Walking performance, oxygen uptake kinetics and resting muscle pyruvate dehydrogenase complex activity in peripheral arterial disease

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ABSTRACT

In the present study, we tested the hypothesis that walking intolerance in intermittent claudication (IC) is related to both slowed whole body oxygen uptake ($\dot{V}O_2$) kinetics and altered activity of the active fraction of the pyruvate dehydrogenase complex (PDCa) in skeletal muscle. Ten patients with IC and peripheral arterial disease [ankle/brachial index (ABI) $= 0.73 \pm 0.13$] and eight healthy controls (ABI $= 1.17 \pm 0.13$) completed three maximal walking tests. From these tests, averaged estimates of walking time, peak $\dot{V}O_2$ and the time constant of $\dot{V}O_2$ ($\tau$) during submaximal walking were obtained. A muscle sample was taken from the gastrocnemius medialis muscle at rest and analysed for PDCa and several other biochemical variables. Walking time and peak $\dot{V}O_2$ were approx. 50% lower in patients with IC than controls, and $\tau$ was 2-fold higher ($P < 0.05$). $\tau$ was significantly correlated with walking time ($r = -0.72$) and peak $\dot{V}O_2$ ($r = -0.66$) in patients with IC, but not in controls. PDCa was not significantly lower in patients with IC than controls; however, PDCa tended to be correlated with $\tau$ ($r = -0.56, P = 0.09$) in patients with IC, but not in controls ($r = -0.14$). A similar correlation was observed between resting ABI and $\tau$ ($r = -0.63, P = 0.05$) in patients with IC. These data suggest that the impaired $\dot{V}O_2$ kinetics contributes to walking intolerance in IC and that, within a group of patients with IC, differences in $\dot{V}O_2$ kinetics might be partly linked to differences in muscle carbohydrate oxidation.

INTRODUCTION

Walking intolerance is a major problem faced by people who suffer with peripheral arterial disease (PAD) and its common symptom intermittent claudication (IC). On average, walking performance and peak oxygen uptake ($\dot{V}O_2$) are less than 50% of those values observed in healthy controls [1], and the maximal exercise duration during treadmill testing is usually restricted to approx. 2–6 min, depending upon the protocol used [2]. Within a group of patients with IC there is usually a large range of performances, with up to a 6-fold difference between the lowest and highest values [1,3].

Although the restriction of blood flow to active muscles is the primary cause of walking intolerance in IC, various measurements of ‘haemodynamic limitations’ in the ischaemic limbs often fail to correlate with walking performance [4]. In contrast, several studies [5–7] have reported significant correlations between biochemical measurements in ischaemic calf muscle and walking...
performance in patients with IC [5]. Brass and Hiatt [5] suggested that a metabolic myopathy exists in IC and that this contributes to exercise intolerance. This idea was partly founded upon their own findings of (i) abnormally high levels of short-chain acylcarnitine content found in the calf muscle of the ischaemic limb in patients with unilateral PAD [6], (ii) an inverse correlation between these acylcarnitine levels and walking performance [6], as well as (iii) a significant correlation between the decline in resting muscle short-chain acylcarnitine levels and the improvement in walking performance induced by exercise training [7]. They did not, however, offer an explanation as to how these biochemical changes could alter muscle and exercise performance.

Recently, we [3] isolated mitochondria from the calf muscles of patients with IC and showed that the rates of mitochondrial oxidation of carbohydrate, but not fatty acid, were positively correlated with walking performance and were elevated in the trained state. Increasing muscle glycogen levels through carbohydrate supplementation also improved exercise performance in a limited study of patients with IC [8]. A possible link between these data and those reported by Hiatt and co-workers [5–7] is the active fraction of the pyruvate dehydrogenase complex (PDCa) [3]. In resting skeletal muscle, an increase in the activity of PDCa is associated with an increase in muscle glycogen and a concomitant decrease in acetyl-CoA [9], an inhibitor of PDCa. Since acetyl-CoA is in equilibrium with acetylcarnitine, a major short-chain acylcarnitine, variations in muscle acetylcarnitine and glycogen in the ischaemic muscles of patients with IC might lead to alterations in PDCa activity. Therefore one aim of the present study was to compare the levels of acetylcarnitine, glycogen and PDCa in the calf muscles of patients with IC and healthy controls.

