30% of the maximum assessed as the peak reached during five previous maximal compressions on a hydraulic dynamometer (MAP 1.1, Kern, Balingen, Germany). Exercise was followed by 3 min of PEMI on the exercised arm obtained by rapidly inflating a tourniquet to 50 mmHg above peak exercise systolic pressure at the end of exercise (in <3 s). The duration of circulatory occlusion was 3 min. The cuff was then deflated, and a further period of 3 min of recovery was allowed. Thus, the total recovery lasted 6 min. This protocol has been used several times in the past in similar experimental settings to study hemodynamic consequences of metaboreflex recruitment in both healthy subjects and patients (14, 15, 29, 30, 33, 49, 50).

There was also a control exercise recovery (CER) session. The same rest-exercise protocol used for PEMI was used, but ischemia after exercise was not applied in this session. Instead, a period of 6-min recovery was conducted. The CER test allowed for a control exercise-recovery situation without metaboreflex activation to compare data obtained from the PEMI test.

The PEMI and CER tests were assigned in random order. All experiments were carried out in a temperature-controlled, air-conditioned room (22°C, relative humidity: 50%) between 10.00 AM and 01.00 PM. Subjects were asked to consume a light meal at least 3 h

before the experiments and to refrain from caffeine ingestion for at least 6 h.

Hemodynamic assessment. Throughout the PEMI and CER tests, hemodynamics were measured using the transthoracic impedance cardiography method, which has been previously used in similar experimental settings dealing with the metaboreflex activation in both healthy subjects and disease states (14, 16, 18, 29, 32, 33, 49, 50). The impedance method allows for noninvasive calculation of SV on the basis of the Sramek-Bernstein equation (5). Subjects were connected with eight spot electrodes to an impedance cardiograph (NCCOM 3, BoMed, Irvine, CA) able to provide data of thorax impedance (Z_0) and the Z_0 first derivative, which were acquired using a digital chart recorder (PowerLab 8sp, AD Instruments) and analyzed offline. Moreover, NCCOM 3 was able to provide an analog ECG trace, which was also recorded and analyzed offline.

HR was calculated as the reciprocal of the electrocardiogram RR interval, and CO was obtained as SV $\times$ HR. The preejection period (PEP) and LV ejection time (LVET) were also calculated from impedance traces, as described in a previous study (17). Diastolic time (DT) was measured by subtracting the sum of PEP and LVET from the cardiac cycle total period, and the ventricular filling rate (VFR), a measure of the mean rate of diastolic blood flux, was calculated by dividing SV by DT (17, 31). A manual sphygmomanometer was placed in the nondominant arm, and systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by the same physician throughout all protocol sessions. Mean arterial blood pressure (MAP) was calculated using conventional formulae (52). SVR was obtained by multiplying MAP/CO by 80, where 80 is a conversion factor to convert units to standard resistance units.

During the PEMI and CER sessions, end-diastolic volume (EDV) and end-systolic volume (ESV) were also measured with two-dimensional echocardiography (M5 Diagnostic Ultrasound System, Mindray Bio-Medical Electronics, Shenzen, China) equipped with a handheld 3.5-MHz ultrasound probe. Echocardiography images were collected in the apical four-chamber view with subjects in the sitting position. We were not able to take good echocardiography images during exercise phases; thus, measures were performed at rest and during the last minute of the PEMI period and the corresponding period of the CER test (i.e., at the third minute of recovery). When images were considered of good quality, a 6-s frame was recorded and then analyzed offline by an operator who was unaware of the purpose of the study. At least three beats were taken into consideration for each analysis (range: 3–6 beats). Individual values in each beat were calculated as the average of three trials of the same beat, i.e., each beat value was the average from three measures. EDV and ESV were calculated using the following formula: $8A^2/3\pi L$, where A is the LV area and L is the longest ventricular length (8). Ventricular area was determined by tracing along the inner edge of the endocardial targets, and length was obtained by measuring the distance from the LV apex to the midpoint of the mitral annulus. Moreover, LV EF was calculated as (EDV $-$ ESV/EDV) $\times$ 100 to have a measure of myocardial performance.

In the same beats used for the assessment of EDV, ESV, and EF, early and atrial transmitral filling peak velocities (E_{vel} and A_{vel}, respectively) and their ratio (E/A) were collected using a pulse-wave Doppler recording to evaluate LV diastolic functions (10, 22). Measures were obtained from the apical four-chamber view with a 5-mm pulse-wave Doppler sample volume placed distal to the mitral annulus, between the mitral leaflets. The interrogation beam was aligned with mitral flow.

Data analysis. Data are shown as means $\pm$ SD. Hemodynamic data during PEMI and CER tests were averaged over 1 min. Descriptive statistics were performed on each variable to confirm the assumptions of normality by means of the Kolmogorov-Smirnov test. The α level was set at $P < 0.05$. Values at rest, at the third minute of exercise, and at the third minute of recovery from both tests (when a steady state in metaboreflex activity was expected to be reached) were taken into account for statistical analysis. To further assess the metaboreflex activity, the following procedure was used. The difference in variable level between the PEMI and CER at the third minute of recovery was calculated. This procedure enabled metaboreflex response to be assessed, i.e., the response attributable to the metaboreflex activity (18, 32, 33). Differences in levels of parameters between groups were assessed by means of two-way ANOVA for repeated measures (factors were group and condition) followed by Tukey's post hoc test when appropriate, whereas differences in response variables were assessed by a *t*-test for unpaired data. Statistics were carried out by means of commercially available software (Prism, GraphPad, San Diego, CA). Significance was set at a P value of <0.05 in all cases.

