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【 ORIGINAL ARTICLE 】

Diagnostic Values of Venous Peak Lactate, Lactate-to-pyruvate Ratio, and Fold Increase in Lactate from Baseline in Aerobic Exercise Tests in Patients with Mitochondrial Diseases

Masanori Kurihara[1], Yusuke Sugiyama[1], Masaki Tanaka[1,2], Kenichiro Sato[1], Akihiko Mitsutake[1], Hiroyuki Ishiura[1], Akatsuki Kubota[1], Kaori Sakuishi[1], Toshihiro Hayashi[1], Atsushi Iwata[1], Jun Shimizu[1,3], Kei Murayama[4], Shoji Tsuji[1,2,5] and Tatsushi Toda[1]

Abstract:

Objective Although aerobic exercise tests on cycle ergometry have long been used for initial assessments of cases of suspected mitochondrial disease, the test parameters in patients with final diagnoses of other diseases via the widely used 15 W for 15 minutes exercise protocol have not been fully characterized.

Methods We retrospectively reviewed all patients who underwent the test at our institution. We classified the patients with genetic diagnoses or those who met previously reported clinical criteria as having mitochondrial diseases and those with a final diagnosis of another disease as having other diseases. Results were available from 6 patients with mitochondrial disease and 15 with other diseases.

Results During the test, elevated venous peak lactate above the upper normal limit of healthy controls at rest [19.2 mg/dL (2.13 mM)] was observed in 3 patients with mitochondrial diseases (50.0%) and 5 with other diseases (33.3%). In the group of patients with elevated venous peak lactate, a lactate-to-pyruvate ratio of >20 was observed in all 3 patients with mitochondrial disease but in only 1 of the 5 with other diseases. More than a 2-fold increase in venous lactate from baseline was observed in 4 patients with mitochondrial disease (66.7%) and 1 with another disease (6.7%).

Conclusion Elevated venous peak lactate levels were observed in patients with final diagnoses of other diseases, even under a low 15-minute workload at 15 W. The lactate-to-pyruvate ratio and increase in lactate level from baseline may add diagnostic value to venous peak lactate levels alone.

Key words: mitochondria, ergometry, Ergometer, lactate, lactic acid, literature review

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Introduction

Mitochondrial diseases are among the most common inherited metabolic disorders, and atypical cases are often difficult to diagnose because of broad clinical presentations and heterogeneities in etiology (1, 2). Simple initial testing is useful before further diagnostic testing, such as tissue biopsies. However, although new biomarkers (e.g., FGF-21 and GDF-15) have been shown to be useful in screening for mitochondrial diseases (3-5), the measurement of these markers remains difficult in daily clinical practice.

[1]Department of Neurology, Graduate School of Medicine, The University of Tokyo, Japan, [2]Institute of Medical Genomics, International University of Health and Welfare, Japan, [3]Department of Physical Therapy, School of Health Science, Tokyo University of Technology, Japan, [4]Department of Metabolism, Chiba Children's Hospital, Japan and [5]Department of Molecular Neurology, Graduate School of Medicine, The University of Tokyo, Japan

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Correspondence to Dr. Masanori Kurihara, mkurihara-tky@umin.ac.jp

Aerobic exercise tests on cycle ergometry have long been performed as an initial diagnostic procedure for mitochondrial disease, and their utility has been reported in many studies (6-19). Because of defects in mitochondrial respiratory chain enzymes, pyruvate accumulates, and the intramitochondrial NADH/NAD ratio increases, which leads to an increase in venous lactate levels; aerobic exercise sensitively reflects this defect in mitochondrial oxidative phosphorylation (7, 9). Although some studies have implemented exercise protocols with incremental workload, studies using a constant workload are easier to perform and may even have greater diagnostic value (20). However, the interpretation of results obtained in clinical practice can be difficult, as in previous studies, a wide variety of exercise protocols and cut-off values of venous lactate have been applied; furthermore, patients with other neurological and muscular diseases may show abnormal results during such tests. Although an exercise protocol comprising a constant workload of 15 W for 15 minutes has been implemented widely across Japan and demonstrated abnormal results in patients with mitochondrial diseases compared with those obtained from healthy individuals (21-25), the results in patients with other neurological and muscular diseases have not been fully characterized. In addition, although reference values of several parameters, including the venous peak lactate, venous lactate-to-pyruvate ratio (L/P), and fold increase in lactate from baseline, have been obtained, the diagnostic values of these parameters have not yet been fully evaluated.

