

Alteration of Lactate Production During Incremental Exercise in Myotonic Dystrophy Is Not Dependent by Catecholamine Increase

Gabriele Siciliano, Sonia Tovani, Livia Paquali, Michela Falorni, Fabio Galluzzi, Maria Laura Manca, Anna Rocchi, Ornella Baglini, Concetta Prontera⁽¹⁾, Michele Emdin⁽¹⁾

Neurological Clinics, Department of Neuroscience, University of Pisa, and (1) Institute of Clinical Physiology, CNR, Pisa, Italy

Abstract

Abnormalities of mitochondria have been reported in Myotonic Dystrophy (MD). Adrenergic activation is one of the major factors influencing exercise lactate production in healthy subjects. In order to assess the role of such activation in MD, we compared blood catecholamine levels to those of lactate during an incremental bicycle exercise in 9 MD patients and 6 controls.

The lactate values reached significantly higher levels in patients than in controls at rest (2.91 ± 0.58 vs 1.44 ± 1.14 mmol/l, $p < 0.01$, in average), at the anaerobic threshold (4.43 ± 1.11 vs 2.62 ± 0.73 mmol/l, $p < 0.05$), at exercise peak (6.64 ± 1.34 vs 3.90 ± 0.99 mmol/l, $p < 0.05$) and at recovery (3.21 ± 0.94 vs 1.91 ± 0.19 , $p < 0.01$). Furthermore, the anaerobic lactate threshold (LT) in MD was acquired at lower workload (mostly in a range between 30% and 50% of the predicted normal maximal power output) compared to controls (60-70%), this suggesting an early activation of the anaerobic metabolism in MD patients. On the contrary, catecholamine levels were not significantly different between patients and controls. These results indicate that abnormal lactate production in MD is independent of the adrenergic response to exercise and suggest a direct involvement of skeletal muscle oxidative metabolism in MD.

Keywords: catecholamine, exercise, lactate, lactate threshold, myotonic dystrophy.

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Myotonic dystrophy (MD), an autosomal dominant disease, is the most common muscular dystrophy in adulthood. MD is a multisystemic disease characterised by a wide range of neurological, ophthalmological, cardiological, endocrinological and gastro-intestinal manifestations. The muscle impairment consists of myotonia, weakness and wasting of the facial and limb muscles, being this latter usually distal in the early stages. MD is due to an unstable trinucleotide (CTG)_n expansion located in the 3' noncoding region of the chromosome 19q13.3. The gene, whose function is still unknown, encodes for a protein with putative serine-threonine protein kinase activity (MDPK: Myotonic Dystrophy Protein Kinase), and is expressed in various tissues, such as skeletal muscle, heart and brain. Despite advances in genetics, the specific pathophysiological mechanism of MD remains unclear. Some authors have found mitochondrial abnormalities in muscle biopsy of MD subjects, such as ragged red fibres [16], cytochrome c oxidase-negative fibres, subsarcolemmal proliferation, and DNA mitochondrial deletions [1, 3, 20]. Furthermore, it

has been observed a deficit of plasma levels of coenzyme Q10 in MD patients [7, 11, 26]. These data support the probable occurrence of a mitochondrial functional impairment in the pathogenesis of the disease. Also autonomic nervous system can be a target of this disease. Sympathetic system activation is considered one of the main factors influencing muscle lactate production during both incremental [14, 18] and constant [29] exercise workload in normal individuals. To date no study has evaluated the role of the adrenergic response during prolonged exercise in MD.

Therefore, aims of the study have been: 1) to indirectly evaluate the oxidative metabolism in exercising muscle, by the assessment of blood lactate kinetics during an incremental exercise test, and 2) to assess the level of sympathetic system activation by relating plasma catecholamine levels to lactate under such exercise conditions, in MD patients.

Materials and Methods

Patients

The study has been performed in nine MD patients (five M and four F, mean age $\pm$ SD: 39.8 ± 7.3 years), diagnosed with molecular analysis. Based on the number of CTG expansion, each subject was included in one of the four classes (E1-E2-E3-E4), according to Tsilfidis et al. [28] (Table 1).

The study was approved by the Ethical Committee of our Institution and all subjects gave their informed consent, after being explained the purposes and procedures of the study.