RESULTS

All subjects completed the protocol, and none reported problems and/or signs of exercise intolerance during both the CPX and metaboreflex test. In particular, none of the patients from the CAD group showed any signs or symptom of myocardial ischemia during the incremental exercise test. The Kolmogorov-Smirnov test confirmed the normal distribution for all parameters examined. Therefore, they were not destined to nonparametric analysis. Statistics did not reveal any difference in terms of age ($P = 0.825$), body mass ($P = 0.052$), or height ($P = 0.057$) between the two groups. Results from the CPX test showed that the $\dot{V}O_{2max}$ reached was 26.09 $\pm$ 6.76 versus 39.43 $\pm$ 11.38 ml·min^{-1}·kg^{-1} for the CAD and CTL groups, respectively ($P = 0.002$). The maximum workload during the handgrip test was 25.71 $\pm$ 5.51 versus 27.38 $\pm$ 5.31 kg for the CAD and CTL groups, respectively ($P = 0.468$).

The values of data recorded during rest periods preceding handgrip strains are shown in Table 2. There was no condition effect for any of the recorded variables. Groups significantly affected VFR, MAP, EDV, E_{vel}, and E/A, which were higher in the CTL group than in the CAD group, whereas A_{vel} was higher in the CAD group with respect to the CTL group.

Table 3 shows the values of hemodynamic parameters at the third minutes of exercise. Because we were not able to collect data with echocardiography and transmitral Doppler during handgrip strains, Table 3 only shows data gathered with impedance cardiography. Statistics reveal that condition did not affect parameters, whereas the group did influence all variables, with the exception of SV. In particular, the CTL group showed higher HR, CO, VFR, and MAP and lower SVR values compared with the CAD group.

Figures 1–4 show the hemodynamic levels and responses at the third minute of recovery after the handgrip of the PEMI and CER sessions in both groups. In detail, Fig. 1 shows that HR was similar between groups both in terms of absolute level (Fig. 1*A*) and response (Fig. 1*B*). SV absolute value was not influenced by group or condition (Fig. 1*C*). However, the PEMI maneuver induced a higher SV response in the CTL group than in the CAD group. In particular, the SV response was positive in the CTL group and negative in the CAD group (Fig. 1*D*). Resulting from the HR and SV behavior, CO (Fig. 1*E*) was significantly higher in the CTL group than in the CAD group considering the absolute values (Fig. 1*E*) and the response (Fig. 1*F*), which was positive in the CTL group and negative in the CAD group.

Figure 2*A* shows that EDV was higher in the CTL group than in the CAD group. Furthermore, statistics demonstrated that the EDV response was positive in the CTL group, whereas it was negative in the CAD group (Fig. 2*B*). Likewise, Fig. 2*C*

Table 2. *Hemodynamic data values during rest periods preceding PEMI and CER tests in CTL and CAD groups*

	CTL (n = 11)	CAD (n = 12)	P Value Group Effect	P Value Condition Effect
Heart rate, beats/min				
PEMI	66.40 ± 8.50	62.80 ± 8.30	0.164	0.970
CER	66.70 ± 11.50	62.70 ± 7.80		
Stroke volume, ml				
PEMI	65.30 ± 22.60	59.40 ± 26.90	0.329	0.712
CER	70.20 ± 31.00	60.40 ± 26.80		
Cardiac output, l/min				
PEMI	4.22 ± 1.02	3.70 ± 1.73	0.115	0.670
CER	4.58 ± 1.47	3.71 ± 1.51		
Ventricular filling rate, ml/s				
PEMI	132.30 ± 39.20	111.90 ± 56.00	0.046	0.611
CER	148.90 ± 50.90	110.10 ± 47.60		
Mean arterial pressure, mmHg				
PEMI	90.70 ± 10.20	87.00 ± 9.70	0.025	0.928
CER	93.70 ± 9.10	84.50 ± 8.70		
Systemic vascular resistance, $dyn \cdot s^{-1} \cdot cm^{-5}$				
PEMI	1,817.30 ± 482.20	2,172.00 ± 807.80	0.193	0.826
CER	1,853.80 ± 799.60	2,044.30 ± 648.30		
End-diastolic volume, ml				
PEMI	129.60 ± 22.10	104.20 ± 19.90	0.002	0.937
CER	128.30 ± 22.30	106.40 ± 12.00		
End-systolic volume, ml				
PEMI	64.30 ± 14.30	44.70 ± 30.50	0.068	0.746
CER	55.40 ± 18.70	49.10 ± 25.70		
Ejection fraction, %				
PEMI	49.60 ± 10.80	58.6 ± 19.4	0.406	0.766
CER	55.90 ± 14.70	55.3 ± 20.5		
E_{vel}, cm/s				
PEMI	57.00 ± 7.50	49.90 ± 8.70	0.023	0.917
CER	58.10 ± 6.90	49.50 ± 17.90		
A_{vel}, cm/s				
PEMI	37.20 ± 4.10	45.40 ± 10.80	0.001	0.884
CER	37.70 ± 5.40	45.60 ± 9.60		
E/*A*				
PEMI	1.53 ± 0.17	1.12 ± 0.16	<0.001	0.973
CER	1.56 ± 0.22	1.09 ± 0.35		

Values are means ± SD; n, number of subjects. PEMI, postexercise muscle ischemia; CER, control exercise recovery; CTL, control; CAD, coronary artery disease; *E*/*A*, E wave velocity (E_{vel})-to-A wave velocity (A_{vel}) ratio.

shows that ESV absolute values were higher in the CTL group than in the CAD group, but the response in this parameter was unaffected by group (Fig. 2*D*). Figure 2 also shows that EF was not different between groups, either in terms of absolute values or in terms of response (Fig. 2, *E* and *F*, respectively).