We also aimed to explore the functional relevance of differences in these aspects of muscle biochemistry, with a particular focus on the link between the levels of PDCa in resting muscle, the kinetic response of VO2 during walking and walking performance. During exercise that can only be sustained for several minutes, the rate at which VO2 increases towards its asymptotic value is a major determinant of performance [10]. This kinetic response of VO2 is impaired in IC [11], although the degree to which it relates to walking performance is not known. There is evidence in a human model of limb ischaemia that anaerobic ATP synthesis in exercised muscle is reduced by raising the resting level of PDCa through the administration of dichloroacetate [12]. Assuming that this reduction in anaerobic ATP synthesis reflected an increase in VO2 kinetics [13], these data suggest that differences in the levels of PDCa in resting skeletal muscle might affect VO2 kinetics and performance in patients with IC. Therefore the second main aim of the present study was to explore the inter-relationships between PDCa activity in resting skeletal muscle, VO2 kinetics and walking performance in IC.

**METHODS**

**Subjects**

Eight elderly healthy controls (five males and three females; age, 66 ± 7.5 years) and ten patients who displayed IC (seven males and three females; age 62 ± 11 years) participated in this study. Body weight was similar (P > 0.05) between controls (73.1 ± 13.4 kg) and patients with IC (82.5 ± 19.8 kg) and remained stable over the testing period. PAD was diagnosed based on clinical history, duplex scanning results and a resting ankle/brachial index (ABI) < 0.9. Exclusion criteria for PAD patients included diabetes mellitus, ischaemic pain at rest, hospitalization during the 6 months prior to the study or where exercise performance was limited by any symptom other than IC. All patients in the present study reported calf muscle pain as the limiting symptom. Healthy control subjects were recruited from the local community and their exercise performance was usually limited by ‘general fatigue’. All subjects were maintained on prescribed medications and no subject had received an experimental drug immediately prior to or during the study. One patient with IC had been routinely taking a β-blocker (atenolol), but exclusion of this subject had no effect on the outcomes of data analyses and the subject was retained in the sample. The study was conducted in accordance with the National Health and Medical Research Council guidelines and was approved by the Research Ethics Committees of the participating institutions. All subjects gave their written informed consent prior to participation.

**Experimental overview**

Following an initial screening examination, which included an ECG treadmill stress test, all subjects reported to the laboratory once a week for 6 weeks. Subjects performed a maximal incremental treadmill test once a week for the first 5 weeks and in week 6 a sample of the medial gastrocnemius muscle was taken using a percutaneous biopsy needle. Prior to any test, subjects were weighed and then rested in a supine position for 20 min before systolic blood pressures were measured in the arm and at each ankle. Subjects then stood quietly on the treadmill for 4 min and respiratory gases and heart rate were collected over the last 2 min of this resting period. All subjects then performed a maximal treadmill test. Upon termination of the treadmill test, subjects were placed in a supine position and recovery blood pressures at the arm and both ankles were monitored. The first two sessions were used to familiarize the subjects with these procedures. Data collected only during subsequent sessions were used in our analyses.
Walking performance
Maximal walking time was assessed using a maximal graded treadmill test that was a modification of that described previously [7]. This test was performed on a wide-bodied motorized treadmill (Payne, NSW, Australia) operating at constant speed of 3.2 km·h⁻¹. The first stage of the test was performed at a zero gradient for 5 min, and then the gradient was increased by 3.5 % every 3 min. Subjects were instructed not to hold the handrails during the test and to walk as long as possible. Subjects were given verbal encouragement throughout the test and the test was terminated at their request. The total time spent walking was recorded and was equal to maximal walking time.

