

Aerobic Conditioning in Patients with Mitochondrial Myopathies: Physiological, Biochemical, and Genetic Effects

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Aerobic training has been shown to increase work and oxidative capacity in patients with mitochondrial myopathies, but the mechanisms underlying improvement are not known. We evaluated physiological (cycle exercise, ³¹P-MRS), biochemical (enzyme levels), and genetic (proportion of mutant/wild-type genomes) responses to 14 weeks of bicycle exercise training in 10 patients with heteroplasmic mitochondrial DNA (mtDNA) mutations. Training increased peak work and oxidative capacities (20–30%), systemic arteriovenous O₂ difference (20%), and ³¹P-MRS indices of metabolic recovery (35%), consistent with enhanced muscle oxidative phosphorylation. Mitochondrial volume in vastus lateralis biopsies increased significantly (50%) and increases in deficient respiratory chain enzymes were found in patients with Complex I (36%) and Complex IV (25%) defects, whereas decreases occurred in 2 patients with Complex III defects (~20%). These results suggest that the cellular basis of improved oxygen utilization is related to training-induced mitochondrial proliferation likely resulting in increased levels of functional, wild-type mtDNA. However, genetic analysis indicated the proportion of wild-type mtDNA was unchanged (3/9) or fell (6/9), suggesting a trend toward preferential proliferation of mutant genomes. The long-term implications of training-induced increases in mutant relative to wild-type mtDNA, despite positive physiological and biochemical findings, need to be assessed before aerobic training can be proposed as a general treatment option.

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Since the first identification of mutations in the mitochondrial genome (mtDNA) in 1988,^{1,2} respiratory chain defects attributable to mtDNA mutations have increasingly been recognized as a cause of disease in humans. In adults, the majority of respiratory chain disorders are attributable to large-scale mtDNA deletions or to point mutations that affect the 22 tRNA or 13 protein coding genes within mtDNA, leading to impaired function of one or more of the respiratory chain complexes (I, III, IV, and V) that contain mtDNA-encoded subunits.^{3,4} Pathogenic mutations are typically heteroplasmic, and the level of oxidative impairment is linked to the proportion of cellular heteroplasmy. As the ratio of mutant to wild-type mtDNA increases beyond a critical threshold, the capacity to express mtDNA-encoded respiratory chain subunits declines to a level that is insufficient to ade-

quately meet cellular requirements for oxidative phosphorylation.^{3,4}

A typical consequence of declining oxidative capacity in patients with mitochondrial myopathies is exercise intolerance, such that activities easily tolerated by healthy individuals provoke muscle fatigue. Exercise intolerance in turn encourages a sedentary lifestyle that may promote a further lowering of aerobic capacity through physical deconditioning. In healthy subjects, deconditioning lowers cardiovascular fitness and thus reduces the maximal capacity to deliver oxygen and blood-borne fuels to working muscle, and it reduces mitochondrial mass and the enzymatic machinery necessary for peak rates of oxidative phosphorylation.⁵

We postulated that if deconditioning contributes to exercise intolerance in mitochondrial myopathy patients, aerobic training might be a therapeutic interven-

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tion.⁶ Consistent with this hypothesis, we showed that short-term aerobic training improved exercise and oxidative capacity in patients with mtDNA mutations and those with suspected nuclear mutations.⁶ However, that study did not assess the mechanisms underlying observed improvements or the relative contribution of cardiovascular versus skeletal muscle adaptation. In particular, training effects on mitochondrial numbers and enzyme activities were not assessed. In patients with heteroplasmic mtDNA mutations, the effect of training on proliferation of mutant versus wild-type mitochondrial genomes could have important implications for the long term effects of training on cellular oxidative capacity.

To address these questions, we evaluated the effect of training on oxidative capacity and attempted to determine the contribution of cardiovascular versus muscle mitochondrial adaptations to improved exercise capacity. We utilized objective measures obtained from exercise testing, phosphorus magnetic resonance spectroscopy (³¹P MRS), and skeletal muscle biopsies to monitor training effects. To our knowledge, this study is the first attempt to assess the effects of a potentially effective therapeutic intervention on combined physiological, biochemical, and genetic indices of skeletal muscle oxidative capacity in patients with heteroplasmic mtDNA mutations.

Patients and Methods

The patients consisted of 6 women and 4 men (mean age: 39.3 ± 9.5 years) with heterogeneous clinical, biochemical,

and molecular features (Table 1). Each had progressive, and often lifelong, exercise intolerance where modest exercise typically provoked heaviness, weakness, and aching of active muscles, a rapid heartbeat, and a sense breathlessness. Other symptoms varied (see Table 1). Three patients had experienced bouts of recurrent myoglobinuria.