All patients underwent physical and neurological examination including MDRS (Muscular Disability Rating Scale) and MRC (Medical Research Council index scores) scores on 11 muscles (Table 1), as well as electromyography. In order to assess the extramuscular involvement, a careful cardiological, pneumological, ophthalmological, endocrinological and gastrointestinal evaluation was performed.

Inclusion criteria were:

- i. mild degree of muscle impairment, as established by MDRS score ≤ 3 [13], Activity of Daily Living score ≤ 2 for each item [25], MRC score ≥ 4 [5] and functional testing score ≤ 2 [25, 8]. The patients could therefore manage an independent life and were capable of performing the required exercise test.
- ii. absence of overt cardiac/respiratory involvement, assessed by ECG, cardiac Echo Doppler evaluation and spirometry;
- ii. absence of joint or bone deformities;
- iv. body weight not exceeding 20% of the theoretical anthropometric value.

Three different phenotypical subclasses of patients were identified, according to clinical parameters, as suggested by Gennarelli et al [9] (Table 1).

Myotonia was present both clinically and electrically in all cases. None of the patients had abnormalities in

motor-sensitive nerve conduction velocity study. Electrophysiological recording of cutaneous sympathetic response and ECG R-R interval were performed to exclude autonomic nervous system involvement. As far as the phenotype was concerned, we found two patients in class 1, five in class 2 and two in class 3. Extramuscular involvement consisted of ovarian cyst in one patient, cataract in two, biliary lithiasis in three, nodular disease of thyroid in one. Concerning the cardiac involvement, one patient had first degree atrio-ventricular block, one left anterior hemiblock. At 2-D Echo-doppler cardiac examination cardiac diameters and biventricular function were normal in all subjects.

Exercise protocol

An electrically braked pedal-rate bicycle ergometer (Ergocard III, Esaote Biomedica SpA) was used to have subjects perform a series of 3-min exercise bouts starting at a minimum pedaling rate of 70 rpm and increasing the workload after each 2-min rest intervals. The predicted normal maximal power output (pnPOMax) was defined for each patient on the basis of sex, age, weight and height. The exercise started with a first bout at 10% of the pnPOMax and then, through successive increments of 10% of pnPOMax, brought to the highest work level at which cycling could be maintained for 3-min; this figure, when expressed in Watts, was taken as the actual or real maximum power output (rPOMax).

Anaerobic lactate threshold (LT) was assessed as previously reported [21] and expressed as percentage of the pnPOMax. Briefly, we determined the best-fit curve for lactate values during the exercise, then calculated the exercise power output level at which the slope of this best-fit curve began to rise exponentially.

Consecutive blood samples were collected from an antecubital vein under basal conditions, midway through each resting period, and during recovery at 1, 15 and 30 minutes after the end of exercise.

As the blood samples were drawn, heart and ventilation rate, and capillary hemoglobin O₂ saturation, pO₂

Table 1. Clinical characteristics of MD patients

Patients	Age (years)	Sex	Class of expansion*	MDRS [°]	MRC [•]	Phenotypic class [∇]
1	30	F	E2	2	51	1
2	33	M	E3	3	42	2
3	35	F	E3	3	49	2
4	38	M	E2	2	53	2
5	51	F	E3	3	43	2
6	37	M	E2	2	51	1
7	50	M	E2	3	40	2
8	45	M	E2	3	48	3
9	40	F	E2	3	42	3

* According to Tsilfidis et al. [26].

[°]MDRS: Muscular Disability Rating Scale

[•]MRC: Medical Research Council index scores

[∇] According to Gennarelli et al. [9].

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and pCO₂ were recorded as previously reported [21].

Six untrained healthy volunteers, 3 male and 3 female, mean age $\pm$ SE: 34.2 ± 12.5 yrs, were recruited from our laboratory staff as a comparison group for exercise testing, lactate and plasma catecholamine measurements.

Laboratory

Venous lactate levels were assessed spectrophotometrically on an ERIS Analyzer 6170 (Eppendorf Geratebau, Hamburg, Germany) (laboratory range: 0.67-2.47 mmol/l). As far as plasma catecholamines were concerned, free epinephrine (EP) and norepinephrine (NEP) levels were determined by a fully automated, high-performance liquid chromatography analyzer for catecholamines (HLC 725), developed by Tosoh (Tosoh Co, Tokyo, Japan) and distributed by Eurogenetics (Eurogenetic, Tessenderlo, Belgium). The laboratory ranges* for catecholamines were < 80 pg/ml and < 500 pg/ml for EP and NEP respectively.