We herein report the values of these parameters in patients with mitochondrial disease as well as in those with other diseases and investigate the diagnostic value of the aerobic exercise test on cycle ergometry under a constant workload of 15 W for 15 minutes as an initial diagnostic test for patients with suspected mitochondrial disease.

Materials and Methods

Subjects

We retrospectively reviewed all patients who underwent aerobic exercise tests on cycle ergometry as part of a diagnostic evaluation for patients with suspected mitochondrial disease in our department (Department of Neurology, the University of Tokyo Hospital, Tokyo, Japan) from January 2001 to March 2017. This retrospective study was approved by the institutional review board of the University of Tokyo (2399). Genetic analyses of patients were approved by the institutional review board of the University of Tokyo (G 1396).

At the time of the chart review, patients with a diagnosis of mitochondrial disease by genetic testing [pathogenic mitochondrial or nuclear DNA mutations listed in MITOMAP (https://www.mitomap.org/MITOMAP) confirmed via the Sanger sequencing or detection of large mitochondrial DNA deletions confirmed via the Southern blot hybridization analysis] or those who were classified as definite (i.e., fulfilled two major criteria or one major and two minor criteria) according to previously reported diagnostic criteria for respiratory chain disorders using clinical information, muscle histology, enzyme activity, and genetic testing (26, 27) (Table 1) were enrolled in the study as patients with mitochondrial diseases. Patients with a diagnosis of other diseases at the time of the chart review were classified as patients with other diseases (Table 2 lists the diagnostic basis for each patient).

Aerobic exercise test protocol

A standardized protocol was used, comprising 15 minutes of exercise with a constant workload of 15 W using cycle ergometry (21-25) (StrengthErgo240; Mitsubishi Electric Engineering, Tokyo, Japan). An intravenous heparinized catheter was inserted into a vein on either side of the upper extremities. The exercise was started after 30 minutes of rest. Venous blood was obtained immediately before starting the exercise and at 10, 15, 20, 30, and 60 minutes after the start of the test.

Blood and cerebrospinal fluid analyses

Blood and cerebrospinal fluid (CSF) were each mixed immediately with 1 mol/L perchloric acid for protein precipitation. Lactate and pyruvate were measured using the enzymatic method using lactate oxidase and pyruvate oxidase, respectively (Determiner LA and Determiner PA; Kyowa Medex, Tokyo, Japan). The reference range was predetermined as 4.0-19.2 mg/dL (0.44-2.13 mM) for lactate and 0.3-0.9 mg/dL (0.03-0.10 mM) for pyruvate by the manufacturer based on the results obtained from 164 healthy individuals at rest. L/P was calculated by dividing the lactate concentration (mg/dL) by the pyruvate concentration (mg/dL) at each time point. The fold increase in lactate from baseline during the aerobic exercise test was calculated by dividing the lactate concentration by the lactate concentration at rest.

Statistical analyses

All statistical analyses were conducted using the software programs R version 3.4.1 and EZR, a graphical interface (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (28). Categorical variables are presented as percentages, and differences between groups were evaluated using Fisher's exact test. Because a normal distribution was not confirmed, nominal variables are presented as medians [interquartile ranges (IQR)], and differences between groups were evaluated using the Mann-Whitney U-test. P values of <0.05 were considered statistically significant. Because some patients had missing data at some time points, values with the highest venous lactate concentration (peak value) were used to develop criteria to minimize sample size reduction.

Table 1. Clinical Information and Representative Laboratory Test Results of Patients with Mitochondrial Diseases.