All patients had evidence of a mitochondrial myopathy by histological and biochemical criteria. In 9 patients, the molecular defect in mtDNA was identified; in 1 patient (Patient MB)⁷ the presence of abundant cytochrome oxidase (COX) negative/succinate dehydrogenase (SDH) positive fibers with severe cytochrome oxidase deficiency suggests an underlying mtDNA mutation. In 5 patients, the mtDNA mutation affected coding genes with selective deficiency of Complex I (SL), Complex III (EO and FB), or Complex IV (CW and RW). In 4 patients, large-scale deletions (TK, RJ) or tRNA mutations (PS, WB) resulted in deficiency of multiple respiratory chain complexes. Many of the patients in this study have previously been described^{8–12} (see Table 1). The study was approved by the institutional review boards of 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 their written consent to participate.

All patients underwent heart-rate monitored exercise on a stationary bicycle for a total of 50 sessions in 14 weeks (3–4 times per week). Exercise was performed at an intensity corresponding to 70 to 80% of maximal heart rate for 30 minutes per session for the first 7 weeks and 40 minutes per session for the last 7 weeks of training. Heart rates and exercise duration were continuously recorded on a Polar NV[®] chest band and watch, allowing for subsequent analysis using a Polar/PC computer interface.

Table 1. Clinical and Molecular Characteristics of Patients

Patient	Age/Sex	Clinical Symptoms	MtDNA Defect	Histochemistry Findings
MB	41/F	EI with prominent dyspnea, weakness	Unidentified ⁷	RRFs, COX ⁻
PS	39/F	EI, muscle pain	TRNAGlu (Anitori et al., in preparation)	RRFs, COX ⁻
WB	53/M	EI with prominent dyspnea, tachycardia	TRNATrp ¹¹	RRFs, COX ⁻
TK	32/F	CPEO, EI, weakness	4.3 kb deletion	RRFs, COX ⁻
RJ	32/F	CPEO, EI, mild weakness	5 kb deletion	RRFs, COX ⁻
CW	24/F	Muscle pain, dyspnea, myoglobinuria	15 bp microdeletion in COX III ¹⁰	RRFs, COX ⁻
RW	34/M	EI, dyspnea, myoglobinuria	COX I G5920A ¹²	RRFs, COX ⁻
SL	38/M	Severe EI, dyspnea	ND4 G11832A ⁹	RRFs, COX ⁺
EO	53/F	Severe EI with prominent dyspnea	Cyt b G14846A ⁸	RRFs, COX ⁺
FB	47/M	EI, myoglobinuria, weakness	Cyt b microdeletion of 8 aa ⁸	RRFs, COX ⁺

EI = exercise intolerance; CPEO = chronic progressive external ophthalmoplegia; Cyt b = cytochrome b; RRFs = ragged-red fibers; COX⁻ = cytochrome oxidase deficient fibers; COX⁺ = cytochrome oxidase positive fibers.

Pretraining Evaluation

MAXIMAL EXERCISE TESTING. All patients were familiarized with the experimental setup and studied on an electronically braked, pedal rate-independent cycle ergometer (MedGraphics 2000) on two separate occasions. Gas exchange and cardiac output were determined at rest and during an incremental workload test, which ended when the patient reached maximal heart rate ($220 - \text{age}$) or exhaustion. Peak oxygen uptake (VO_2) was determined at the final peak workload. Expired air was collected in Douglas bags for 120 seconds at rest and 60 seconds at various submaximal exercise workloads and final workload. The fractions of O_2 , CO_2 , and N_2 in each bag were analyzed with a Marquette 1100 Medical Gas Analyzer and ventilation was measured with a Tissot spirometer for calculation of VO_2 . Cardiac output (Q) was measured utilizing acetylene rebreathing in which the rate of disappearance of C_2H_2 from a rebreathing bag is proportional to pulmonary blood flow and Q.¹³ Determination of VO_2 and Q allows for the calculation of systemic arteriovenous oxygen difference, indicated by the Fick equation: $\text{VO}_2 = \text{Q} \times \text{systemic a-vO}_2 \text{ difference}$.

Another index, $\Delta\text{Q}/\Delta\text{VO}_2$ was used to assess the integrity of muscle oxidative metabolism, as this ratio normally reflects a tightly coupled relationship between O_2 delivery and uptake. In healthy individuals, Q increases approximately 5 liters for each liter of increase in O_2 consumption ($\Delta\text{Q}/\Delta\text{VO}_2 = 5$) from rest to maximal exercise, irrespective of age, sex, body weight, or level of conditioning.¹⁴ This relationship provides valuable clues as to whether a low VO_2 is attributable simply to deconditioning ($\Delta\text{Q}/\Delta\text{VO}_2 = 5$) or whether VO_2 is limited by impaired muscle oxidative phosphorylation ($\Delta\text{Q}/\Delta\text{VO}_2 \gg 5$).

