

# The spectrum of exercise tolerance in mitochondrial myopathies: a study of 40 patients

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## Summary

Impaired skeletal muscle oxidative phosphorylation in patients with severe mitochondrial respiratory chain defects results in disabling exercise intolerance that is associated with a markedly blunted capacity of muscle to increase oxygen utilization in relation to circulatory and ventilatory responses that increase oxygen delivery to muscle during exercise. The range of oxidative limitation and the relationship between the severity of oxidative defects and physiological responses to exercise among a broader spectrum of mitochondrial respiratory chain defects has not been defined. We evaluated oxidative capacity and circulatory and ventilatory responses to maximal cycle exercise in 40 patients with biochemically and/or molecularly defined mitochondrial myopathy (MM) associated with varying levels of exercise tolerance, and compared responses with those in healthy sedentary individuals. In the MM patients, mean peak work capacity ( $0.88 \pm 0.6$  W/kg) and oxygen uptake ( $\text{VO}_2$ ,  $16 \pm 8$  ml/kg/min) were significantly lower ( $P < 0.01$ ) than in controls (mean work capacity =  $2.2 \pm 0.7$  W/kg;  $\text{VO}_2 = 32 \pm 7$  ml/kg/min), but the patient range was broad ( $0.17$ – $3.2$  W/kg;  $6$ – $47$  ml/kg/min). Oxidative capacity in patients was limited by the ability of muscle to extract available oxygen from blood [mean peak systemic arteriovenous  $\text{O}_2$  difference (a-v $\text{O}_2$ ); patients =  $7.7 \pm 3.5$ , range  $2.7$ – $17.6$  ml/dl, controls =

$15.2 \pm 2.1$  ml/dl], as indicated by a linear correlation between peak  $\text{VO}_2$  and peak systemic a-v $\text{O}_2$  difference ( $r^2 = 0.69$ ). In the patients, the increase in cardiac output relative to  $\text{VO}_2$  (mean  $\Delta Q/\Delta \text{VO}_2 = 15.0 \pm 13.6$ ; range  $3.3$ – $73$ ) and ventilation (mean peak  $\text{VE}/\text{VO}_2 = 65 \pm 24$ ; range  $21$ – $104$ ) were exaggerated compared with controls (mean  $\Delta Q/\Delta \text{VO}_2 = 5.1 \pm 0.7$ ;  $\text{VE}/\text{VO}_2 = 41.2 \pm 7.4$ ,  $P < 0.01$ ). There was a negative exponential relationship between  $\Delta Q/\Delta \text{VO}_2$  and peak systemic a-v $\text{O}_2$  difference ( $r^2 = 0.92$ ) and between peak  $\text{VE}/\text{VO}_2$  and systemic a-v $\text{O}_2$  difference ( $r^2 = 0.53$ ). In patients with heteroplasmic mtDNA mutations, we found an inverse relationship between the proportion of skeletal muscle mutant mtDNA and peak extraction of available oxygen during exercise ( $r^2 = 0.70$ ). We conclude that the degree of exercise intolerance in MM correlates directly with the severity of impaired muscle oxidative phosphorylation as indicated by the peak capacity for muscle oxygen extraction. Exaggerated circulatory and ventilatory responses to exercise are direct consequences of the level of impaired muscle oxidative phosphorylation and increase exponentially in relation to an increasing severity of oxidative impairment. In patients with mtDNA mutations, muscle mutation load governs mitochondrial capacity for oxidative phosphorylation and determines exercise capacity.

**Keywords:** mitochondrial myopathies; exercise; oxidative metabolism

**Abbreviations:** MET = metabolic equivalent; MM = mitochondrial myopathy; RER = respiratory exchange ratio

## Introduction

Exercise intolerance is a well-recognized clinical feature of mitochondrial respiratory chain defects due to pathogenic mutations of mitochondrial or nuclear DNA. Severely impaired muscle oxidative phosphorylation results in disabling exercise limitations in which trivial exertion produces muscle fatigue and lactic acidosis. In such patients, low levels of exercise cause prominent tachycardia and dyspnoea due to increases in cardiac output and ventilation that exceed the capacity of skeletal muscle to utilize the increase in oxygen delivery mediated by these physiological responses (Haller and Bertocci, 1994).

This pattern of exercise pathophysiology in mitochondrial myopathies has been defined in case reports or small series which are probably skewed to the most severe examples of oxidative limitations (Carroll *et al.*, 1979; Elliot *et al.*, 1989; Haller *et al.*, 1989, 1991; Vissing *et al.*, 1996; Taivassalo *et al.*, 2001). Clinical, biochemical and molecular studies suggest that the range of oxidative impairment in mitochondrial myopathies (MMs), especially those attributable to heteroplasmic mtDNA mutations, is broad. However, detailed assessment of exercise and oxidative capacity and of the physiological components of oxygen utilization in a broad range of MM patients has not been undertaken heretofore. Accordingly, we have evaluated exercise and oxidative capacities as well as circulatory and ventilatory responses to exercise in 40 patients with biochemically and/or molecularly defined MMs. The primary objectives were to define the spectrum of exercise capability in this patient population and to illuminate the relationship between molecular and cellular features of respiratory chain defects and cardiopulmonary responses to exercise that normally are closely linked to muscle oxygen utilization.

## Methods

### Subjects

Forty patients (22 females, 18 males;  $37 \pm 12$  years) with heterogeneous clinical, biochemical and molecular features of MM associated with electron transport chain defects were evaluated (Table 1). Clinically, patients had varying levels of exercise tolerance ranging from severe fatigue, weakness and occasional aching of active muscles with a rapid heartbeat and a sense of breathlessness provoked by trivial exercise, to no apparent exercise limitation. There was no evidence of a cardiomyopathy or impaired cardiac conduction in any patient. The range of clinical symptoms is summarized in Table 1. Thirty of the 40 patients had defined mtDNA mutations. In 23 of these 30 patients, the mtDNA mutation loads, defined as the percentage of mutant relative to total mtDNA in skeletal muscle, was quantified. In cases where the molecular mutation has not yet been identified, biochemical and histochemical features in the muscle biopsy were indicative of either a mitochondrial or nuclear-encoded gene mutation (Table 1). A group of 32 healthy sedentary

individuals (nine females, 23 males,  $39 \pm 8$  years) were used as controls for comparison of exercise responses.

The experimental protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center and Presbyterian Hospital of Dallas as well as the ethics committee of the Copenhagen Muscle Research Center, Denmark. Informed consent to participate was obtained from each patient.

### Physiological exercise testing

All individuals were familiarized with the experimental set-up prior to testing. Gas exchange and cardiac output determinations were performed at rest and during an incremental workload exercise test on an electronically braked, pedal rate-independent cycle ergometer (MedGraphics 2000) in which the workload increased 5 or 10 W every 1–2 min. The total duration of the cycle test typically was 10–15 min, and ended when the individual reached maximal heart rate ( $220 - \text{age}$ ) or exhaustion, as indicated by maximal levels of self-perceived exertion using the validated Borg Scale (Borg, 1982).

