Sildenafil improves skeletal muscle oxygenation during exercise in men with intermittent claudication

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Sildenafil improves skeletal muscle oxygenation during exercise in men with intermittent claudication (IC). In the present study, we evaluated the acute effects of sildenafil, a phosphodiesterase type 5 inhibitor that acts by prolonging NO-mediated cGMP signaling in vascular smooth muscle, on blood pressure (BP), skeletal muscle oxygenation, and walking tolerance in patients with IC. A randomized, double-blind, crossover study was conducted in which 12 men with stable IC received two consecutive doses of 50 mg of sildenafil or matching placebo and underwent a symptom-limited exercise test on the treadmill. Changes in gastrocnemius deoxygenation by near-infrared spectroscopy estimated peripheral muscle O2 delivery-to-utilization matching. Systolic BP was significantly lower during the sildenafil trial relative to placebo during supine rest (P < 0.05). Diastolic BP was also lower after sildenafil during upright rest (P < 0.05) and during recovery from exercise (P < 0.05). Gastrocnemius deoxygenation was consistently reduced during submaximal exercise (~41%) and at peak exercise (~34%) following sildenafil compared with placebo (P < 0.05). However, pain-free walking time (placebo: 355 ± 42 s vs. sildenafil: 294 ± 35 s) and maximal walking time (placebo: 701 ± 58 s vs. sildenafil: 716 ± 62 s) did not differ between trials. Acute administration of sildenafil lowers BP and improves skeletal muscle oxygenation during exercise but does not enhance walking tolerance in patients with IC. Whether the beneficial effects of sildenafil on muscle oxygenation can be sustained over time and translated into positive clinical outcomes deserve further consideration in this patient population.

intermittent claudication; sildenafil; blood flow; exercise

ATHEROSCLEROTIC PERIPHERAL artery disease (PAD) is an escalating, highly debilitating disease that affects ~202 million people worldwide (15). The most common clinical presentation of PAD is intermittent claudication (IC), defined as pain in the legs during ambulatory activity (63). Impaired leg function in these individuals leads to reduced mobility and impaired exercise capacity, which negatively affect quality of life and contribute to an accelerated decline in functional capacity (29, 30, 43, 46). Individuals with PAD perform simple functional tasks at slower rates than individuals without the disease (31). These patients are also at greater risk of dying from cardiovascular events (3). Thus, prevention of mobility loss and reduction of cardiovascular risk are current major goals in the management of PAD (28).

Endothelial dysfunction caused, in part, by deficient nitric oxide (NO) production and/or increased bioactivation is a central feature in the pathophysiology of IC (7, 25). Systemic biomarkers of NO metabolism and endothelial-dependent dilation of the calf resistance vessels are known to be severely reduced in these patients (1, 7, 25). Among other consequences, impaired NO signaling and reduced vascular responsiveness may contribute to the impairment in leg blood flow responses to exercise in these individuals (58). A recent study that employed magnetic resonance imaging showed that calf muscle blood flow is reduced by as much as 40% during peak plantar flexion exercise in claudicants compared with healthy controls (41). At the microcirculatory level, numerous reports have documented that near-infrared spectroscopy (NIRS)-derived measures of muscle deoxygenation increase prematurely and to a greater extent during treadmill walking in patients with IC compared with their healthy counterparts (22, 23, 27).

Restoring the functionality of the NO pathway has long been considered an attractive therapeutic approach to potentially improve muscle blood flow and functional capacity in PAD (61). Phosphodiesterase (PDE) inhibitors have received particular attention and cilostazol, an inhibitor of PDE type III, is one of the few drugs approved for the treatment of claudication (26). Surprisingly, the impact of selective PDE type 5 (PDE5) inhibitors has not been investigated in this population. Sildenafil is a potent PDE5 inhibitor that acts by amplifying NO effects via an elevation in the available pool of cyclic guanosine monophosphate (cGMP) (53). This drug has been successfully used to treat a number of conditions, including erectile dysfunction, pulmonary hypertension, and congestive heart failure (CHF) (18, 42). Emerging evidence also indicates that sildenafil may be effective in lowering blood pressure (BP) in patients with essential hypertension (35, 36). Recently, our group has shown that a single oral dose of sildenafil markedly
METHODS

