

# Venous Oxygen Levels During Aerobic Forearm Exercise: An Index of Impaired Oxidative Metabolism in Mitochondrial Myopathy

Tanja Taivassalo, PhD,<sup>1</sup> Amy Abbott, RN,<sup>1</sup> Phil Wyrick, MS,<sup>1</sup> and Ronald G. Haller, MD<sup>1,2</sup>

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A cardinal feature of impaired skeletal muscle oxidative metabolism in mitochondrial myopathies is a limited ability to increase the extraction of O<sub>2</sub> from blood relative to the increase in O<sub>2</sub> delivery by the circulation during exercise. We investigated whether aerobic forearm exercise would result in an abnormal increase in venous effluent O<sub>2</sub> in patients with impaired skeletal muscle oxidative phosphorylation attributable to mitochondrial disease. We monitored the partial pressure of O<sub>2</sub> (PO<sub>2</sub>) in cubital venous blood at rest, during handgrip exercise, and during recovery in 13 patients with mitochondrial myopathy and exercise intolerance and in 13 healthy control and 11 patient control subjects. Resting and recovery venous effluent PO<sub>2</sub> were similar in all subjects, but during exercise venous PO<sub>2</sub> paradoxically rose in mitochondrial myopathy patients from 27.2 ± 4.0mmHg to 38.2 ± 13.3mmHg, whereas PO<sub>2</sub> fell from 27.2 ± 4.2mmHg to 24.2 ± 2.7mmHg in healthy subjects and from 27.4 ± 9.5mmHg to 22.2 ± 5.2mmHg in patient controls. The range of elevated venous PO<sub>2</sub> during forearm exercise in mitochondrial myopathy patients (32 to 82mmHg) correlated closely with the severity of oxidative impairment as assessed during cycle exercise. We conclude that measurement of venous PO<sub>2</sub> during aerobic forearm exercise provides an easily performed screening test that sensitively detects impaired O<sub>2</sub> use and accurately assesses the severity of oxidative impairment in patients with mitochondrial myopathy and exercise intolerance.

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Mitochondrial disorders due to nuclear and especially mitochondrial DNA mutations are now recognized to be among the most common causes of metabolic myopathy.<sup>1</sup> The clinical phenotype is remarkably variable and includes purely myopathic disorders as well as multisystem disease in which myopathy occurs in association with symptoms of brain, heart, endocrine or other organ involvement.<sup>2</sup> Muscle symptoms also are diverse and include weakness (especially of the extraocular muscles), exercise intolerance, and myoglobinuria. The protean manifestations of mitochondrial disease thus challenge the clinician to consider a mitochondrial cause in a variety of clinical settings.

The capacity to diagnose mitochondrial myopathy using muscle histochemistry, biochemistry, and particularly molecular genetic analysis has advanced rapidly in the past decade. However, relatively few noninvasive diagnostic tests are available to assist in deciding whether such analyses are warranted. In particular, patients who complain of easy fatigability illustrate the

problem facing the clinician. Exertional fatigue is a cardinal feature of impaired oxidative phosphorylation in mitochondrial myopathies, but fatigability is a nonspecific symptom that accompanies a variety of medical and psychological conditions, as well as being a normal consequence of physical deconditioning. It would therefore be desirable to have an easy screening test to determine whether there is evidence of an underlying defect of mitochondrial function, especially when other typical features of mitochondrial disease such as ophthalmoplegia or evidence of maternal inheritance are lacking. Elevated resting blood lactate levels provide indirect evidence of a mitochondrial defect, but preceding exercise or diet can result in false positive results in normal subjects, and false negative results are common with normal blood lactate levels found in more than 50% of patients with proven mitochondrial disorders.<sup>3,4</sup> Monitoring oxidative metabolism during exercise using <sup>31</sup>P-magnetic resonance spectroscopy, near-infrared spectroscopy, or sophisticated cycle ergometry

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From the <sup>1</sup>Neuromuscular Center, Institute for Exercise and Environmental Medicine, Presbyterian Hospital; the <sup>2</sup>University of Texas Southwestern Medical Center; and the VA Medical Center, Dallas, TX.

