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Sildenafil improves microvascular O_2 delivery-to-utilization matching and accelerates exercise O_2 uptake kinetics in chronic heart failure

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Sperandio PA, Oliveira MF, Rodrigues MK, Berton DC, Treptow E, Nery LE, Almeida DR, Neder JA. Sildenafil improves microvascular O_2 delivery-to-utilization matching and accelerates exercise O_2 uptake kinetics in chronic heart failure. *Am J Physiol Heart Circ Physiol* 303: H1474–H1480, 2012. First published September 28, 2012; doi:10.1152/ajpheart.00435.2012.—Nitric oxide (NO) can temporally and spatially match microvascular oxygen (O_2) delivery ($\dot{Q}o_{2mv}$) to O_2 uptake ($\dot{V}o_2$) in the skeletal muscle, a crucial adjustment-to-exercise tolerance that is impaired in chronic heart failure (CHF). To investigate the effects of NO bioavailability induced by sildenafil intake on muscle $\dot{Q}o_{2mv}$-to-O_2 utilization matching and $\dot{V}o_2$ kinetics, 10 males with CHF (ejection fraction = 27 ± 6%) undertook constant work-rate exercise (70–80% peak). Breath-by-breath $\dot{V}o_2$, fractional O_2 extraction in the vastus lateralis {~deoxygenated hemoglobin + myoglobin ([deoxy-Hb + Mb]) by near-infrared spectroscopy}, and cardiac output (CO) were evaluated after sildenafil (50 mg) or placebo. Sildenafil increased exercise tolerance compared with placebo by ~20%, an effect that was related to faster on- and off-exercise $\dot{V}o_2$ kinetics ($P < 0.05$). Active treatment, however, failed to accelerate CO dynamics ($P > 0.05$). On-exercise [deoxy-Hb + Mb] kinetics were slowed by sildenafil (~25%), and a subsequent response "overshoot" ($n = 8$) was significantly lessened or even abolished. In contrast, [deoxy-Hb + Mb] recovery was faster with sildenafil (~15%). Improvements in muscle oxygenation with sildenafil were related to faster on-exercise $\dot{V}o_2$ kinetics, blunted oscillations in ventilation ($n = 9$), and greater exercise capacity ($P < 0.05$). Sildenafil intake enhanced intramuscular $\dot{Q}o_{2mv}$-to-$\dot{V}o_2$ matching with beneficial effects on $\dot{V}o_2$ kinetics and exercise tolerance in CHF. The lack of effect on CO suggests that improvement in blood flow to and within skeletal muscles underlies these effects.

sildenafil; blood flow; heart failure; hemodynamics; near-infrared spectroscopy; oxygen consumption; kinetics

THE INABILITY TO MAINTAIN an adequate driving pressure for blood-myocite oxygen (O_2) diffusion [i.e., microvascular partial pressure of O_2 (Po_{2mv})] is paramount to explain the slowness of exercise O_2 uptake ($\dot{V}o_2$) kinetics in patients with chronic heart failure [CHF; as recently reviewed by Poole and colleagues (34)]. To keep a sufficiently high Po_{2mv}, however, O_2 delivery should be spatially and temporally matched to $\dot{V}o_2$ of individual fibers. In this context, seminal studies found that intramuscular Po_{2mv} in rodents with CHF was critically low either at rest-to-contractions transition (7, 14) or during early recovery (12), i.e., when $\dot{V}o_2$ should be increasing or decreasing most rapidly, respectively. Importantly, it was demonstrated that reduced nitric oxide (NO) bioavailability exerted a key mechanistic role on on- and off-exercise O_2 delivery-to-utilization uncoupling in these animal preparations (24, 25).

In intact humans with CHF, previous studies have concomitantly assessed the rate of change in *phase II* ("muscle") $\dot{V}o_2$ (39) and intramuscular fractional O_2 extraction {~deoxygenated hemoglobin + myoglobin ([deoxy-Hb + Mb]) by near-infrared spectroscopy (NIRS)} (18) to gain insight into the dynamic matching of O_2 delivery to utilization (9, 10, 37). This analysis is based on the widely accepted concept that Po_{2mv} and changes thereof reflect the delivery/utilization ratio, i.e., $\dot{V}o_2$/microvascular O_2 delivery ($\dot{Q}o_{2mv}$) as deoxygenation (18). In fact, decreases in O_2 delivery relative to O_2 needs speeded (and heightened) on-exercise [deoxy-Hb + Mb] kinetics but slowed postexercise [deoxy-Hb + Mb] recovery in patients with CHF (9, 10, 37). The therapeutic potential of increasing NO bioavailability to improve the dynamic coupling of $\dot{Q}o_{2mv}$ to $\dot{V}o_2$, thereby accelerating $\dot{V}o_2$ kinetics and enhancing exercise tolerance, however, remains unexplored in these patients. Moreover, considering that stimulation of muscle metaboreceptors by hypoxia-related by-products might further increase the ventilatory drive, thereby predisposing to oscillatory breathing, it could be hypothesized that better O_2 delivery-to-utilization matching induced by NO could contribute to breathing stability in CHF (23).