Subjects

Twelve men with a history of IC and an ankle-brachial index (ABI) lower than 0.9 in at least one leg were recruited from the Claudication Outpatient Clinic at the Sao Paulo Hospital, a university-based teaching hospital. Exclusion criteria were I) diabetes; 2) use of cilostazol, pentoxifylline, nitrates, and metformin; 3) inability to walk on the treadmill, and 4) presence of exercise-limiting comorbidity (e.g., angina, dyspnea, etc.). All of the patients had significant aortoiliac or femoropopliteal disease, as confirmed by duplex ultrasonography scanning. Written informed consent was obtained from all study participants, and the protocol was approved by the Institutional Review Board (CAAE no. 14586313.9.0000.5505).

Protocol

In the first visit to the laboratory, the patients underwent a physical examination and were familiarized with the study protocol. After recording the anthropometric characteristics, the participants rested for 15 min in the supine position and the systolic pressures in the arms and legs were measured for the calculation of the ABI, as described below. Patients then performed a progressive, symptom-limited exercise test on the treadmill. Individuals that were not familiar with the treadmill test were required to complete at least two practice sessions. These patients were only allowed to commence the experimental protocol when the difference in maximal walking time between two consecutive tests was less than 20%. Next, in a randomized, crossover design, the patients were asked to report to the laboratory twice for the experimental sessions, at least 72 h apart. Subjects took their usual medication and were instructed to refrain from smoking for at least 4 h prior to the experiments. Upon arrival to the laboratory, the patients rested in the supine position for 15 min, after which systolic pressure measurements were taken. The patients then received two consecutive doses of 50 mg of sildenafil (Viagra, Pfizer) or matching placebo with a 30-min interval between each dose. Both patients and investigators were blinded to the assigned treatment. Systolic pressure measurements were repeated 30 min after the final dose following a period of 15 min of resting in the supine position. The patients were then instrumented and completed a symptom-limited exercise test on the treadmill as described below. The test began ~90 min after ingestion of the first sildenafil/placebo capsule.

Measurements

ABI. Systolic pressures were measured in duplicate with a handheld Doppler system (Micromed, Brasilia, Brazil) in the right and left brachial and in the dorsalis pedis and posterior tibial arteries. The ABIs were calculated by dividing the higher ankle pressure (obtained from the dorsalis pedis or posterior tibial arteries) by the higher of the two brachial artery pressures.

Treadmill Exercise Test

Patients performed a graded, symptom-limited exercise test on a treadmill (Super ATL, Inbrasport, Brazil) following the Gardner protocol (treadmill speed of 2 mph, 0% grade with increments of 2% every 2 min). During the test, patients were asked to inform us when the leg symptoms first appeared, which was defined as the pain-free walking time, and were encouraged to walk until voluntary exhaustion, defined as maximal walking time. Verbal encouragement was given by the same investigator on all exercise tests. During the practice sessions, the intraclass correlation coefficients were $R = 0.79$ for the pain-free walking time and $R = 0.89$ for the maximal walking time. When the patients indicated that they could no longer continue walking, the treadmill was turned off, and the subjects recovered standing on the treadmill for 10 min. Gas exchange and ventilatory variables were measured breath-by-breath during exercise and recovery using a computer-based system (Caro Systems Medical Graphics, Saint Paul, MN), as described previously (51). The following variables were determined: pulmonary oxygen uptake (V\(\text{\text{O}}_2\), ml/min), carbon dioxide output (V\(\text{\text{CO}}_2\), ml/min), respiratory exchange ratio (RER), minute ventilation (V\(\text{\text{E}}\), l/min), and ventilatory equivalents for O\(\text{\text{2}}\) and CO\(\text{\text{2}}\) (Ve/V\(\text{\text{O}}_2\) and Ve/V\(\text{\text{CO}}_2\)). Heart rate (HR) was determined by using the R-R interval from a 12-lead electrocardiogram. BP was measured prior to exercise onset and every 2 min during the test and during the recovery period by the conventional auscultatory method.