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Address correspondence to Dr Haller, Neuromuscular Center, Institute for Exercise and Environmental Medicine, Presbyterian Hospital, 7232 Greenville Avenue, Dallas, TX 75231.  
E-mail: ronald.haller@UTSouthwestern.edu

Table 1. Clinical, Molecular, and Physiological Characteristics of Patients with Mitochondrial Myopathies

Patient	Age (yr)/ Sex	Clinical Features	Genetic Defect	% Mutant mtDNA	Reference	Indices of Oxidative Capacity During Cycle Exercise			Symbols in Fig 1
						Peak $\dot{V}O_2$ (ml/kg/min)	Peak a- $\dot{v}O_2$ Diff (ml/dl)	$\Delta Q/\Delta \dot{V}O_2$	
EO	53/F	Pure EI	Cyt b G14846A	>98	15	5.9	2.7	73	▲
FB	46/M	EI, myoglobinuria	Cyt b microdeletion of 8 aa	36	15	11.7	8.7	10	▽
SL	38/M	Pure EI	ND4 G11832A	31	16	11.8	3.8	25	■
RW	35/M	EI, myoglobinuria	COX I G5920A	68	18	16.0	7.5	10	⊕
CW	23/F	EI, myoglobinuria	15-bp deletion in COX III	36	14	22.5	8.6	9	♣
TK	31/F	CPEO, EI	4.3-kb deletion	78	—	14.7	7.3	15	☆
RJ	30/F	CPEO, EI	5-kb deletion	78	—	18.4	6.8	13	⊗
PS	38/F	EI, myalgia	tRNAGlu	67	27	10.2	8.5	8	⊠
WB	55/M	Pure EI	tRNATrp	95	17	10.9	5.0	18	*
MB	40/F	EI, pancreas failure	Unidentified COX deficiency	—	8	9.0	5.1	11	□
JD	36/M	PEO	AD multiple deletions	—	—	24.7	16.3	5	⊕
EK	27/M	EI, myoglobinuria	SDH/aconitase deficiency	—	9	12.2	3.9	30	▼
CS	35/F	EI, myoglobinuria, seizures	CoQ deficiency	—	10	5.9	3.5	36	◆

mtDNA = mitochondrial DNA (% mutant mtDNA was determined from biopsy analysis at the time of cycle exercise testing, as reported by Taivassalo and colleagues<sup>27</sup>);  $\dot{V}O_2$  = oxygen consumption; a- $\dot{v}O_2$  diff = systemic arteriovenous oxygen difference;  $\Delta Q/\Delta \dot{V}O_2$  = level of increase in cardiac output relative to increase in  $O_2$  utilization; EI = exercise intolerance; CPEO = chronic progressive external ophthalmoplegia; Cyt b = cytochrome b; AD = autosomal dominant; COX = cytochrome oxidase; SDH = succinate dehydrogenase.

facilitates diagnosis, but these tests are not widely available.<sup>5</sup>

Physiological investigations indicate that the block in oxidative phosphorylation in mitochondrial myopathies impairs the ability of muscle to increase the rate of extraction of available  $O_2$  from blood during exercise.<sup>6</sup> As a result, the exercise increase in  $O_2$  delivery by the circulation is exaggerated relative to  $O_2$  use<sup>7-10</sup>; muscle  $O_2$  levels during exercise are abnormally high<sup>10-12</sup>; and peak levels of arteriovenous  $O_2$  difference remain low.<sup>7-10,13</sup> On the basis of these results, we attempted to develop a screening test for patients with exercise intolerance attributable to mitochondrial myopathy analogous to the ischemic forearm test used to detect muscle glycolytic defects. We postulated that deficient  $O_2$  use relative to  $O_2$  delivery during forearm exercise would result in abnormally high levels of  $O_2$  in effluent venous blood, and that the level of increase in exercise  $PO_2$  would be proportional to the severity of the underlying defect in oxidative phosphorylation.

### Patients and Methods

The study was approved by the Institutional Review Boards of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas. Each subject was informed of the nature and risks of the study and gave written consent to participate.