Expired air was collected in Douglas bags for 120 s at rest, and for 60 s at submaximal workloads and at peak exercise workloads. The fractions of  $\text{O}_2$ ,  $\text{CO}_2$  and  $\text{N}_2$  in each bag were analysed in a Marquette 1100 Medical Gas Analyzer. Respiratory minute volume was measured with a balanced Tissot spirometer, allowing for determination of oxygen uptake ( $\text{VO}_2$ ), carbon dioxide production ( $\text{VCO}_2$ ), the respiratory exchange ratio (RER, i.e.  $\text{VCO}_2/\text{VO}_2$ ), ventilation (VE) and the ventilatory equivalent for oxygen ( $\text{VE}/\text{VO}_2$ ). Cardiac output (Q) was measured non-invasively utilizing acetylene rebreathing in which the rate of disappearance of  $\text{C}_2\text{H}_2$  from a rebreathing bag is proportional to pulmonary blood flow and cardiac output (Triebwasser *et al.*, 1977). As indicated by the Fick equation,  $\text{VO}_2$  is the product of Q and oxygen extraction [systemic arteriovenous oxygen difference ( $a-v\text{O}_2$ )]. Accordingly, the measurement of  $\text{VO}_2$  and Q allows for calculation of  $a-v\text{O}_2$  difference, thus providing an estimate of muscle oxygen extraction (Mitchell and Blomqvist, 1971).

The final workload (peak W/kg) achieved by patients was considered their peak work capacity. Peak oxygen consumption ( $\text{VO}_{2\text{max}}$ ) denotes cardiovascular or 'aerobic fitness'. It reflects both Q, i.e. peak capacity of the heart to pump oxygen and blood to skeletal muscle, and the capacity of muscle to extract available oxygen from blood ( $a-v\text{O}_2$  difference). Cardiac output and, to a lesser extent, ventilation normally are closely coupled to increases in muscle oxygen utilization during exercise, irrespective of age, sex, body weight or level of conditioning (Wasserman and Whipp, 1975; Faulkner *et al.*, 1977). For each subject, the increase in cardiac output relative to the increase in oxygen utilization ( $\Delta Q/\Delta \text{VO}_2$ ) was calculated from the slope of the linear regression between cardiac output (Q; l/min) and oxygen utilization ( $\text{VO}_2$ ; l/min) from rest, submaximal and maximal exercise data. In healthy

**Table 1** Mitochondrial myopathy patient characteristics

Patient	Sex	Age (years)	Other clinical features	Nature of mutation	Genome	Mutation load	
1	JL	M	24	CPEO	5 kb deletion	mtDNA	29%
2	SL	F	39	CPEO	Single deletion	mtDNA	37%
3	JF	M	38	CPEO	Single deletion	mtDNA	40%
4	JL2	M	37	CPEO	6 kb deletion	mtDNA	45%
5	LJ	F	48	CPEO	1 kb deletion	mtDNA	50%
6	CP	F	57	CPEO	Single deletion	mtDNA	53%
7	UI	F	53	CPEO	5 kb deletion	mtDNA	63%
8	LJ2	F	67	CPEO	1 kb deletion	mtDNA	67%
9	TK	F	30	CPEO	4.3 kb deletion	mtDNA	78%
10	RJ	F	30	CPEO	5 kb deletion	mtDNA	78%
11	PB	F	43	CPEO	Single deletion	mtDNA	80%
12	PS	F	38	Myalgia	tRNAglu G14710A	mtDNA	67%
13	WB	M	53	Pure EI	tRNAtrp T5543C (Anitori <i>et al.</i> , 1999)	mtDNA	95%
14	PL	F	35	MELAS	tRNAleu 3243 (Hammans <i>et al.</i> , 1995)	mtDNA	83%
15	RH	F	29	Ataxia, hearing loss, epilepsy	A3243G	mtDNA	80%
16	SF	M	46	Diabetes mellitus, hearing loss, migraine	A3243G	mtDNA	89%
17	SL2	M	38	Pure EI	ND4 point mutation (Andreu <i>et al.</i> , 1999b)	mtDNA	31%
18	SS	M	28	Pure EI	2 bp microdeletion ND2 (Schwartz and Vissing, 2002)	mtDNA	90%
19	RK	F	35	Growth retardation	T4409C point mutation (Vissing <i>et al.</i> , 1998)	mtDNA	91%
20	FB	M	46	Myoglobinuria, weakness	24 bp deletion Cytb (Andreu <i>et al.</i> , 1999a)	mtDNA	36%
21	EO	F	56	Pure EI	G14846A Cytb (Andreu <i>et al.</i> , 1999a)	mtDNA	98%
22	CW	F	23	Myoglobinuria	15 bp microdeletion COXIII (Keightley <i>et al.</i> , 1996)	mtDNA	36%
23	RW	M	35	Myoglobinuria	COXI G5920A (Karadimas <i>et al.</i> , 2000)	mtDNA	68%
24	CP2	F	45	CPEO	Single deletion	mtDNA	
25	JH	M	34	CPEO	Single deletion	mtDNA	
26	HB	M	11	CPEO	Single deletion	mtDNA	
27	MK	F	17	CPEO	Deletion/duplication	mtDNA	
28	BB	M	42	MERRF	tRNAlys 8344	mtDNA	
29	DB	F	55	MERRF	tRNAlys 8344	mtDNA	
30	GM	M	28	MELAS	A3243G	mtDNA	
31	KH	F	31	CPEO	?	mtDNA*	
32	LH	M	29	EI, cardiac hypertrophy	?	mtDNA*	
33	MB	F	40	Exocrine pancreatic failure	Severe COX deficiency (Haller <i>et al.</i> , 1989)	mtDNA*	
34	SS2	F	49	Migraines, stroke	?	mtDNA*	
35	LL	F	14	Ataxia, myoclonus	?	mtDNA*	
36	JD	M	38	Autosomal dominant CPEO	Multiple mtDNA deletions	nDNA	
37	BD	M	35	Autosomal dominant CPEO	Multiple mtDNA deletions	nDNA	
38	CS	F	35	Myoglobinuria, migraines, seizures	CoQ deficiency (Sobreira <i>et al.</i> , 1997)	nDNA*	
39	EK	M	23	Myoglobinuria	SDH/aconitase deficiency (Haller <i>et al.</i> , 1991)	nDNA*	
40	JZ	M	38	Pure EI	Pyruvate oxidation defect	nDNA*	

Pure EI = pure exercise intolerance with no other signs or symptoms of mitochondrial disease; CPEO = chronic progressive external ophthalmoplegia; Cytb = cytochrome *b*; MELAS = mitochondrial encephalomyopathy, lactic acidosis and strokes; MERRF = myoclonus epilepsy with ragged red fibres; mtDNA = mitochondrial DNA; nDNA = nuclear DNA; \* = suspected, ? = unknown.

individuals, cardiac output increases ~5 l for each litre of increase in oxygen consumption. Since arterial blood normally contains ~200 ml O<sub>2</sub>/l, a  $\Delta Q/\Delta VO_2$  of 5 indicates a virtual 1:1 relationship between oxygen delivery and oxygen utilization during exercise. Oxidative limitation due to deconditioning maintains a  $\Delta Q/\Delta VO_2 \cong 5$ . In contrast, when VO<sub>2</sub> is limited by impaired muscle oxidative phosphorylation, the normal coupling between O<sub>2</sub> delivery and utilization is disrupted and  $\Delta Q/\Delta VO_2 \gg 5$  (Haller and Bertocci, 1994). VE/VO<sub>2</sub> is a more complex relationship, with VE linearly related to VO<sub>2</sub> at moderate workloads but rising disproportionately to VO<sub>2</sub> above the so-called 'anaerobic' threshold (Wasserman, 1986).