Pulmonary gas exchange
During all walking tests, pulmonary gas exchange variables [i.e. \( \dot{V}_\text{O}_2 \) and \( \dot{V}_\text{CO}_2 \) production (\( \dot{V}_\text{CO}_2 \)) were measured breath-by-breath using an automated gas analysis system (CPX; Medical Graphics, St Paul, MN, U.S.A.). Prior to each test, the machine was manually calibrated using gases of known concentrations prepared to the highest (i.e. alpha) standard. The pneumotachometer was calibrated manually using a 3 litre syringe with flow rates that simulated the range expected during exercise. Peak data were derived from the average of the peaks attained during each test. Other pulmonary data were averaged over consecutive 20 s intervals (except at peaks attained during each test). The respiratory exchange ratio (RER) was calculated as the ratio of \( \dot{V}_\text{CO}_2 \) to \( \dot{V}_\text{O}_2 \).

\( \dot{V}_\text{O}_2 \) kinetics
The kinetic response of \( \dot{V}_\text{O}_2 \) was measured during the first stage (5 min) of the three graded walking tests (i.e. tests three to five). \( \dot{V}_\text{O}_2 \) was measured breath-by-breath and interpolated to 1 s intervals. Data from the three tests were then time-aligned to the start of walking and averaged to produce a single response. Phase II (fast component) kinetics were then assessed using the equation:

\[
\dot{V}_\text{O}_2(t) = \dot{V}_\text{O}_2(a) + b[1 - e^{-(t-TD)/\tau}]
\]

where \( \dot{V}_\text{O}_2(t) \) is \( \dot{V}_\text{O}_2 \) at time \( t \), \( \dot{V}_\text{O}_2(a) \) is the resting baseline \( \dot{V}_\text{O}_2 \), \( b \) is the amplitude of the \( \dot{V}_\text{O}_2 \) response from rest to steady state, TD is the time delay from the onset of exercise to the initiation of phase II, and \( \tau \) is the time constant. We excluded phase I of the \( \dot{V}_\text{O}_2 \) response, which does not represent muscle \( \text{O}_2 \) consumption, from our model using two approaches. For approach 1, we assumed that phase I lasted 15 s, and this was based on the average duration of phase I for young subjects reported in the literature that was determined through curve fitting [14]. It is also similar to the phase I duration (i.e. 20 s) that other investigators have assumed and applied to the study of \( \dot{V}_\text{O}_2 \) kinetics in young and old subjects [15]. For approach 2, two observers independently estimated the time point corresponding to the end of phase I by visual inspection of the plotted data. Both observers were familiar with the conceptual representation of phase I/phase II as illustrated previously [14], were blinded to which individual the raw data belonged to and had data plots presented to them in a random order. In over 80 % of cases, the difference between the two observers was less than 10 s and the difference in average values for both groups was less than 2 s. The average of the two observations for each subject was recorded and all data prior to this point were discarded. For both approaches the equation was fitted, 95 % prediction intervals were plotted and any outlying data were excluded. This fitting and exclusion process was repeated (average of six iterations) until all data were within a 95 % prediction interval. Curve fitting and parameter estimation was made using weighted least-squares non-linear regression techniques and TableCurve software.

Systolic pressures and ABI
During the familiarization phase, brachial systolic pressure was determined three times in both arms using a fully automated measuring device (Criticon, Sydney, Australia). The arm with the highest average pressure was used on all subsequent visits. Systolic arterial pressures within the dorsalis pedis and posterior tibial arteries at the ankles of both legs were measured three times each at rest using a continuous wave Doppler probe and a manual sphygmomanometer. In each leg, the artery with the highest mean resting systolic pressure was used for all subsequent visits. ABI was calculated as the ratio of the ankle systolic pressure to the brachial systolic pressure. At rest, all sites were measured three times and the average value was used in calculations. Post-exercise systolic pressures in the arm and both ankles were obtained 2, 4 and 6 min after finishing the treadmill test. In all patients there was agreement between the leg with the lowest ABI and the most severe pain experienced during walking, and so it was termed the ‘low ABI leg’.