Statistical analysis

All data were reported as mean and standard deviation (SD).

In order to reduce inter-subject data variability, all parameters were normalized for each subject and expressed as percentages of the baseline.

Goodness-of-fit models, in terms of minimal square residuals, were utilized to fit all curves. After a Kolmogorov-Smirnov test confirmed that the data did not present a Gaussian distribution, non-parametric analysis was selected. In particular, the Mann Whitney test between patient and control groups, and the Spearman test to analyse correlations were applied. In all tests, we have considered a significance level of $p = 0.05$.

Results

Resting conditions

Mean basal lactate value was significantly higher in patients than in controls (2.91 ± 0.58 vs. 1.44 ± 1.14 mmol/l; $p < 0.01$) (Table 2). Both mean basal values* of norepinephrine (496.1 ± 200.3 pg/ml) and epinephrine (44.9 ± 16.1 pg/ml) were under the limit of laboratory reference value. When considering the single cases, basal lactate was above the mean + 1 SD of controls in seven cases; norepinephrine was increased in four patients, epinephrine in none.

Exercise protocol

In all patients the exercise was interrupted at a workload level under the predicted normal maximum power output, due to exhaustion: two patients reached 40% of pnPOMax, two other 60% of it, while the remaining five 70% of pnPOMax.

Exercise anaerobic lactate threshold (LT) was achieved at 40 or 50% of the pnPOMax in six MD individuals, 30% in two cases, and at 70% of the pnPOMax in the last one (Table 2). On the contrary, LT in normal controls ranged from 60 to 70% of pnPOMax. When plotting average lactate values, mean LT corresponded to 40% of pnPOMax, compared to 70% of controls (Figure 1). While mean normalised lactate values of both LT and peak lactate were not significantly different between the groups ($142.2 \pm 32.9\%$ in MD vs. $182 \pm 27.8\%$ in controls, for LT lactate; 232.1 ± 49.9 in MD vs $271 \pm 25.3\%$ in controls, for peak lactate), the corresponding absolute levels resulted significantly higher in MD patients compared to controls (4.43 ± 1.11 vs. 2.62 ± 0.73 mmol/l for LT lactate, $p < 0.05$, 6.64 ± 1.34 vs. 3.90 ± 0.99 mmol/l for peak lactate, $p < 0.05$) (Figure 2, table 2).

Thirty minutes after the end of the exercise the mean absolute level of lactate was 3.21 ± 0.94 mmol/l ($1.91 \pm$

Table 2. Plasmatic lactate values at resting, at anaerobic lactate threshold (LT) and during recovery in MD patients.

Patients (sex/age)	Basal lactate (mmol/l)	LT* as % of PnPOmax°	Lactate at LT (mmol/l)	Lactate at peak (mmol/l)	Lactate at recovery (mmol/l)
F/30	2.67	40%	3.14	6.12	2.46
M/33	1.89	70%	2.92	4.04	1.33
F/35	3.48	30%	4.36	5.63	3.46
M/38	2.89	50%	3.90	6.75	4.07
F/51	3.10	40%	5.20	7.69	3.67
M/37	3.05	50%	6.48	8.84	3.53
M/50	2.26	50%	4.77	7.25	3.62
M/45	3.80	30%	5.20	6.80	4.30
F/40	3.10	50%	3.90	6.60	2.50
Mean values	2.91 ± 0.58		4.43 ± 1.11	6.64 ± 1.34	3.11 ± 0.95
Mean values in controls	1.44 ± 1.14	Range: 30-50%	2.24 ± 0.39	3.90 ± 0.99	1.91 ± 0.19

* LT: Lactate Threshold

° pnPOMax: predicted normal maximal power output.