Skeletal Muscle Oxygenation

The microvascular oxygenation status of the medial gastrocnemius muscle of the most symptomatic leg was monitored using a commercially available NIRS system (NIRO 300; Hamamatsu Photonics, Hamamatsu City, Japan), as described previously (51). Initially, the skin in the medial portion of the calf was shaved and cleaned, and the anatomical location for the optode placement was determined while the patient was standing on tiptoe. The optode holder was secured to the skin with adhesive tape and covered with aluminum foil. An elastic bandage was then wrapped around the entire calf. To ensure that the probe was positioned in the same location during both experimental trials, pen marks were made around the probe holder. The variables assessed by the NIRS system are the concentration of oxygenated [oxy-Hb+Mb] and deoxygenated [deoxy-Hb+Mb] hemoglobin + myoglobin, as well as total Hb. As described in detail by our group (50, 51) and others (14, 17), [deoxy-Hb+Mb] was selected as the preferred indicator of muscle oxygenation during exercise, as this signal is relatively insensitive to blood volume changes and is interpreted, therefore, as a proxy for muscle fractional oxygen extraction (14, 17). The responses of [deoxy-Hb+Mb] reported herein are expressed as relative changes from the baseline value.

Data Analysis

Breath-by-breath gas exchange and ventilation responses were visually inspected, and breaths outside four SDs of the local mean were deleted. The responses were time-aligned at exercise onset, interpolated second by second, and averaged into 5-s bins. Gas exchange and NIRS variables were studied at baseline, defined as the last 20 s preceding exercise onset, during the last 20 s of stages 1–3 of the exercise test, and at peak (isotime) exercise. Peak (isotime) was defined as the last 20 s of the test with the shortest duration. Average values for both NIRS and gas exchange variables were also calculated every 2 min (last 20 s) during 10 min of recovery. The area under the curve for the entire recovery period was calculated for $\Delta$[Deoxy-Hb+Mb].

Statistical Analysis

Data are presented as means $\pm$ SE. Sample size was estimated on the basis of a recent study by our group that demonstrated that acute oral sildenafil improved exercise tolerance by $\sim$21% in patients with CHF (51). Gas exchange, BP, and NIRS variables during exercise and recovery were compared between conditions with a two-way repeated-measures ANOVA followed by Tukey’s post hoc test when ap-
propriate. Walking tolerance and the area under the curve for [Deoxy-Hb/Hb] were compared between conditions with two-tailed, paired \(t\)-tests. Statistical significance was accepted when \(P < 0.05\).

RESULTS

Patient Characteristics

Anthropometric and clinical characteristics of the participants are shown on Table 1. Sildenafil was well tolerated, and none of the patients experienced adverse reactions to the drug.

Table 1. Demographic and clinical characteristics of patients with IC

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>12</td>
</tr>
<tr>
<td>Age, yr</td>
<td>64 ± 2</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24 ± 1</td>
</tr>
<tr>
<td>ABI most affected leg</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td>ABI least affected leg</td>
<td>0.71 ± 0.07</td>
</tr>
<tr>
<td>Race, % White</td>
<td>83</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>33</td>
</tr>
<tr>
<td>Past smoker, %</td>
<td>67</td>
</tr>
<tr>
<td>Medication, %</td>
<td></td>
</tr>
<tr>
<td>Antiplatelet agents</td>
<td>66</td>
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<tr>
<td>Statins</td>
<td>66</td>
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<tr>
<td>Antihypertensive agents</td>
<td></td>
</tr>
<tr>
<td>(\beta)-blocker</td>
<td>41</td>
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<td>Diuretics</td>
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<td>Calcium channel blocker</td>
<td>25</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>75</td>
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<tr>
<td>ANG II receptor antagonist</td>
<td>8</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SE or percentages. IC, intermittent claudication; ABI, ankle-brachial index; ACE, angiotensin-converting enzyme.