The PEMI maneuver significantly increased MAP with respect to the CER session (Fig. 3*A*). The MAP increment was similar between groups. Moreover, there was no difference in the MAP response between groups (Fig. 3*B*). Figure 3*C* shows that there was no group or condition effect for absolute SVR values, although a P value very close to significance (0.0573) was reached for group. The SVR response was higher in the CAD group compared with the CTL group (Fig. 3*D*). Absolute values of VFR were not affected by group or condition (Fig. 3*E*). However, the VFR response was, on average, positive in the CTL group and negative in the CAD group, reaching statistical significance (Fig. 3*F*).

Finally, Fig. 4 shows results from the transmitral Doppler analysis. E_{vel} absolute values were higher in the CTL group than in the CAD group (Fig. 4*A*). Conversely, A_{vel} was higher in the CAD group compared with the CTL group (Fig. 4*C*). There was no difference between groups in the responses of these parameters (Fig. 4, *B* and *D*, respectively). Figure 4*E* shows that, on average, *E*/*A* was higher in the CTL group than in the CAD group. Moreover, the *E*/*A* response was significantly different between groups, as it was positive in the CTL group and negative in the CAD group (Fig. 4*F*).

DISCUSSION

The present study was conducted on individuals with a diagnosis of CAD but without overt signs of heart failure and without symptoms of reduced exercise capacity. In fact, according to the Weber classification of heart failure, none of the patients with CAD was below the $\dot{V}O_{2max}$ cutoff (i.e., 20 $ml \cdot min^{-1} \cdot kg^{-1}$) to be included in the mild-moderate stage of deterioration of functional capacity. The purpose was to test whether or not the presence of CAD per se caused any abnormal hemodynamic response during the metaboreflex activation obtained by the PEMI method. Our reasoning was that the presence of any initial subclinical impairment in systolic and/or diastolic functions precluded the possibility to increase SV and CO in response to the PEMI maneuver. As a consequence, this occurrence would lead to an exaggerated SVR increment in the mechanism through which the target blood pressure was reached during the metaboreflex. The results confirmed our initial hypothesis that CAD per se alters hemodynamics during the metaboreflex obtained by PEMI.

Table 3. *Hemodynamic data values during the third minute of exercise preceding PEMI and CER maneuvers in CTL and CAD groups*

	CTL (n = 11)	CAD (n = 12)	P Value Group Effect	P Value Condition Effect
Heart rate, beats/min				
PEMI	75.50 ± 11.20	68.80 ± 8.40	0.010	0.810
CER	77.30 ± 11.10	68.40 ± 8.60		
Stroke volume, ml				
PEMI	73.30 ± 29.70	59.30 ± 20.90	0.061	0.842
CER	75.20 ± 27.60	60.40 ± 22.90		
Cardiac output, l/min				
PEMI	5.34 ± 1.55	4.07 ± 1.56	0.003	0.735
CER	5.60 ± 1.22	4.11 ± 1.58		
Ventricular filling rate, ml/s	PEMI CER	PEMI CER		
PEMI	179.20 ± 82.40	129.50 ± 54.20	0.010	0.732
CER	190.40 ± 82.00	130.80 ± 56.80		
Mean arterial blood pressure, mmHg				
PEMI	112.10 ± 17.50	102.00 ± 11.30	0.022	0.787
CER	114.20 ± 19.80	102.40 ± 12.90		
Systemic vascular resistance, $dyn \cdot s^{-1} \cdot cm^{-5}$				
PEMI	1,804.40 ± 542.40	2,219.40 ± 698.90	0.015	0.741
CER	1,695.90 ± 425.60	2,206.20 ± 739.00		

Values are means ± SD; n, number of subjects. PEMI, postexercise muscle ischemia; CER, control exercise recovery; CTL, control; CAD, coronary artery disease.

To the best of our knowledge, the present is the first study that has investigated hemodynamics during the metaboreflex in humans with a CAD diagnosis; thus, our results cannot be compared with similar investigations. The only studies we were able to find on the consequence of coronary flow reduction during the metaboreflex were conducted in animal models of CAD or in instrumented dogs with pacing-induced heart failure (2, 11, 12, 28, 43), whereas only one study was conducted on humans who received a coronary bypass, a situation quite different from that of the present investigation (34). Thus, any comparison between our results and those from other studies is problematic.

In experiments conducted on dogs, it has been found that the activation of the metaboreflex during dynamic exercise can induce significant coronary vasoconstriction (2, 43). This phenomenon suggests that the metaboreflex may restrain coronary vasodilation and cause reduction in the coronary flow. The sympathetic restraint of coronary vasodilation may in turn limit increases in ventricular contractility (11). Moreover, in dogs with heart failure, it has been reported that during the metaboreflex the inability to raise ventricular contractility was not solely due to ventricular dysfunction but it was also the consequence of coronary vasoconstriction, which limited myocardial perfusion (12). Taken together, these results indicate that, in the presence of cardiac disorders (heart failure and reduced coronary reserve), the metaboreflex-induced coronary vasoconstriction exerts detrimental effects on hemodynamics. However, to the best of our knowledge, there is no study that has investigated the effect of metaboreflex activation on the coronary circulation during heart failure in the human setting. Thus, it is not known whether these results in a canine model of metaboreflex can also be applied to humans.