### Subjects

Thirteen patients (7 females and 6 males; mean age,  $37.5 \pm 9.4$  years) with heterogeneous clinical, biochemical, and molecular features of mitochondrial myopathy associated with nuclear or mitochondrial DNA defects were studied. Results from 10 of these patients have previously been reported (Table 1).<sup>8-10,14-18</sup> Most of these patients had purely myopathic features marked by exercise intolerance alone or in association with recurrent myoglobinuria or ophthalmoplegia (see Table 1). One patient had multisystem involvement.

Thirteen healthy control subjects (6 females, 7 males; mean age,  $36.4 \pm 9.2$  years) and 11 patient control subjects (5 females, 6 males; mean age,  $34.2 \pm 18.0$  years) were studied for comparison. The patient controls were referred to our clinic for evaluation of fatigability and found not to have a mitochondrial myopathy on the basis of exercise testing, muscle histology, or muscle biochemical testing.

### Aerobic Forearm Exercise Protocol

Maximal voluntary contraction (MVC) using a custom-built dynamometer was assessed as the highest of three brief maximal hand grip efforts. Subjects performed continuous submaximal, aerobic hand grip exercise consisting of 5 seconds of isometric grip force alternating with 5 seconds of rest for 6 to 7 minutes. For the first 3 minutes of exercise, the target grip force was 30% MVC, and for the last 3 minutes of exercise it was 50% of MVC. A similar exercise paradigm has previously been employed to assess low and moderate levels of aerobic exercise as monitored by <sup>31</sup>P-magnetic resonance

Table 2. Mean Venous Blood PO<sub>2</sub>, PCO<sub>2</sub>, Lactate, and pH During Rest, Exercise, and Recovery in Mitochondrial Myopathies, Normal and Patient Controls

Group		PO <sub>2</sub> (mmHg)					PCO <sub>2</sub> (mmHg)					Lactate (mmol/L)					pH				
		Rest	Exercise		Recovery		Rest	Exercise		Recovery		Rest	Exercise		Recovery		Rest	Exercise		Recovery	
			30%	50%	Post -5	Post -10		30%	50%	Post -5	Post -10		30%	50%	Post -5	Post -10		30%	50%	Post -5	Post -10
MM	AV	27.2	38.2	41.9	36.6	33.5	48.7	50.7	53.0	46.8	46.1	3.1	3.8	4.5	4.8	4.0	7.35	7.33	7.30	7.33	7.36
	SD	4.0	13.3 <sub>ab</sub>	16.5 <sub>ab</sub>	9.9	6.9	6.0	7.9 <sub>a</sub>	9.6 <sub>ab</sub>	5.2	4.8 <sub>b</sub>	2.6 <sub>a</sub>	2.4 <sub>ab</sub>	2.5 <sub>ab</sub>	2.4 <sub>ab</sub>	2.3 <sub>ab</sub>	0.03 <sub>a</sub>	0.03	0.03 <sub>b</sub>	0.02 <sub>a</sub>	0.02
NC	AV	27.2	24.2	26.8	37.3	30.5	52.0	57.9	68.2	48.8	48.5	0.9	1.5	2.9	2.8	1.8	7.37	7.34	7.28	7.35	7.37
	SD	4.2	2.7 <sub>b</sub>	2.6	5.6 <sub>b</sub>	5.7 <sub>b</sub>	5.1	6.5 <sub>b</sub>	9.0 <sub>b</sub>	4.8	5.7 <sub>b</sub>	0.2	0.5 <sub>b</sub>	1.1 <sub>b</sub>	1.0 <sub>b</sub>	0.6 <sub>b</sub>	0.02	0.02 <sub>b</sub>	0.02 <sub>b</sub>	0.02 <sub>b</sub>	0.02
PC	AV	27.4	22.2	23.1	37.8	31.5	49.1	56.4	61.2	47.2	47.6	1.0	1.3	2.0	2.1	1.7	7.39	7.35	7.32	7.37	7.38
	SD	9.5	5.2 <sub>b</sub>	4.9	13.0 <sub>b</sub>	8.7 <sub>b</sub>	7.2	7.2 <sub>b</sub>	9.6 <sub>b</sub>	7.3	7.2 <sub>b</sub>	0.2	0.6 <sub>b</sub>	0.8 <sub>b</sub>	1.0 <sub>b</sub>	0.7 <sub>b</sub>	0.04	0.03 <sub>b</sub>	0.04 <sub>b</sub>	0.03 <sub>b</sub>	0.03

MM, mitochondrial myopathy group; NC, normal control group; PC, patient control group.