Muscle biochemistry
All subjects were asked to refrain from eating and exercising for the 2 h prior to their appointment. After 20 min of rest, a muscle biopsy was taken from the medial gastrocnemius of the low ABI leg in PAD patients and a leg selected at random in the controls. A small incision was made under local anaesthetic (1 % xylocaine) through which a muscle sample was taken using a biopsy needle [16]. The sample was pulled from the needle and immediately frozen in liquid nitrogen. Time from sampling to freezing was less than 10 s.
A total of 10–30 mg was chipped from each muscle sample under liquid nitrogen and analysed for the activity of PDCa [9,17]. The remaining muscle was freeze-dried, powdered and dissected free of blood and connective tissue. For the determination of glycogen, 2 mg of the powdered muscle was extracted in 2 M HCl for 2 h at 95 °C [18]. Glucosyl units were determined fluorimetrically following enzymic coupling to NADPH production [19]. The remaining muscle powder was extracted in a solution of 0.5 M perchloric acid containing 1 mM EDTA and neutralized to pH 7 with 2.2 M KHCO3. Acetylcarnitine was measured using a 14C radiolabelling technique [20]. Phosphocreatine and creatine were analysed fluorimetrically following enzymic coupling to NADH production or consumption [21]. Protein content was determined spectrophotometrically, according to the method of Lowry et al. [22], in the homogenates used for the determination of PDCa after they had been weighed and incubated for 18 h in a 0.1 M solution under liquid nitrogen and analysed for the activity of PDCa [9,17]. The remaining muscle was divided by the mean of an individual’s data from weeks 3, 4 and 5 only. Differences in single variables between the two groups were analysed using Student’s t test. Relationships between variables were assessed using Pearson’s product-moment correlations. Results were expressed as means ± S.D. and considered significant at \( P \leq 0.05 \).

**Data treatment and statistics**

Of the five treadmill tests performed, the first two were regarded as familiarization tests and thus the data were not used. For all variables measured during the final three treadmill tests there was no significant main effect of time for controls or patients with IC (between-within ANOVA), and so each subject’s average response across the 3 weeks was used in all statistical analyses. The individual coefficient of variation was defined as the S.D. divided by the mean of an individual’s data from weeks 3, 4 and 5 only. Differences in single variables between the two groups were analysed using Student’s t test. Relationships between variables were assessed using Pearson’s product-moment correlations. Results were expressed as means ± S.D. and considered significant at \( P \leq 0.05 \).

**RESULTS**

**Maximal walking time and peak variables**

During the walking tests all patients with PAD developed claudication pain in the calf muscle and all cited this as the sole reason for stopping the test. Control subjects nominated a variety of reasons for stopping the test, with the most common symptom reported being general fatigue or shortness of breath; no subject cited localized leg pain as a limiting symptom. Over the three tests there was no significant change in maximal walking time for either group and the coefficients of variation for controls and patients with IC were 3.0 ± 3.3 and 6.0 ± 3.3 % respectively. Peak \( \dot{V}_O_2 \) was also stable over this same period and the coefficients of variation for controls and patients with IC were 6.9 ± 2.9 and 4.1 ± 3.0 %. Peak responses are shown in Table 1. Maximal walking time, peak \( \dot{V}_O_2 \) and peak heart rate were significantly lower in patients with IC.

**End-stage I responses**

Pulmonary gas-exchange variables and heart rate during the final minute of the first stage of the walking test were not different between the groups (Table 2).

**\( \dot{V}_O_2 \) kinetics**

Parameter estimates using approach 1 and 2 are shown in Table 3. The estimated duration of phase I using approach 2 was 40 ± 5 s for controls and 44 ± 15 s for patients with IC. Figure 1 illustrates the \( \dot{V}_O_2 \) response to the first stage of walking in a control and a patient with IC who best represented the average response observed in their corresponding groups. These data illustrate the significant slowing of the \( \dot{V}_O_2 \) response (i.e. higher \( \tau \)) in a patient with IC compared with a control.