In the present study, therefore, we aimed to investigate the effects of increased NO bioavailability through acute pharmacological inhibition of muscle cGMP-specific phosphodiesterase-5 (PDE_5) (32) by sildenafil intake (20) on peripheral muscle $\dot{Q}o_{2mv}$-to-$\dot{V}o_2$ matching and $\dot{V}o_2$ kinetics at the transition to and from constant work-rate exercise in patients with stable CHF. We hypothesized that compared with placebo, sildenafil would improve muscle oxygenation and accelerate $\dot{V}o_2$ kinetics with positive consequences on exercise tolerance in these patients.

METHODS

Subjects. This was a prospective study involving 10 nonsmoking men recruited from the CHF Outpatients Clinic of The São Paulo Hospital, Federal University of São Paulo. Patients had an established diagnosis of CHF (ischemic or idiopathic cardiomyopathy) for at least 4 yr, three-dimensional echodopplercardiography showing left-ventricle ejection fraction <35%, and New York Heart Association functional *classes II–III* (Table 1). No patient had cardiac resynchroniza-

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Table 1. *Resting and peak exercise data (n = 10)*

Variables	Values
Anthropometrics/Demographics	
Age, yr	57.2 ± 12.3
Body mass, kg	72.5 ± 11.0
Body mass index, kg/m^2	25.2 ± 4.3
Etiology	
Ischemic	4
Idiopathic dilated cardiomyopathy	6
Echocardiography	
Left-ventricle ejection fraction, %	27.4 ± 6.6
Drugs	
Diuretics	9
Spironolactone	9
Digitalis	2
Carvedilol	10
ACE inhibitors/AR blockers	10
Incremental exercise	
At peak	
Work rate, W	82 ± 12
$\dot{V}O_2$, ml/min	1,069 ± 261
$\dot{V}O_2$, ml · min^{-1} · kg^{-1}	14.7 ± 2.7
$\dot{V}O_2$, % pred	56.3 ± 12.6
HR, beats/min	116 ± 28
HR, % pred	75.2 ± 12.8
O_2 pulse (ml · min^{-1} · beat^{-1})	9.6 ± 3.0
Mean arterial pressure, mmHg	102 ± 19
Submaximal variables	
$\Delta\dot{V}E/\Delta\dot{V}CO_2$ slope	46.3 ± 7.4
$\dot{V}O_2$/work rate (ml · min^{-1} · W^{-1})	8.2 ± 1.9
$\dot{V}O_{2GET}$, ml/min	653 ± 131

ACE, angiotensin-converting enzyme; AR, angiotensin receptor; $\dot{V}O_2$, oxygen (O_2) uptake; pred, predicted; HR, heart rate; Δ, variation; $\dot{V}E$, minute ventilation; $\dot{V}CO_2$, carbon dioxide (CO_2) output; GET, gas exchange threshold. Values are means ± SD.

tion therapy or a left-ventricle assist device. Patients were excluded from the study if they had clinical and/or functional evidences of chronic expiratory flow limitation (forced expiratory volume in 1 s/forced vital capacity ratio <0.7), anemia (actual Hb values as 15.5 ± 1.4 g%), unstable angina or significant cardiac arrhythmias, and myocardial infarction within the previous 12 mo. Renal function parameters were: serum creatinine = 1.2 ± 0.2 g/dl, estimated glomerular filtration rate (Cockroft-Gault) = 67.1 ± 8.7 ml·min^{-1} ·1.73 m^{-2}, and urea = 48.4 ± 17.2 mg/dl. Study participants gave a written, informed consent, and the study protocol was approved by the Institutional Medical Ethics Committee.

Study protocol. After familiarization, subjects performed an individualized ramp-incremental exercise test (5–10 W/min) to determine the difference between $\dot{V}O_2$ at the gas exchange threshold (GET; by the V-slope method) and $\dot{V}O_2$ at peak exercise ($\Delta\dot{V}O_{2peak\text{-}GET}$). On different days, subjects underwent a constant work-rate exercise test to the limit of tolerance (Tlim; s) at a $\dot{V}O_2$ equivalent to 40–50% of the $\Delta\dot{V}O_{2peak\text{-}GET}$ (~70–80% peak work rate if the GET was not identified) 1 h after sildenafil (50 mg) or placebo intake.

Cardiopulmonary exercise testing. The tests were performed on an electronically braked cycle ergometer (Corival 400, Lode, The Netherlands) at 50 ± 5 rpm, and they were preceded and followed by unloaded pedaling at 0 W for 3 min. $\dot{V}O_2$ (ml/min), carbon dioxide (CO_2) output ($\dot{V}CO_2$; ml/min), minute ventilation ($\dot{V}E$; l/min), and end-tidal partial pressures for CO_2 (mmHg) were measured breath by breath (CardiO_2 System, Medical Graphics, St. Paul, MN). During the incremental test, values were averaged as arithmetic mean of 20 s, and peak $\dot{V}O_2$ was the highest mean value recorded. The relationship between the rates of changes in $\dot{V}E$ and $\dot{V}CO_2$ ($\Delta\dot{V}E/\Delta\dot{V}CO_2$) was established from unloaded exercise to the respiratory compensation point. Exercise oscillatory breathing, either during incremental or constant work-rate tests, was assumed as present when oscillations occurred ≥60% of exercise data at an amplitude >15% of resting values with minimal average amplitude of 5 l/min, and there was a regular oscillation (SD of three consecutive cycle lengths within 20% of the average) (31).