<sup>a</sup>Different from control groups (NC and PC).

<sup>b</sup>Different from rest, *p* < 0.05 or <0.01, see text.

spectroscopy.<sup>19</sup> All subjects had an intravenous catheter inserted in a medial cubital vein of the exercised arm from which venous blood was drawn for analysis at rest (before maximal force determination), during the last 30 seconds of exercise at each workload, and at 5 and 10 minutes postexercise.

### Blood Analysis

Venous blood was collected in 3ml lithium-heparinized syringes (with care taken to avoid air bubbles in the sample), mixed thoroughly, placed immediately on ice, and assayed for partial pressure of O<sub>2</sub> (PO<sub>2</sub>), partial pressure of CO<sub>2</sub> (PCO<sub>2</sub>), and pH within 30 minutes of collection, using a blood gas analyzer (series 1640; Instrumentation Laboratories, Lexington, MA). A separate 1ml sample of whole blood was assayed for lactate, using a commercially available analyzer (Yellow Springs Instruments, Yellow Springs, OH).

### Cycle Ergometry

Mitochondrial myopathy (MM) patients and patient controls performed cycle ergometry with measurement of peak O<sub>2</sub> consumption (Douglas bags, mass spectrometry), noninvasive determination of cardiac output (acetylene rebreathing) and calculation of systemic arteriovenous O<sub>2</sub> difference, from the Fick equation:

$$V_{O_2} = Q \times a-vO_2 \text{ difference}$$

as previously described.<sup>8,9</sup> Two indices of impaired oxidative metabolism in cycle exercise—peak systemic a-vO<sub>2</sub> difference and the level of mismatch between the increase in systemic O<sub>2</sub> delivery (cardiac output, Q) relative to the increase in O<sub>2</sub> use (V<sub>O<sub>2</sub></sub>; expressed as ΔQ/ΔV<sub>O<sub>2</sub></sub>)—were correlated with cubital venous PO<sub>2</sub> obtained during forearm exercise.<sup>5</sup>

### Statistics

Repeated measures analysis of variance was performed to detect differences in blood gas measurements between the 3 groups at rest, during exercise, and during recovery. Post hoc testing was performed on significant interactions. To determine the strength of the relationship between the venous effluent PO<sub>2</sub> during forearm exercise and peak a-vO<sub>2</sub>diff and ΔQ/ΔV<sub>O<sub>2</sub></sub> during cycle exercise, regression analysis was performed on data from MM patients and patient controls. To assess the effectiveness of the aerobic forearm test in discriminating between subjects with and without mitochondrial defects, a receiver operating characteristic curve was constructed. The area under the curve (AUC)<sup>20</sup> and its standard deviation<sup>21</sup> were estimated. Logistic regression was employed to obtain a discriminant function. A classification table was constructed to obtain representative values of sensitivity (number of MM patients correctly identified relative to the total number of patients), specificity (number of control subjects below the discriminant function relative to the total number of control subjects), and accuracy (number of MM subjects correctly identified relative to the total number of subjects tested).

### Results

#### Hand Grip Force

Grip MVC (mean ± SD) was higher in healthy control subjects than in MM patients and patient controls (MM, 21.6 ± 10.4kg; normal, 38.2 ± 8.9kg; patient control, 30.3 ± 12.3kg; *p* < 0.01). Accordingly, the absolute force during exercise at 30% and at 50% of MVC was higher in healthy subjects. Exercise grip force in MM patients did not differ from the patient controls. All subjects completed the exercise without premature fatigue or unusual discomfort.

