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Effects of sildenafil on maximum walking time in patients with arterial claudication: The ARTERIOFIL study

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ABSTRACT

Background: Patients with lower extremity peripheral artery disease (PAD) frequently experience claudication, a clinical symptom indicative of reduced walking capacity. Recommended care consists of exercise rehabilitation combined with optimal medical treatment and surgery. The effects of a single oral dose of sildenafil, a phosphodiesterase type-5 inhibitor, on patients with claudication are discussed. The aim of this study was to test the efficacy of a single 100 mg dose of sildenafil compared to placebo in terms of maximal walking time (MWT) in patients with claudication.

Methods: The ARTERIOFIL study is a crossover, double-blind, prospective, randomized, single-center study conducted at Angers University Hospital in France. MWT (primary endpoint) was assessed using a treadmill test (10% incline; 3.2 km/h). Secondary endpoints (pain-free walking time (PFWT), transcutaneous oximetry during exercise and redox cycle parameters and safety) were also studied.

Results: Fourteen patients were included of whom two were ultimately excluded. In the 12 remaining patients, the MWT was significantly improved during the sildenafil period compared with the placebo period (300 s [95% CI 172 s–428 s] vs 402 s [95% CI 274 s–529 s] $p < 0.01$). Sildenafil had no significant effect on pain-free walking time or skin tissue oxygenation during exercise. According to redox cycle parameters, sildenafil significantly reduced blood glucose and pyruvate levels and the 3-hydroxybutyrate/acetoacetate ratio, while there was no significant effect on lactate, 3-hydroxybutyrate, acetoacetate and free fatty acid levels. Symptomatic transient hypotension was observed in two women.

Conclusions: The ARTERIOFIL study has shown that a single 100 mg oral dose of sildenafil had a significant effect on increase in MWT but had no significant effects on PFWT and oxygenation parameters in patients with

Abbreviations: 3OHB, 3-Hydroxybutyrate; AA, Acetoacetate.; ABI, Ankle-brachial index.; ACEi, Angiotensin-converting enzyme inhibitors.; cGMP, Cyclic guanosine monophosphate.; DROP, Decrease from resting of oxygen pressure.; mITT, Modified intention-to-treat.; MWT, Maximum walking time.; NO, Nitric oxide.; OMT, Optimal medical treatment.; PAD, Peripheral artery disease.; PDEi, Phosphodiesterase inhibitors.; PFWT, Pain-free walking time.; TcPO2, Transcutaneous oxygen pressure measurement.

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claudication. A double-blind, prospective, randomized, multicenter study (VIRTUOSE©) is ongoing to evaluate the chronic effect of six month-long sildenafil treatment on MWT in PAD patients with claudication.
Clinical trial registration: This clinical trial was registered at clinicaltrials.gov, registration. number: NCT02832570, (https://clinicaltrials.gov/ct2/show/NCT02832570).

1. Introduction

Lower extremity peripheral arterial disease (PAD) is a highly debilitating disease that affects 202 million people around the world [1]. Morbidity and mortality from cardiovascular events is greater in this population [2]. Claudication is defined as discomfort or pain or fatigue in the lower limbs while walking that subsides with rest [3]. Claudication is caused by arterial obstruction proximal to affected muscle beds, thereby lessening exercise-induced increase in blood flow and leading to transient muscle ischemia [4–6]. In symptomatic PAD patients, claudication numbers among the symptoms [3]. Multiple studies have objectively documented patient functional status impairment with respect to claudication [7–10].

Claudication seriously affects quality of life [11] and is associated with severe functional impairment that can however be significantly improved by intervention in PAD patients [9,10]. Thus, prevention of mobility loss and cardiovascular risk reduction are major ongoing goals in the management of PAD [12]. This fact, along with impact on lifestyle and the large population affected by PAD, have caused it to be identified as an area where new treatments are urgently needed [10]. According to recommendations, establishing appropriate medical treatment and lifestyle changes for a minimum of 3 to 6 months [13] should always precede contemplation of invasive procedures [13].

In patients with claudication, the primary therapeutic approach is optimal medical treatment (OMT) including antiplatelet agents, lipid lowering drugs, angiotensin converting enzyme inhibitors (ACEi) and recommended walking programs [13]. Revascularization is only proposed when this therapeutic strategy has failed to improve symptoms and walking ability for a minimum of 3 to 6 months [13].