Skeletal muscle oxygenation. Skeletal muscle oxygenation profiles of the left vastus lateralis were evaluated with a commercially available NIRS system (Hamamatsu NIRO-200, Hamamatsu Photonics, Japan). The methodology of continuous-wave NIRS has been described elsewhere (16). Among the NIRS variables, [deoxy-Hb + Mb] has been used as a proxy of fractional O_2 extraction in the microcirculation, reflecting the balance between O_2 delivery and utilization (18, 37). To improve intra- and intersubject comparability, values (μM/cm) were recorded as a delta (Δ) and expressed relative (%) to the amplitude of variation from baseline to the steady state (within 2 SD of the local mean) with placebo.

Central hemodynamics. Cardiac output (CO) was measured throughout the constant work-rate tests using a calibrated signal-morphology impedance cardiography device (PhysioFlow PF-05, Manatec Biomedical, France). Values were recorded as mean of seven beats, as indicated by the manufacturer. The PhysioFlow device and its methodology have also been described in detail elsewhere (11). In preliminary experiments, the coefficient of variation (CV) for changes in CO during exercise was 3.3%, and stepwise changes in CO were consistent with those predicted from $\dot{V}O_2$ values, as described previously in CHF patients (27). Values (l/min) were recorded as a delta (Δ) from baseline and expressed relative (%) (27) to amplitude of variation from baseline to the steady state (within 2 SD of the local mean) after placebo.

Data analysis. The breath-by-breath $\dot{V}O_2$, [deoxy-Hb + Mb], and CO data were time aligned to the start of exercise to 180 s after exercise cessation and interpolated second by second. Breaths outside 4 SD of the local $\dot{V}E$ mean were deleted, and $\dot{V}O_2$ was averaged into 5-s bins to further improve signal stability (26). The kinetics of these responses were determined by nonlinear regression using a least-square technique (Marquardt-Levenberg; SigmaPlot 10.0, Systat Software, San Jose, CA).

Exercise onset. $\dot{V}O_2$ was fitted from 30 s of baseline pedaling to 180 s after the onset of exercise. For [deoxy-Hb + Mb] kinetics, the analyses were conducted on data from 30-s baseline cycling to the steady-state response. The model used for fitting the kinetic response of $\dot{V}O_2$ and Δ[deoxy-Hb + Mb] without an "overshoot" (see below) was

$$[Y]_{(t)} = [Y]_{(b)} + A \cdot \left[1 - e^{-(t-TD)/\tau}\right]^6 \quad (1)$$

where *b* refers to baseline-unloaded cycling, and A, TD, and τ are the amplitude, time delay, and time constant of the exponential response (i.e., time to reach 63% of the final value), respectively. For $\dot{V}O_2$ analysis, we deleted the data relative to the cardiodynamic phase (9). The overall kinetics of $\dot{V}O_2$ and [deoxy-Hb + Mb] were determined by the mean response time (MRT) = τ + TD. For CO, we calculated the one-half time ($t_{1/2}$; s), and an estimate of MRT was obtained as $t_{1/2} \times 1.44$. A two-component, monoexponential model was applied to [deoxy-Hb + Mb] data with an overshoot (4, 10, 18)

$$[Y]_{(t)} = [Y]_{(b)} + A_1 \cdot \left[1 - e^{-(t-TD_1)/\tau_1}\right] - A_2 \cdot \left[1 - e^{-(t-TD_2)/\tau_2}\right] \quad (2)$$

where the subscripts *1* and *2* correspond to the two sequential components (upward and downward, respectively). The area under the [deoxy-Hb + Mb] overshoot (AUC; a.u.) and a longer τ_2 (*Eq. 2*) were used as additional indicators of impaired $\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching (4) (Fig. 1). On preliminary trials, the CV for the kinetic parameters of the [deoxy-Hb + Mb] response ranged between 5% and 11% [first test − second test mean bias and range: τ = 0.3 s (−0.3 s to 0.8 s), and TD = 0.1 s (−0.4 s to 0.5 s)].

Exercise recovery. We used data from the last 30 s of exercise to 180 s of recovery to calculate the kinetics of the primary component

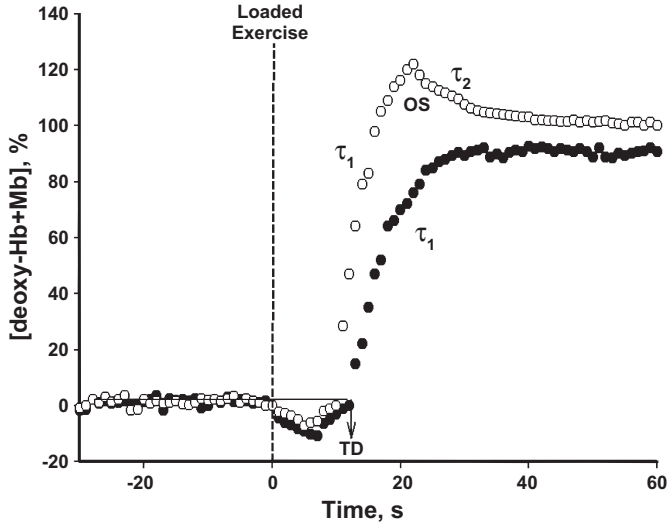

Fig. 1. Skeletal muscle deoxygenated hemoglobin + myoglobin ([deoxy-Hb + Mb]) profiles at the onset of constant work-rate exercise after placebo (○) and sildenafil (●) intake in a representative patient with chronic heart failure (CHF). Values are expressed relative to the amplitude of the coefficient of variation from baseline to the steady state with placebo. TD, time delay; τ_1, time constant of the upward component; τ_2, time constant of the downward component; OS, overshoot.

of response. The model used for fitting the kinetic response of $\dot{V}O_2$ and [deoxy-Hb + Mb] was $[Y]_{(t)} = [Y]_{(ss)} - A \cdot [1 - e^{-(t - TD)/\tau}]$.