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

³¹P MRS. Nine patients underwent MR examination before and after aerobic training. For technical reasons, the pretraining evaluation could not be performed in 1 patient (WB).

Acquisition: Spectroscopic studies of the vastus lateralis were performed on a Philips Gyroscan NT whole-body MRI/MRS system operating at 1.5 T (Philips Medical Systems, Best, The Netherlands). All individuals lay supine with feet first on a custom-built exercise apparatus in the magnet. A 6-cm diameter surface coil was positioned approximately 1/3 the distance between the lateral epicondyle and greater trochanter on the lateral thigh. Routine spin echo T1-weighted images were obtained to ensure proper surface coil placement. Magnetic field homogeneity was optimized over the sensitive volume of the surface coil by shimming on the proton signal of water in a rectangular volume approximately $20 \times 60 \times 60$ mm, positioned over the vastus lateralis with little or no contribution from fat or bone.

Resting free-induction decays (FIDs) from muscle were acquired using two separate sequences: (1) 64 signal averages

under partially saturated conditions, with an interpulse delay (TR) of 2 seconds; (2) 16 signal averages under fully relaxed conditions with a TR of 30 seconds. This allowed for saturation of resonances during the rapid pulse (TR = 2 seconds) acquisition during exercise and recovery. 150 FIDs, each consisting of 4 signal averages, were collected over 20 minutes (time resolution per spectrum = 8 seconds). In all sequences, a block detection pulse angle of 15 degrees was applied, and a decoupling factor of 0.8 Hz was used to increase signal intensity.

Exercise Protocol: Short-arc knee extension exercise was performed inside the magnet by rhythmically pulling against bungee cords of various tensions that were attached to a strap around the ankle. A strain gauge connected to the bungee cords allowed for force measurements. The exercise protocol consisted of contractions that were held for 3 to 5 seconds with 1 to 2 seconds of relaxation. The intensity corresponded to 30% of maximum voluntary contraction for the first minute followed by maximal intensity contractions until exhaustion. This nonischaemic exercise paradigm led to pronounced metabolic changes within 1 to 3 minutes of exercise, allowing a minimum of 15 minutes of acquisition during the recovery period.

Spectral Analysis and Quantitation: Calculation of metabolite concentrations (phosphocreatine [PCr], inorganic phosphate [Pi], adenosine diphosphate [ADP]), recovery kinetics, and intracellular pH was performed as previously reported.^{15,16} We used two derived indices of metabolic recovery from exercise to assess mitochondrial function in vivo, the initial rate of PCr resynthesis (V), and the apparent maximum rate of oxidative ATP synthesis (Q_{max}).¹⁷ To establish a range of normal values, 18 individuals (5 females, 13 males, mean age: 37 ± 11.2 years) underwent similar MR examination.

MUSCLE NEEDLE BIOPSY. A needle biopsy of one vastus lateralis muscle was performed to obtain on average a 150-mg sample. The sample was immediately divided into two sections for biochemical and genetic determinations, and frozen and stored in liquid nitrogen until the posttraining sample was obtained so that the samples could be analyzed concurrently. Controls for biochemical studies were patients undergoing muscle biopsy who ultimately were deemed to be free of neuromuscular disease.

Posttraining Evaluation

MAXIMAL EXERCISE TESTING. In each patient, posttraining cycle testing was performed at least twice on separate days. The incremental workload protocol was identical to each patient's initial evaluation until the final pretraining workload was reached. After this point, exercise intensity was increased until the same absolute pretraining heart rate was attained, and the corresponding maximal workload was noted. At submaximal intensities, heart rate and blood lactate levels were compared at the same absolute workload.

³¹P MRS. Because the spectra were continuously monitored, changes in PCr and Pi peak heights could be followed. For each individual, exercise was stopped at a PCr:Pi ratio similar

to that of the pretraining evaluation to ensure similar metabolic depletion for recovery kinetics analysis.

MUSCLE NEEDLE BIOPSY. A needle biopsy was performed on the opposite leg.

Biochemical measures: The activities of mitochondrial enzymes were determined by spectrophotometric analysis of freeze-thawed muscle extracts adapted from published methods.^{18–21} Rotenone-sensitive NADH ubiquinone reductase was assayed for the patient with Complex I defect, ubiquinone-cytochrome c reductase was assayed for 2 patients with defects in cytochrome b, cytochrome c oxidase activity was determined for 7 patients (2 large-scale deletions, 2 tRNA point mutations, point mutation in subunit I of Complex IV, microdeletion in subunit III of Complex IV, and 1 unidentified defect). Citrate synthase (nuclear encoded, index of mitochondrial volume) and succinate dehydrogenase (Complex II) activities were assayed in all patients.