In contrast, the only study performed on humans enrolled patients that had a coronary bypass. The authors reported that coronary vasoconstriction did not take place during the metaboreflex (34). We do not know the reasons for this difference between studies in dogs and in the human setting. Possible explanations include differences in exercise mode (static vs. dynamic), recruited muscle mass, and, of course, differences attributable to species. However, the quoted study on humans was conducted only on six subjects who had a coronary bypass. Thus, definitive conclusions cannot be drawn from such a small and particular sample.

In our study, we observed several hemodynamic alterations during the PEMI-induced metaboreflex activation. The first abnormality was the lack of any SV increment in response to the PEMI maneuver in the CAD group. Inasmuch as HR did not show any appreciable response, the impaired SV response in turn led to the incapacity to sustain CO and caused a functional shifting from a CO-mediated (i.e., flow-mediated) mechanism to a SVR-mediated (i.e., vasoconstriction-mediated) mechanism through which the target blood pressure response was obtained during the metaboreflex. This kind of functional shift has been reported several times both in humans and animals studies dealing with heart failure. The precise mechanism through which this phenomenon occurs remains speculative, although a reduced capacity of the arterial baroreflex to buffer the metaboreflex activity has been demonstrated in animal models of heart failure (27). To the best of our knowledge, to date, no one has investigated this phenomenon in humans. It should be underscored that both MAP absolute values and MAP response were quite similar between groups, thereby indicating that the CAD group was still able to have a normal blood pressure regulation even though SV could not be increased. This intact ability to increase blood pressure even in the presence of hemodynamic abnormalities was very similar to what was previously observed in patients suffering from several cardiovascular, metabolic, and neurological diseases as well as in normal aging (16, 18, 29–33, 50) and supports the concept that blood pressure is defended by a mechanism controlling the cardiovascular system even when one or more of the cardiovascular modulators (i.e., chronotropism, inotropism, preload, and afterload) are impaired.

In our investigation, several phenomena may account for the absence of any PEMI-mediated SV increase in the CAD group. The first could be the incapacity to enhance myocardial per-

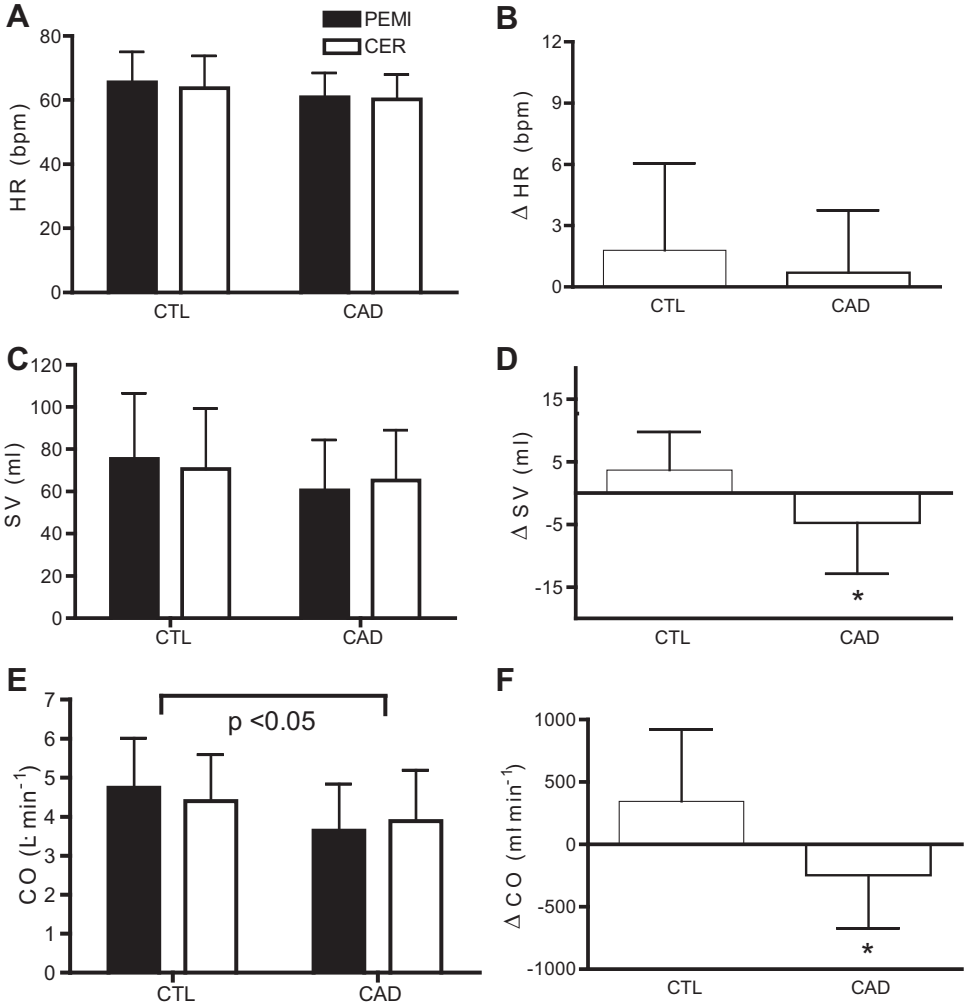

Fig. 1. Absolute values during postexercise muscle ischemia (PEMI) and control exercise recovery (CER) tests and response in heart rate (HR; *A* and *B*), stroke volume (SV; *C* and *D*), and cardiac output (CO; *E* and *F*) in the control (CTL) group and coronary artery disease (CAD) group. Values are means ± SD. A horizontal bracket indicates the overall main effect of groups. There was no interaction effect. *$P < 0.05$ vs. the CTL group.

formance. However, this hypothesis should be rejected, as statistics did not find any difference in EF absolute values and the EF response between groups, although the EF response was positive in the CTL group and negative in the CAD group. However, there was not significant effect between groups ($P = 0.074$).