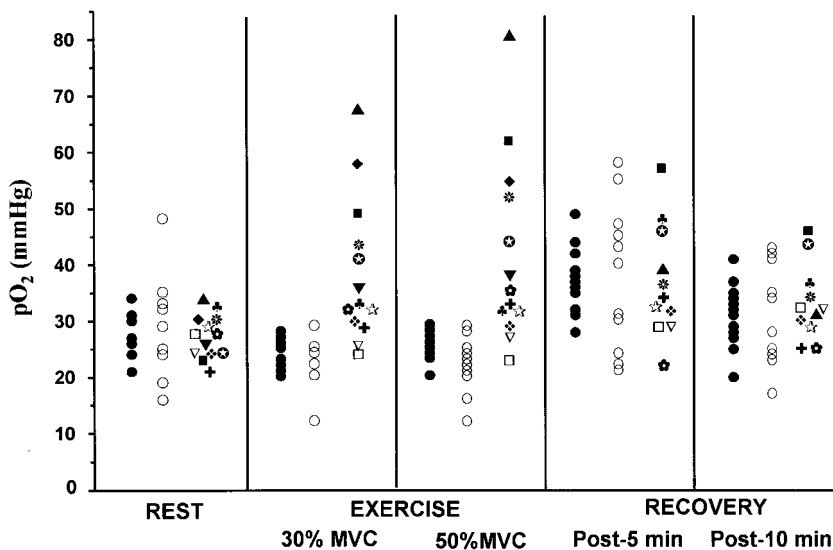


Fig 1. Venous  $PO_2$  at rest and during both exercise and recovery in normal control subjects (solid circles), in patient control subjects (open circles), and in patients with mitochondrial myopathy (for a description of each symbol, see Table 1). Some control values overlap.

#### Venous $PO_2$ During Rest, Exercise, and Recovery

At rest, there were no differences in venous effluent  $PO_2$  among the subject groups (Table 2, Fig 1). During exercise at 30% MVC,  $PO_2$  increased significantly in MM patients from  $27.2 \pm 4.0$  to  $38.2 \pm 13.3$  mmHg ( $p < 0.01$ ). In contrast,  $PO_2$  decreased in the healthy subjects from  $27.2 \pm 4.2$  to  $24.2 \pm 2.7$  mmHg ( $p < 0.05$ ), and in patient controls from  $27.4 \pm 9.5$  to  $22.2 \pm 5.2$  mmHg ( $p < 0.05$ ). At 50% MVC, venous  $PO_2$  in the MM patients rose significantly to  $41.9 \pm 16.5$  mmHg ( $p < 0.05$ ). In contrast,  $PO_2$  at 50% MVC did not differ from rest in healthy subjects ( $26.8 \pm 2.6$  mmHg) or in patient controls ( $23.1 \pm 4.9$  mmHg). A striking feature of the exercise results in MM patients was the large range in venous  $PO_2$  responses to exercise. Four patients achieved  $PO_2$  levels of more than 50 mmHg, including 1 patient who reached a venous  $PO_2$  of more than 80 mmHg (see Fig 1). This contrasted with the relatively small variation in venous  $PO_2$  response that occurred in healthy subjects and patient controls despite substantial differences in absolute workloads in these subjects (see Fig 1).

During postexercise recovery, venous  $PO_2$  was similar in all subject groups. In MM patients, mean  $PO_2$  at 5 minutes ( $36.6 \pm 9.9$  mmHg) and at 10 minutes postexercise ( $33.5 \pm 6.9$  mmHg) did not differ significantly from exercise levels. In contrast, venous  $PO_2$  rose significantly from exercise levels in both healthy subjects (5 minutes post,  $37.3 \pm 5.6$  mmHg; 10 minutes post,  $30.5 \pm 5.7$  mmHg;  $p < 0.05$ ) and patient controls (5 minutes post,  $37.8 \pm 13$  mmHg; 10 minutes post,  $31.5 \pm 8.7$  mmHg;  $p < 0.05$ ).

#### Venous $PCO_2$ , Lactate, and pH During Rest, Exercise, and Recovery

Resting  $PCO_2$  did not differ among subject groups (see Table 2). During exercise at 30% and 50% MVC,

$PCO_2$  in effluent venous blood increased similarly in both control groups ( $p < 0.01$ ). In contrast, the increase in venous  $PCO_2$  was absent or blunted in MM patients. Venous  $PCO_2$  postexercise was similar in all patient groups.

Mean venous lactate at rest was higher in patients with mitochondrial myopathy compared with both control groups ( $p < 0.01$ ). Correspondingly, venous pH was significantly lower in MM patients than in both control groups ( $p < 0.01$ ). Mean blood lactate was higher during exercise at both 30% and 50% MVC in the MM group compared with both control groups ( $p < 0.01$ ), but the level of lactate increase from rest did not differ among subject groups.