MRT of [deoxy-Hb + Mb] and CO was determined as described above. On preliminary trials, the CV for the kinetic parameters of the [deoxy-Hb + Mb] response ranged between 3% and 6% [first test − second test mean bias and range: τ = 3.4 s (−1.9 s to 7.9 s), and TD = 1.2 s (−1.4 s to 2.7 s)].

Statistical analysis. Results were summarized as mean ± SD. The primary end point of the study was changes in on-exercise MRT-[deoxy-Hb + Mb] after sildenafil when compared with placebo. Secondary end points included Tlim, AUC (*Eq. 2*), τ-$\dot{V}O_2$/MRT-Δ[deoxy-Hb + Mb], $\dot{V}O_2$ kinetics, and amplitude of oscillatory breathing. To contrast within-subject resting and exercise responses, paired *t*- or Wlicoxon tests were used as appropriate. Pearson's product moment correlation was used to assess the level of association between continuous variables. The level of statistical significance was set at $P < 0.05$ for all tests.

RESULTS

Peak exercise capacity. Peak work rate and $\dot{V}O_2$ of all patients were below the age- and gender-corrected lower limits of normality. Eight patients were on Weber's *class C* and two on *class B*. The GET was identified in all but two subjects. As anticipated by the long-term β-blocker therapy, patients showed impaired peak heart rate (Table 1). Exercise oscillatory breathing (31) was present in all but one patient during the incremental and constant work-rate exercise tests.

On- and off-exercise response dynamics after placebo. All fitted data were included in the kinetics analysis, as coefficient of determination values ranged from 0.90 to 0.99. On- and off-exercise $\dot{V}O_2$ and CO kinetics with placebo were slower than reported previously in healthy males of similar age (10) (Table 2). On-exercise CO kinetics were consistently slower than $\dot{V}O_2$ dynamics, whereas off-exercise rates of change were remarkably similar (Fig. 2). There were significant relationships between these variables both at the onset of and during recovery from exercise (R = 0.69, and R = 0.81, respectively; $P < 0.05$).

At the onset of exercise, [deoxy-Hb + Mb] increased rapidly with a rate of change that was faster than the $\dot{V}O_2$ and CO responses. In contrast, MRT-[deoxy-Hb + Mb] during recovery did not differ from MRT-$\dot{V}O_2$ and MRT-CO (Fig. 2). An overshoot in [deoxy-Hb + Mb] was identified in eight of 10 patients (see Fig. 1 for a representative patient). AUC and the kinetics of its downward component were inversely related to on-exercise τ-$\dot{V}O_2$ (R = 0.80, and R = 0.73, respectively; $P < 0.05$).

Effects of sildenafil on exercise tolerance and physiological responses. Sildenafil increased Tlim compared with placebo by ~20% ($P < 0.05$; Table 3). Despite a lack of effect on the cardiopulmonary responses at exercise cessation (Table 3), sildenafil significantly decreased the amplitude of $\dot{V}E$ oscillations (% of mean) and their cycle length (21.4 ± 15.2% vs.

Table 2. *On- and off-exercise kinetic parameters for $\dot{V}O_2$, [deoxy-Hb/Mb], and cardiac output after placebo or sildenafil intake (n = 10)*

	Placebo		Sildenafil	
	On-Exercise	Off-Exercise	On-Exercise	Off-Exercise
$\dot{V}O_2$				
Baseline, ml/min	512 ± 149	985 ± 204*	544 ± 174	1,015 ± 201*
Amplitude, ml/min	459 ± 187	361 ± 168	427 ± 103	432 ± 119
τ, s	54.3 ± 10.4	62.9 ± 19.4*	41.3 ± 12.1†	52.1 ± 15.7*,†
TD, s	14.1 ± 8.7	16.4 ± 12.2	15.7 ± 9.9	13.7 ± 10.1
[deoxy-Hb/Mb]				
Baseline, %	−1.8 ± 3.3	101.1 ± 14.4*	1.2 ± 2.9	90.4 ± 10.9*,†
Amplitude, %	118.8 ± 10.7	60.6 ± 18.3*	89.1 ± 11.3†	40.7 ± 15.1*,†
τ, s	5.7 ± 2.1	58.9 ± 20.3*	10.2 ± 2.8†	50.1 ± 17.0*,†
TD, s	10.9 ± 4.7	13.8 ± 9.7	12.4 ± 5.1	12.7 ± 8.9
Cardiac output				
Baseline, %	0.9 ± 5.4	103.4 ± 11.9*	1.3 ± 6.1	103.4 ± 11.9*
Amplitude, %	103.4 ± 11.9	69.5 ± 15.7*	101.1 ± 14.3	70.9 ± 12.1*
$t_{1/2}$, s	53.6 ± 9.4	58.3 ± 12.2	55.9 ± 10.7	53.1 ± 11.9

[deoxy-Hb/Mb], deoxygenated hemoglobin/myoglobin; τ, time constant; TD, time delay; $t_{1/2}$, 1/2-time. Values are means ± SD. [deoxy-Hb/Mb] and cardiac output values are expressed relative to steady state − baseline variation on placebo. *$P < 0.05$ for on- vs. off-exercise within a given treatment; †$P < 0.05$ for between-treatment differences within a given time point.