Resting Systolic Pressure

Supine systolic pressures in the arms and ankles measured with the hand-held Doppler prior to and 1 h after placebo or sildenafil ingestion are shown in Fig. 1. Baseline pressures were similar before placebo or sildenafil. There were significant differences in systolic pressures between the placebo and sildenafil conditions after 1 h in the right arm (\(P = 0.002\), left arm (\(P = 0.01\)) and right ankle (\(P = 0.002\)) (Fig. 1). The calculated ABIs for both legs remained unaltered (\(P > 0.05\)) after placebo (right ankle, before: 0.60 ± 0.05 and after: 0.60 ± 0.04; left ankle, before: 0.65 ± 0.06 and after: 0.66 ± 0.06) or sildenafil (right ankle, before: 0.57 ± 0.05 and after: 0.59 ± 0.05 and left ankle, before: 0.64 ± 0.07 and after: 0.67 ± 0.06).

Blood Pressure Responses to Exercise and Recovery

Systolic (SBP) and diastolic (DBP) blood pressure values before, during and after the treadmill walking test are shown in Fig. 2. Baseline DBP but not SBP was significantly lower (\(P < 0.05\)) after sildenafil ingestion relative to placebo. During the first three stages of the test, SBP was on average \(\sim 14\) mmHg lower after sildenafil compared with the placebo condition (\(P < 0.05\)), while DBP responses were not different between the two trials (Fig. 2). There were no differences between the two conditions for peak SBP and DBP. During the recovery period from exercise, sildenafil ingestion markedly impacted the BP responses. SBP and DBP were on average \(\sim 18\) and \(\sim 7\) mmHg lower (\(P < 0.05\)) in the sildenafil trial compared with placebo during the first 10 min of recovery (Fig. 2).

Ventilatory and Gas Exchange Responses

\(\dot{V}\text{O}_2, \dot{V}\text{CO}_2, \text{RER}, \dot{V}\text{E}, \dot{V}\text{E}/\dot{V}\text{O}_2\) and \(\dot{V}\text{E}/\dot{V}\text{CO}_2\) values during the treadmill test and during the recovery period are given in Tables 2 and 3. Because of technical difficulties, gas exchange...
data from one patient were excluded from the analysis. The profile of $\dot{V}O_2$ responses during exercise and recovery are depicted in Fig. 3. There were no differences between the two experimental conditions for ventilatory and gas exchange variables.

Table 2. Ventilatory and gas exchange parameters during the first three stages of the treadmill test and at peak exercise (isotime)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Peak (Isotime)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>PLAC</td>
<td>SILD</td>
<td>PLAC</td>
<td>SILD</td>
<td>PLAC</td>
</tr>
<tr>
<td>$\dot{V}O_2$, ml/min</td>
<td>230 ± 16</td>
<td>240 ± 19</td>
<td>609 ± 56</td>
<td>597 ± 53</td>
<td>733 ± 59</td>
</tr>
<tr>
<td>$\dot{V}O_2$, ml·min$^{-1}$·kg$^{-1}$</td>
<td>3.4 ± 0.1</td>
<td>3.6 ± 0.2</td>
<td>8.9 ± 0.6</td>
<td>8.7 ± 0.5</td>
<td>10.8 ± 0.7</td>
</tr>
<tr>
<td>$\dot{V}CO_2$, ml/min</td>
<td>189 ± 13</td>
<td>206 ± 17</td>
<td>459 ± 41</td>
<td>453 ± 41</td>
<td>591 ± 55</td>
</tr>
<tr>
<td>RER</td>
<td>0.8 ± 0.01</td>
<td>0.8 ± 0.04</td>
<td>0.7 ± 0.02</td>
<td>0.7 ± 0.01</td>
<td>0.8 ± 0.02</td>
</tr>
<tr>
<td>$V_e$, l/min</td>
<td>7.9 ± 0.3</td>
<td>8.9 ± 0.7</td>
<td>15.9 ± 1.1</td>
<td>15.6 ± 1.1</td>
<td>19.8 ± 1.8</td>
</tr>
<tr>
<td>$V_e/\dot{V}O_2$</td>
<td>35.4 ± 1.8</td>
<td>37 ± 2.2</td>
<td>27 ± 1.4</td>
<td>26.8 ± 1.6</td>
<td>27.1 ± 1.3</td>
</tr>
<tr>
<td>$V_e/\dot{V}CO_2$</td>
<td>43.1 ± 2.2</td>
<td>44.9 ± 2.5</td>
<td>35.6 ± 1.4</td>
<td>35.2 ± 1.6</td>
<td>33.8 ± 1</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. PLAC, placebo; SILD, sildenafil; $\dot{V}O_2$, pulmonary oxygen uptake; $\dot{V}CO_2$, carbon dioxide output; RER, respiratory exchange ratio; $V_e$, minute ventilation; $V_e/\dot{V}O_2$, ventilatory equivalent for $O_2$; $V_e/\dot{V}CO_2$, ventilatory equivalent for $CO_2$.