To date, no other drug has provided consistent evidence of functional improvement in claudication, except for Cilostazol, a type-3 phosphodiesterase inhibitor (PDEi) [14]. Sildenafil is a type-5 PDEi that acts by amplifying the effects of nitric oxide (NO) via an increase in the available cyclic guanosine monophosphate (cGMP) pool [15]. This drug has been successfully used to treat a number of conditions including erectile dysfunction, pulmonary hypertension and congestive heart failure (CHF) [16,17], but to date few results regarding its use in claudication have been published [18–20]. In terms of pharmacodynamics, in addition to the vasodilator effect of sildenafil due to its positive impact on vascular endothelial function via the NO/cGMP pathway [21], data from the literature suggest that sildenafil has an opioid-like analgesic effect via this pathway [21]. Previous research work has shown that a single oral dose of sildenafil markedly reduces skeletal muscle microvascular deoxygenation and increases exercise tolerance in patients with congestive heart failure and claudication [18,22]. The present study was designed to test the efficacy of a single 100 mg dose of sildenafil compared with placebo in terms of maximum walking time (MWT) in patients with claudication.

2. Materials and methods

2.1. Study design and oversight

The ARTERIOFIL study is a crossover, double-blind, prospective, randomized, single-center study conducted at Angers University Hospital in France. The study was designed to test the hypothesis that a single 100 mg oral dose of sildenafil plus OMT would correlate with greater improvement in maximum walking time (MWT) in treadmill tests than placebo plus OMT in PAD patients with claudication. Secondarily, ARTERIOFIL tested improvement in pain-free walking time (PFWT) and exercise transcutaneous oxygen pressure (Exercise-TcPO2) measurement during treadmill testing with sildenafil compared with placebo. Redox cycle parameters (blood glucose, lactate, and pyruvate levels, lactate/pyruvate ratio, 3-hydroxybutyrate (3OHB) and acetoacetate (AA) levels, 3OHB/AA ratio and free fatty acid (FFA) levels) were also tested during fasting (T1), after taking sildenafil or placebo (T2), immediately (T3), 5 min (T4) and 15 min (T5) after the treadmill test. Finally, the safety and tolerance profile of sildenafil was verified according to its summary of product characteristics. The trial protocol was approved by the local research ethics committee (CPP Ouest II, Angers, France) and was implemented according to the most recent amendments to the Declaration of Helsinki and in keeping with good clinical practice guidelines. Written informed consent was obtained from all patients prior to enrolment. The study has been registered under reference number NCT02832570 with Clinicaltrials.gov since July 142,016, and has been overseen by an independent data safety and monitoring committee. The lead author wrote the first draft of the manuscript, and all co-authors participated in and approved subsequent revisions.

2.2. Subjects

Fourteen patients with a history of PAD were recruited from the Department of Vascular Investigation at Angers University Hospital. Eligibility criteria were: 1) 18 years of age and over; 2) male or female; 3) patients with claudication (ankle-brachial index (resting-ABI) ≤ 0.90 or TcPO2 of buttock < -15 mmHg in isolated proximal claudication); 4) patients in a fit state to perform treadmill test; 5) patients with no contraindications to sildenafil; 6) patients under stable optimal medical treatment (combining ACEi + antiplatelet agents + lipid-lowering drugs) since a minimum of 1 month.

Inclusion criteria were: 1) walking time on treadmill (3.2 km/h, 10% incline) of < 5 min; 2) patient comprehension of protocol and participant consent.

Non-inclusion criteria were: 1) patients with critical limb ischemia; 2) history of myocardial infarction or unstable angina; 3) amblyopia; 4) patients treated with nitrates and related derivatives or medication causing drug interaction with the substance under investigation; 5) patients with severe renal impairment (creatinine clearance < 30 ml/min); 6) patients with severe hepatic impairment; 7) patients with hypotension (blood pressure $< 90/50$ mmHg).

2.3. Protocol

The patients included in this study were randomized to receive either sildenafil or placebo at two visits spaced one week apart. At each visit, MWT was measured on a treadmill.

Patients arrived on an empty stomach at the Clinical Research Center of Angers University Hospital at least 2 h before the treadmill test. A resting electrocardiogram was performed to screen for myocardial ischemia and arrhythmias or conduction disorders. A 20G catheter was inserted into a vein to measure redox cycle parameters. Once blood samples were obtained, patients were provided with a breakfast that was standardized in terms of calories and nutrients.

Treatment was then allocated (in line with order of administration determined by randomization): patients received either 100 mg of sildenafil, or a placebo administered by a clinical research center nurse in compliance with double-blind conditions. Patients remained under the

supervision of a physician. After drug absorption, each patient was monitored using a Mobilograph® (device for continuous automated measurement of blood pressure) for 2 h. Next exercise-TcPO2 testing was performed. A further blood sample was taken before the treadmill test as previously described. Subsequently, an Ishihara test was conducted by a nurse to detect any color vision deficiency that is an infrequent side effect of sildenafil.