#### Correlation with Systemic Oxidative Capacity

Patients with mitochondrial myopathies demonstrated a substantial range of oxidative impairment as assessed during maximal cycle exercise. Mean peak  $\dot{V}O_2$  of the MM group was low ( $13.4 \pm 5.8$  ml/kg/min; range, 5.6 to 24.7 ml/kg/min) compared with patient controls, in whom mean peak  $\dot{V}O_2$  was  $26.0 \pm 9.6$  ml/kg/min, and with previously reported levels for healthy sedentary subjects.<sup>8,9</sup> The increase in cardiac output relative to  $O_2$  uptake during exercise was exaggerated in the MM group (MM mean  $\Delta Q/\Delta \dot{V}O_2$ ,  $20.1 \pm 18.3$ ; range, 4.6–73.0; patient control mean  $\Delta Q/\Delta \dot{V}O_2$ ,  $5.8 \pm 1.4$ ; range, 4.4–9.4). In contrast to the threefold increase in systemic a-v $O_2$  difference from rest to maximal exercise observed in healthy sedentary individuals and in our patient controls (mean peak a-v $O_2$  diff,  $13.8 \pm 2.2$  ml/dl; range, 10.6–16.7 ml/dl), the peak systemic a-v $O_2$  diff was low in most MM patients (mean a-v $O_2$  diff,  $6.7 \pm 3.6$  ml/dl) ranging from a severely low peak a-v $O_2$  difference of 2.7 ml/dl to a normal value of 16.3 ml/dl in 1 MM patient.

There was a highly significant exponential relationship between venous effluent  $PO_2$  during aerobic forearm exercise and both peak systemic  $a-vO_2$  difference ( $R^2 = .62$ ;  $p < .0001$ ) and peak cardiac output relative to  $O_2$  consumption ( $R^2 = 0.75$ ;  $p < .0001$ ), indicating that the severity of oxidative defect determined by the aerobic forearm test correlates with the severity of oxidative impairment measured during cycle exercise testing (Fig 2). A receiver operating characteristic curve was constructed for the venous  $PO_2$  variable (AUC, 0.93; SD, 0.05). As a point of reference, an AUC of 1 represents perfect capacity to discriminate, while an AUC of 0.5 represents no ability to discriminate between 2 patient populations. Discriminant analysis was performed using logistic regression. A simple linear function of venous  $PO_2$  effluent was found to be significant ( $p = 0.02$ ). Based on this analysis, selecting a  $PO_2$  threshold value of 30mmHg has an associated sensitivity of 77% (at 50% MVC), a specificity of 100%, and an accuracy of 88%.

### Discussion

We introduce the aerobic forearm exercise test as a novel and sensitive screening tool to detect and quantify impaired muscle oxidative metabolism in patients with mitochondrial myopathies. We found that venous effluent  $PO_2$  paradoxically increased during aerobic forearm exercise in most patients with mitochondrial myopathies, consistent with a defect in muscle oxidative phosphorylation. Furthermore, we found that the severity of the muscle oxidative defect as indicated by the level of venous  $PO_2$  in exercise correlated closely with the severity of oxidative impairment as assessed using cycle exercise.

Efficient extraction of available  $O_2$  from blood as indicated by a fall in the  $O_2$  content of venous effluent from working muscle is a fundamental physiological feature of increased muscle  $O_2$  use during exercise.<sup>22</sup> A decline in venous effluent  $O_2$  levels is paralleled by a decrease in tissue  $O_2$  levels as monitored by tissue oximetry,<sup>11,23</sup> indicating that increased  $O_2$  use by respiring mitochondria in working muscle normally results in a net increase in the level of  $O_2$  extraction relative to increased  $O_2$  delivery by circulation. A cardinal feature of mitochondrial myopathies is a blunted ability of working muscle to extract  $O_2$ , resulting in high  $O_2$  delivery relative to use, high tissue  $O_2$  content, and elevated  $O_2$  levels in venous effluent from working muscle. The classic description of this exercise response derives from studies of Larsson and Linderholm and colleagues<sup>7,13</sup> of patients who have since been shown to have an unusual mitochondrial myopathy associated with deficiency of multiple iron-sulfur proteins including aconitase and succinate dehydrogenase.<sup>9,24,25</sup> In these patients, cycle exercise resulted in paradoxical arterialization of femoral venous blood and low peak lev-