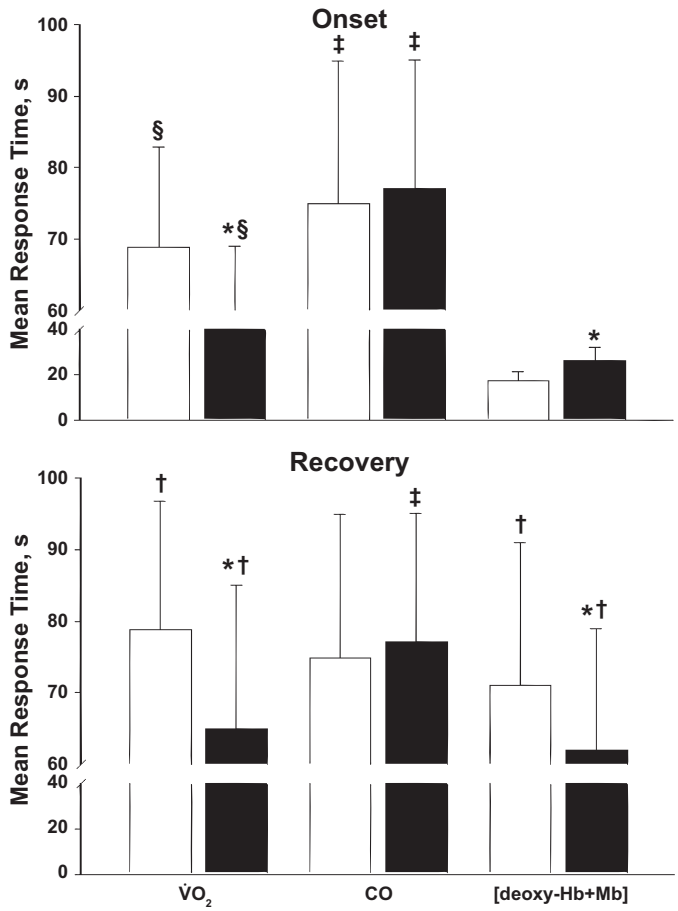

Fig. 2. Mean response time (MRT) of oxygen (O_2) uptake ($\dot{V}O_2$), cardiac output (CO), and [deoxy-Hb + Mb] at the onset of and recovery from exercise after placebo (open bars) and sildenafil (solid bars) intake in patients with CHF. Data are mean (SD). *$P < 0.05$ for between-treatment comparisons on a given time point; †$P < 0.05$ for onset vs. recovery on a given treatment; ‡$P < 0.05$ for CO vs. $\dot{V}O_2$ and [deoxy-Hb + Mb] at a given time point; §$P < 0.05$ for $\dot{V}O_2$ vs. [deoxy-Hb + Mb] at a given time point.

50.8 ± 10.4% and 19.9 ± 10.7% vs. 48.3 ± 12.1% for sildenafil and placebo, respectively). In addition, the number of cycles was reduced in two patients, and oscillatory breathing disappeared in other two patients.

Table 3. *Main physiological responses just prior to exercise cessation after placebo or sildenafil intake (n = 10)*

	Placebo	Sildenafil
Tlim, s	435 ± 267	529 ± 405*
$\dot{V}O_2$, ml/min	972 ± 212	1,008 ± 208
$\dot{V}CO_2$, ml/min	1,113 ± 260	1,112 ± 262
RER	1.15 ± 0.15	1.10 ± 0.14
$\dot{V}E$, l/min	49.4 ± 14.5	46.8 ± 12.1
$\dot{V}E/\dot{V}O_2$	51.0 ± 11.6	46.8 ± 8.2
$\dot{V}E/\dot{V}CO_2$	44.5 ± 8.3	42.6 ± 7.1
$PETCO_2$, mmHg	27.4 ± 5.7	28.0 ± 4.6
HR, beats/min	117 ± 25	114 ± 18
HR, % pred	74.4 ± 11.8	73.7 ± 14.1
Systolic blood pressure, mmHg	128 ± 22	124 ± 18
Diastolic blood pressure, mmHg	75 ± 11	79 ± 11
Mean arterial pressure, mmHg	103 ± 20	97 ± 15

Tlim, time-to-exercise intolerance; RER, respiratory exchange ratio; $PETCO_2$, end-tidal partial pressure for CO_2. Values are means ± SD. *$P < 0.05$.

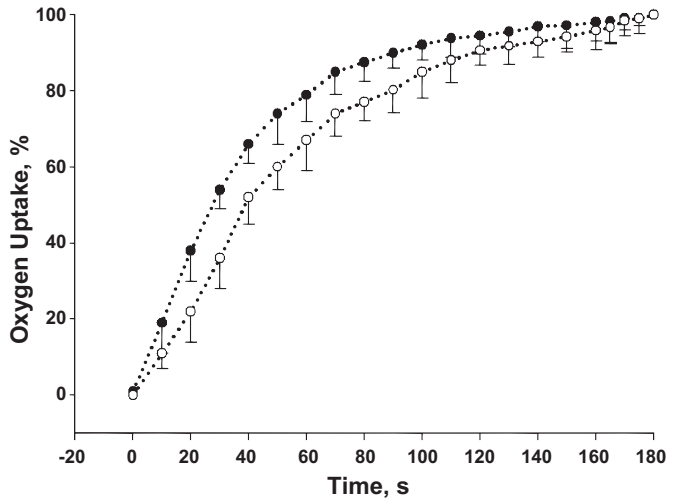

Fig. 3. Time course of $\dot{V}O_2$ at the onset of exercise after placebo (○) and sildenafil intake (●) in patients with CHF. Values were averaged in 10-s bins [mean (SE)] and expressed relative (%) to the amplitude of variation from start of loaded exercise to the 3rd min.