	Age/ Sex	Diagnosis	Venous blood				Fold increase in LA	CSF		Known pathogenic mutation	Diagnostic criteria for respiratory chain disorders (26, 27)								
			At rest		At peak LA						Classification	Major criteria				Minor criteria				
			LA mg/dL	L/P	LA mg/dL	L/P		LA mg/dL	L/P			Clinical	Histology	Enzymology	Functional	Clinical	Histology	Enzymology	Functional	Genetic	Metabolic
1	34/ M	MERRF	17.4	13.4	78.1	26.9	4.49	25.8	19.8	m.8344A>G	Definite	Yes	n/a	n/a	n/a	n/a	n/a	n/a	n/a	Yes	Yes
2	53/ M	Adult Leigh disease	16.7	18.6	108.5	37.4	6.5	46.6	23.3	None	Definite	Yes	Yes	Yes	n/a	No	n/a	n/a	n/a	Yes	Yes
3	47/ M	KSS	13.5	27	28.3	31.4	2.1	14.7	18.4	Large deletion	Definite	Yes	Yes	Yes	n/a	Yes	n/a	n/a	n/a	n/a	Yes
4	46/ M	NARP	5.3	10.6	16.7	15.2	3.15	24.4	22.2	m.8993T>G	Definite	Yes	n/a	n/a	n/a	Yes	n/a	n/a	n/a	n/a	Yes
5	71/ M	Mitochondrial disease*	15.6	19.5	17.8	19.8	1.14	36.5	21.5	m.3243A>G	Definite	Yes	No	No	n/a	Yes	n/a	Yes	n/a	n/a	Yes
6	22/ M	Leber-plus disease	10.4	9.5	13.2	13.2	1.27	17.6	19.6	m.14487T>C	Definite	Yes	n/a	n/a	n/a	Yes	n/a	n/a	n/a	n/a	Yes

M: male, F: female, MERRF: myoclonus epilepsy associated with ragged-red fibers, KSS: Kearns-Sayre syndrome, NARP: neuropathy, ataxia, and retinitis pigmentosa, LA: lactate, L/P: lactate-to-pyruvate ratio, CSF: cerebrospinal fluid, n/a: not available

*symptoms were hearing impairment, ataxia, and chorea

Results

Patient demographic information

Test results were available from 6 patients classified as having mitochondrial diseases (Table 1) and 15 patients classified as having other diseases (Table 2). Clinical information on patient 9 has been reported previously (29).

Five of the six patients with mitochondrial diseases had previously described pathogenic variants in their mitochondrial DNA, and one patient with clinical presentations compatible with adult Leigh disease had positive findings on two tests, supporting the presence of mitochondrial disease (muscle histology and decreased respiratory chain enzyme activity). The clinical subtypes were all different and included myoclonus epilepsy associated with ragged-red fibers (MERRF), adult Leigh disease, Kearns-Sayre syndrome (KSS), neuropathy, ataxia, and retinitis pigmentosa (NARP), mitochondrial disease with hearing impairment, ataxia, chorea [m.3243A>G], and Leber-plus disease (Table 1). These six patients were classified as definite according to previously reported diagnostic criteria for respiratory chain disorders (26, 27) (Table 1). Patients with other diseases had various final diagnoses, which included other genetic, autoimmune, neoplastic, and vascular diseases (Table 2).

Baseline characteristics (age, sex, height, body weight, body mass index, and muscle weakness) were similar across the two groups (Table 3). The CSF lactate and pyruvate concentrations were available in all patients with mitochondrial diseases and in 10 of the 15 patients with other diseases.

Results of the aerobic exercise test on cycle ergometry

The Figure plots the time courses of venous lactate concentration of each patient during the study. Individual venous lactate and pyruvate concentration values at each time point are described with baseline characteristics in Supplementary material. The median and interquartile ranges of venous lactate or L/P at rest and peak and fold increase in lactate from baseline are summarized in Table 4.

The median venous peak lactate value was 23.1 (IQR 17.0-65.7) mg/dL in patients with mitochondrial diseases and 15.9 (IQR 13.5-20.6) mg/dL in patients with other diseases. Three of the 6 patients with mitochondrial diseases (50.0%) showed an increase in the venous peak lactate concentration during exercise above the upper normal limit (UNL) at rest provided by the manufacturer [19.2 mg/dL (2.13 mM); patients 1, 2, and 3 in Table 1]. The remaining three patients with mitochondrial diseases showed venous lactate below the UNL throughout the test (patients 4, 5, and 6 in Table 1). Five of the 15 patients with other diseases (33.3%) also showed a venous peak lactate concentration above the UNL during the test (patients 7, 9, 10, 17, and 21 in Table 2). A venous peak lactate value above 40.0 mg/dL was observed only in patients with mitochondrial diseases

Table 2. Clinical Information and Representative Laboratory Test Results of Patients with a Final Diagnosis of Other Diseases.