Another possible explanation could be an impairment in the diastolic function. As a matter of fact, several clues support this hypothesis. First, the EDV response was different between groups. It increased in the CTL group, whereas it decreased in the CAD group, during the PEMI maneuver. Likewise, VFR, a measure of diastolic flux, increased during the metaboreflex in the CTL group and decreased in the CAD group. However, *E*/*A*, a measure that reflects ventricle stiffness and compliance and that is often used to investigate diastolic function (9), increased during the PEMI session in the CTL group and decreased in the CAD group. Taken together, all these results support the hypothesis that the main hemodynamic consequence of CAD was on diastolic rather than systolic myocardial functions. To further support this hypothesis, there was the observation that diastolic function appeared impaired already at rest in the CAD subjects compared with CTL subjects. Interestingly, a group effect for E_{vel}, A_{vel}, and *E*/*A* was discovered. Reduced E_{vel} and *E*/*A* and increased A_{vel} are all signs of impaired myocardial diastolic function (9). In particular, these indexes reflect increased myocardial stiffness and reduced relaxation, which are compensated with a shift of diastolic volumes from early to late diastole, i.e., to the atrial contraction.

The hemodynamic results of the present investigation closely mirrored what has been recently reported in subjects suffering from overt heart failure with preserved EF, where the impaired diastolic function was responsible for an abolished EDV, VFR, and E_{vel} response during the metaboreflex elicited by PEMI (50). Moreover, similar results were reported by Sala-Mercado et. al (54), who demonstrated impaired metaboreflex responses, in part attributable to impaired diastolic dysfunction, in hypertensive canines. As a consequence, SV decreased, and this phenomenon did not allow for a CO-mediated MAP response during PEMI. Instead, there was an exaggerated SVR-mediated mechanism (i.e., exaggerated vasoconstriction) through which the target blood pressure response was obtained during the metaboreflex.

It remains to be explained how the presence of CAD without any overt symptoms of heart failure impacted the cardiac diastolic function. In our opinion, one possible explanation could be that the observed diastolic impairment was the consequence of a low myocardial energetic in the CAD group. Indeed, ventricular relaxation is an energy-consuming process, as ATP hydrolysis is required for myofilament detachment and

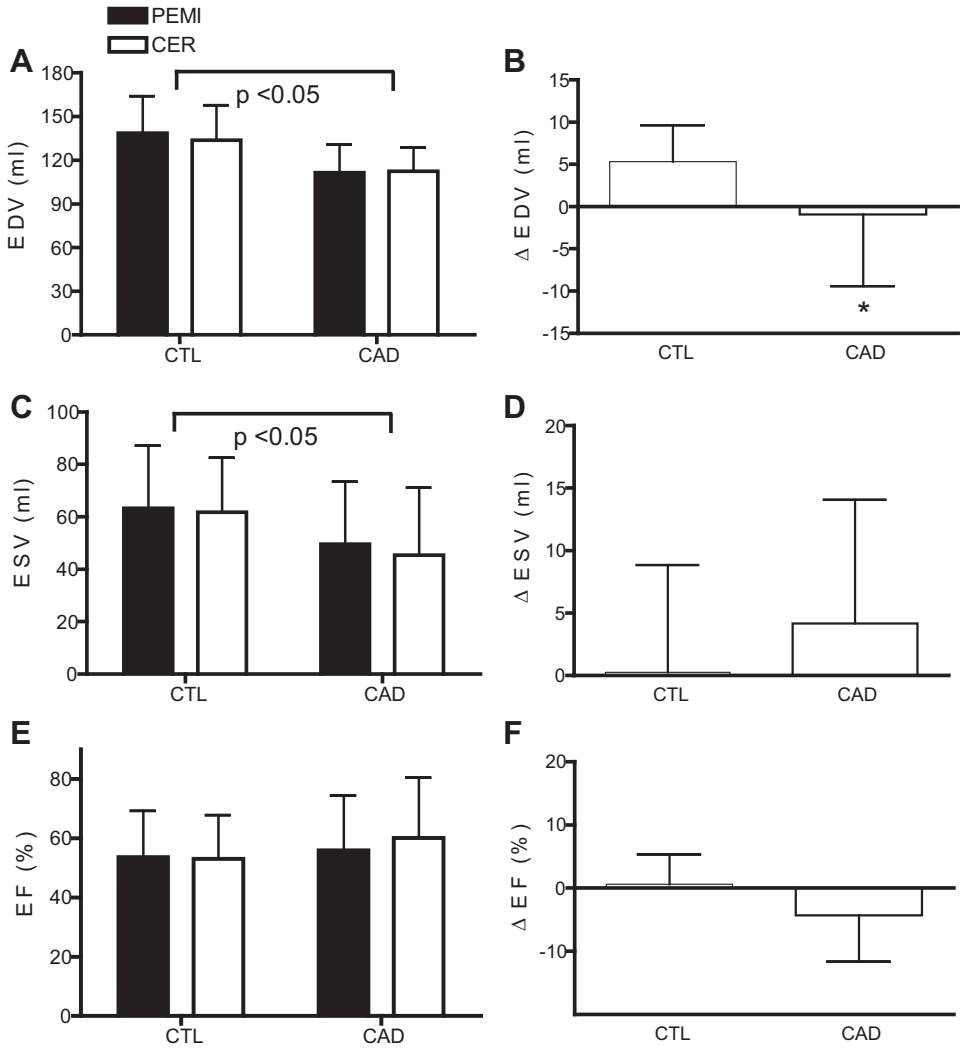

Fig. 2. Absolute values during postexercise muscle ischemia (PEMI) and control exercise recovery (CER) tests and response in end-diastolic volume (EDV; *A* and *B*), end-systolic volume (ESV; *C* and *D*), and ejection fraction (EF; *E* and *F*) in the control (CTL) group and coronary artery disease (CAD) group. Values are means ± SD. A horizontal bracket indicates the overall main effect of group. There was no interaction effect. $*P < 0.05$ vs. the CTL group.