Heart rate was monitored continuously during rest and exercise with a 12-lead ECG (Quinton 3040 ECG monitor). All subjects had intravenous catheters inserted in a cubital vein from which blood was drawn for lactate analysis at rest, at various submaximal exercise levels and at the maximal workload. Whole blood samples were assayed using a commercially available analyser (Yellow Springs Instruments).

### Statistical analysis

For comparison of physiological data, unpaired *t* tests were performed to determine significant differences between

patient and control group means when assumptions of normality and equal variances were met. In comparisons where data were not normally distributed and variances were not equal (peak  $\text{VO}_2$ ,  $\Delta\text{Q}/\Delta\text{VO}_2$  and  $\text{VE}/\text{VO}_2$ ), the Mann-Whitney rank sum test was applied to determine differences in median values between the two groups. Differences were considered statistically significant when  $P < 0.05$ . To determine the strength of the relationship between variables, regression analysis was performed on data from MM patients.

## Results

The average weight of MM patients was lower than controls ( $64.4 \pm 19$  versus  $78.5 \pm 14$  kg,  $P < 0.01$ ). Indices reflecting oxidative capacity are therefore normalized to body weight.

### Physiology at rest

Resting oxygen consumption was similar in both the MM patients and control subjects (MM group =  $4.0 \pm 0.9$ ; control =  $3.7 \pm 0.8$  ml/kg/min). Cardiac output was slightly higher in the patient group (MM =  $88.0 \pm 22$ ; control =  $66.0 \pm 20$  ml/kg/min,  $P < 0.01$ ) and systemic a- $\text{VO}_2$  difference was slightly lower (MM =  $4.8 \pm 1.3$ ; controls =  $5.5 \pm 1.4$  ml/dl,  $P < 0.05$ ). Resting ventilation (MM =  $172.4 \pm 65$ ; control =  $146.2 \pm 50$  ml/kg/min), RER (MM =  $0.82 \pm 0.1$ ; control  $0.85 \pm 0.1$ ) and heart rate (MM =  $79 \pm 15$ ; control =  $75 \pm 15$ ) did not differ between the two groups. Blood lactate levels at rest were elevated ( $>2.0$  mM) in 14/40 mitochondrial patients (mean =  $2.1 \pm 1.1$  mM, range =  $0.9$ – $5.4$  mM).

### Peak exercise response

In MM patients, the average peak work capacity ( $0.88 \pm 0.6$  W/kg) and  $\text{VO}_2$  ( $16 \pm 8$  ml/kg/min) were significantly lower ( $P < 0.01$ ) than in control subjects (work,  $2.2 \pm 0.7$  W/kg;  $\text{VO}_2$ ,  $32 \pm 7$  ml/kg/min) with a broad range in both groups (MM peak work capacity,  $0.17$ – $3.2$  W/kg; peak  $\text{VO}_2$ ,  $6$ – $47$  ml/kg/min; controls peak work capacity,  $1.2$ – $3.5$  W/kg;  $\text{VO}_2$ ,  $19.5$ – $46.3$  ml/kg/min). Mean peak cardiac output did not differ between the two groups (MM =  $212 \pm 56$ , range  $123$ – $368$  ml/kg/min; controls =  $212 \pm 37$ , range  $128$ – $295$  ml/kg/min). However, the capacity to increase oxygen extraction during exercise was severely attenuated in the MM group, as indicated by a low peak systemic a- $\text{VO}_2$  difference ( $7.7 \pm 3.5$ , range  $2.7$ – $17.6$  ml/dl) compared with healthy subjects ( $15.2 \pm 2.1$ , range  $10.5$ – $18.7$  dl/ml) (Fig. 1A–D).

Peak work capacity and peak  $\text{VO}_2$  were directly proportional in both patients ( $r^2 = 0.94$ ) and healthy subjects ( $r^2 = 0.83$ ), consistent with the normal linear relationship between oxygen utilization and cycle ergometer work. The slopes of the regression depicting this relationship differed slightly (MM slope =  $12.9$ , control slope =  $9.6$ ,  $P = 0.053$ ), indicating that the oxygen cost at a given workload was

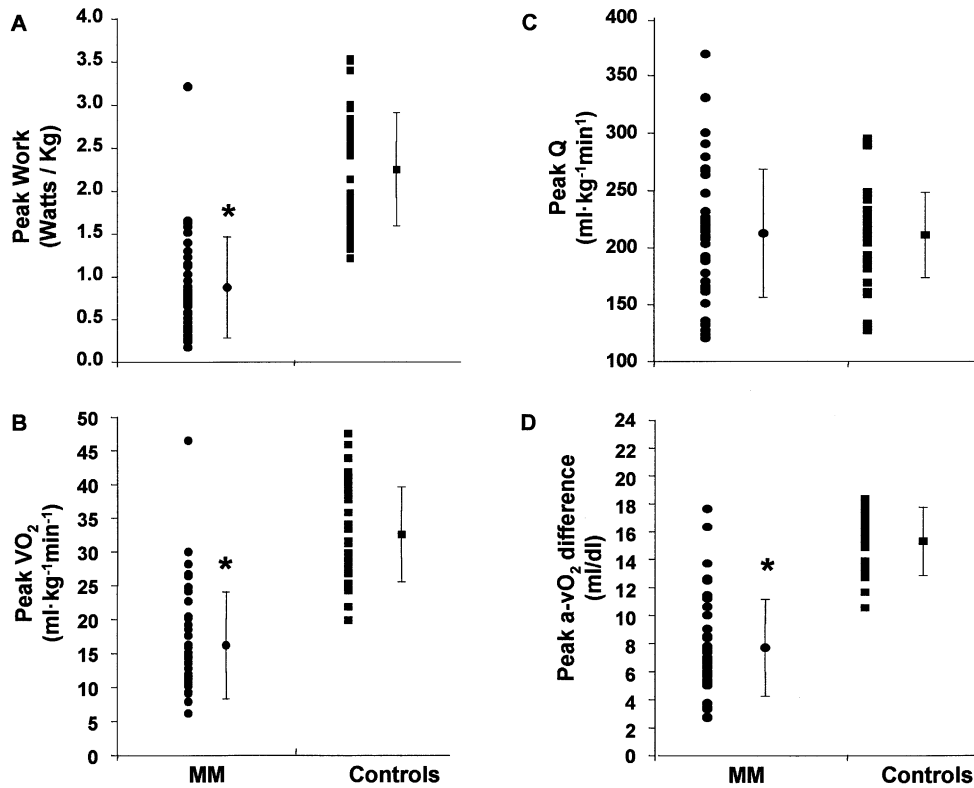
slightly higher in the MM group compared with the control group (Fig. 2).