**Skeletal Muscle Deoxygenation Responses**

Figure 4, A and B depicts the profile of [Deoxy-Hb+Mb] of a representative subject during the treadmill test and during the recovery period in the sildenafil and placebo trials. Relative changes in [Deoxy-Hb+Mb] were significantly reduced at the end of the first ($P = 0.046$) and third ($P = 0.048$) stages of exercise after sildenafil relative to placebo (Fig. 4C). At peak exercise (isotime), $\Delta[\text{Deoxy-Hb+Mb}]$ was ~34% lower ($P = 0.015$) during the sildenafil trial compared with the placebo condition. During the recovery period, there was a tendency for lower $\Delta[\text{Deoxy-Hb+Mb}]$ at the end of 2nd minute after sildenafil (placebo: $-24 ± 17$ vs. sildenafil: $-5 ± 18$, $P = 0.055$) (Fig. 4D). Calculation of the area under the curve for the entire recovery period also revealed a trend for lower $\Delta[\text{Deoxy-Hb+Mb}]$ in the sildenafil trial compared with the placebo condition (placebo: $43,028 ± 6,116$ vs. sildenafil: $38,878 ± 5,096$ μM·cm$^{-1}$; $P = 0.08$).

**Walking Tolerance**

Pain-free walking time (placebo: $335 ± 42$ s vs. sildenafil: $294 ± 35$ s) and maximal walking time (placebo: $701 ± 58$ vs. sildenafil: $716 ± 62$ s) were not different ($P > 0.05$) in the placebo and sildenafil trials. Peak $\dot{V}O_2$ (placebo: $14.9 ± 1.2$ vs. sildenafil: $15.1 ± 1.1$ ml·kg$^{-1}$·min$^{-1}$; $P = 0.78$) and the HR at the onset of pain (placebo: $89 ± 4$ vs. sildenafil: $87 ± 5$ bpm; $P = 0.87$) and at peak exercise (placebo: $105 ± 5$ vs. sildenafil: $105 ± 5$ bpm, $P = 0.43$) also did not differ in the two experimental conditions.

**DISCUSSION**

This is the first study to investigate the acute effects of the PDE5 inhibitor sildenafil on resting blood pressure and the physiological responses to exercise in patients with IC. We found that oral sildenafil reduced BP at rest, during submaximal exercise, as well as during the recovery period. The drug also evoked a marked reduction on NIRS-derived $\Delta[\text{Deoxy-Hb+Mb}]$ in the gastrocnemius muscle during exercise, a finding that indicates improved microvascular $O_2$ delivery-to-utilization matching. Despite these salutary effects, sildenafil also evoked a marked reduction in NIRS-derived $\Delta[\text{Deoxy-Hb+Mb}]$ in the gastrocnemius muscle during exercise, a finding that indicates improved microvascular $O_2$ delivery-to-utilization matching. Despite these salutary effects, sildenafil failed to affect the time to onset of pain and overall walking tolerance in these patients.
Effects of Sildenafil on Blood Pressure