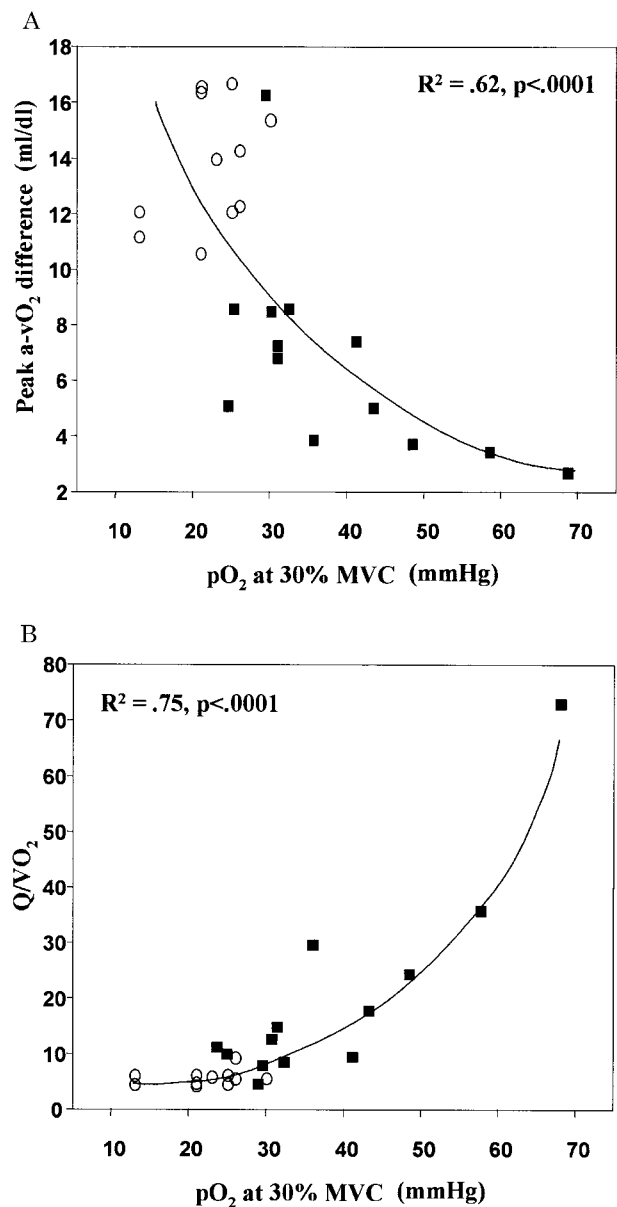


Fig 2. Exponential fit regression analysis performed on data obtained from patients with mitochondrial myopathy (solid squares) and patient controls (open circles) demonstrates a highly significant relationship ( $p < 0.0001$ ) between venous effluent  $PO_2$  during forearm exercise and peak systemic  $a-vO_2$  difference obtained from cycle exercise (A) and between venous  $PO_2$  effluent during forearm exercise and the level of increase in cardiac output (systemic oxygen transport) relative to the increase in  $O_2$  utilization (expressed as  $\Delta Q/\Delta VO_2$ ) (B).

els of systemic arteriovenous  $O_2$  difference, in conjunction with increases in blood flow and systemic  $O_2$  delivery relative to  $O_2$  uptake during cycle exercise that were 4- to 6 times the normal value.<sup>7,13</sup> Our patients include a man from northern Sweden with the clinical and physiological features of the disease described by Larsson and Linderholm.<sup>9</sup> As previously reported, at

rest,  $PO_2$  in femoral venous blood in this patient was similar to healthy subjects (patient, 47mmHg; control subjects,  $43 \pm 3$ mmHg). However, during cycle exercise, femoral venous  $PO_2$  rose paradoxically to 70mmHg, in contrast to a fall in  $PO_2$  to  $20 \pm 2$ mmHg observed in healthy men.<sup>9</sup> A similar paradoxical rise in cubital venous  $PO_2$  during forearm exercise occurred in this patient (see Table 1 and Fig 1).