A direct relationship between peak capacity for  $\text{O}_2$  delivery (cardiac output) and  $\text{O}_2$  utilization is characteristic of healthy individuals and was present in our control subjects ( $r^2 = 0.61$ ,  $P < 0.01$ ). In contrast, there was no correlation between peak cardiac output and  $\text{VO}_2$  in the patients ( $r^2 = 0.08$ ) (Fig. 3A). Instead, peak  $\text{VO}_2$  in MM patients correlated directly with peak systemic a- $\text{VO}_2$  difference ( $r^2 = 0.69$ ,  $P < 0.01$ ), thus linking the range of oxygen utilization in patients to the capacity for oxygen extraction within skeletal muscle (Fig. 3B). This relationship differs from the normal physiological response to exercise in healthy individuals, as indicated by the lack of correlation between peak  $\text{VO}_2$  and systemic a- $\text{VO}_2$  difference in healthy subjects ( $r^2 = 0.18$ ).

During exercise, the increase in cardiac output relative to increase in oxygen uptake was exaggerated in most patients. The mean slope of  $\Delta\text{Q}/\Delta\text{VO}_2$  in the MM group was 3-fold greater than normal (MM =  $15.0 \pm 13.6$ , controls =  $5.1 \pm 0.7$ ). Also, the range was markedly variable in patients (MM =  $3.3$ – $73$ ) in contrast to healthy subjects ( $4.3$ – $6.7$ ) (Fig. 4A). Ventilation during peak exercise was also in excess relative to  $\text{VO}_2$  in patients, as indicated by an elevated ventilatory equivalent for oxygen (peak  $\text{VE}/\text{VO}_2$  in MM =  $65.1 \pm 24.6$ , range  $35$ – $135$ ; controls =  $41.2 \pm 7.4$ , range  $23$ – $55$ ;  $P < 0.01$ ) (Fig. 4B). Likewise, maximal RER was abnormally high in the MM group compared with controls (MM =  $1.31 \pm 0.31$ , range  $0.94$ – $2.4$ ; controls =  $1.14 \pm 0.06$ , range  $1.06$ – $1.24$ ;  $P < 0.01$ ).

In healthy individuals,  $\Delta\text{Q}/\Delta\text{VO}_2$  is  $\sim 5$  irrespective of the peak capacity for oxygen utilization. In contrast, in the MM patients, an increasingly severe mismatch between oxygen delivery and utilization accompanied more severely impaired muscle oxidative phosphorylation, as indicated by a negative exponential relationship between  $\Delta\text{Q}/\Delta\text{VO}_2$  and both peak oxygen utilization ( $r^2 = 0.69$ ,  $P < 0.01$ ) and peak systemic a- $\text{VO}_2$  difference ( $r^2 = 0.92$ ,  $P < 0.01$ ; Fig. 5A). Similarly, a negative exponential correlation was evident for peak  $\text{VE}/\text{VO}_2$  relative to peak  $\text{VO}_2$  ( $r^2 = 0.58$ ,  $P < 0.01$ ) and peak systemic a- $\text{VO}_2$  difference ( $r^2 = 0.53$ ,  $P < 0.01$ ; Fig. 5B). These results suggest that more exaggerated cardiovascular and ventilatory responses were related to lower peak capacity for oxidative phosphorylation as reflected in peak capacity for extraction of available oxygen from blood.

Blood lactate levels at peak exercise were high relative to peak workload and  $\text{VO}_2$  in MM patients compared with control subjects (MM exercise lactate/W =  $0.26 \pm 0.29$  mM/W, controls =  $0.05 \pm 0.02$  mM/W,  $P < 0.01$ ; MM exercise lactate/ $\text{VO}_2$  =  $0.60 \pm 0.38$  mM/ml/kg/min, controls =  $0.28 \pm 0.06$  mM/ml/kg/min,  $P < 0.01$ ). In the MM patients, the ratio of lactate (mM) to  $\text{VO}_2$  (ml/kg/min) at peak exercise correlated closely with both peak  $\text{O}_2$  utilization ( $r^2 = 0.78$ ,  $P < 0.01$ ) and peak systemic a- $\text{VO}_2$  difference ( $r^2 = 0.60$ ,  $P < 0.01$ ; Fig. 6). In contrast, there was only a weak correlation between blood lactate levels at rest and both peak



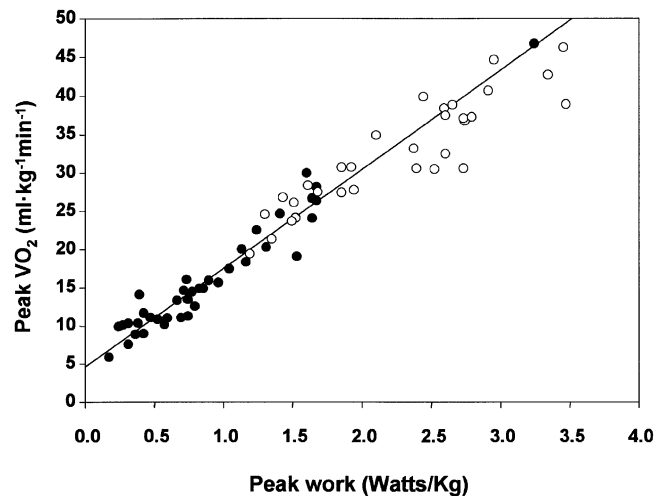
**Fig. 1** Individual values for peak work (A), oxygen uptake (B), cardiac output (C) and systemic arteriovenous  $\text{O}_2$  difference (D) obtained during maximal cycle exercise testing in 40 patients with MM (filled circles) and 32 healthy control individuals (filled squares). The mean values  $\pm$  SD are shown to the right of the individual data for each group. \* = significantly different from the control mean,  $P < 0.01$ .

oxygen utilization ( $r^2 = 0.20$ ,  $P < 0.05$ ) and peak systemic a- $\text{vO}_2$  difference ( $r^2 = 0.22$ ,  $P < 0.05$ ) in the MM patients.

The mutation load in 23 patients with heteroplasmic mtDNA defects ranged from 29 to 98%. Analysis of genotype-phenotype relationships in these patients revealed that increasing mutation load was associated with decreasing capacity for oxidative phosphorylation, as suggested by a negative exponential relationship between percentage mutation and peak  $\text{O}_2$  uptake ( $r^2 = 0.65$ ,  $P < 0.01$ ), and between percentage mutation and peak systemic a- $\text{vO}_2$  difference ( $r^2 = 0.70$ ,  $P < 0.01$ ; Fig. 7). One patient (no. 17) harbouring a low mutation load despite severe physiological oxidative impairment was determined to be a statistical outlier (Hedges and Olkin, 1985) and was excluded from the regression analysis.