**Systolic pressures and ABI**

Resting brachial and ankle systolic pressures and ABI remained stable over the course of the study in controls and patients with IC and coefficients of variation were, on average, between 4 and 8 %. Compared with controls, patients with IC had significantly higher resting arm systolic pressures (136 ± 12 compared with 124 ± 6 mmHg) and lower leg systolic pressures (98 ± 20 compared with 145 ± 17 mmHg) and ABIs (Table 4).
Table 3  Kinetic parameters for \( \dot{V}O_2 \) during stage 1 in controls (\( n = 8 \)) and patients with IC (\( n = 10 \)) determined using approaches 1 and 2

Baseline \( \dot{V}O_2 \), parameter \( a \) in the Methods section; amplitude, parameter \( b \) in the Methods section. *\( P < 0.05 \) compared with control value for the same curve-fitting approach; †\( P < 0.05 \) compared with approach 1 within a given group.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients with IC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Approach 1</td>
<td>Approach 2</td>
</tr>
<tr>
<td>Baseline ( \dot{V}O_2 ) (ml · min(^{-1}))</td>
<td>240 ± 26</td>
<td>241 ± 41</td>
</tr>
<tr>
<td>( \tau ) (s)</td>
<td>26.7 ± 7.6</td>
<td>18.2 ± 6.8†</td>
</tr>
<tr>
<td>Amplitude (ml · min(^{-1}))</td>
<td>561 ± 118</td>
<td>548 ± 149</td>
</tr>
<tr>
<td>Final ( \dot{V}O_2 ) (ml · min(^{-1}))</td>
<td>800 ± 134</td>
<td>790 ± 133</td>
</tr>
</tbody>
</table>

Table 4  ABI in low ABI leg at rest and following exercise in controls and patients with IC

See the Methods section for the definition of low ABI leg. *\( P < 0.05 \) compared with control. All values obtained during exercise are significantly different from those at rest in both groups.

<table>
<thead>
<tr>
<th>ABI</th>
<th>Controls</th>
<th>Patients with IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>1.17 ± 0.13</td>
<td>0.73 ± 0.14*</td>
</tr>
<tr>
<td>Post-exercise (min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.90 ± 0.16</td>
<td>0.29 ± 0.20*</td>
</tr>
<tr>
<td>4</td>
<td>0.94 ± 0.14</td>
<td>0.34 ± 0.19*</td>
</tr>
<tr>
<td>6</td>
<td>0.99 ± 0.14</td>
<td>0.39 ± 0.14*</td>
</tr>
</tbody>
</table>

Table 5 Resting muscle biochemical variables in controls and patients with IC

PDCA activity is expressed in mmol of acetyl-CoA · min\(^{-1}\) · kg of wet weight\(^{-1}\). Other variables are expressed in mmol · kg of dry weight\(^{-1}\), as well as in mmol · kg of wet weight\(^{-1}\) in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Patients with IC</th>
</tr>
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<tbody>
<tr>
<td>PDCA</td>
<td>0.53 ± 0.27</td>
<td>0.40 ± 0.17</td>
</tr>
<tr>
<td>Glycogen</td>
<td>330 ± 156 (80 ± 39)</td>
<td>325 ± 93 (82 ± 26)</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>91.9 ± 16.4 (21.9 ± 3.9)</td>
<td>81.2 ± 13.5 (20.4 ± 3.5)</td>
</tr>
<tr>
<td>Total creatine</td>
<td>138.1 ± 20.2 (33.0 ± 4.9)</td>
<td>126.9 ± 16.8 (32.1 ± 4.5)</td>
</tr>
<tr>
<td>Acetylcarnitine</td>
<td>1.49 ± 0.04 (0.35 ± 0.20)</td>
<td>1.68 ± 1.27 (0.42 ± 0.31)</td>
</tr>
</tbody>
</table>

Figure 1  \( \dot{V}O_2 \) responses to walking at 3.2 km · h\(^{-1}\) (zero grade) in a healthy control subject (A) and a patient with IC (B)

Post-exercise ABI was significantly decreased from that at rest in both groups at 2, 4 and 6 min and was significantly lower in patients with IC compared with controls at all time points (Table 4). In controls, the post-exercise drop in ABI was due to a significant rise in arm systolic pressure only, whereas in patients with IC, it was due to both a rise in arm pressure and a decline in leg pressure.