subsequent myocardial relaxation (64). Furthermore, the rate of relaxation is determined also by active Ca^{2+} pumping through sarco(endo)plasmic reticulum Ca^{2+}-ATPase (7). All these processes require ATP consumption. The low ventricular energetics could be in turn responsible for a preclinical diastolic dysfunction (PDD), which is an emerging cardiac condition characterized by LV diastolic dysfunction without the symptoms of congestive heart failure and with normal systolic function (61). In individuals suffering from PDD, there is an impaired diastolic function but without any overt reduced cardiac functionality, such as early exertional fatigue and low exercise capacity. Given the difficulties in the identification and definition of PDD, the exact measurement of its prevalence is difficult, although a study has suggested that its prevalence is ~20−30% in the general adult population (61). Moreover, its progression to overt heart failure remains to be elucidated.

Thus, our findings may be explained with a PDD attributable to low ventricular energetics. This occurrence could be the consequence of a reduced coronary reserve during the metaboreflex in the CAD group. Although we did not observe any sign or symptom of myocardial ischemia during the incremental test and during the PEMI and CER test, the possibility that a coronary vasoconstriction took place cannot be ruled out, as coronary vasoconstriction has been observed even in healthy individuals during maneuvers causing sympathetic activation (35). With this in mind, it further remains to be explored whether or not the metaboreflex test may be dangerous for individuals with severe CAD, as some studies in the animal setting have demonstrated exaggerated metaboreflex-induced constriction in the coronary vasculature in heart failure and hypertension (12, 60).

Our data also suggest that, from a clinical point of view, the diastolic phase could be impaired earlier than the contraction phase in individuals diagnosed with CAD. Moreover, diastolic abnormalities may develop before any sign of overt heart failure. Several pieces of evidence support the hypothesis that the capacity to recruit the Frank-Starling mechanism is pivotal in the hemodynamics during the metaboreflex. In health, a central blood volume mobilization and significant cardiac preload recruitment were reported (4, 14, 56, 57). Furthermore, reduction in the capacity to sustain cardiac preload and/or impairment in the diastolic proprieties of the heart were all found to negatively impact on the hemodynamic responses to the metaboreflex (14, 31, 32, 50). The present investigation is in line with the concept that diastole is particularly sensitive in detecting heart abnormalities and that the PEMI test is highly