### Discussion

Exercise intolerance is a fundamental consequence of impaired respiratory chain function due to pathogenic mutations of mitochondrial or nuclear DNA in skeletal muscle. It may be the sole manifestation of a respiratory chain defect (Andreu *et al.*, 1999a; DiMauro, 1999; Pulkes *et al.*, 2000) or it may occur in combination with muscle weakness and dysfunction of other organ systems. Exercise intolerance



**Fig. 2** The relationship between peak workload and oxygen uptake during cycle exercise in patients with MM (filled circles) and control individuals (open circles). The regression line for patients ( $r^2 = 0.94$ ) is shown.

is difficult to assess clinically. Thus, determination of the prevalence and range of exercise limitations in MMs requires exercise testing. Furthermore, differentiating exercise limi-

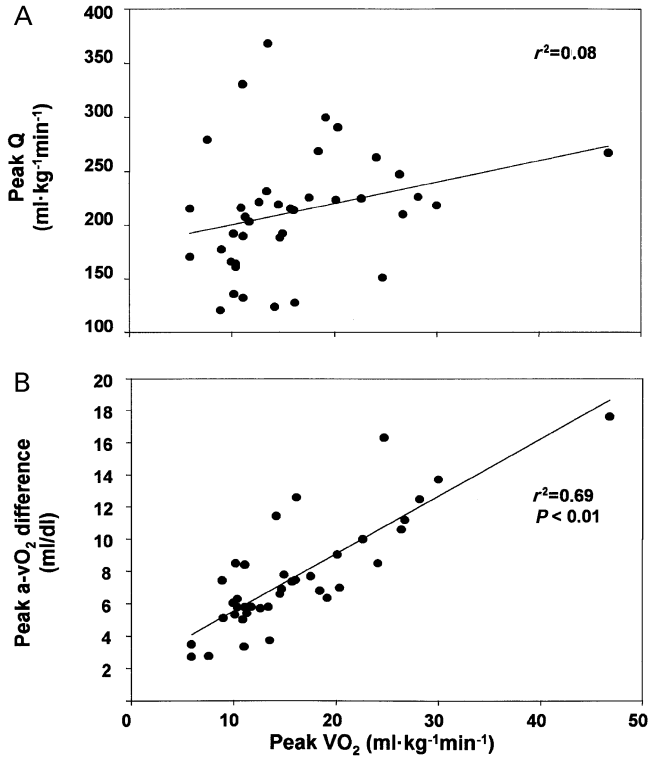
tation that is attributable to physical deconditioning from that which is due to muscle respiratory chain dysfunction necessitates the independent assessment of cardiovascular

fitness as well as an evaluation of the ability of working muscle to extract available oxygen. This study assesses each of these elements of exercise capacity in a large group of MMs.

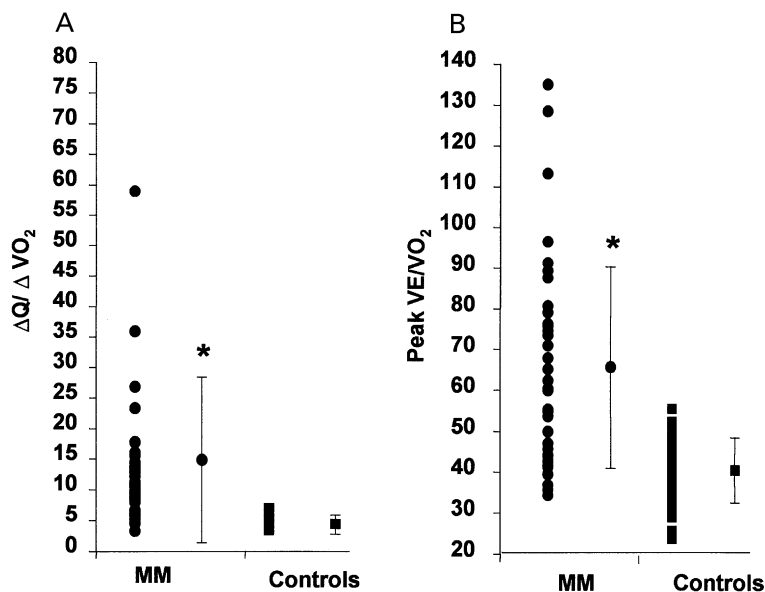
The major findings of this study were that, in patients with MMs: (i) exercise capacity varied widely, attributable to varying levels of oxidative impairment; (ii) oxidative capacity was directly proportional to peak levels of extraction of available oxygen from blood (systemic a-vO<sub>2</sub> difference), representing a surrogate marker of muscle capacity for oxidative phosphorylation; (iii) exaggerated circulatory and ventilatory responses to exercise were governed by skeletal muscle oxidative capacity, in which more severely impaired oxidative phosphorylation elicited more exaggerated systemic responses to exercise; and (iv) in patients with defined mtDNA mutations, peak oxygen uptake and mitochondrial capacity for oxidative phosphorylation decreased in proportion to increasing mutation load in muscle.

**Exercise capacity and oxygen utilization**

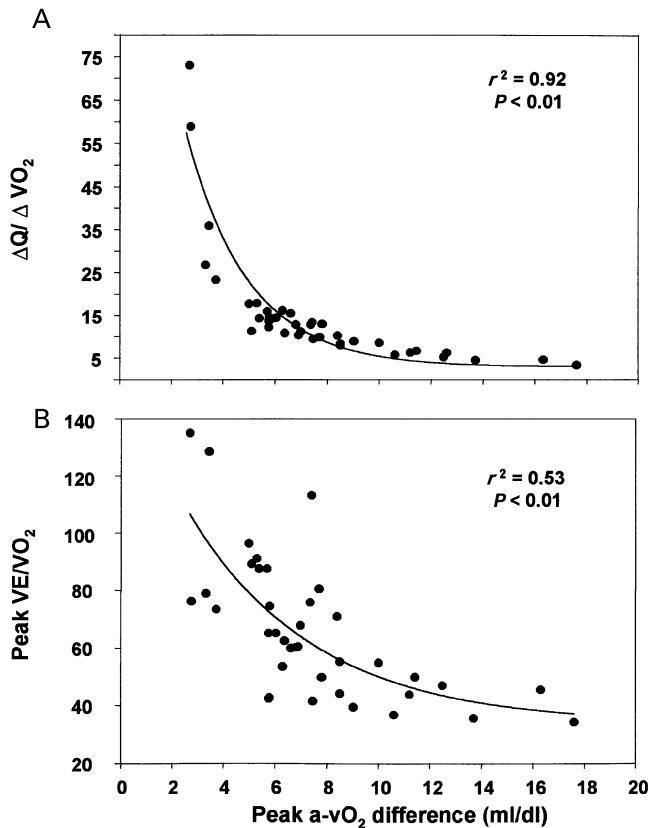
The wide range of exercise and oxidative capacities in MM patients accounts for the clinical variability in severity of exercise intolerance in this patient population. Peak VO<sub>2</sub> was low in the majority of patients, and in some cases barely increased above the resting metabolic rate, commonly designated 1 metabolic equivalent (MET), defined as an oxygen utilization rate of 3.5 ml/kg/min (Wasserman, 1986). Patient functional capacity is often described in terms of METs. For example, activities of daily living including washing, driving and very slow walking (3.2 km/h) typically require a metabolic cost of ~4 METs (14 ml/kg/min), whereas



**Fig. 3** In MM patients, (A) peak oxygen uptake does not correlate directly with peak cardiovascular capacity for oxygen delivery (cardiac output) but does correlate closely with peak a-vO<sub>2</sub> difference (B).



**Fig. 4** The cardiovascular (A) and ventilatory (B) response to maximal exercise are depicted for 40 patients with MM (filled circles) and 32 healthy control individuals (filled squares). The mean values ± SD are shown to the right of the individual data for each group. \* = significantly different from the control mean, P < 0.01.