Muscle biochemistry

Muscle PDCA and glycogen, acetylcarnitine, phosphocreatine and total creatine concentrations were not significantly different between controls and patients with IC (Table 5), although PDCA was 25% lower in patients with IC. Data on muscle non-collagenous protein (NCP) content were not available for all subjects; however, based on the samples available, there was no difference in NCP content between groups (controls, \( n = 5 \) out of seven, 0.14 ± 0.03 g of NCP · kg\(^{-1}\); patients with IC, \( n = 7 \)
Correlations

Maximal walking time was significantly correlated with \( \tau \) (approach 2) in patients with IC, but not in controls (Figure 2). \( \dot{V}O_2 \) peak was also significantly correlated with \( \tau \) (approach 2) in patients with IC \((r = -0.66, P < 0.05)\), but not in controls \((r = 0.32)\).

Resting ABI was not correlated with maximal walking time or peak \( \dot{V}O_2 \) in either group \((r < 0.33)\), but it significantly correlated with \( \tau \) (approach 2) only in patients with IC (Figure 3). In contrast, in patients with IC, none of the muscle metabolic measurements were significantly correlated with maximal walking time, peak \( \dot{V}O_2 \) or \( \tau \), although the correlation between PDCa and \( \tau \) (approach 2) approached significance (Figure 4). In controls, maximal walking time was significantly correlated with muscle glycogen \((r = 0.71)\) and acetylcarnitine \((r = -0.73)\), and similar correlations were observed for peak \( \dot{V}O_2 \). \( \tau \) was not correlated with any muscle metabolic variable in controls. Despite the large variation in age within both groups, age was not correlated with any exercise or muscle variable.

**DISCUSSION**

The main findings of the present study were: (i) the time constant of the \( \dot{V}O_2 \) response was impaired and significantly correlated with maximal walking time in patients with IC, (ii) PDCa was not significantly lower in patients with IC than controls, and (iii) PDCa was moderately correlated with \( \dot{V}O_2 \) kinetics, but not maximal walking time, in patients with IC. These data suggest that the impairment in \( \dot{V}O_2 \) kinetics during submaximal exercise contributes to exercise intolerance in IC, and that differences in \( \dot{V}O_2 \) kinetics amongst patients with IC might be linked, at least in part, to differences in the activation state of PDC prior to exercise.

The aetiology of walking intolerance in IC is multifactorial, and understanding it is complicated further by the heterogeneity of disease and the very focused limitation to sustaining walking performance (i.e. calf pain). From an energetic perspective only, increasing \( \dot{V}O_2 \) to meet the metabolic needs of the active, and particularly ischaemic, muscles is of primary importance to sustaining walking performance in IC [2]. The 2-fold higher value for \( \tau \) in patients with IC compared with controls (Table 3) is almost identical with that shown previously [11] and, in both studies, is comparable with the 2-fold difference in maximal walking time and peak \( \dot{V}O_2 \) between patients with IC and healthy controls.
these groups. In addition, the present findings show that $\tau$ is negatively correlated with maximal walking time and peak $V_{O2}$ in IC, indicating that individuals with a slower rise in $V_{O2}$ to the onset of exercise display a low exercise tolerance. This is distinct from healthy individuals (i.e. controls) for whom there is no correlation between maximal walking time and $V_{O2}$ kinetics. These data suggest that an impaired kinetic response of $V_{O2}$ is an important feature of walking intolerance in IC.