2.4. Measurement of ankle brachial index (resting ABI)

Measurement of resting-ABI was conducted in accordance with American Heart Association recommendations using a hand-held Doppler probe by trained vascular medicine physicians [23].

2.5. Treadmill exercise test

Standard treadmill tests in our laboratory are performed at a 10% incline, and a speed of 3.2 km/h (transition phase 1 min) [24]. Measurement of TcPO2 was performed using calibrated TcPO2 electrodes (TCOM/TcPO2; PF6000; Perimed; Jarfalla, Sweden). Measurements were automatically recorded on a 1 Hz basis with home-made software (M Feuilloy, ESEO, Angers, France). The temperature of each electrode was set at 44.5 °C, enabling maximal skin vasodilation. A reference electrode (chest electrode) was placed between the scapulae to measure systemic changes in TcPO2 during exercise [24–26]. One electrode was positioned on each buttock, 4 to 5 cm behind the bony prominence of the trochanter, and one on each calf. Once in place, a period of 10 min in standing position was required to stabilize the electrodes, thus obtaining baseline values. Exercise was performed on a treadmill at a 10% incline and a speed of up to 3.2 km/h[25]. A 12-lead ECG was used to monitor heart rate and rhythm during the exercise test procedure.

Patients were encouraged to walk for the longest time possible to mimic symptoms of claudication. Exercise was discontinued at the patient's request. Measurements from TcPO2 electrodes were used to calculate the decrease from resting of oxygen pressure (DROP) index (expressed in mm Hg), absolute changes in TcPO2 from resting value in each of the 4 limb electrodes, corrected for absolute changes in TcPO2 in the chest electrode. The specific advantages of the DROP calculation, particularly its independence from absolute value, have been widely reported [24,27,28]. The lowest DROP value (DROPmin) was retrieved from the total recordings. A DROPmin index below −15 mmHg was taken as indicative of ischemia occurring during exercise (positive test) as previously validated [24]. Four different DROPmin values were analyzed depending on their skin location: right buttock DROPmin, left buttock DROPmin, right calf DROPmin, and left calf DROPmin [26].

2.6. Endpoint evaluation

The primary efficacy endpoint was an improvement in MWT two hours after taking a single oral dose of sildenafil compared with placebo using a recognized, standardized treadmill test on the same patient at a one-week interval (Visit 1 and Visit 2).

Secondary endpoints were: i) improvement in PFWT and DROPmin under sildenafil compared with placebo; ii) difference between redox cycle parameters during fasting, after breakfast and after taking sildenafil or placebo, before and after treadmill test; iii) safety and tolerance profile of sildenafil.

2.7. Statistical analysis

Continuous variables were described as mean and confidence of interval 95% (IC 95%) or median and interquartile range (IQR) values, and categorical variables were expressed as numbers (percentages) with respect to the modified intention-to-treat (mITT) population. The mITT population was defined as all randomized subjects who received one dose of study medication and completed one post-randomization treadmill test.

Mixed models were used to test the effects of the treatment administered, taking into account subject as a random effect, sequence effect (placebo/sildenafil or sildenafil/placebo) and period effect (Visit 1 or Visit 2) as fixed effects. Normal distribution of the measured variables was verified. Where the hypothesis was not confirmed, gamma distribution or rank transformation were applied.

A significance threshold of 0.05 was used in all statistical tests. Statistical analysis was performed using SAS® 9.4 software (SAS Institute, Cary, NC, USA).

Unblinding was carried out on April 162,018 by the Data Management and Evaluation Unit of Angers University Hospital.

2.8. Sample size calculation

For this cross-over study, the sample size needed to detect a mean difference of 2.5 min (from 4.5 min to 7 min) in MWT assuming a SD of 2.8 min with a power of 90% and a significance level of 5% using a one-sided paired *t*-test was 14 patients.