Hypertension is a major risk factor for PAD and an important contributor to the increased likelihood of cardiovascular events in individuals with this condition (49, 54). A particularly clinically relevant feature of PAD is an exaggerated BP response to exercise that is evident even at relatively mild workloads (5, 32). This abnormal BP response to walking has been shown to be an independent predictor of all-cause mortality and adverse cerebrovascular and cardiac events in these patients (13). In the present study, despite the fact that the vast majority of the patients were on antihypertensive treatment, several individuals were hypertensive at rest and showed pronounced changes in BP during the treadmill test (Fig. 2). In this regard, it is worth highlighting that oral sildenafil produced a modest reduction in BP at rest, as well as during submaximal exercise intensities (Fig. 2). The degree of BP reduction observed herein is similar to that reported in a number of studies in healthy controls, hypertensive individuals, and patients with cardiac disease following acute sildenafil administration (10, 19, 57). The hypotensive effect of sildenafil was especially noticeable during recovery from exercise (Fig. 2). This observation might also be clinically meaningful, as previous studies have shown that the behavior of BP during recovery from exercise is an important marker of cardiovascular risk in hypertensive individuals (64). If the BP-lowering effects of sildenafil reported here are sustained over time during regular administration, it is fair to speculate that this drug might be a useful aid in the pharmacological arsenal designed to prevent cardiovascular events in this population. Nevertheless, our sample size is small, and larger trials are warranted to document the hemodynamic effects of regular sildenafil in patients with IC. Finally, it must be emphasized that since the antihypertensive medication was not withheld prior to the experiments in the present study, it is impossible to partition the exclusive effects of sildenafil on blood pressure in these patients. Numerous reports indicate that the combination of sildenafil with other pharmacological agents may yield small additive decreases in blood pressure (21). Thus, the isolated acute and chronic effects of sildenafil on BP in patients with IC remain to be determined.

Effects of Sildenafil on Muscle Oxygenation

NIRS has long been considered a useful tool to discriminate individuals with and without PAD (11, 27) and to grade the severity of the disease in affected patients (22, 23). Larger increases in skeletal muscle deoxygenation during exercise have been found in patients with IC compared with healthy counterparts, a finding that has been ascribed largely to the hemodynamic limitations associated with this condition (11, 22, 27). Of note, few therapeutic strategies to date have been shown to effectively improve muscle oxygenation during exercise in patients with IC. For example, Beckitt et al. (6) recently reported that angioplasty, but not supervised exercise training, modestly improved gastrocnemius muscle oxygenation during submaximal exercise in claudicants (6). In the present study, we observed that sildenafil reduced Δ[Deoxy-Hb+Mb] by ~40% relative to the placebo condition during the graded treadmill test (Fig. 4C). This finding is in line with a recent observation from our group that sildenafil reduced Δ[Deoxy-Hb+Mb] during high-intensity exercise in patients with CHF (51). The relative changes in [Deoxy-Hb+Mb] during exercise have been interpreted by several laboratories as a reflection of the dynamic balance between local O2 supply and utilization (14, 17). For a given VO2, it is anticipated that