Theoretically, the slowing of $V_{O2}$ kinetics is caused by a reduction in $O_2$ supply to, and/or $O_2$ consumption by, active skeletal muscle. Since the primary problem in IC is an impaired blood flow to the lower limbs, an impaired $O_2$ flow to active muscles is likely to be the primary explanation for the slowed $V_{O2}$ kinetics in IC. Although this has yet to be tested in IC, it is supported by the findings that reducing the arterial flow into active muscles slows the kinetic response of $V_{O2}$ in healthy individuals [24] and that the $V_{O2}$ response after 1 min of exercise is increased after vascular surgery and an improvement in resting ABI in patients with IC [25]. There is also limited support from the present data in so much as the severity of PAD, as reflected by the ABI, was negatively correlated with the time constant of the $V_{O2}$ response (Figure 3).

In addition to the influence of blood flow on $V_{O2}$ kinetics, the inertia of $O_2$ consumption within active muscles could be important. It has been suggested that a locus of such inertia could be PDCa and that increasing its activation prior to exercise might increase the supply of acetyl-CoA for mitochondrial respiration and accelerate $O_2$ consumption during the early period of exercise [12,13]. In healthy humans, complete activation of PDC at rest through dichloroacetate administration increased glucose oxidation and reduced lactate accumulation and phosphocreatine hydrolysis during submaximal exercise (65 % $V_{O2}$ max) [13]. In a human model of limb ischaemia, complete activation of PDC at rest also reduced phosphocreatine hydrolysis and lactate accumulation during submaximal exercise [12]. Although $O_2$ uptake was not measured in these studies, these data suggested that the $V_{O2}$ response to exercise is accelerated in response to increases in resting muscle PDCa activity [12,13]. These data also raised the possibility that differences in PDCa activity in resting skeletal muscle might contribute to the differences observed in $V_{O2}$ kinetics during submaximal walking between controls and patients with IC and/or differences within the group of patients with IC.

In the present study, PDCa activities in resting muscle of the older healthy controls (0.5 mmol · min$^{-1}$ · kg of body weight$^{-1}$) lay within the range of values observed in the quadriceps muscle of young healthy individuals [9]. PDCa activity was not significantly different between patients with IC and controls and so, unlike ABI (Figure 3), it does not appear to explain the large difference in $V_{O2}$ kinetics observed between these groups. However, it is worth noting that PDCa was 25 % lower in patients with IC (and 32 % lower when expressed relative to NCP content) and that the failure of this difference to be significant ($P = 0.25$) may reflect a type-II error. Moreover, PDCa was negatively correlated ($P = 0.09$) with the $V_{O2}$ time constant in patients with IC (Figure 4), suggesting that variations in PDCa activity might contribute to differences in $V_{O2}$ kinetics amongst patients with IC. This seems to be distinct from the situation in healthy individuals as, in the present study, there was no correlation between PDCa and $V_{O2}$ kinetics (controls in Figure 4). Further work is needed to clarify this potential link between PDCa and $V_{O2}$ kinetics in patients with IC.

The present data support the growing recognition of the importance of assessing $V_{O2}$ kinetics in patient populations. In the present study, we contrasted two approaches to describing the $V_{O2}$ response during submaximal exercise that only differed with respect to how the duration of phase I was determined. This phase represents the delay in deoxygenated blood from active muscle reaching the lungs soon after the onset of exercise [26] and, thus, should be excluded from the determination of a time constant that reflects the kinetics of muscle $V_{O2}$. Phase I duration in young and healthy subjects is approx. 15–20 s, and this value has been applied to the description of $V_{O2}$ kinetics in older people [15]. We adopted this approach (approach 1) and contrasted it with an estimation of the phase I duration through visual inspection (approach 2). Using this latter approach, the average duration of phase I was 40–45 s in both groups and was similar to the corresponding value obtained through curve-fitting in patients with IC and controls [11]. Importantly, when comparing approach 2 (visual inspection) with approach 1, $\tau$ was approx. 40 % lower, it yielded time constants for patients with IC and controls similar to those obtained through curve-fitting [11], and correlations between $\tau$ and other variables were consistently higher. These data suggest that the assumption that phase I lasts for a set period of time can lead to incorrect estimation of the $V_{O2}$ kinetics of phase II in older people, and that for more accurate estimation the duration of phase I should be determined for each individual.