3. Results

3.1. Patient demographic and clinical characteristics

From November 2016 to October 2017, fourteen patients were

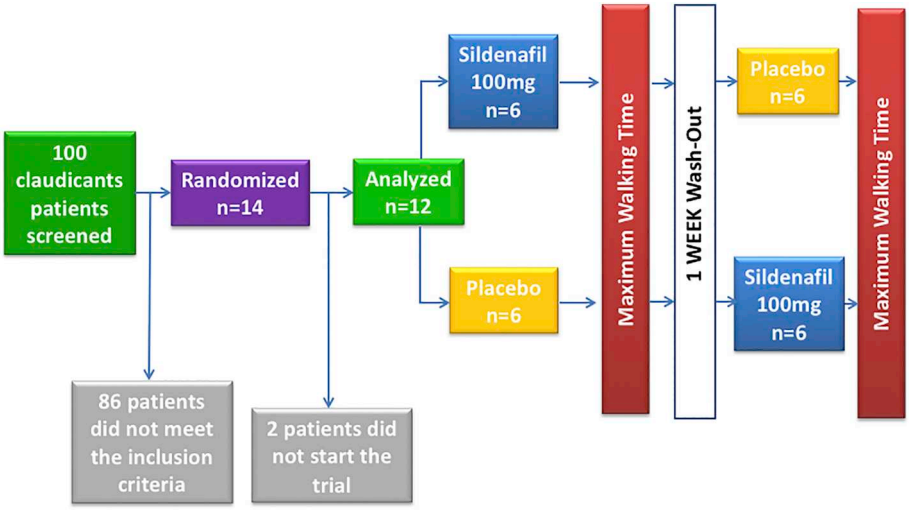

Fig. 1. Flowchart of the ARTERIOFIL trial.

Table 1
Demographic and Clinical Characteristics in modified intention-to-treat (mITT) population at baseline (n = 12).

Patients demographic and clinical characteristics	All	Sequence		p
		Placebo/Sildenafil	Sildenafil/Placebo	
	n = 12	n = 6	n = 6	
Age (years) [median (Q1;Q3)]	61.0 (59.0; 66.0)	59.0 (57.0; 66.0)	62.0 (61.0; 66.0)	0.2
Male [n (%)]	8 (66.7)	4 (66.7)	4 (66.7)	1.00
BMI (Kg/m2) [median (Q1;Q3)]	29.6 (27.5; 31.8)	28.1 (22.0; 29.3)	30.7 (29.8; 32.0)	0.09
Previous history	11 (91.7)	5 (83.3)	6 (100)	1.00
Diabetes [n (%)]	2 (16.7)	0 (0.0)	2 (33.3)	0.45
Hypertension [n (%)]	10 (83.3)	4 (66.7)	6 (100)	0.45
Hypercholesterolemia [n (%)]	3 (25.0)	2 (33.3)	1 (16.7)	1.00
Myocardial Infarction [n (%)]	0 (0.0)	0 (0.0)	0 (0.0)	
Stroke [n (%)]	0 (0.0)	0 (0.0)	0 (0.0)	
Current smoker [n (%)]	5 (41.7)	3 (50.0)	2 (33.3)	1.00
ABI (Right Leg) [median (Q1;Q3)]	0.8 (0.6; 0.9)	0.8 (0.7; 0.9)	0.7 (0.5; 0.9)	0.38
ABI (Left Leg) [median (Q1;Q3)]	0.7 (0.5; 0.7)	0.7 (0.7; 1.1)	0.5 (0.5; 0.7)	0.07
Arterial Revascularization (Aortoiliac) [n (%)]	3 (25.0)	1 (16.7)	2 (33.3)	1.00
Arterial Revascularization (Femorotibial) [n (%)]	3 (25.0)	1 (16.7)	2 (33.3)	1.00
MWT (s) [median (Q1;Q3)] at baseline	179.5 (154.0; 269.0)	213.5 (129.0; 295.0)	175.5 (169.0; 195.0)	0.81
DROPmin [median (Q1;Q3)]				
Right buttock	−16.0 (−22.5; −11.5)	−16.0 (−24.0; −9.0)	−16.0 (−21.0; −12.0)	0.94
Left buttock	−20.5 (−25.5; −8.0)	−15.5 (−27.0; −7.0)	−21.0 (−24.0; −11.0)	0.87
Right calf	−24.5 (−32,5; −16.0)	−25.5 (−35.0; −21.0)	−22.0 (−30.0; −13.0)	0.69
Left calf	−26.0 (−34.0; −11.5)	−17.5 (−35.0; −7.0)	−28.0 (−33.0; −14.0)	0.47
Blood-Pressure (mmHg) [median (Q1;Q3)] at baseline				
Systolic Blood-Pressure (mmHg)	141.5 (123.0; 161.0)	128.0 (110.0; 148.0)	161.0 (135.0; 177.0)	0.08
Diastolic Blood-Pressure (mmHg)	76.5 (70.0; 80.0)	73.0 (70.0; 80.0)	78.5 (71.0; 87.0)	0.37

BMI: body mass index; ABI: Ankle-brachial index; MWT: Maximum Walking Time; DROP: Decrease From Resting of Oxygen Pressure; Q1: First Quartile; Q3: Third Quartile.

included and randomized. Of these fourteen patients, two were ultimately excluded due to epistaxis in one patient and fluctuation in arterial blood pressure in the other (Fig. 1). Anthropometric and clinical characteristics of the participants are presented in Table 1.