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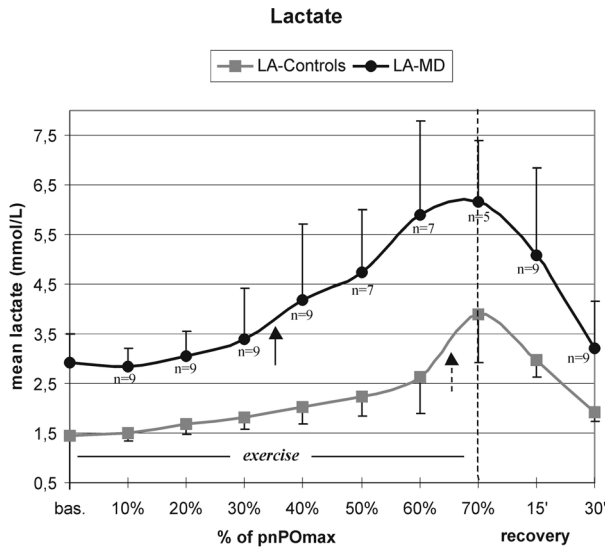


Figure 1. Lactate kinetics: anaerobic lactate threshold (arrows) in MD patients and controls.

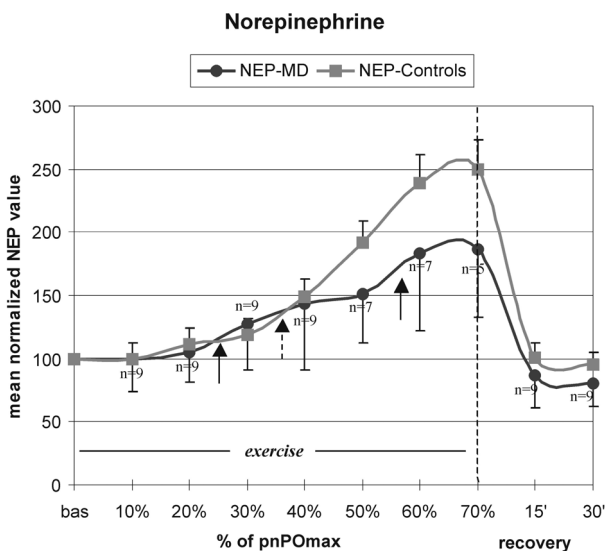


Figure 2. Kinetics of normalized norepinephrine (NEP) in MD patients and in controls. Arrows indicate curve thresholds.

0.19 mmol/l in controls). When considering single cases, two MD patients still exhibited lactate levels clearly above the baseline, while in the other seven as well as in norms lactate approached the resting condition value.

As far as NEP plasma levels were concerned a double threshold curve model was observed in MD patients, with two inflection points of the normalized incremental values corresponding, in average, to 30% and 60% of pnPOmax. NEP threshold in controls was at 40% of pnPOmax (Figure 2). Step by step comparison between the two groups indicated that NEP mean values were higher in controls than MD starting from 50% of pnPOmax till the exercise peak, without this difference reaching, however, a statistical level (Figure 2). Similar figures of exercise related modifications were obtained for EP plasma values in both MD patients and controls,

with the exception that in the former the first inflection point of incremental curve was at 20% of pnPOmax. No statistical difference was found in the step by step mean value comparison between the two groups (Figure 3).

Heart and respiratory rates, capillary hemoglobin O₂ saturation, pO₂ and pCO₂ were not different between patients and controls in basal conditions, as well as during exercise and recovery.

Statistical correlations

In MD patients, the correlation between normalized lactate value and EP and NEP was significant ($R = 0.83$ and $R = 0.82$, respectively) (Figure 4), while in controls it was borderline ($R = 0.75$ and $R = 0.68$, respectively). No significant correlation was found between lactate values and clinical characteristics of patients.

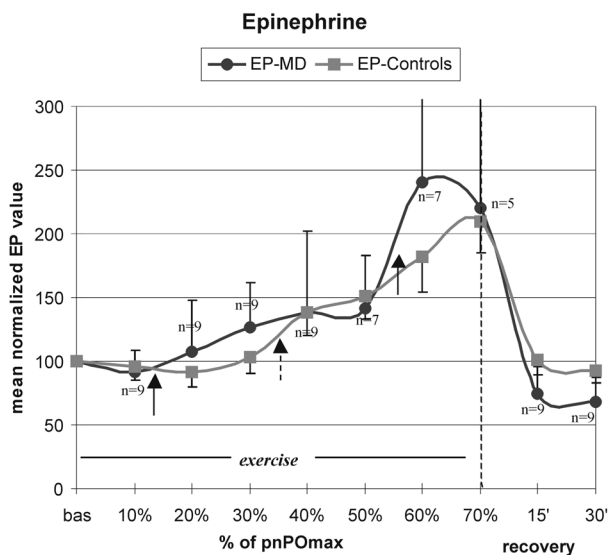


Figure 3. Kinetics of normalized epinephrine (EP) in MD patients and in controls. Arrows indicate curve thresholds.