A limitation of our approach to assessing $V_{O2}$ kinetics is the failure to include a second term in the model that describes the 'slow component' (phase III) of $O_2$ uptake. During exercise for 5 min, this slow component of $V_{O2}$ is absent at mild to moderate intensities of exercise and present at higher intensities [14]. The first stage of the graded walking test in the present study was relatively mild for the controls (mean intensity = 41 % peak $V_{O2}$), and the $V_{O2}$ response illustrated in Figure 1(A), which shows no evidence of a slow component, was typical of this group. For the patients with IC, this same stage represented a relatively higher intensity (mean = 61 % peak $V_{O2}$) and, for individuals with a relatively low peak
\( \dot{V}O_2 \) might have been intense enough to induce the slow component of \( \dot{V}O_2 \). Unfortunately, we could not discern and, thus, adequately model a slow component of \( \dot{V}O_2 \), because the breath-by-breath noise was too high relative to the amplitude of the response (compare and contrast Figures 1A and 1B). Failure to account for the presence of a slow component, which has a longer time constant than the phase II response [14], will lead to an overestimate of \( \tau \) as measured in the patients with IC. In this case, the impairment in \( \dot{V}O_2 \) kinetics in IC that we and others [11] have observed will be due, at least in part, to a greater influence of the slow component on the overall \( \dot{V}O_2 \) response in patients with IC compared with controls. This clearly warrants further study, so as to shed light on the mechanisms that lead to an impairment in \( \dot{V}O_2 \) kinetics and exercise performance in IC.

Brass and Hiatt [5] have proposed that a metabolic myopathy exists in IC, partly on the basis that patients with IC have an abnormally high short-chain acylcarnitine content expressed relative to lactate levels [6] and a reduction in its concentration that is related to the improvement in peak \( \dot{V}O_2 \) [7]. Acetyl carnitine is a major short-chain acylcarnitine that is in equilibrium with acetyl-CoA, which inhibits PDCa. In healthy individuals, exercise and dietary-induced decreases in PDCa in resting skeletal muscle are associated with elevated acetylcarnitine and acetyl-CoA and with a decrease in muscle glycogen [9]. Since glycogen levels in the vastus lateralis muscle of individuals with severe PAD are less than 50% of those observed in controls [27], we reasoned that alterations in muscle glycogen in patients with IC might be associated with functionally relevant changes in acetylcarnitine and PDCa. However, in the present study, muscle glycogen and acetylcarnitine levels were not different in patients with IC compared with controls and they were not correlated with each other or PDCa. In addition, none of these variables were correlated with maximal walking time or peak \( \dot{V}O_2 \) in IC. On this basis, it is difficult to postulate a mechanism that underpins the relationships observed between short-chain acylcarnitines and walking performance or peak \( \dot{V}O_2 \) in the studies of Hiatt and co-workers [5]. We took care in resting the patients for a long enough period before the muscle was sampled, because of our concerns that ischaemic muscle work in getting to the laboratory would raise acetylcarnitine [28] and that it would then remain elevated for a prolonged period [29]. If, in this situation, recovery is insufficient, then differences in muscle acetylcarnitine levels between subjects could simply reflect different haemodynamic, rather than metabolic, limitations.

In conclusion, the present study has shown that the kinetic response of \( \dot{V}O_2 \) was correlated with muscle PDCa activity, maximal walking time and peak \( \dot{V}O_2 \) in patients with IC, but not in healthy controls. These data extend previous evidence [3,6,7,11] and suggest that the impaired \( \dot{V}O_2 \) response to submaximal exercise is an important feature of exercise intolerance in IC and that, to some extent, it is linked to alterations in muscle carbohydrate metabolism. However, further study of the metabolic response in calf muscle during exercise is required to clarify this.

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REFERENCES

Exercise, oxygen uptake and pyruvate dehydrogenase complex activity in intermittent claudication


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