**Fig. 5** Regression analysis demonstrates a negative exponential relationship ( $P < 0.01$ ) between the hyperdynamic cardiovascular response to exercise (A) and peak systemic a-vO<sub>2</sub> difference in MM patients, suggesting that at greater levels of oxidative impairment (lower peak a-vO<sub>2</sub> difference) the mismatch between oxygen delivery and utilization ( $\Delta Q/\Delta VO_2$ ) becomes progressively worse. A negative exponential regression is also evident in patients between peak ventilatory response to exercise (B) and peak systemic a-vO<sub>2</sub> difference.

walking at a moderate pace (5–6.5 km/h), slow stair climbing and skating require 5–7 METs (18–25 ml/kg/min), and slow jogging, bicycling and carrying heavy objects require >7 METs. Approximately two-thirds of our patient group could not exercise above 5 METs and would be classified in the ‘low’ fitness category (Wasserman, 1986). One-third of patients demonstrated exercise capacities within the range of our healthy sedentary controls (>20 ml/kg/min), including one patient who had a fitness level comparable with that of a healthy, conditioned individual.

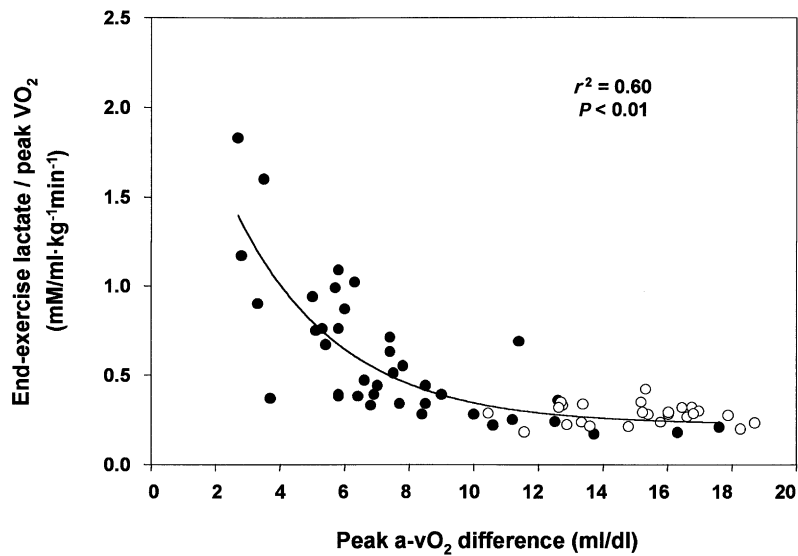
This study illuminates the physiological mechanism that limits oxidative metabolism during physical activity in patients with MMs. Normally, from rest to peak exercise, the increased demand for oxygen by working muscle for aerobic energy production is met by both an increase in delivery of oxygenated blood to muscle by the circulation and an increase in the level of muscle O<sub>2</sub> extraction from blood. In healthy individuals, there is a net increase in level of O<sub>2</sub> extraction relative to O<sub>2</sub> delivery during exercise. This is indicated by an exercise-related fall in O<sub>2</sub> levels in venous

blood (Taivassalo *et al.*, 2002) and working muscle (Wariar *et al.*, 2000), consistent with increased utilization of O<sub>2</sub> by respiring mitochondria relative to the rate of increase in O<sub>2</sub> delivery. Systemic a-vO<sub>2</sub> difference therefore represents a marker of the capacity for mitochondrial oxidative phosphorylation. In healthy individuals, a-vO<sub>2</sub> difference increases from 5 ml/dl at rest to ~15 ml/dl at peak exercise and does not limit oxygen utilization during exercise. This view is supported by the fact that peak a-vO<sub>2</sub> difference does not correlate directly with peak oxidative capacity. Rather, oxygen delivery by the circulation is believed to be the principal determinant of aerobic performance, as indicated by findings that peak VO<sub>2</sub> among healthy subjects correlates directly with peak cardiac output.

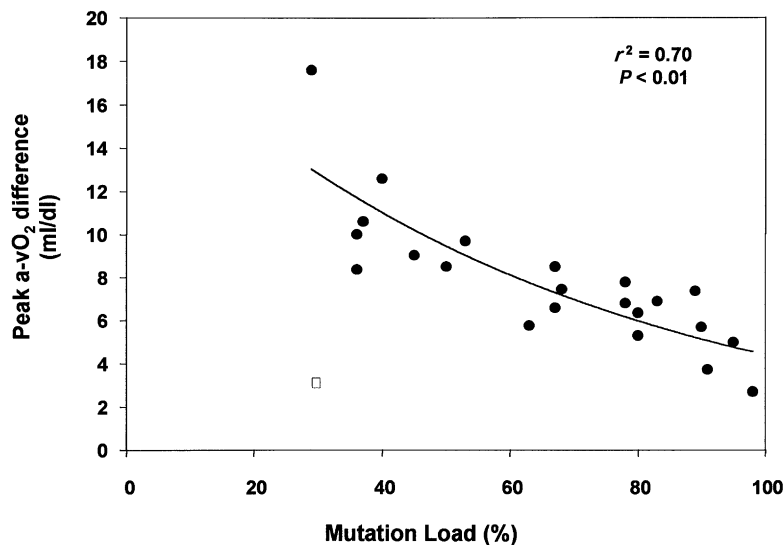
The majority of MM patients exhibited a blunted ability to increase systemic a-vO<sub>2</sub> difference with exercise, consistent with previous findings of elevated O<sub>2</sub> levels in venous blood from working muscle (Taivassalo *et al.*, 2002) and high tissue O<sub>2</sub> content (Bank and Chance, 1994). Furthermore, our data indicate that the degree of oxidative limitation during exercise in MMs relates directly to the capacity of muscle to extract available oxygen from blood. In other words, whereas peak work capacity in healthy individuals is limited by cardiovascular capacity to deliver O<sub>2</sub> to working muscle, peak exercise capacity in patients with MMs is limited by muscle respiratory chain function. Our data indicate that an inability to increase systemic a-vO<sub>2</sub> difference above 10 ml/dl is highly sensitive (80%) and specific (100%) for attributing low oxygen uptake to impaired mitochondrial function in patients with exercise intolerance. Cardiovascular capacity for oxygen delivery in most patients included in this investigation is comparable with that of healthy individuals.

### **Circulatory and ventilatory responses to exercise**

These data demonstrate that skeletal muscle oxidative phosphorylation is a critical component in circulatory regulation during exercise. The exact mechanism responsible for the tight matching of oxygen delivery to oxygen utilization in healthy individuals is unclear but probably involves activation of neural reflexes via metaboreceptors in working muscle that are responsive to metabolites that reflect muscle oxidative demand (Haller and Vissing, 2000). Previous studies have indicated that an exaggerated increase in O<sub>2</sub> delivery relative to muscle metabolic rate in exercise is a consistent feature of deficient muscle oxidative phosphorylation (Haller *et al.*, 1989; Vissing *et al.*, 1996; Haller and Vissing, 2000; Taivassalo *et al.*, 2002). The prevalence of such a ‘hyperkinetic’ circulatory response to exercise in most of our MM patients underscores the requirement for a preserved capacity for muscle oxidative phosphorylation for normal coupling of oxygen delivery and utilization during exercise. Although our patients did not have evidence of cardiac involvement, combined myopathy and cardiomyopathy is common in mitochondrial disorders. In the setting of cardiac disease, a hyperkinetic circulatory response would be



**Fig. 6** The level of blood lactate relative to oxygen uptake at peak exercise correlates exponentially with peak systemic a-vO<sub>2</sub> difference in the MM patients (filled circles). In contrast, no such relationship exists in the control subjects (open circles).