On- and off-exercise $\dot{V}O_2$ kinetics were accelerated by sildenafil intake (Table 2 and Figs. 2–4). There was a significant relationship between Δ (sildenafil-placebo) Tlim with treatment-induced decrements in τ-$\dot{V}O_2$ ($R = -0.68$; $P < 0.05$). In contrast, sildenafil failed to accelerate CO dynamics either at the onset of or recovery from exercise (Table 2 and Fig. 2). Consequently, $\dot{V}O_2$ and CO kinetics were no longer correlated after sildenafil treatment ($P > 0.05$).

On-exercise Δ[deoxy-Hb + Mb] kinetics were slowed by sildenafil (+25%; Table 2 and Fig. 2), and the AUC was lessened [median (range) = 2,085 (857–3,115) a.u. vs. 428 (125–628) a.u.; $P < 0.05$] or even abolished ($n = 2$; Fig. 1). Consequently, τ-$\dot{V}O_2$/MRT-[deoxy-Hb + Mb] after sildenafil was lower at the onset of exercise compared with placebo (1.77 ± 0.89 s vs. 3.31 ± 1.0 s, respectively; $P < 0.05$). In addition, [deoxy-Hb + Mb] recovery was faster with sildenafil (−15%; Table 2 and Fig. 2). Interestingly, improvement in on-exercise muscle oxygenation

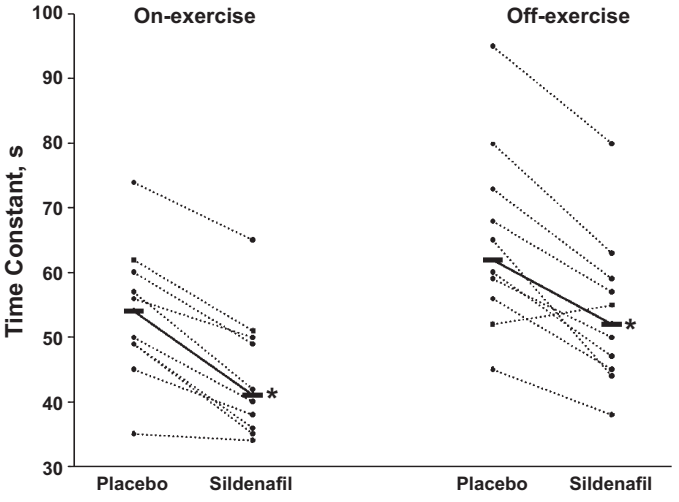

Fig. 4. Individual effects of placebo and sildenafil intake in τ of $\dot{V}O_2$ at the onset (On-exercise; *left*) and recovery (Off-exercise; *right*) from exercise in patients with CHF. *$P < 0.05$.

with active treatment {i.e., higher Δ (sildenafil-placebo) MRT-[deoxy-Hb + Mb]} was related to larger decrements in the amplitude of $\dot{V}_E$ oscillations ($R = -0.66$; $P = 0.045$), faster $\dot{V}O_2$ kinetics (Fig. 5, *top*), and greater increases in exercise tolerance (Fig. 5, *bottom*).

DISCUSSION

This study is the first to demonstrate that compared with placebo, acute PDE_5 inhibition with sildenafil led to a closer matching between intramuscular O_2 delivery and utilization during the transition to and from constant work-rate exercise in patients with CHF. These beneficial consequences of active treatment speeded pulmonary $\dot{V}O_2$ kinetics, which suggests that peripheral muscle $\dot{V}O_2$ kinetics were faster, reduced the amplitude of oscillatory breathing, and increased exercise capacity. Improvements in muscle oxygenation and aerobic metabolism were not related to changes in CO dynamics, thereby suggesting that blood flow redistribution to and within the working muscles underlies these effects.

CHF-related O_2 delivery-to-utilization mismatching. Disease-induced decreases in O_2 delivery relative to muscle O_2 demands have been found to accelerate and amplify rest-to-exercise decrements in $P_{O_{2mv}}$ and to slow its recovery after exercise (8, 12, 24, 25). This is physiologically equivalent to faster and heightened on-exercise increases in microvascular fractional O_2 extraction (~[deoxy-Hb + Mb], the mirror image of $P_{O_{2mv}}$) and slower postexercise [deoxy-Hb + Mb] recovery (9, 10, 18, 37). Moreover, fractional O_2 extraction transiently overshoots the subsequent steady-state value (Fig. 1) when O_2 delivery is markedly delayed relative to O_2 needs (4, 9, 10, 18). All of these derangements are expected to increase O_2 deficit and promote metabolic abnormalities associated with muscle fatigability and poor exercise tolerance (34). The effects of sildenafil on these abnormal patterns of intramuscular (de)oxygenation have not yet been determined in patients with CHF.