	Age/ Sex	Diagnosis	Diagnostic basis	Venous blood at rest		Venous blood at peak LA		Fold increase in LA	CSF	
				LA (mg/dL)	L/P	LA (mg/dL)	L/P		LA (mg/dL)	L/P
7	73/F	OPMD	Genetic*	11.0	10.0	22.8	17.5	2.07	15.8	14.4
8	76/F	OPMD	Genetic*	8.1	16.2	10.9	18.2	1.35	14.4	18.0
9	16/M	Krabbe disease	Genetic*	17.7	17.7	19.4	21.6	1.10	n/a	n/a
10	57/M	SBMA	Genetic*	13.9	10.7	27.3	17.1	1.96	22.5	15.0
11	34/M	Multiple sclerosis	Clinical	14.4	14.4	17.3	17.3	1.20	16.1	14.6
12	22/M	Multiple sclerosis	Clinical	16.4	27.3	15.9	31.8	0.97	11.6	23.2
13	27/M	Rasmussen encephalitis	Clinical	10.7	8.9	12.0	15.0	1.12	14.5	20.7
14	41/M	Autoimmune encephalitis	Clinical	10.8	10.8	17.5	12.5	1.62	n/a	n/a
15	16/F	SCA7	Genetic*	9.6	19.2	13.9	15.4	1.45	n/a	n/a
16	30/M	SCA19	Genetic*	10.3	20.6	13.0	26.0	1.26	13.7	17.1
17	41/F	Dopa-responsive dystonia	Clinical**	12.1	13.4	21.8	16.8	1.80	19.2	16.0
18	41/M	HDLS	Genetic*	13.1	11.9	14.3	17.9	1.09	21.7	14.5
19	47/M	CNS lymphoma	Pathological	11.5	16.4	15.3	21.9	1.33	29.1	18.2
20	54/M	Ischemic stroke	Clinical	9.4	23.5	11.2	22.4	1.19	n/a	n/a
21	70/M	Peripheral artery disease	Clinical	16.9	12.1	31.2	13.6	1.85	n/a	n/a

M: male, F: female, OPMD: oculopharyngeal muscular dystrophy, SBMA: spinal and bulbar muscular atrophy, SCA: spinocerebellar ataxia, HDLS: hereditary diffuse leukoencephalopathy with spheroids, CNS: central nervous system, LA: lactate, L/P: lactate-to-pyruvate ratio, CSF: cerebrospinal fluid, n/a: not available

*mutational analysis of causative genes confirmed pathogenic variants

**diagnosed based on treatment response.

Table 3. Baseline Characteristics of the Two Groups.

		Mitochondrial disease (n=6)	Other diseases (n=15)
Age (years)		46.5 (37.0-51.5)	41.0 (28.5-55.5)
Male (%)		100	73
Height (cm)		172 (167-173)	169 (163-172)
Body weight (kg)		55.0 (50.5-61.8)	57.0 (50.4-69.0)
Body mass index		19.8 (18.8-21.0)	20.0 (19.2-24.2)
MMT score (0-5)	Iliopsoas	4 (4.0-4.75)	5 (4.5-5.0)
	Quadriceps	5 (5.0-5.0)	5 (4.5-5.0)
	Hamstrings	4 (4.0-4.75)	5 (4.5-5.0)

Categorical variables are presented as percentages, and nominal variables are presented as medians (interquartile ranges). MMT: manual muscle testing

(patients 1 and 2 in Table 1).

The median venous L/P at the peak lactate point was 23.4 (IQR 16.4-30.3) in patients with mitochondrial diseases and 17.5 (16.1-21.8) in patients with other diseases. An L/P >20 at peak venous lactate was observed in 3 patients with mitochondrial diseases (50.0%) and 5 patients with other diseases (33.3%). Of the 8 patients with venous peak lactate values>UNL, an L/P>20 was observed in all 3 patients with mitochondrial diseases (patients 1, 2, and 3 in Table 1) and in only 1 of the 5 patients with other diseases (patient 9 in Table 2).

The median fold increase in venous lactate from baseline was 2.62 (IQR 1.48-4.15) in patients with mitochondrial diseases and 1.33 (1.16-1.71) in patients with other diseases, showing a significant increase with mitochondrial disease ($p=0.045$). An over 2-fold increase in venous lactate from baseline was observed in 4 (66.7%) of the patients with mitochondrial diseases (patients 1-4 in Table 1) and 1 (6.7%) of the patients with other diseases (patient 7 in Table 2). The venous peak lactate value in patient 4 (a 46-year-old man with NARP) was below the UNL (16.7 mg/dL) but showed a 3.15-fold increase from the venous rest lactate value (5.3 mg/dL).