Molecular genetic analysis: Mitochondrial DNA was extracted from skeletal muscle and the percentage of mutant relative to total mtDNA was quantified using polymerase chain reaction (PCR)^{9–12} and Southern blot analysis.²² The precision of these assays to quantify proportions of mtDNA in a muscle sample is $\pm 2\%$ in this laboratory.

STATISTICS. Differences between the patients and controls were analyzed using an unpaired *t* test. A paired *t* test was used to detect changes within the mitochondrial patient group for all outcome measures except $\Delta Q/\Delta VO_2$, in which case a nonparametric Wilcoxon signed rank test was applied. Statistical significance was determined at $p < 0.05$.

Results

All patients were able to tolerate exercise without complications and completed the required cycle exercise sessions.

Pretraining Evaluation

PHYSIOLOGIC MONITORING: CYCLE TEST. At rest, mean heart rate (78 ± 7 bpm), VO_2 (0.29 ± 0.07 L/min), cardiac output (4.8 ± 1.1 L/min), and a- VO_2 difference (5.8 ± 0.9 ml/dl) in patients were within normal limits.²³ Blood lactate levels at rest were elevated (>2.0 mM) in 5 patients, ranging from 2.3 to 8.2 mM. Peak VO_2 during maximal exercise was low in all patients, ranging from 5.6 to 22.1 ml/kg/min (Table 2), which contrasts to that of healthy sedentary men (39 ml/kg/min)²⁴ and women (32 ml/kg/min).⁷ The increase in cardiac output relative to oxygen uptake during exercise was exaggerated in all patients. In 9 of the patients, $\Delta Q/\Delta VO_2$ ranged from 7.9 to 30. In 1 patient (EO) $\Delta Q/\Delta VO_2$ was 59, ie, approximately tenfold greater than normal (see Table 2). In contrast to a threefold increase in systemic a- VO_2 difference from rest to maximal exercise observed in healthy sedentary individuals (peak value ≈ 15 ml/dl), this increase was attenuated in all patients (see Table 2), ranging from a severely low peak a- VO_2 difference of 3.1 ml/dl to 10.4 ml/dl.

PHYSIOLOGIC MONITORING: ³¹P MRS RECOVERY KINETICS. Both *V* and *Q*_{max} were low ($V_{\text{patient group}} = 10.0 \pm 4.7$, $V_{\text{control group}} = 30.2 \pm 8.3$ mM/min, $Q_{\text{max patient group}} = 12.7 \pm 6.1$, $Q_{\text{max control group}} = 38.5 \pm 9.9$, $p < 0.05$), with values of all patients outside the normal range ($V_{\text{normal}} = 21.1 - 48.9$, $Q_{\text{max normal}}: 24.5 - 59.6$ mM/min).

MUSCLE BIOPSY. Prior to training, patient levels of citrate synthase and SDH (16.8 ± 6.0 and 2.8 ± 0.8 $\mu\text{mol/min per g tissue}$, respectively) were high com-

Table 2. In Vivo Physiological Monitoring: Effects of Aerobic Training on Exercise Capacity and ³¹P MRS Indices of Metabolic Recovery

Pt	Work Capacity (watts)									Cardiac Output (L/min)									³¹ P MRS Indices								
	VO ₂ (ml/kg/min)			a-vO ₂ (ml/dl)			ΔQ/ΔVO ₂			V (mM/min)			Q _{max} (mM/min)														
	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff									
MB	20	30	50%	8.9	10.3	16%	9.4	7.0	-26%	5.9	8.5	44%	15.0	9.5	-37%	12.3	14.8	20%	18.1	19.5	8%						
PS	37.5	50	33%	10.3	14.3	39%	8.8	11.7	33%	9.4	9.1	-3%	7.9	10.2	29%	10.7	17.4	62%	16.9	20.9	23%						
WB	37.5	52.5	40%	10.2	11.9	17%	16.2	15.1	-7%	4.9	5.9	20%	26.9	18.2	-32%	np	np	np	np	np	np						
TK	60	70	17%	14.9	16.4	10%	14.0	14.0	0%	7.8	8.5	9%	12.9	10.4	-19%	13.8	18.0	30%	16.0	21.6	35%						
RJ	52.5	67.5	29%	18.7	21.7	16%	9.9	11.5	16%	8.6	9.4	9%	10.3	7.6	-26%	8.0	15.1	90%	10.4	20.0	93%						
CW	80	97.5	22%	22.1	23.9	8%	15.3	16.6	8%	10.4	10.6	2%	8.2	7.6	-7%	11.9	13.7	15%	13.7	16.9	24%						
RW	70	102	46%	16.3	21	29%	12.9	13.1	2%	7.7	12.0	56%	8.2	6.2	-24%	7.5	21.2	183%	8.8	23.1	161%						
SL	35	45	29%	9.6	13.3	39%	21.1	16.9	-20%	3.1	4.8	55%	29.4	20.4	-31%	5.7	9.2	61%	6.3	10.6	67%						
EO	15	13.3	-11%	5.6	6.3	13%	12.3	11.8	-4%	3.1	3.0	-3%	59	102	73% ^b	1.7	2.4	36%	2.0	2.7	36%						
FB	45	60	33%	11.4	13.9	22%	12.7	14.8	17%	8.5	8.8	4%	10.1	9.7	-4%	18.7	15.2	-19%	22.4	19.3	-14%						
Avg	45	59 ^a	30%	13	15.3 ^a	20%	13.3	13.3	0%	6.9	8.0 ^a	16%	14.2	11.3	-20%	10.0	14.1 ^a	40%	12.7	17.2 ^a	35%						
SD	21	27		5.1	5.5		3.7	2.9		2.6	2.7		16.1	29.1		4.7	5.5		6.0	6.5							