**Fig. 7** Analysis of molecular genotype and physiological phenotype associations in 23 patients with heteroplasmic mtDNA mutations. A negative exponential relationship is indicated between the percentage mutation and peak systemic a-vO<sub>2</sub> difference. The outlier is identified (open square).

expected to have deleterious effects by increasing cardiac work.

This study is the first to relate the severity of the skeletal muscle oxidative defect to the severity of mismatch between the exercise increase in cardiac output and oxygen uptake ( $\Delta Q/\Delta VO_2$ ). The data indicate that this normal close coupling becomes progressively distorted as the capacity for oxygen uptake and for muscle oxygen extraction becomes more impaired. Given the remarkably constant relationship between the exercise increase in oxygen uptake and corresponding increase in cardiac output in healthy individuals

irrespective of age, gender or conditioning level and as reflected in our results from control subjects, we consider the cardiovascular exercise response to be consistent with an underlying defect in muscle oxidative phosphorylation when  $\Delta Q/\Delta VO_2 \geq 7.0$  (100% specificity, 83% sensitivity).

The data also confirm previous reports that the ventilatory response to exercise is abnormal in many patients with MMs, as indicated by a level of pulmonary ventilation relative to peak oxygen uptake ( $VE/VO_2$ ) that is excessive compared with control subjects (Haller *et al.*, 1989; Flaherty *et al.*, 2001). The ventilatory equivalent for oxygen ( $VE/VO_2$ ) is an

indicator of breathing economy. During exercise,  $VE/VO_2$  typically ranges between 30 and 40 l of air per litre of oxygen utilization in healthy individuals, with lower values in athletes and more conditioned individuals (Wasserman, 1986). This study demonstrates for the first time that exaggerated ventilation relative to oxygen utilization (higher  $VE/VO_2$ ) in patients with MMs is related to the degree of oxidative impairment. Hyperventilation was more pronounced in patients with more severe oxidative defects, consistent with symptoms of exertional dyspnoea experienced by many patients. The mechanism underlying this hyperventilatory exercise response may relate to excess carbon dioxide production due to lactate buffering, as suggested by the finding of a correspondingly exaggerated respiratory exchange ratio (RER or  $VCO_2/VO_2$ ). Alternatively, hyperventilation, like the hyperdynamic circulatory response, may be regulated by metabolic feedback from skeletal muscle that reflects limited oxidative phosphorylation relative to oxidative demand in working muscle.

### **Oxygen utilization and lactic acidosis**

Lactic acidosis has often been used as an indicator of impaired oxidative metabolism and as a clinical marker for mitochondrial disorders. Although elevated lactate values at rest strengthen the possibility of a mitochondrial disorder, our data indicate diagnostic sensitivity to be only 35% at 100% specificity ( $>2.0$  mM). Furthermore, our findings demonstrate that resting lactic acidosis is not a strong indicator of the degree of skeletal muscle oxidative impairment. In contrast, higher levels of lactate in relation to oxygen utilization during exercise in MM patients correlated closely with the severity of deficiency of mitochondrial oxidative phosphorylation as reflected in peak  $a-vO_2$  difference.

### **Oxygen utilization and mutation load**

While a direct correlation between impaired respiratory chain function and limited capacity to extract oxygen is implied by our results, insufficient biochemical data were available to evaluate the relationship between biochemical and physiological phenotypes. However, data regarding the mutation load were available in 23 of 30 patients with mtDNA mutations. A major finding of this study relates molecular genotype to physiological and clinical phenotype in patients with characterized heteroplasmic mutations of mtDNA. While there is evidence that the mutation load is inversely related to mitochondrial respiratory chain function *in vitro* (Chomyn *et al.*, 1994), the correlation *in vivo* has been weak at best (Chinnery *et al.*, 1997, 2000, 2001; Morgan-Hughes and Hanna, 1999). This is the first study to demonstrate that the mutation load in patients with various respiratory chain defects governs peak oxygen utilization and determines exercise capacity in patients with MMs. The data demonstrate an inverse relationship between the proportion of mutant

mtDNA in skeletal muscle and muscle capacity for oxidative phosphorylation as reflected in peak systemic  $a-vO_2$  difference.

### **Conclusion**

This study underscores the utility of physiological exercise testing in the assessment of exercise tolerance and in the determination of functional severity of a skeletal muscle oxidative defect in patients with MMs. The non-invasive assessment of cardiac output during exercise is critical for revealing a hyperdynamic cardiovascular exercise response as well as for determining the capacity for skeletal muscle oxygen extraction. These measurements permit differentiation of reduced peak oxygen uptake that is due to poor physical conditioning from that due to an underlying genetic defect in mitochondrial oxidative metabolism. Particularly in the absence of other characteristic signs and symptoms, the exaggerated cardiovascular and ventilatory responses during exercise are sensitive indicators of an underlying oxidative impairment and account for the clinical prominence of tachycardia and dyspnoea that have been described in MM patients.

In conclusion, this study reveals a wide spectrum of oxidative limitations and exercise capacities in a heterogeneous group of mitochondrial myopathies. It illuminates the relationship between severity of muscle oxidative defects and symptoms of exercise intolerance in MM patients. Furthermore, it provides insight into the regulatory mechanisms responsible for characteristic physiological responses to exercise in these patients, as well as evidence linking skeletal muscle mutation load to capacity for mitochondrial oxidative phosphorylation.

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### **References**

- Andreu AL, Hanna MG, Reichmann H, Bruno C, Penn AS, Tanji K, et al. Exercise intolerance due to mutations in the cytochrome b gene of mitochondrial DNA. *N Engl J Med* 1999a; 341: 1037–44.
- Andreu AL, Tanji K, Bruno C, Hadjigeorgiou GM, Sue CM, Jay C, et al. Exercise intolerance due to a nonsense mutation in the mtDNA ND4 gene. *Ann Neurol* 1999b; 45: 820–3.
- Anitori RP, Quan F, Buist NRM, Shoubridge EA, Kennaway, NG. A novel mutation in the mitochondrial tRNA<sup>Trp</sup> gene in a sporadic case of mitochondrial myopathy [abstract]. In: Proceedings of the