3.2. Primary endpoint analysis

The MWT was significantly improved during the sildenafil period compared with the placebo period (300 s [95% CI 172 s–428 s] vs 402 s [95% CI 274 s–529 s] $p < 0.01$). Fig. 2 shows MWT in relation to treatment. Fig. 3 shows MWT in relation to treatment according to sequence for every patient. There were no significant sequence or period effects ($p = 0.15$ and $p = 0.08$ respectively).

3.3. Secondary endpoint analysis

Sildenafil had no significant effect on PFWT, ABI, or DROPmin. However, with respect to redox cycle parameters when fasting (T1), after taking sildenafil or placebo (T2), immediately (T3), 5 min (T4) and 15 min (T5) after the treadmill test, sildenafil significantly reduced blood-glucose and pyruvate levels and the 3OHB/AA ratio whereas there was no significant effect on lactate levels, the lactate/pyruvate ratio, and 3OHB, AA and FFA levels (Table 2).

3.4. Safety analysis

A significant decrease in systolic (SBP) and diastolic (DPB) blood pressure values before and after the treadmill walking test, and prior to leaving the hospital was found with sildenafil compared to placebo ($p = 0.01$ and $p = 0.02$) (Table 2).

Symptomatic hypotension (vertigo, faintness) as a known side effect was documented in two patients from the sildenafil group after the treadmill test whereas no side effects were documented in the placebo group. This condition was transient and was resolved by the time the patient was discharged from the hospital.

Lastly, no color vision deficiency was detected by the Ishihara test conducted at both visits and no other side effects were reported.

4. Discussion

The ARTERIOFIL study has shown that a single 100 mg oral dose of sildenafil taken 2 h before the treadmill test was effective in increasing MWT compared to placebo in PAD patients with claudication. This result contrasts with a previous negative double blind study that found no difference between sildenafil and placebo in terms of MWT[18]. There are several differences between our study and the *Roseguini* et al. study that possibly account for these conflicting results [18]. Firstly, patient severity in our study was dissimilar to that of the *Roseguini* et al. study, since mean MWT was 300 s vs 701 s and 401 s vs 716 s in the placebo and sildenafil groups respectively [18]. In our study MWT at inclusion

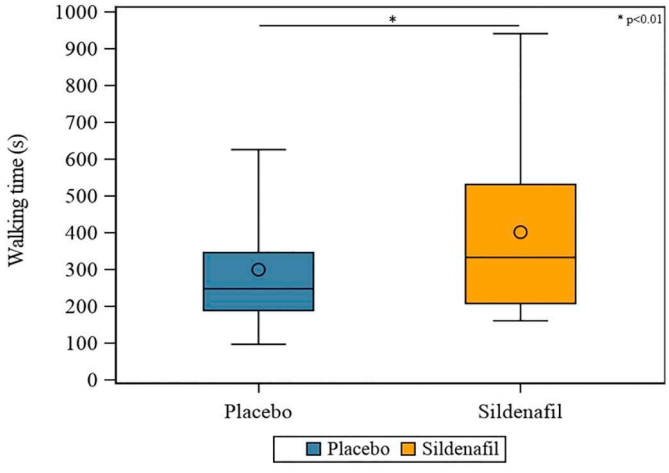

Fig. 2. Acute effect of sildenafil vs placebo on maximum walking time (s).

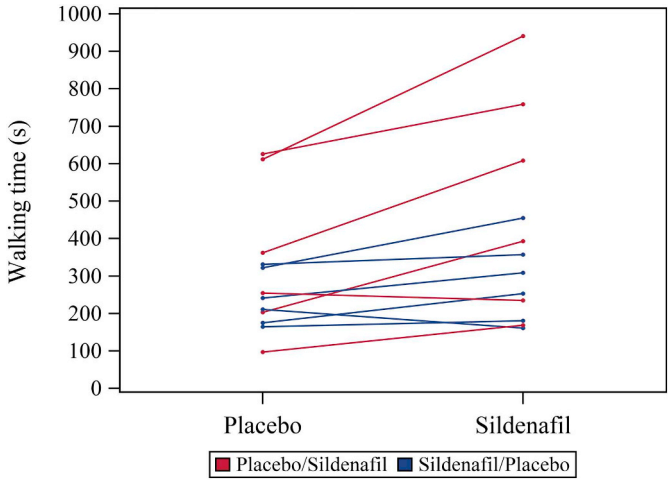

Fig. 3. Maximum walking time (s) in relation to treatment according to sequence for each patient.

was 199.8 ± 70.2 s in the population under investigation. In general, the beneficial effects of treatments that improve MWT are greater in severe patients than in less severe patients [14]. Secondly, *Roseguini* et al. employed the Gardner protocol whereas we used a modified Strandness test (10% incline and 3.2 km/h). This meant that for equal walking time, the distance covered on the treadmill was not the same. Thirdly, their patients received two consecutive 50 mg doses of sildenafil with a.