Discussion

In the present study, we retrospectively reviewed venous peak lactate, L/P, and fold increase in lactate from baseline obtained from all patients who underwent aerobic exercise tests on cycle ergometry under a constant workload of 15 W for 15 minutes performed as an initial test for a suspected mitochondrial disease.

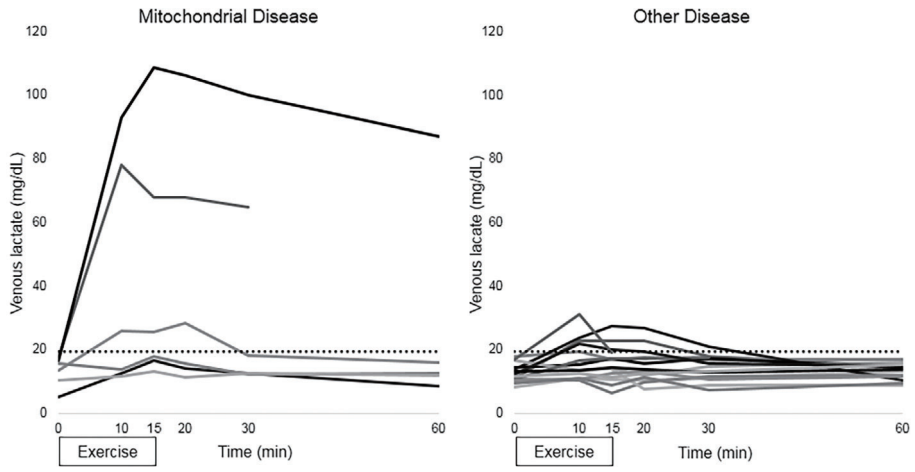

Figure. **Time course of venous lactate values in each patient during the study. The upper normal limit of the rest venous lactate value provided by the manufacturer (19.2 mg/dL) is shown as a dotted line.**

Table 4. **Test Parameters of the Two Groups.**

	Mitochondrial disease (n=6)	Other diseases (n=15)	p value
Venous lactate at rest	14.6 (11.2-16.4)	11.5 (10.5-14.2)	0.57
Venous L/P at rest	16.0 (11.3-19.3)	13.4 (11.4-18.5)	0.94
Venous peak lactate (mg/dL)	23.1 (17.0-65.7)	15.9 (13.5-20.6)	0.11
Venous L/P at peak lactate	23.4 (16.4-30.3)	17.5 (16.1-21.8)	0.38
Fold increase in venous lactate from baseline	2.62 (1.48-4.15)	1.33 (1.16-1.71)	0.045*

Nominal variables are presented as medians (interquartile ranges). L/P: lactate-to-pyruvate ratio, *p<0.05

Elevated venous peak lactate above the UNL [19.2 mg/dL (2.13 mM)] was observed in half of the patients with mitochondrial diseases. However, one-third of the patients with other diseases also showed mildly elevated venous peak lactate levels, even under a relatively low workload of 15 W for 15 minutes. Previous studies using other exercise protocols with higher workloads have suggested that patients with nonmitochondrial neurological and muscular diseases, such as amyotrophic lateral sclerosis, hereditary spastic paraplegia, Huntington's disease, myotonic dystrophy, and oculopharyngeal muscular dystrophy (OPMD), may show blood lactate elevation (13-17, 19, 20, 30-32). In our study using a workload of 15 W for 15 minutes, mild blood lactate elevation above the UNL was observed in patients with a final diagnosis of OPMD, as previously described in a study using a higher workload (13), and also in patients with final diagnoses of Krabbe disease, spinal and bulbar muscular atrophy, and dopa-responsive dystonia. Although previous research has suggested that healthy individuals do not show elevated lactate levels during aerobic exercise tests using such low workloads, our results suggest that patients with other diseases can exhibit mildly elevated venous peak lactate values.