^aMean change is significant; $p < 0.05$.

^bValue not included in the average and SD that are presented in table.

Pt = patient; Diff = % difference between pre- and post-evaluation; np = not performed; VO_2 = peak oxygen uptake; a- VO_2 = arteriovenous oxygen difference; $\Delta Q/\Delta VO_2$ = change in cardiac output relative to change in oxygen consumption; *V* = initial rate of phosphocreatine resynthesis; *Q*_{max} = apparent maximum rate of ATP synthesis, SD = standard deviation.

pared to control values (10.1 ± 2.0 and 1.5 ± 0.3 , respectively). COX activity was low in all patients with deletions, tRNA, and COX subunit mutations (1.6 ± 0.7 versus normal mean of $3.0 \pm 1.4 \mu\text{mol}/\text{min per g}$, $p < 0.01$). Complex I activity was low in the patient with ND4 mutation (1.4 vs. normal $5.1 \pm 0.8 \mu\text{mol}/\text{min per g}$). Likewise, Complex III activity was low in both patients with mutations in cytochrome b (8.5 and 3.9 versus normal activity of $12.1 \pm 1.2 \mu\text{mol}/\text{min per g}$).

The proportion of mutant mtDNA ranged from 36% to 98% in the 9 patients with known mtDNA mutations.

Posttraining Evaluation

PHYSIOLOGIC MONITORING: CYCLE TEST. After training, there was a small but significant decrease in heart rate at rest (78 ± 7 to 74 ± 8 bpm, $p < .05$). There was no change in resting VO_2 , cardiac output, or a-v O_2 difference. Mean blood lactate was unchanged (pre = 2.9 ± 1.9 , post = 3.1 ± 2.5 mmol/L), although 3 of 5 patients with elevated levels demonstrated 25% decreases after training.

Heart rates were lower in each patient at comparable absolute workloads after training. There was an average decrease of 9 bpm ($p < 0.01$) at the workload corresponding to 70% to 80% of pretraining maximal workload. Blood lactate was on average 1.5 mM lower ($p < 0.01$) at exercise corresponding to the pretraining maximum.

Significant increases ($p < 0.01$) in peak work capac-

ity (from 45.3 ± 21 to 59 ± 27 watts) and VO_2 (from 0.90 ± 0.40 to 1.10 ± 0.40 L/min) occurred after training. There was no consistent effect on cardiac output, but peak a-v O_2 difference was higher ($p < 0.05$), suggesting improved ability of muscle to extract available O_2 . Mean $\Delta\text{Q}/\Delta\text{VO}_2$ decreased 20%, suggesting a trend toward normalizing the mismatch between O_2 delivery and uptake (see Table 2). This ratio increased posttraining in 2 patients (PS = 7.9 to 10.2, EO = 59 to 102).

PHYSIOLOGIC MONITORING: ^{31}P MRS RECOVERY KINETICS. There was a significant increase ($p < 0.05$) in V (40%) and Q_{max} (35%) suggesting improved mitochondrial oxidative capacity in most patients (see Table 2). Marked improvements were detected in 5 patients, whereas in the 2 patients with Complex III defects, training effects were small, with decreases occurring in 1 patient (FB).

MUSCLE BIOPSY ANALYSIS. There was an almost 50% increase in CS activity (16.8 ± 6.0 to 25.1 ± 7.6) and a more than 40% increase in SDH activity (2.8 ± 0.8 to 4.0 ± 1.5 ; $p < 0.005$), indicating that training induced substantial mitochondrial proliferation (Table 3).