30 min interval between each dose, whereas our patients received a single 100 mg oral dose in the present study. This may have affected the comparison between pharmacokinetic parameters. We cannot exclude the eventuality that a synergistic effect could have occurred in our study due to the combination of OMT that was absent from the *Roseguini* et al.study [18].

Sildenafil has interesting pharmacokinetic properties. Indeed, improvement in MWT is conceivably attributable to improvement in muscle oxygenation and to this drug's analgesic effects during exercise. A daily 100 mg oral intake of sildenafil is absorbed rapidly (peak blood concentration in under an hour) and it has a half-life of approximately 4 to 6 h, allowing the patient to carry out his/her daily activities such as walking and avoiding side effects at night such as hypotension. This in all likelihood enhances quality of life, canceling out a vicious circle of sedentary or even bed-ridden lifestyle and creating a virtuous circle involving an active lifestyle [29]. The decision to opt for a 100 mg dose is justified in this context by the fact that it is the maximum recommended dosage for treatment of erectile dysfunction [30]. Furthermore, this dosage is frequently prescribed by urologists and sexologists and no major adverse effects have been reported [31]. Finally, an attempt at therapeutic de-escalation involving a dose of 50 mg led to an increase in claudication in a patient previously prescribed 100 mg of sildenafil [19].

4.1. Effects of sildenafil on pain induced by exercise ischemia

In the present study, there was no significant increase in PFWT whereas a significant increase in MWT was observed. The absence of significance is not particularly surprising since in a meta-regression analysis, *Nicolaï* et al.[32] claim that the coefficient of variation for PFWT is estimated as higher than for MWT (20% vs 12%) implying that MWT should be prioritized especially in studies where the number of participants is low [32]. Potential mechanisms that could account for the positive acute effects of sildenafil beyond its vasodilator effect are as follows: sildenafil was believed to produce a direct anti-nociceptive effect likely to entail positive repercussions for claudication in humans via the L-arginine/nitric oxide/cyclic guanosine monophosphate pathway [19]. *Alves* et al. have shown that the L-arginine/NO/cGMP pathway acts as an endogenous modulator of peripheral inflammatory hyperalgesia [21]. Furthermore, *Lee* et al. have demonstrated that sildenafil acts as effective treatment for acute spinal pain in pain-induced models [33]. Additionally, the subtypes A1, A2A, A2B, and A3 of spinal adenosine receptors may play a role in sildenafil-induced anti-nociception [33]. *Rocha* et al. have shown that tadalafil, another PDE5i, dose-dependently inhibits hyperalgesia in zymosan-induced arthritis models [34]. Therapeutic oral administration of tadalafil was shown to have provided analgesia mediated by guanylyl cyclase and was not implicated in the release of endogenous opioids [34]. This effect of tadalafil was associated with an anti-inflammatory effect entailing a decrease in neutrophil influx and TNF-alpha release in inflamed joints [34]. Finally, daily administration of low-dose sildenafil is an easy, well-tolerated, and effective treatment for interstitial cystitis in women [35] and diminishes pain in patients with irritable bowel syndrome [36].

4.2. Effects of sildenafil on muscle oxygenation

An increase in skeletal muscle deoxygenation during exercise has been found in patients with claudication compared with healthy controls, accounting for the hemodynamic limitations associated with this condition [18]. In the present study, transcutaneous oxygenation assessed by exercise-TcPO2 and quantified by DROP showed no sildenafil-related improvement compared to placebo. This outcome contrasts with a previous study that used near-infrared spectroscopy (NIRS) [18]. NIRS and exercise-TcPO2 have long been considered as useful tools for distinguishing exertional limb symptoms of vascular origin from other causes [24,27,37,38]. Nevertheless, the depth of exploration of NIRS is approximately 1.5 cm whereas TcPO2 explores skin microcirculation alone [39]. This could account for the difference between the two studies [18]. Furthermore, despite no evidence of exercise-TcpO2 improvement, we found that a single oral dose of sildenafil resulted in a significant increase in blood glucose and pyruvate concentrations, and in the 3OHB/AA ratio. This reduced glucose and pyruvate consumption may be due to an improved rate of mitochondrial oxidation in the skeletal muscle, thus providing a better yield of ATP production. Reduction in the 3OHB/AA ratio may also corroborate this relative improvement in mitochondrial redox equilibrium. However, under such conditions of increased aerobic glycolysis, sildenafil should diminish post-exercise lactate production. Absence of significant reduction in lactate production is likely due to the wide inter-individual variations of blood lactate concentrations observed in a relatively small sample of individuals [40].