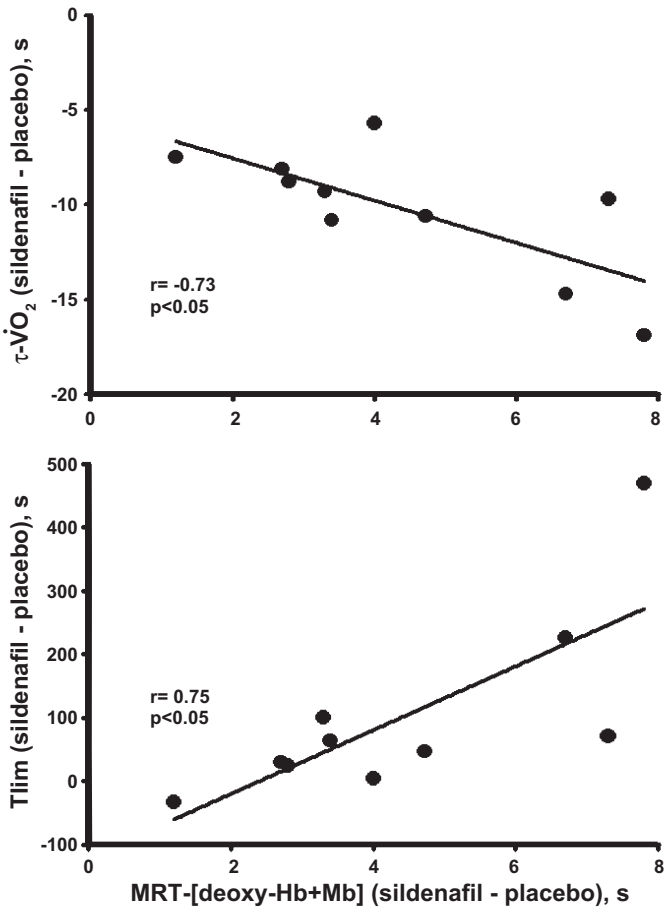

Fig. 5. Correlations between sildenafil-related changes in on-exercise MRT-[deoxy-Hb + Mb] with τ-$\dot{V}O_2$ kinetics (*top*) and time-to-exercise intolerance (Tlim; *bottom*) in patients with CHF.

Sildenafil on muscle $\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching. The present study describes a novel mechanism (improved $\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching) to explain the ergogenic properties of sildenafil (5, 9a, 22) in patients with CHF. The first hypothetical explanation for this finding is an increase in bulk muscle blood flow due to faster CO dynamics. However, our data indicate that this was not the case (Tables 2 and 3 and Fig. 2). In fact, Guazzi et al. (22) were the first to show that increased $\dot{V}O_2$/work-rate slope and faster postexercise $\dot{V}O_2$ kinetics with sildenafil were loosely related to improvements in central hemodynamics. These findings led the authors to hypothesize that CO had been redistributed to skeletal muscles after sildenafil administration, probably due to enhanced shear stress vasodilation in local conduit vessels. This hypothesis makes sense under the view that CHF is associated with impaired endothelial function and reduced NO bioavailability (15, 28), and sildenafil can improve flow-mediated vasodilation and local NO levels (21). Although we did not measure blood flow through these vessels, this might help to explain our findings of improved on- and off-exercise muscle oxygenation despite unaltered CO dynamics. However, vasodilation of feeding arteries is not an obligatory requisite for a better dynamic coupling between $\dot{Q}O_2$ and $\dot{V}O_2$ at the capillary level; in fact, the time course of skeletal muscle capillary hyperemia at the onset of exercise might differ markedly from the increased blood flow through larger conduit arteries (18, 35). Moreover, based on the stability of mean arterial pressure (Table 3) and CO, it is unlikely that there was a substantial decline in total peripheral vascular resistance after sildenafil. It is conceivable, therefore, that even if bulk blood flow increased with sildenafil (5, 21), it had the additional effect of more precisely matching intramuscular $\dot{Q}O_{2mv}$ to $\dot{V}O_2$; i.e., active treatment might have redistributed blood flow within the working muscles (34).

Role of NO on $\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching. Diffusion of NO to surrounding vascular smooth muscle cells activates local cGMP, which induces vasodilation by inhibition of calcium influx into the cell, activation of calcium-ATPase pumps, and opening of potassium channels, thereby leading to hyperpolarization and relaxation (15, 38). Interestingly, however, NO can modulate not only $\dot{Q}O_{2mv}$ but also, through inhibition of cellular respiration, muscle $\dot{V}O_2$ (36). Therefore, by acting on both determinants of tissue oxygenation (delivery and utilization), NO is the "ideal" candidate to exert a commanding role in muscle $\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching (34). In line with the extensive evidence derived from animal studies (17, 24, 25), increased muscle NO bioavailability might have not only increased $\dot{Q}O_{2mv}$ but also concomitantly decreased local O_2 needs, thereby allowing better temporal and spatial $\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching and a faster rate of transcapillary O_2 flux.