Table 3. Ventilatory and gas exchange parameters during recovery from exercise

<table>
<thead>
<tr>
<th></th>
<th>2 min</th>
<th></th>
<th>4 min</th>
<th></th>
<th>6 min</th>
<th></th>
<th>8 min</th>
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<th>10 min</th>
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<tbody>
<tr>
<td>PLAC</td>
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<td>PLAC</td>
<td>SILD</td>
<td>PLAC</td>
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<td>SILD</td>
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<td>PLAC</td>
</tr>
<tr>
<td>VO2, ml/min</td>
<td>428 ± 30</td>
<td>422 ± 27</td>
<td>269 ± 14</td>
<td>296 ± 15</td>
<td>243 ± 17</td>
<td>257 ± 14</td>
<td>224 ± 18</td>
<td>241 ± 13</td>
<td>228 ± 18</td>
<td>239 ± 22</td>
</tr>
<tr>
<td>VO2, ml·min⁻¹·kg⁻¹</td>
<td>6.4 ± 0.4</td>
<td>6.5 ± 0.3</td>
<td>4 ± 0.2</td>
<td>4.4 ± 0.1</td>
<td>3.6 ± 0.1</td>
<td>3.8 ± 0.2</td>
<td>3.3 ± 0.2</td>
<td>3.5 ± 0.1</td>
<td>3.4 ± 0.2</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>Vco2, ml/min</td>
<td>514 ± 50</td>
<td>520 ± 45</td>
<td>304 ± 24</td>
<td>327 ± 19</td>
<td>247 ± 18</td>
<td>256 ± 13</td>
<td>206 ± 18</td>
<td>224 ± 12</td>
<td>205 ± 17</td>
<td>210 ± 18</td>
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<tr>
<td>RER</td>
<td>1.1 ± 0.05</td>
<td>1.1 ± 0.04</td>
<td>1.1 ± 0.04</td>
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<td>1 ± 0.06</td>
<td>1 ± 0.02</td>
<td>0.8 ± 0.05</td>
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<td>0.8 ± 0.02</td>
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<tr>
<td>Ve, l/min</td>
<td>19 ± 1.6</td>
<td>19.6 ± 1.9</td>
<td>12.9 ± 0.8</td>
<td>13.6 ± 0.4</td>
<td>10.7 ± 0.7</td>
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<td>Ve/Vo2</td>
<td>44.6 ± 2.5</td>
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<td>42.7 ± 3</td>
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<tr>
<td>Ve/Vco2</td>
<td>38 ± 1.8</td>
<td>38.7 ± 2.4</td>
<td>43.6 ± 2.1</td>
<td>42.8 ± 2.4</td>
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<td>44.7 ± 2.1</td>
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<td>46.9 ± 2.7</td>
<td>46.8 ± 2.3</td>
<td>48.1 ± 3.1</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SE. PLAC, placebo; SILD, sildenafil; VO2, pulmonary oxygen uptake; VCO2, carbon dioxide output; RER, respiratory exchange ratio; VE, minute ventilation; VE/VO2, ventilatory equivalent for O2; VE/VCO2, ventilatory equivalent for CO2.

Fig. 3. Group mean VO2 responses during the first three stages of the treadmill test and at peak exercise (isotime) (A) and throughout 10 min of recovery (B) after placebo (○) and sildenafil (●). Data are expressed as means ± SE.
an improvement in muscle oxygen delivery would translate into a reduced fractional oxygen extraction (i.e., a reduction in Δ[Deoxy-Hb+Mb]), as shown in the present study. This is not surprising given that sildenafil is a well-known potent vasodilator that acts by increasing the intracellular pool of cGMP, which prolongs the signaling action of NO in the vasculature (53). Overall, this observation strengthens the concept that enhancing NO functional activity can be a potentially useful strategy to improve leg hemodynamics in patients with IC. Along these lines, a recent study documented that acute ingestion of beetroot juice, which is associated with increased circulating levels of bioactive nitrite, also evoked marked improvements in muscle oxygenation during exercise in claudicants (20). Altogether, these observations provide compelling evidence that dietary and pharmacological (or a combination of both) manipulation of the NO pathway are promising therapeutic targets to alleviate skeletal muscle ischemia in patients with IC (1, 61).

**Clinical implications.** An unexpected finding of this study was that despite the significant improvement in muscle oxygenation during exercise after sildenafil intake, pain-free and total walking time did not increase compared with placebo. These findings underscore the complexity of the genesis of functional impairment in individuals with IC (2). It is well established that walking impairment in these patients stem from a combination of several mechanisms that range from intrinsic skeletal muscle abnormalities to poor walking economy (2, 16, 40). In essence, targeting only one of the multiple mechanisms that underlie the exercise intolerance in these individuals might be insufficient to promote an acute improvement in performance (8). An alternative explanation is that the magnitude of the improvement in muscle oxygenation evoked by sildenafil was insufficient to be translated into a measurable change in walking tolerance. However, this seems unlikely as the improvement observed herein is comparable to that achieved after angioplasty (6), which typically enhances walking tolerance in patients with IC (6, 62). Lastly, it is worth highlighting that NIRS measurements were performed in a single site in the gastrocnemius muscle, and it is unclear whether the observed local improvement in microvascular O2 delivery-to-utilization matching after sildenafil extends to the other regions of the muscle, as well as to other muscles in the leg recruited during walking. Using mathematical simulations, Sprague and co-workers have shown that a potential caveat of the administration of vasodilators as a means to restore tissue oxygenation is that blood flow theoretically increases nonselectively within the microvascular network and, therefore, this strategy may fail to fully correct potential mismatches between O2 supply and demand (52). If true for the exercising muscles, it is conceivable that undersupplied regions would still be present in the tissue after sildenafil administration, therefore, precluding a measurable impact on exercise tolerance. This scenario is nonetheless hypothetical, and future studies with multisite NIRS measurements that encompass a larger area of the active muscle mass are required to determine the impact of sildenafil on the spatial heterogeneity of muscle deoxygenation during exercise in patients with IC.