4.3. Safety and tolerance of sildenafil

Before the treadmill test, the hypotensive effect of sildenafil was recorded using a Mobilograph® during the two hours following oral intake in patients who were asymptomatic. In the present study, 83.3% of patients were hypertensive. The extent of blood pressure reduction induced by sildenafil observed herein is similar to that reported in previous studies in healthy controls, hypertensive patients, and patients with cardiac disease subsequent to acute sildenafil administration [41–43]. After the treadmill test, two women sustained symptomatic but transient hypotension. *Roseguini* et al. have shown that the hypotensive effect of sildenafil is noticeable during recovery from exercise [18]. It is well documented that the blood pressure of PAD patients increases continuously and significantly when walking to the maximum distance [44]. Blood pressure has been shown to be an independent risk factor for major adverse cardiovascular events and for all-cause mortality [45]. Finally, previous studies have reported the use of sildenafil in patients with hypertension taking several antihypertensive agents, in whom there were commonly small additive decreases in blood pressure without a significant increase in adverse events [46].

Table 2
Effect of Sildenafil on secondary endpoints.

Variable	Placebo	Sildenafil	p[a]
Maximum walking time (s)	300.0 [95% CI 172 s–428 s]	402.0 [95% CI 274 s–529 s]	0.01
Secondary endpoints			
Pain-Free walking time (s) [median (Q1;Q3)]	136.0 [101.5; 197.0]	133.5 [96.0; 220.0]	0.94
DROPmin (mmHg) [median (Q1;Q3)]			
Right buttock	−21.5 [−29.5; −12.0]	−13.5 [−27.5; −8.5]	0.12
Left buttock	−22.0 [−28.5; −15.5]	−14.0 [−25.5; −9.5]	0.59
Right calf	−25.0 [−37.0; −12.5]	−19.0 [−32.0; −15.5]	0.31
Left calf	−23.5 [−38.0; −13.0]	−26.5 [−32.0; −18.0]	0.61
Redox cycle parameters[b] [median (Q1;Q3)]			
Blood-Glucose (mmol/l)			0.02
T1	5.5 [5.2; 6.0]	5.8 [5.3; 6.6]	
T2	7.7 [6.2; 9.6]	6.7 [6.3; 8.2]	
T3	5.8 [5.4; 6.3]	6.0 [5.6; 6.8]	
T4	5.3 [5.0; 6.0]	5.8 [5.6; 6.5]	
T5	5.6 [5.2; 5.9]	5.9 [5.6; 6.4]	
Lactate (mmol/l)			0.60
T1	1.2 [1.1; 1.5]	1.3 [1.0; 1.4]	
T2	1.6 [1.4; 2.0]	1.7 [1.4; 2.1]	
T3	3.4 [2.2; 4.6]	4.0 [2.4; 5.0]	
T4	3.5 [2.9; 4.7]	4.2 [2.4; 4.4]	
T5	2.4 [1.5; 3.6]	3.3 [1.7; 4.0]	
Pyruvate (mmol/l)			0.03
T1	83.0 [70.5; 117.5]	103.0 [84.0; 115.0]	
T2	111.5 [96.5; 135.0]	129.0 [96.5; 164.5]	
T3	112.0 [99.5; 179.5]	135.0 [119.0; 165.5]	
T4	141.0 [119.0; 220.0]	184.0 [148.0; 237.0]	
T5	124.0 [105.0; 202.0]	180.5 [131.5; 217.0]	
Lactate/Pyruvate			0.68
T1	1.3 [1.1; 1.5]	1.3 [1.1; 1.6]	
T2	1.4 [1.3; 1.6]	1.3 [1.2; 1.5]	
T3	2.6 [2.0; 3.4]	2.7 [2.0; 3.8]	
T4	2.1 [2.0; 2.9]	2.0 [1.6; 2.5]	
T5	1.8 [1.6; 2.2]	1.9 [1.4; 2.3]	
3-Hydroxybutyrate (mmol/l)			0.26
T1	58.0 [48.0; 106.0]	55.5 [37.5; 87.5]	
T2	40.0 [25.5; 51.0]	38.0 [25.0; 57.0]	
T3	39.0 [25.0; 65.0]	27.0 [25.0; 58.0]	
T4	30.0 [25.0; 105.0]	39.0 [25.5; 53.5]	
T5	52.0 [27.0; 109.0]	46.0 [34.0; 53.0]	
Acetoacetate (mmol/l)			0.51
T1	123.5 [99.0; 138.0]	121.5 [98.0; 153.5]	
T2	111.0 [99.5; 135.5]	129.0 [99.0; 185.0]	
T3	138.5 [101.5; 182.0]	125.0 [96.0; 168.0]	
T4	142.0 [121.0; 225.0]	154.0 [125.0; 223.0]	
T5	146.0 [111.0; 234.0]	163.5 [115.0; 211.0]	
3-Hydroxybutyrate/Acetoacetate			0.02
T1	59.6 [46.3; 105.2]	50.9 [27.2; 63.8]	
T2	30.1 [25.2; 49.0]	24.8 [19.4; 45.7]	
T3	27.8 [18.4; 54.6]	27.5 [20.0; 48.0]	
T4	25.0 [20.0; 44.6]	22.1 [16.7; 35.5]	
T5	37.2 [22.1; 60.4]	29.5 [21.3; 36.7]	
Free fatty acid (mmol/l)			0.43
T1	543.5 [427.5; 685.5]	547.0 [467.5; 632.0]	
T2	136.5 [107.5; 215.5]	181.5 [115.0; 251.0]	
T3	220.0 [137.5; 296.0]	218.0 [151.5; 269.0]	
T4	319.0 [196.0; 424.0]	339.0 [248.0; 371.0]	
T5	366.0 [238.0; 448.0]	345.5 [274.0; 364.5]	
Blood pressure (mmHg)[2] [median (Q1;Q3)]			
Systolic blood-Pressure			0.01
Before treadmill exercise test	140.0 [130.0; 165.0]	130.0 [110.0; 135.0]	
After treadmill exercise test	121.5 [105.0; 132.0]	109.0 [98.0; 119.5]	
Before leaving hospital	125.5 [109.0; 130.0]	115.0 [108.5; 133.5]	
Diastolic blood-Pressure			0.02
Before treadmill exercise test	80.0 [80.0; 80.0]	80.0 [70.0; 80.0]	
After treadmill exercise test	68.0 [57.5; 69.0]	58.0 [55.5; 69.5]	
Before leaving hospital	62.0 [55.5; 67.5]	70.0 [63.0; 75.0]	