Study implications and directions for future research. The novel mechanistic explanation for the effects of sildenafil on exercise tolerance in CHF provided in the present study ($\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching) may also help explain why measurements of central hemodynamics are poorly predictive of the ergogenic effects of sildenafil in these patients (21, 22). Moreover, our results open the perspective to combine sildenafil with other strategies known to increase muscle NO bioavailability [e.g., exercise training (2, 13) and L-arginine (30) or dietary nitrate supplementation (3)] in future trials in CHF. Considering that the speeding effect of sildenafil on postexercise muscle deoxygenation might have accelerated the rate of oxidative rephosphorylation (39), it seems interesting to investigate its value in improving patients' ability to perform repetitive activities, which are probably relevant to patients' daily functioning. Our sample comprised middle age subjects not older than 65 yr, and senescence is associated with further reductions in muscle NO bioavailability in CHF (7, 34). Therefore, we may have underestimated the beneficial effects of sildenafil on $\dot{Q}O_{2mv}$-to-$\dot{V}O_2$ matching, an issue to be investigated in older patients.

Confirming the reports of Guazzi and coworkers (22), we found a marked decrease in exercise-related oscillatory breathing with sildenafil, which was largely independent of changes in CO. Although we are uncertain whether changes in pulmonary vascular resistance and/or facilitation of intrapulmonary gas diffusion after sildenafil played a mechanistic role in this regard, improvements in oscillatory breathing and intramuscular oxygenation were significantly correlated. It is possible, therefore, that less accumulation of metabolic by-products due to better muscular O_2 delivery-to-utilization matching reduced activation of *groups III* and *IV* afferents and the ventilatory drive, thereby contributing to breathing stability (9a, 19, 23, 33). Larger trials specifically powered to this issue are needed to further investigate the potential relationships among improved intramuscular O_2 delivery-to-utilization coupling, enhanced muscle bioenergetics, decreased ergoreflex activation, and lower exercise ventilatory stimuli in CHF.

Study limitations. We recognize that the small sample size and the acute nature of the present study indicate that it remains to be experimentally demonstrated whether the observed beneficial effects of sildenafil could be extrapolated to less-severe patients and whether they would be long lasting. These positive results, albeit obtained in a selected group of stable patients, now justify larger longitudinal investigations with a sizeable number of patients with different degrees of disease severity to look specifically at these physiological outcomes. It also remains to be tested whether these effects of sildenafil intake can also be reproduced in normal subjects.

As a noninvasive method, NIRS has a number of limitations (as discussed extensively elsewhere) (16); however, it remains the only available approach to interrogate the microcirculation (small arterioles, capillary, and venules) during whole-body exercise in intact humans. We restrained our analysis to [deoxy-Hb + Mb], as this variable is insensitive to blood volume changes, and its time course has been found to be remarkably equivalent to fractional O_2 extraction when muscle venous outflow is carefully isolated in animal preparations [as discussed at length in Barbosa et al. (4) and Ferreira et al. (17)]. A caveat particularly pertinent to the present study, however, is its inability to differentiate Hb from Mb with regard to light absorption. However, by comparing muscle deoxygenation with $P_{O_{2mv}}$, determined by phosphorescence quenching within the same muscle region in rat preparations, Koga et al. (29) confirmed that the deoxygenation signal did provide a valid index of local fractional O_2 extraction kinetics during exercise transients.

In the present study, we relied on a single exercise transition to extract the parameters of $\dot{V}O_2$ kinetics to avoid repetitive tests (and drug intake) in a very disabled population. Although we recognize that the signal-to-noise ratio would have been improved by averaging multiple transitions (1, 26, 39), the supra-GET bout provided a response amplitude from baseline (~0.5 l/min of $\dot{V}O_2$ and ~30 l/min of $\dot{V}E$), which was large enough for fidelity of parameters estimation while avoiding the confounding effects of the ventilatory oscillations. Also, importantly, the effects of sildenafil on on-exercise τ-$\dot{V}O_2$ were commensurate with those found in response to interventions in CHF (1) and above the test-to-test variation in our laboratory during supra-anaerobic threshold exercise (10.2%).

Conclusions. Oversignaling of the NO pathway by PDE_5 inhibition through a single dose of sildenafil enhanced on- and off-exercise O_2 delivery-to-utilization matching at the microcirculatory level, reduced the amplitude of oscillatory breathing, and accelerated $\dot{V}O_2$ kinetics with positive effects on tolerance to endurance exercise in CHF. The lack of effect of sildenafil on CO suggests that improvement in blood flow to and within skeletal muscles is mechanistically linked to these actions. Our data indicate that sildenafil is a drug particularly suited to pathophysiology of CHF, as it enhances muscle oxygenation and pulmonary $\dot{V}O_2$ kinetics during exercise without the need of major functional improvements in the failing heart. Results of this cross-sectional investigation, however, should be confirmed in larger longitudinal studies to assess the long-term effects of sildenafil intake on these outcomes in patients with different degrees of disease severity.

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AUTHOR CONTRIBUTIONS

Author contributions: L.E.N., D.R.A., and J.A.N. conception and design of research; P.A.S., M.F.O., M.K.R., D.C.B., and E.T. performed experiments; P.A.S., M.F.O., M.K.R., D.C.B., E.T., L.E.N., D.R.A., and J.A.N. analyzed data; P.A.S., M.F.O., E.T., L.E.N., D.R.A., and J.A.N. interpreted results of experiments; P.A.S., M.K.R., and J.A.N. prepared figures; J.A.N. drafted manuscript; P.A.S., D.C.B., E.T., L.E.N., D.R.A., and J.A.N. edited and revised manuscript; P.A.S., M.F.O., M.K.R., D.C.B., E.T., L.E.N., D.R.A., and J.A.N. approved final version of manuscript.

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