Two patients with MERRF and adult Leigh disease showed markedly high venous peak lactate levels [78.1 mg/dL (8.67 mM), 108.5 mg/dL (12.04 mM)]. Venous peak lactate levels above 40.0 mg/dL (4.44 mM) were not observed in patients with other diseases, being specific for patients with mitochondrial diseases. Patient 3 with KSS also showed an increased venous peak lactate level at 28.3 mg/dL (3.14 mM). However, this value was near the range of mildly increased venous peak lactate observed in patients with other diseases [19.4 mg/dL (2.15 mM) to 31.2 mg/dL (3.46 mM)]. Although increasing the cut-off value of venous peak lactate (e.g., 27.4-28.2 mg/dL determined retrospectively in this study) may improve the diagnostic specificity of the test, caution should be exercised to ensure that the sensitivity of the test is not decreased further.

An elevated venous L/P at the venous peak lactate level was not superior to the venous peak lactate level in our study in regard to its ability to differentiate mitochondrial diseases from other diseases. However, an elevated venous L/P value had added diagnostic value for patients with elevated venous lactate levels, as suggested in current recommendations (1). Although all 3 patients with mitochondrial diseases with elevated venous peak lactate levels showed a venous L/P>20, only 1 of the 5 patients with other diseases with elevated venous peak lactate levels showed a venous L/P>20. An over 2-fold increase in venous lactate values from baseline was observed more often in patients with mitochondrial diseases than was a venous peak lactate value above the UNL [4/6 (66.7%) vs. 3/6 (50.0%)], and was observed

Table 5. Summary of Studies Assessing the Diagnostic Role of Aerobic Exercise Tests on Cycle Ergometry under Constant Workload Including This Study.

Reference	Patient	Control	Protocol	Criteria for positive	Se	Sp
7	Mitochondrial diseases (29)	Healthy or disease control (14)	Workload to produce heart rate ≈ 150/min 15 min	Peak lactate >2.0 mM (18.0 mg/dL)	66%	(100%)
				Post-exercise lactate >1.5 mM (13.5 mg/dL)	69%	(100%)
23	Mitochondrial diseases (9) [KSS (7)]	Healthy control (6)	15W 15 min	Lactate+pyruvate area >upper normal limit*	100%	(100%)
				L/P area >upper normal limit*	44%	(100%)
8	CPEO-plus (6)	Healthy control (29)	90% of the predicted workload 15 min	Peak lactate >5.0 mM (45.0 mg/dL)	100%	(93%)
9	Mitochondrial diseases (30) [CPEO (27)]	Healthy control (12) nonmitochondrial diseases (14)	30W 15 min	Peak lactate >2.0 mM (18.0 mg/dL)	83%	(100%)
				L/P>max and mean+2SD of healthy controls (≈ 23)	80%	(100%)
10	CPEO (20)	Healthy control (25)	30W 15 min	"Rest lacatate >1.4 mM (12.6 mg/dL)" or "peak lactate >1.9 mM (17.1 mg/dL)"	75%	(100%)
18	Mitochondrial diseases (155)	Various nonmitochondrial diseases (31)	30W 15 min	More than 2 of the 5 lactate>UNL [1.8-2.1 mM (16.2-18.9 mg/dL)]	67%	94%
21	Mitochondrial myopathy (15) [CPEO (7)]	MyD (10) Healthy control (18)	60% VO_{2max} 20 min	Lactate "increase" >4.5 mM (40.5 mg/dL)	27%	(86%)
19	Mitochondrial myopathy (9) [CPEO (4)]	Healthy control (9)	(90% of the predicted workload) × (relative muscle strength) 15 min	Peak lactate >2.9 mM (26.1 mg/dL)	78%	(100%)
		Neuromuscular diseases (10)				60%
20	Mitochondrial diseases (24) [CPEO (19)]	Healthy control (37) + Neuromuscular diseases (26)	30 W 15 min	Peak lactate >2.9 mM (26.1 mg/dL)	58%	(92%)
				Post-exercise lactate >1.8 mM (16.2 mg/dL)	67%	(92%)
				"Peak lactate >2.9 mM (26.1 mg/dL)" or "post-exercise lactate >1.8 mM (16.2 mg/dL)"	71%	(92%)
Present study	Mitochondrial diseases (6)	Various nonmitochondrial diseases (15)	15 W 15 min	Peak lactate ≥2.13 mM (19.2 mg/dL)	50%	67%
				"Peak lactate ≥2.13 mM (19.2 mg/dL)" and "L/P>20 at peak lactate"	50%	93%
				More than a twofold increase in lactate from baseline	67%	93%