The effects of training on the activities of deficient enzymes were more variable (Table 4). In the COX-deficient patients, mean COX activity was 25% higher after training ($p < 0.05$), increasing in 6 of 7 patients. In the patient with the ND4 defect, training resulted

Table 3. Biochemical Analysis of Skeletal Muscle Mitochondrial Volume Before and After Endurance Training in Patients with Defects in mtDNA^a

Patient	CS ^b			SDH ^c		
	Pre	Post	Diff	Pre	Post	Diff
MB	16.6	25.1	51%	3.1	5.2	67%
PS	10.3	17.7	72%	1.4	1.8	29%
WB	27.4	31.5	15%	3.1	3.9	26%
TK	19.1	23.8	25%	3.3	4.4	33%
RJ	22.4	32.6	46%	2.9	4.3	49%
CW	8.5	12.7	49%	1.5	1.8	20%
RW	16.0	30.8	93%	2.6	3.7	42%
SL	13.6	35.6	162%	4.1	7.1	73%
EO	22.2	24.1	9%	3.0	3.3	10%
FB	12.5	17.1	37%	3.6	4.2	17%
Avg	16.8 ^d	25.1 ^e	49%	2.8 ^d	4.0 ^e	43%
SD	6.0	7.6		0.8	1.5	

^aEnzyme activities are obtained from muscle homogenates, expressed in $\mu\text{mol}/\text{min per g}$ tissue.

^bNormal control range: 6.1–13.2; Avg: 10.1; SD: 2.0; n = 15.

^cNormal control range: 0.9–1.9; Avg: 1.5; SD: 0.3; n = 15.

^dMean of patient group is significantly different from normal controls.

^eMean change is significantly different, $p < 0.05$.

SD = standard deviation.

Table 4. Biochemical Analysis of Mitochondrial Respiratory Chain Function and Molecular Genetic Analysis of the Relative Proportion of Mutant mtDNA Before and After Endurance Training in Patients with Defects in mtDNA^a

Pt	Respiratory Chain Complex Activity									% Mutant mtDNA		
	Complex IV (COX) ^b ($\mu\text{mol}/\text{min}/\text{g}$)			Complex I ^c ($\mu\text{mol}/\text{min}/\text{g}$)			Complex III ^d ($\mu\text{mol}/\text{min}/\text{g}$)			(% of total mtDNA)		
	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff	Pre	Post	Diff
MB	0.5	0.7	40%							nq	nq	
PS	2.7	3.6	33%							67	76	9
WB	1.0	1.3	30%							95	95	0
TK	2.0	1.8	-10%							78	89	10
RJ	1.6	1.9	19%							78	79	1
CW	1.3	2.3	77%							36	41	5
RW	1.9	2.2	16%							68	74	6
SL				1.4	1.9	36%				31	43	12
EO							3.9	3.1	-21%	>98	>98	
FB							8.5	7.0	-18%	5	55	50
										36 ^e	73	37
Avg	1.6 ^f	2.0 ^g	25%							65	74 ^g	9
SD	0.7	0.9								25.5	20.5	

^aEnzyme activities are obtained from muscle homogenates, expressed in $\mu\text{mol}/\text{min}$ per g tissue.

^bNormal control range: 1.3–5.0; Avg: 3.0; SD: 1.4; n = 15.

^cNormal control range: 4.1–8.0; Avg: 5.1; SD: 0.8; n = 9.

^dNormal control range: 10.1–14.9; Avg: 12.1; SD: 1.2; n = 9.

^eA second sample of muscle tissue was tested in this patient and used for statistical calculations.

^fMean of patient group is significantly different from normal controls.

^gMean change is significantly different, $p < 0.05$.

SD = standard deviation; nq = % heteroplasmy is nonquantifiable in this patient.

in an increase in Complex I activity (36%). In contrast, Complex III activity decreased by approximately 20% in the two cytochrome b mutation patients.

Enzyme activities were normalized to mitochondrial volume, using CS. In patients with defects affecting Complex IV, COX relative to CS activity decreased (pretraining $0.11 \pm .08$, posttraining $0.09 \pm .07$) in all but 2 patients (WB and CW). Complex I decreased relative to CS in the patient with the ND4 mutation. Similarly, in both patients with cytochrome b defects, Complex III relative to CS activity fell.