DROP: Delta from Resting Oxygen Pressure;
Before the treadmill test: (T1: Fasting; T2: after taking sildenafil or placebo); After the treadmill test: (T3: immediately; T4: 5 min; T5: 15 min).
[a] Mixed models were used. In all types of analysis, period and sequence fixed effects were not significant.
[b] In analysis, sampling time was added as a fixed effect.

5. Study limitations

This study focused solely on the acute effect of sildenafil on MWT. This is a pilot study that paves the way for a larger prospective trial to study long-term effects. Furthermore, this study was conducted on patients with severe claudication whose mean MWT was < 300 s. The efficacy on PAD patients with lesser functional impairment remains to be studied.

Twelve patients were analyzed in mITT on the 14 initially planned patients using a unilateral *t*-test. However, the observed variability is smaller in the current study as compared with the expected variability for the sample size calculation (observed in the current study: ± 1.8 min vs expected ± 2.8 min). This smaller observed difference between placebo and sildenafil on the MWT (Primary Efficacy Endpoint) (expected: +2.5 min; observed: +1.7 min), the significant difference obtained in our study corresponds to a power of 90% using the one-sided paired *t*-test and 81% using the two-sided paired *t*-test.

6. Conclusion

The ARTERIOFIL study has demonstrated that a single 100 mg oral dose of sildenafil had a significant effect on increased MWT but had no significant effects on PFWT and oxygenation parameters. One of the mechanisms likely to account for this positive effect would be a reduction in pain intensity allowing the patient to walk for longer. Given the vicious circle of a sedentary lifestyle on cardiovascular morbidity and mortality and the correlation between maximum walking distance and cardiovascular/all-cause mortality as demonstrated by meta-analysis [47], sildenafil is in all likelihood an effective and safe adjuvant therapy in conjunction with PAD optimal medical treatment, and this combination could represent a new paradigm in the management of patients with claudication. The current results obtained in this pilot study should be confirmed in a larger population. In this aim, a double-blind, prospective, randomized, multicenter study (VIRTUOSE©) is ongoing to evaluate the chronic effect of six month-long sildenafil treatment on MWT in PAD patients with claudication.

7. Clinical perspectives

Compared with placebo, a single oral dose of Sildenafil had a significant effect on increased Maximum Walking Time in patients with arterial claudication without significant adverse effects. Further research is needed to assess the long-term efficacy and safety of Sildenafil in patients with arterial claudication and to evaluate it potential therapeutic utility on Maximum Walking Time and on Major Adverse Cardiovascular Events.

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Disclosures

All authors declare they have nothing to disclose.

Ethics approval and participant consent

All the procedures in this report have been approved by the ethics committee, and performed according to the guidelines of the Helsinki Declaration of 2008.

Permission information

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