The improvement in skeletal muscle oxygenation observed in the present study can be particularly meaningful when considering the deleterious impact of ischemia/reperfusion triggered by exercise in these patients (9). Exercise-induced muscle ischemia in patients with IC evokes a potent increase in local and systemic markers of inflammation and oxidative stress (33, 47, 56), as well as an acute impairment in vascular reactivity (48). Repeated exposure to cycles of ischemia-reoxygenation injury during ambulatory activity in these
patients is thought to contribute, among others, to the development of skeletal muscle abnormalities, including fibrosis and mitochondrial dysfunction (39). Conceivably, the reduction in the magnitude of muscle deoxygenation during exercise achieved after sildenafil administration can potentially abrogate the harmful inflammatory response to exertion in these patients and as a consequence prevent the maladaptations in skeletal muscle. Interestingly, sildenafil has a well-established protective effect against ischemia/reperfusion in the heart (24), and as recently shown by Armstrong et al. (4), might also reduce ischemic damage in skeletal muscle following a period of ischemia (4). These encouraging findings prompt the need for future studies to determine the impact of sildenafil on the inflammatory responses to exercise in patients with IC.

**Study Limitations**

The exercise test started ~60 min after the final oral dose of sildenafil. The rationale behind this choice is based on the observation that sildenafil exhibits rapid oral absorption, and peak plasma concentrations are typically achieved ~1 h after administration (34, 59). However, as we have not studied the pharmacokinetic profile of the dosing regimen employed herein, it is unclear whether the maximal possible physiological inhibition of PDE5 was, indeed, attained in these patients.

Another major limitation of the present study is that we focused only in the acute responses to sildenafil administration. Regular, long-term treatment with the drug might evoke a number of beneficial adaptations that cannot be perceived in a single-dose study. This notion is supported by findings from studies with other pharmacological agents, such as the selective PDE type III inhibitor cilostazol. The clinical benefits of cilostazol increase progressively during 24 wk of treatment in patients with IC (12). As pointed out by Brass (8), this finding suggests that an adaptive process rather than an acute vasodilatory action mediate the positive effects of this drug on walking tolerance in patients with IC. In agreement with this concept, several recent studies have documented beneficial adaptations following repeated, long-term administration of sildenafil. Senthilkumar et al. (44) showed that regular sildenafil administration restored blood flow and increased vascular density in the ischemic skeletal muscle in a preclinical model of PAD. As PDE5 is also expressed in skeletal muscle cells (60), it has been proposed that chronic sildenafil treatment might also trigger beneficial changes in this tissue (37). Per-cival et al. (38) showed that a 14-wk treatment with sildenafil reduced respiratory muscle weakness and fibrosis in the mdx mouse model of Duchenne muscular dystrophy. In humans, a recent study documented that sildenafil administration for 8 consecutive days nearly doubled skeletal muscle protein synthesis and reduced muscle fatigue in healthy subjects (45). On the basis of these observations, future studies are needed to verify the long-term consequences of regular sildenafil administration in patients with IC. Anecdotal observations have been reported that regular sildenafil was associated with alleviation of the leg symptoms in a patient with claudication (55). Whether similar outcomes will hold true in larger, randomized controlled trials remains to be determined.

**Conclusions**

Current pharmacological management of IC is aimed at reducing the risk of cardiovascular events and alleviating the leg ischemia induced by exercise. Results from the present study suggest that sildenafil might fulfill both therapeutic goals as revealed by the marked reduction in blood pressure and improved muscle oxygenation during walking in patients with IC. Nonetheless, the lack of changes in functional performance after acute administration suggests that long-term, regular treatment with this drug may be required to evoke meaningful changes in walking capacity in these individuals.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