Training effects on the proportion of mutant mtDNA were variable (see Table 4). Overall, there was a significant increase in the percent mutant relative to wild-type mtDNA ($p < 0.05$). In 3 patients, there was no detectable change although in 1 of these patients the proportion of mutant mtDNA before training was >98%. In 6 patients, the proportion of mutant mtDNA increased after training. In 2 patients the increase was approximately 5% (CW = 5%, RW = 6%) and in 3 it was approximately 10% (PS = 9%, TK = 10%, SL = 12%). In 1 patient (FB), the posttraining increase in mutant mtDNA was 50%. Reanalysis of another tissue sample confirmed an increase, although the magnitude was less (37%).

Discussion

The major findings of this study were that: (1) exercise training in patients with heteroplasmic mtDNA mutations improved work and oxidative capacity, confirming and extending our previous results; (2) the physiological basis of increased oxidative capacity in these patients was enhanced skeletal muscle oxidative phosphorylation; (3) the cellular basis of improved oxygen utilization was an increase in mitochondrial biogenesis that increased levels of deficient respiratory chain enzymes; and (4) this increase in respiratory chain activity and functional capacity occurred despite an increase in the proportion of mutant mtDNA.

Each of our patients had limited exercise capacity attributable to subnormal muscle oxidative capacity. The level of oxidative impairment ranged from a peak oxygen uptake of $5.6 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, a value barely above resting levels, in a patient with a virtually homoplasmic cytochrome b mutation, to $22.1 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, a level approximately 2/3 of the average for healthy subjects, in a patient with a COX III microdeletion. Consistent with our previous findings, aerobic training significantly increased work and oxidative capacity, lowered resting heart rate, and reduced heart rate and blood lactate levels at the same submaximal exercise workloads.

The absolute increase in oxidative capacity was only 2.3 ml · kg⁻¹ · min⁻¹ on average compared to approximately 7 ml · kg⁻¹ · min⁻¹ in healthy individuals undergoing a similar training program.⁵ However, this represented an approximately 20% improvement over pretraining levels, a percentage increase that is comparable to the effects of training of similar duration and relative intensity in healthy individuals.⁵

In healthy subjects, peak cardiac output correlates directly with peak VO₂ and the major physiological adaptation to aerobic training is an increase in maximal cardiac output.⁵ Training related increases in peak a-vO₂ difference are typically smaller.²⁵ In patients with mitochondrial myopathies, the major physiological limitation to O₂ utilization in exercise is a blunted ability to increase systemic a-vO₂ difference demonstrated in our patients as a peak systemic a-vO₂ difference that is approximately half that of healthy subjects. In contrast, cardiac output during exercise was exaggerated relative to O₂ utilization ($\Delta Q/\Delta VO_2$ was approximately threefold higher than normal) and did not correlate with peak O₂ uptake. Previous studies have indicated that this exaggerated or “hyperkinetic” circulatory response to exercise is a consistent feature of severe muscle oxidative defects and is likely attributable to the activation of neural reflexes via metaboreceptors in working muscle that are responsive to metabolites that reflect muscle oxidative capacity.²⁶

We found that the physiological adaptation responsible for improved aerobic capacity after training in mitochondrial myopathy patients was an increase in peak systemic a-vO₂ difference. Peak systemic a-vO₂ difference rose 16% from 6.9 ± 2.6 to 8.0 ± 2.7 ml/dl. In contrast to the training response in healthy subjects, there was no consistent increase in peak cardiac output with training in our patients. As a result, there was a trend toward normalization of the hyperkinetic circulatory response to exercise. These results are consistent with a training-induced increase in peak rates of muscle oxidative phosphorylation.

³¹P MRS of the trained vastus lateralis confirmed this physiological adaptation. V and Q_{max} have been used as in vivo correlates of mitochondrial oxidative capacity, and are sensitive in detecting mitochondrial dysfunction.^{27–30} In our study, V and Q_{max} were low in all patients. After training, both indices were increased in most patients, indicating improved mitochondrial oxidative phosphorylation.

Muscle biopsy enzyme analysis was also consistent with a training-induced increase in oxidative capacity. Training induced mitochondrial proliferation and increased activities of the deficient respiratory chain enzymes in skeletal muscle in most patients. Despite high baseline levels of CS and SDH, our patients demonstrated a further 40% to 50% increase with training, consistent with a proportional increase in mitochon-

drial volume, an increase that is about 10% to 20% greater than that observed in sedentary healthy subjects undergoing similar training.^{5,31}

In patients with mtDNA deletions, tRNA mutations, and coding region mutations involving subunits of Complex I and IV, the activity of deficient respiratory chain enzymes increased. This implies that training achieved an increase in the levels of wild-type mtDNA necessary for the synthesis of increased levels of functional respiratory chain complexes.