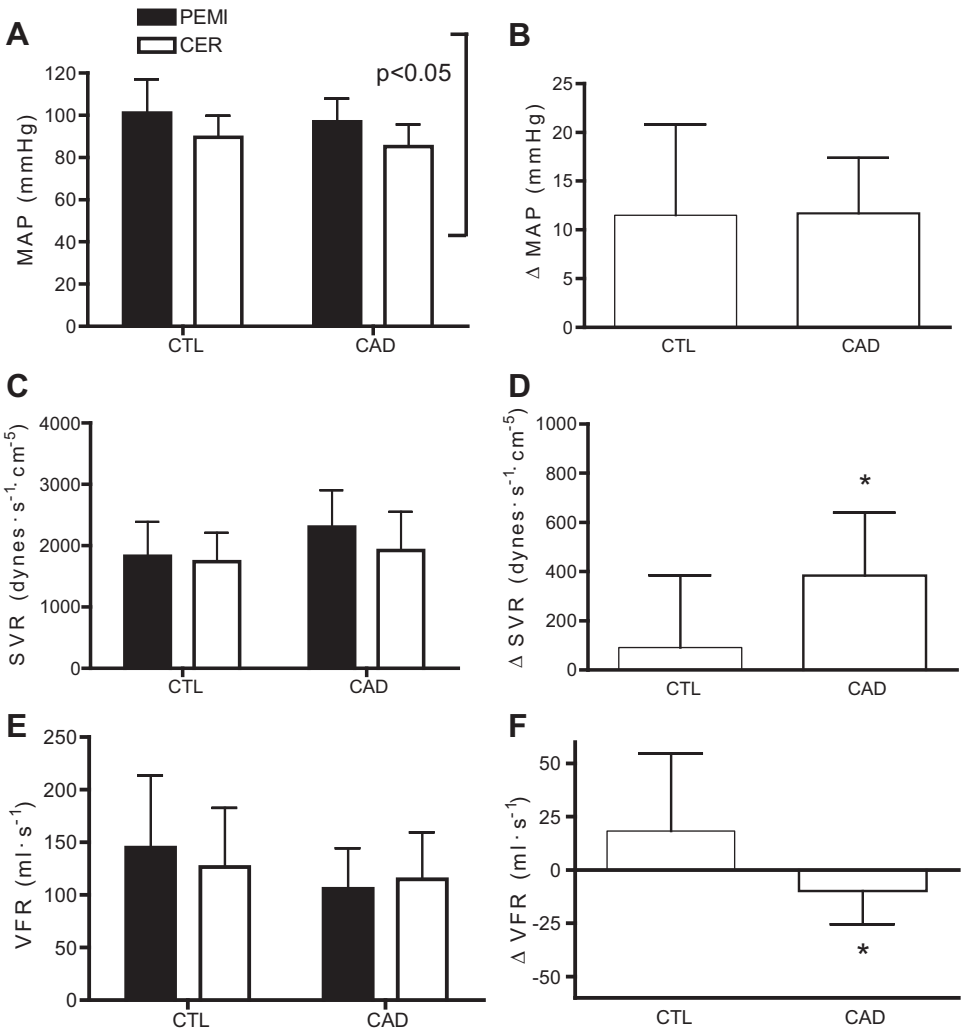

Fig. 3. Absolute values during postexercise muscle ischemia (PEMI) and control exercise recovery (CER) tests and response in mean arterial pressure (MAP; *A* and *B*), systemic vascular resistance (SVR; *C* and *D*), and ventricular filling rate (VFR; *E* and *F*) in the control (CTL) group and the coronary artery disease (CAD) group. Values are means ± SD. A horizontal bracket indicates the overall main effect of group. A vertical bracket denotes a main effect of condition. There was no interaction effect. $^*P < 0.05$ vs. the CAD group.

sensitive in detecting early signs of diastolic impairment. The possibility that diastolic impairment is a harbinger of the deterioration of the cardiac functions deserves further investigation, as it may provide an early hallmark of CAD outcome.

Another finding that deserves consideration was that, although there were no overt symptoms of impaired myocardial function, subjects of the CAD group showed some differences in several diastolic parameters with respect to the CTL group already at rest. Indeed, although in the normal range, *E*vel and *E*/*A* were lower in the CAD group, whereas A_{vel} was higher compared with the CTL group. These parameters are altered when the ventricle stiffness is increased and/or myocardial relaxation is reduced and are conventionally used to characterize diastolic functions (9). Moreover, the CAD group had lower EDV and VFR than the CTL group, thereby reflecting reduced ventricular dimension. These differences in diastolic parameters may be explained with the CAD-induced impaired diastolic function. However, an alternative explanation may also be that the higher level of fitness of the CTL subjects could have positively affected their cardiovascular system. It is a well-known fact that starting from the third decade in life there is a progressive heart stiffening and a decrease in myocardial energetics (23). Moreover, it is also well established that healthy but sedentary subjects exhibit greater LV stiffness compared with active people and that training may be an effective means of preserving cardiac compliance with aging. However, physical training is associated with higher ventricular dimensions (3, 55). Indeed, we enrolled physically active people as the CTL group because a sedentary lifestyle is significantly associated with many metabolic and cardiovascular disorders. Thus, physical inactivity and chronic disease are inevitably linked. Hence, physically active subjects should be a CTL group, especially when aged individuals are under investigation (6).

The active individuals enrolled as controls showed a higher level of physical capacity than the CAD group, as testified by their more elevated $\dot{V}O_{2max}$ during the CPX test. It is possible to speculate that, among other factors, the reduced diastolic function of individuals of the CAD group was at least in part responsible for their lower physical capacity. It has been previously reported that LV diastolic filling is the best predictor of SV at peak exercise in sedentary old people (45). Because exercise capacity is linked to the capacity to increase SV and consequently CO, it is likely that the impaired diastolic function of the patients with CAD reduced their maximal capacity to exercise. It should also be considered that, in patients suffering from chronic heart failure, it has been previously demonstrated that training reduces the activity of