Numbers in parentheses or brackets in the Patient and Control row indicate the number of subjects. KSS: Kearns-Sayre syndrome, CPEO: chronic progressive external ophthalmoplegia, MyD: myotonic dystrophy, W: watts, min: minutes, $VO_{2\,max}$: maximal oxygen uptake, UNL: upper normal limit, L/P: lactate-to-pyruvate ratio. *cut-off illustrated in the figure but the value was unavailable. Percentages in parentheses in the Sp column are specificities calculated against control, including healthy subjects.

less often in patients with other diseases than was a venous peak lactate value above the UNL [1/15 (6.7%) vs. 5/15 (33.3%)]. These results suggest that parameters other than venous peak lactate may add diagnostic value for aerobic exercise tests, and combining venous peak lactate and L/P at peak lactate may increase specificity; furthermore, using the fold increase in venous lactate level from baseline may increase the sensitivity compared with using the venous peak

lactate level alone. However, determining the optimal parameters and cut-off values will require further investigation in a larger sample.

Different mitochondrial disease subtypes may show different results in aerobic exercise tests, depending on the degree of altered mitochondrial oxidative phosphorylation activity. Patients 1, 2, and 3 had MERRF, adult Leigh disease, and KSS, respectively, and showed elevated values of venous peak lactate and L/P. Previous studies have shown that these subtypes often show elevated venous lactate levels (21-23, 33, 34). Patient 4 had NARP and showed a venous peak lactate below the UNL but had a 3.1-fold increase in the lactate value from baseline. There have been previous reports of patients with NARP showing a mild increase in venous lactate levels either below (35) or above (36) the normal limit, a change that may also reflect a defect in the mitochondrial respiratory chain. Patient 5 presented with hearing impairment, ataxia, chorea, and mild muscle weakness without stroke-like episodes and carried the m.3243A>G variant. The patient did not exhibit increased venous peak lactate during the test but showed increased CSF lactate (31.5 mg/dL) and L/P (21.5) values for reasons unknown. Patient 6 had Leber-plus disease without muscle weakness and did not show an increase in venous lactate levels during the test. Patients with Leber disease without muscle symptoms have been shown to have either normal (37) or elevated (38) venous lactate levels.

A literature review of previous studies assessing the diagnostic value of aerobic exercise tests on cycle ergometry under a constant workload showed wide variations in terms of subjects, exercise protocols, and cut-off values (Table 5) (6-9, 13-23). Chronic progressive external ophthalmoplegia (CPEO) and KSS patients constituted the majority of mitochondrial disease patient groups in reports from seven out of nine groups, and healthy individuals were often included as controls (seven out of nine groups). Four out of the 9 groups used an exercise protocol of 30 W for 15 minutes (8, 9, 13-17, 19). Our exercise protocol of 15 W for 15 minutes was initially implemented by Ogasahara et al. (21, 22), and Takahashi et al. reported that all 9 patients with mitochondrial diseases tested showed an increase in the venous lactate-plus-pyruvate area above the UNL of normal subjects using this protocol (23). Abnormalities observed in patients with mitochondrial diseases have subsequently been consistently reported using this exercise protocol (24, 25). However, to our knowledge, no study has summarized the test parameters of patients with a final diagnosis of other diseases using this protocol, so we cannot confirm our results in other datasets.

Several limitations associated with the present study warrant mention. First, this was a retrospective study with a small sample size, which included patients with various diseases and mitochondrial disease subtypes. Second, we used a 15-minute exercise protocol with a constant workload of 15 W and no comparison with other exercise protocols. The ideal parameters and cut-off values may differ among patient populations or with the use of different exercise protocols.

Conclusion

Elevated venous peak lactate above the UNL at rest was observed in patients with a final diagnosis of other diseases during aerobic exercise tests, even under a low workload of 15 W for 15 minutes. Increases in both venous lactate and L/P values may be more specific and the fold increase in venous lactate levels from baseline more sensitive for detecting mitochondrial disease than an increased venous peak lactate level alone. Parameters other than venous lactate, including L/P and the fold increase in lactate from baseline, should also be considered in future studies.

The authors state that they have no Conflict of Interest (COI).

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