However, increases in mitochondrial volume were about twofold higher than the increase in enzyme activity affected by the mutation. The increase in mitochondrial biogenesis in a normal training response preserves the stoichiometry of oxidative enzymes.³² These biochemical results suggest that aerobic training in patients with heteroplasmic mtDNA mutations provokes a disproportionate increase in mutant relative to wild-type mtDNA genomes. This conclusion from biochemical determinations is supported by direct genetic analysis that demonstrated an increase in the proportion of mutant mtDNA in 6 of 9 patients.

Heterogeneous distribution of mutant mtDNA implies that some of the variation in pre- and posttraining muscle samples may be the result of random sampling variations, and repeat analysis using different muscle samples from pre- and posttraining biopsies demonstrated small differences in the proportion of mutant mtDNA in 1 patient (see Table 4). If random, regional variations in the percentage of mutant versus wild-type genomes were a major contributor to our results, decreases as well as increases in the percentage of mutant genomes would be expected in the posttraining biopsies. The fact that the proportion of wild-type mtDNA did not increase in a single case strengthens the notion that a preferential increase in mutant mtDNA occurred with training.

We postulate that the heterogeneous distribution of mtDNA mutations within skeletal muscle fibers plays a role in this apparent preferential proliferation of mutant mtDNA genomes with training. Mitochondrial aggregates in RRFs consist primarily of respiratory incompetent mitochondria containing predominantly mutant mtDNA. These areas are interspersed with fiber segments containing functional mitochondria and more normal levels of wild-type mtDNA.^{33–35}

We propose that the stimulus for mitochondrial proliferation produced by aerobic training in patients with heteroplasmic mtDNA mutations is magnified within areas of muscle that have more compromised oxidative phosphorylation caused by larger percentages of mutant mtDNA. This would in turn promote preferential mitochondrial biogenesis in muscle fiber domains containing high concentrations of mutant mtDNA. The differential segregation and proliferation of mutant mtDNA within muscle fibers could not be assessed in

this study. However, future single-fiber analysis of pre- and posttrained muscle to determine the distribution of mutant and wild-type genomes in unaffected and affected muscle fiber segments would be of great interest. The mechanism by which aerobic training improves oxidative phosphorylation and increases levels of deficient oxidative enzymes despite a trend toward increasing proportions of mutant genomes likely involves a concomitant but lesser increase in absolute numbers of functional, wild-type genomes.

Our data suggest that individual variability conditions the training response in different patients or with respect to specific mutations of mtDNA. This is not surprising given the general heterogeneity of mitochondrial myopathies and that there have been no well-established correlations between the mtDNA mutation (type, size, location), degree of heteroplasmy, or biochemical effect and clinical severity.³⁶ This suggests that other unidentified environmental or genetic factors may play a contributing role in phenotypic expression and may also be involved in the aerobic training response. The degree to which an individual respiratory chain complex is compromised and the level of control that enzyme exerts on mitochondrial flux may also play a role, particularly given that small changes in deficient respiratory enzyme activity have substantial effects on metabolic flux *in vitro*.^{37,38} Last, segregation of a mtDNA mutation throughout the lifespan of an individual can modify the proportions of mutant and wild-type mtDNA dramatically and appears to relate, in part, to the type of molecular defect. In certain mtDNA defects, the ratio of mutant to wild-type mtDNA has been shown to increase with time (deletions,³⁹ certain point mutations (tRNA^{Leu(CUN)35}) while in others the ratio does not appear to change substantially (MERRF tRNA⁸³⁴⁴).^{40,41} It is possible that mutation-specific selection factors are responsible for the negative training response observed in both patients with Complex III mutations.

It is also possible that patients with oxidative defects attributable to nuclear mutations may experience greater relative benefit from aerobic training than patients with heteroplasmic mtDNA mutations, as was suggested in our earlier training study.⁶ Training-induced proliferation of mitochondria harboring partial nuclear-encoded defects, which are uniformly distributed within muscle, may be expected to result in proportional increases in mitochondrial volume and deficient oxidative enzyme activity.

In conclusion, our data demonstrate that aerobic conditioning in patients with mitochondrial myopathies caused by mtDNA mutations can achieve improved exercise and oxidative capacity, attributable to increases in the activity of the respiratory chain. However, in most patients, the beneficial improvement occurs at the cost of expanding the population of mutant

mtDNA. Although there was no worsening of symptoms, this study was short term, and whether aerobic training exacerbates accumulation of mutant mtDNA with time is not presently known. We believe that aerobic training as a general approach to therapy for patients with heteroplasmic mtDNA mutations should be approached with caution. Further studies assessing the long-term implications of training-induced increases in mutant relative to wild-type mtDNA *in vivo* (despite positive physiological and biochemical findings) and factors predisposing patients to respond favorably or unfavorably are necessary to clarify the utility of aerobic training as therapy of mitochondrial myopathy.

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