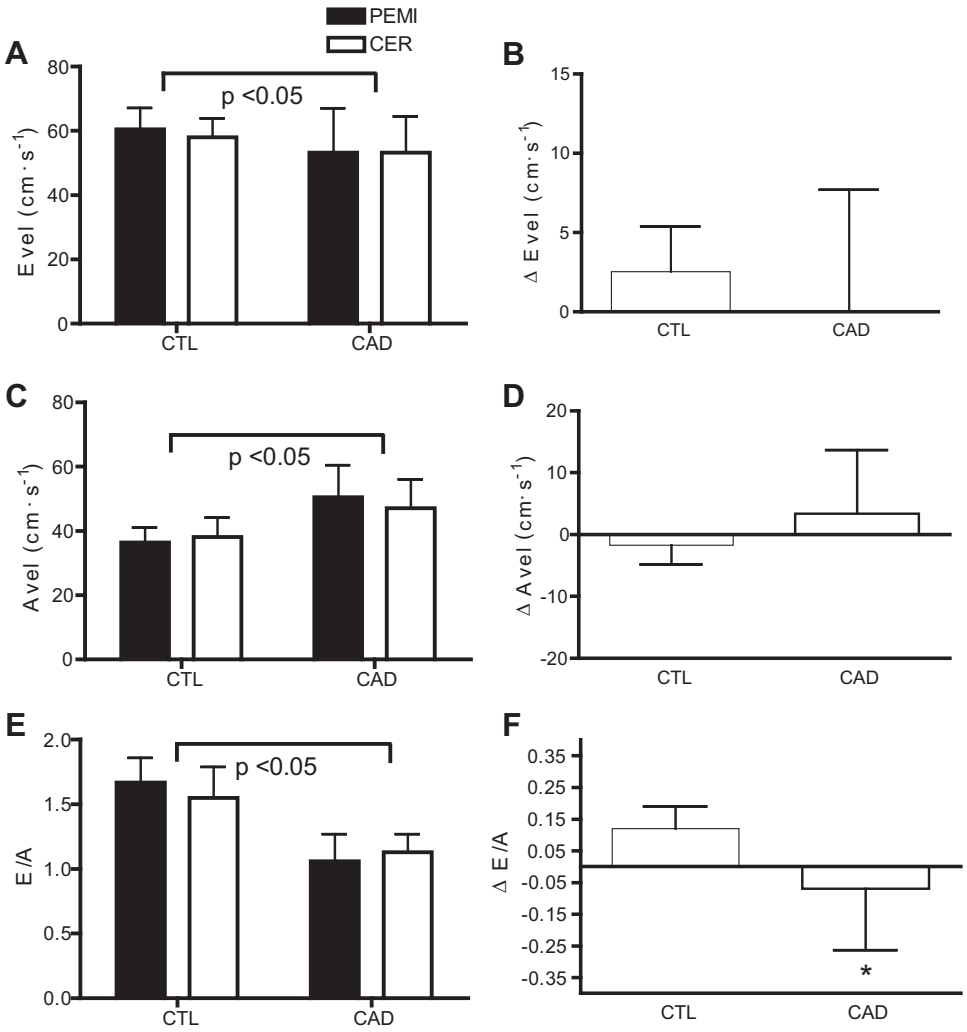

Fig. 4. Absolute values during postexercise muscle ischemia (PEMI) and control exercise recovery (CER) tests and response in E wave velocity (E_{vel}; *A* and *B*), A wave velocity (A_{vel}; *C* and *D*), and *E*-to-*A* ratio (*E*/*A*; *E* and *F*) in the control (CTL) group and the coronary artery disease (CAD) group. Values are means ± SD. A horizontal bracket indicates the overall main effect of group. There was no interaction effect. * $P < 0.05$ vs. the CAD group.

muscle afferents responsible for the metaboreflex activity (46). Because subjects of the CTL group had a higher physical capacity than those of the CAD group, it is possible that the two groups also had different levels of metaboreflex activation, which could in turn negatively affect hemodynamics. Thus, it is possible to speculate that a physical training program, along with its effects on physical capacity, could also counteract the cardiovascular abnormalities observed in patients with CAD. To the best of our knowledge, no one has to date investigated the effects of physical training on the metaboreflex activity in individuals with CAD.

Limitations of the study. One possible limitation of the present study was that we did not gather data on coronary circulation because of technical reasons and the noninvasive nature of the study. Therefore, any hypothesis on the presence of a coronary flow reduction that impaired diastolic functions remains speculative. Further study is warranted to better clarify this point better.

Another potential limitation was that, during the time of the study, subjects enrolled in the CAD group were under medication with several drugs that could affect their hemodynamics. In particular, all were taking β-blockers. It is well known that these kinds of drugs reduce chronotropism and inotropism. However, no difference between groups was detected in HR and EF, thus indicating that parameters related to chronotropism and inotropism were not impaired by the β-blocker administration. Moreover, β-blockers are not expected to influence diastolic functions in the absence of overt congestive heart failure (20). To the best of our knowledge, none of the other drugs that the CAD subjects were taking (i.e., aspirin, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, Ca^{2+} antagonists, diuretics, statins, etc.) has been demonstrated to impair diastolic function. Thus, it is unlikely that these drugs were responsible for the altered hemodynamics shown by the CAD group.

It should also be considered that some of the patients ($n = 7$) were treated for hypertension before infarction. In the recent past, it has been reported that hypertension may negatively affect hemodynamics during the metaboreflex (19, 60). Thus, although blood pressure was well controlled by the therapy, a potential deleterious role of previous hypertension cannot be ruled out in the genesis of hemodynamic abnormalities observed in the present investigation.

Finally, to eliminate any potential effect of hormonal changes during the menstrual cycle that may affect hemodynamics, only male individuals were enrolled in the present study. Hence, the results cannot be applied to female patients.

In conclusion, findings of the present research suggest that diastolic function and the capacity to increase cardiac preload are important for a normal hemodynamic response during the metaboreflex activation. Individuals suffering from CAD without overt signs of heart failure had an impaired diastolic function, which precluded increase in SV and CO during the metaboreflex. As a consequence, the increase in SVR, with exaggerated vasoconstriction, becames pivotal to reach the target blood pressure. Finally, our results also suggest that the PEMI test was able to discriminate between healthy subjects and individuals diagnosed with CAD but without signs of heart failure, thereby providing a potentially useful tool to study the cardiovascular adjustment to sympathetic activation in these subjects.

ACKNOWLEDGMENTS

The authors thank the Language Center at Cagliari University for the revision, completed by a mother-tongue expert, and the editorial assistance.

GRANTS

This study was supported by the University of Cagliari and the Italian Ministry of Scientific Research.

DISCLOSURES

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

AUTHOR CONTRIBUTIONS

S.M., S.R., G.M., and A.C. conceived and designed research; S.M., S.R., G.S., R.M., G.P., L.C., V.P., A.D., S.A.H.K., F.T., G.M., and A.C. performed experiments; S.M., S.R., G.S., R.M., G.P., L.C., V.P., A.D., S.A.H.K., F.T., and A.C. analyzed data; S.M., S.R., G.S., G.P., V.P., F.T., G.M., and A.C. interpreted results of experiments; S.M. and A.C. prepared figures; S.M., S.R., G.S., R.M., L.C., A.D., S.A.H.K., F.T., G.M., and A.C. drafted manuscript; S.M., S.R., G.S., R.M., G.P., L.C., V.P., A.D., S.A.H.K., F.T., G.M., and A.C. edited and revised manuscript; S.M., S.R., G.S., R.M., G.P., L.C., V.P., A.D., S.A.H.K., F.T., G.M., and A.C. approved final version of manuscript.

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