Impaired  $O_2$  extraction relative to  $O_2$  delivery during exercise has now been demonstrated in a variety of mitochondrial myopathies using tissue oximetry<sup>10–12</sup> or noninvasive measurements of systemic  $O_2$  transport.<sup>26,27</sup> These investigations provide strong support for the interpretation that elevated  $PO_2$  in venous effluent blood during forearm exercise in our patients is a direct consequence of limited muscle capacity for oxidative phosphorylation. Our finding of low levels of  $PCO_2$  in venous effluent blood in exercise in MM patients despite a decline in venous pH that was similar to control subjects can also be attributed to a low rate of muscle oxidative metabolism relative to blood flow during forearm exercise.

In contrast to the narrow range of exercise  $PO_2$  found in control subjects,  $PO_2$  responses to forearm exercise in MM patients varied widely with venous  $PO_2$  values greater than 50mmHg in 4 patients, including one who achieved a  $PO_2$  of 82. This range of exercise venous  $PO_2$  is an apparent consequence of differing levels of muscle oxidative impairment in MM patients. In heteroplasmic mitochondrial DNA mutations, the degree of cellular oxidative limitation correlates with the proportion of mutant relative to wild type mtDNA. Nine of our patients had molecularly characterized mtDNA mutations. The 2 patients with 95% or greater abundance of mutant mtDNA experienced large increases in venous  $PO_2$ , whereas 2 of the patients with the lowest abundance of mtDNA (36%) had levels of exercise venous  $PO_2$  within the range of normal subjects, or only slightly elevated. We also found a close correlation between exercise levels of forearm venous  $PO_2$  and peak systemic a-v $O_2$  difference (a measure of impaired  $O_2$  extraction) and with the ratio of increase in cardiac output relative to the increase in  $O_2$  uptake ( $\Delta Q/\Delta V_{O_2}$ , an index of the mismatch between systemic  $O_2$  delivery and  $O_2$  use) in cycle exercise. These results support the conclusion that the aerobic forearm test accurately assesses the severity of impaired muscle oxidative metabolism in mitochondrial myopathies. Although our study focused on patients with heteroplasmic mtDNA mutations associated with skeletal muscle symptoms of exercise intolerance, the results indicate that the forearm exercise response is not mutation-specific, but rather depends on the level of muscle oxidative impairment. This suggests that the aerobic forearm test would be applicable to other clinical syndromes associated with pathogenic heteroplas-

mic mtDNA mutations (including MELAS and MERFF) when the level of mutation within skeletal muscle is sufficient to significantly impair muscle oxidative phosphorylation.

We conclude that the measurement of venous effluent oxygen content during moderate forearm exercise is valuable in screening for mitochondrial myopathies, particularly in patients with prominent exercise intolerance in whom strong clues of mitochondrial disease (history suggesting maternal inheritance, ophthalmoplegia, ragged-red fibers) are absent. This test would assist in indicating the need for more extensive evaluation, including physiological, biochemical, and genetic analyses to investigate the possibility of an underlying mitochondrial defect. On the basis of our results, we consider an effluent venous  $PO_2$  level of more than 30mmHg to be consistent with an underlying oxidative defect and to warrant further evaluation. Furthermore, we suggest that this forearm test may be useful in monitoring disease progression and the effect of therapeutic interventions in affected patients.

The aerobic forearm test can be readily performed with resources available in the clinical setting. The absolute level of exercise intensity is not a crucial variable in such testing, as  $PO_2$  results were similar in healthy subjects and patient controls across a wide range of absolute exercise intensities, and also because paradoxical elevations in  $PO_2$  in mitochondrial myopathy were similar at both low and moderate workloads. The usefulness of this test is emphasized by the fact that 3 of the patients with the most severe oxidative impairment during forearm exercise had pure exercise intolerance (see Table 1) that had been dismissed for many years by friends, teachers, and physicians as being attributable to deconditioning, poor motivation, or both. We therefore recommend that this testing be employed in any patient with symptoms compatible with a mitochondrial myopathy and, in particular, in patients with symptoms of severe exercise intolerance.

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