- 4th European Meeting on Mitochondrial Pathology. Cambridge: Blackwell Scientific Publishing; 1999. p. 73.
- Bank W, Chance B. An oxidative defect in metabolic myopathies: diagnosis by noninvasive tissue oximetry. *Ann Neurol* 1994; 36: 830–7.
- Borg GA. Psychophysical bases of perceived exertion. *Med Sci Sports Exerc* 1982; 14: 377–81.
- Carroll JE, Hagberg JM, Brooke MH, Shumate JB. Bicycle ergometry and gas exchange measurements in neuromuscular diseases. *Arch Neurol* 1979; 36: 457–61.
- Chinnery PF, Howell N, Lightowlers RN, Turnbull DM. Molecular pathology of MELAS and MERRF. The relationship between mutation load and clinical phenotypes. *Brain* 1997; 120: 1713–21.
- Chinnery PF, Taylor DJ, Brown DT, Manners D, Styles P, Lodi R. Very low levels of the mtDNA A3243G mutation associated with mitochondrial dysfunction in vivo. *Ann Neurol* 2000; 47: 381–4.
- Chinnery PF, Taylor DJ, Manners D, Styles P, Lodi R. No correlation between muscle A3243G mutation load and mitochondrial function in vivo. *Neurology* 2001; 56: 1101–4.
- Chomyn A, Lai ST, Shakeley R, Bresolin N, Scarlato G, Attardi G. Platelet-mediated transformation of mtDNA-less human cells: analysis of phenotypic variability among clones from normal individuals—and complementation behavior of the tRNA<sup>Lys</sup> mutation causing myoclonic epilepsy and ragged red fibers. *Am J Hum Genet* 1994; 54: 966–74.
- DiMauro S. Exercise intolerance and the mitochondrial respiratory chain. [Review]. *Ital J Neurol Sci* 1999; 20: 387–93.
- Elliot DL, Buist NR, Goldberg L, Kennaway NG, Powell BR, Kuehl KS. Metabolic myopathies: evaluation by graded exercise testing. *Medicine (Baltimore)* 1989; 68: 163–72.
- Faulkner JA, Heigenhauser GJ, Schork MA. The cardiac output–oxygen uptake relationship of men during graded bicycle ergometry. *Med Sci Sports* 1977; 9: 148–54.
- Flaherty KR, Wald J, Weisman IM, Zeballos RJ, Schork MA, Blaiwas M, et al. Unexplained exertional limitation: characterization of patients with a mitochondrial myopathy. *Am J Respir Crit Care Med* 2001; 164: 425–32.
- Haller RG, Bertocci LA. Exercise evaluation of metabolic myopathies. In: Engel AG, Franzini-Armstrong C, editors. *Myology*. 2nd edn. New York: McGraw-Hill; 1994. p. 807–21.
- Haller RG, Vissing J. Circulatory regulation in muscle disease. In: Saltin B, Bouschel R, Secher N, Mitchell J, editors. *Exercise and circulation in health and disease*. Champaigne (IL): Human Kinetics; 2000. p. 263–73.
- Haller RG, Lewis SF, Cook JD, Blomqvist CG. Hyperkinetic circulation during exercise in neuromuscular disease. *Neurology* 1983; 33: 1283–7.
- Haller RG, Lewis SF, Estabrook RW, DiMauro S, Servidei S, Foster DW. Exercise intolerance, lactic acidosis, and abnormal cardiopulmonary regulation in exercise associated with adult skeletal muscle cytochrome c oxidase deficiency. *J Clin Invest* 1989; 84: 155–161.
- Haller RG, Henriksson KG, Jorfeldt L, Hultman E, Wibom R, Sahlin K, et al. Deficiency of skeletal muscle succinate dehydrogenase and aconitase. Pathophysiology of exercise in a novel human muscle oxidative defect. *J Clin Invest* 1991; 88: 1197–206.
- Hammans SR, Sweeney MG, Hanna MG, Brockington M, Morgan-Hughes JA, Harding AE. The mitochondrial DNA transfer RNA<sup>Leu(UUR)</sup> A→G(3243) mutation. A clinical and genetic study. *Brain* 1995; 118: 721–34.
- Hedges LV, Olkin I. Procedure to identify outliers in regression models. In: *Statistical methods for meta-analysis*. New York: Harcourt Brace Jovanovich Publishers; 1985. p. 248–61.
- Karadimas CL, Greenstein P, Sue CM, Joseph JT, Tanji K, Haller RG, et al. Recurrent myoglobinuria due to a nonsense mutation in the COX I gene of mitochondrial DNA. *Neurology* 2000; 55: 644–9.
- Keightley JA, Hoffbuhr KC, Burton MD, Salas VM, Johnston WS, Penn AM, et al. A microdeletion in cytochrome c oxidase (COX) subunit III associated with COX deficiency and recurrent myoglobinuria. *Nature Genet* 1996; 12: 410–6.
- Mitchell JH, Blomqvist G. Maximal oxygen uptake. *N Engl J Med* 1971; 6: 1018–22.
- Morgan-Hughes JA, Hanna MG. Mitochondrial encephalomyopathies: the enigma of genotype versus phenotype. [Review]. *Biochim Biophys Acta* 1999; 1410: 125–45.
- Pulkes T, Siddiqui A, Morgan-Hughes JA, Hanna MG. A novel mutation in the mitochondrial tRNA(Tyr) gene associated with exercise intolerance. *Neurology* 2000; 55: 1210–12.
- Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA in man. *N Engl J Med* 2002; 347: 576–80.
- Sobreira C, Hirano M, Shanske S, Keller K, Haller RG, Davidson E, et al. Mitochondrial encephalomyopathy with coenzyme Q10 deficiency. *Neurology* 1997; 48: 1238–43.
- Taivassalo T, Shoubridge EA, Chen J, Kennaway NG, DiMauro S, Arnold DA, et al. Aerobic conditioning in patients with mitochondrial myopathies: physiological, biochemical and genetic effects. *Ann Neurol* 2001; 50: 133–41.
- Taivassalo T, Abbott A, Wyrick P, Haller RG. Venous oxygen levels during aerobic forearm exercise: an index of impaired oxidative metabolism in mitochondrial myopathy. *Ann Neurol* 2002; 51: 38–44.
- Triebwasser JH, Johnson RL, Burpo RP, Campbell JC, Reardon WC, Blomqvist CG. Noninvasive determination of cardiac output by a modified acetylene rebreathing procedure utilizing mass spectrometer measurements. *Aviat Space Environ Med* 1977; 48: 203–9.
- Vissing J, Galbo H, Haller RG. Exercise fuel mobilization in mitochondrial myopathy: a metabolic dilemma. *Ann Neurol* 1996; 40: 655–62.
- Vissing J, Salamon MB, Arlien-Søborg P, Nørby S, Manta P, DiMauro S, et al. A new mitochondrial tRNA<sup>Met</sup> gene mutation in a patient with dystrophic muscle and exercise intolerance. *Neurology* 1998; 50: 1875–8.

Vissing J, Gansted U, Quistorff B. Exercise intolerance in mitochondrial myopathy is not related to lactic acidosis. *Ann Neurol* 2001; 49: 672–6.

Wariar R, Gaffke JN, Haller RG, Bertocci LA. A modular system for clinical measurement of impaired skeletal muscle oxygenation. *J Appl Physiol* 2000; 88: 315–25.

Wasserman K. Normal values. In: Wasserman K. *Principles of*

*exercise testing and interpretation*. Philadelphia: Lea & Febiger; 1986. p. 72–86.

Wasserman K, Whipp BJ. Exercise physiology in health and disease. *Am Rev Resp Dis* 1975; 112: 219–49.

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