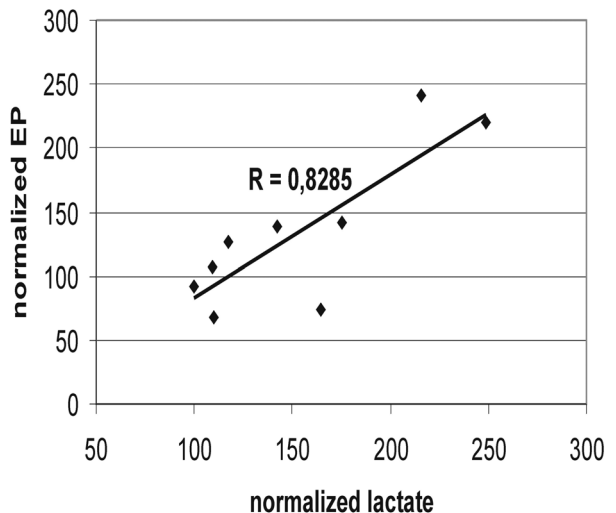


Figure 4. Correlation between normalized lactate value and epinephrine (EP) in MD patients*.

Discussion

The pathophysiology of MD is so far unknown. Among the various hypotheses, as previously reported [22], the possibility of a mitochondrial oxidative dysfunction, probably due to causal mutation, was suggested to explain the skeletal muscle involvement in MD patients. Pathological findings suggestive of mitochondrial abnormalities, such as ragged red fibers [16], cytochrome c oxidase - negative fibres, intramitochondrial inclusions bodies [12], subsarcolemmal proliferation and dense granular matrix materials within enlarged mitochondria [31] have been found in MD patients. In addition to this, some authors have reported on multiple mitochondrial DNA deletions detected by PCR analysis in specimens obtained by muscle biopsy of individuals with MD [20]. Furthermore, some MD patients show clinical feature and histopathological finding overlapping resembling mitochondrial myopathies, such as progressive external ophthalmoplegia, “ragged red fibers” and mitochondrial DNA deletions on muscle biopsy [1, 3]. Recently some authors [7, 11, 26] have reported on reduction, in MD patients, of plasma levels of CoQ10, an endogenously synthesized provitamin involved in a variety of essential cellular processes, most notably the mitochondrial electron-transport chain.

Activation of sympathetic autonomous system is one of the major determinant of muscle exercise, influencing muscle lactate production during both incremental [14, 18] and constant [29] exercise workload in normal individuals. Some studies in healthy subjects show that during exercise EP stimulates β_2 -mediated muscle glycogenolysis [32] and hepatic neoglucogenesis [30], resulting in sustained blood glucose levels and the inducing of muscle lactate production, especially by type 2 fibers [18, 24]. As far as NEP is concerned, its role during exercise is related to its binding to β_3 -receptor and consequent systemic lipolysis during exercise. Furthermore, it may be involved in exercising muscle lactate turnover by inducing vasoconstriction and subsequent redistribution of blood flow away from organs and tissues which normally remove lactate [19].

Symptoms suggestive of sympathetic nervous system impairment, such as orthostatic hypotension, gastrointestinal motility disorders, hyperhidrosis, excessive sialorrhea and lacrimation, sluggish pupillary reactions to light, cardiac arrhythmias, ventilatory impairment, bladder and sexual disturbances, are often reported in MD patients. Nevertheless, some studies seem to excluded a direct involvement of autonomic nervous system in this disease, more likely suggesting that the clinical signs related to its dysfunction in MD could depend on an altered smooth muscle function at the target organs [6, 17, 23]. In agreement to this, our MD patients showed no alteration with cutaneous sympathetic response assessment.

Taking into account these considerations, the aim of this study was to indirectly evaluate, by means of plasma lactate, the skeletal muscle metabolism during

aerobic exercise, as index of mitochondrial function in MD. Furthermore, we have related *catecholamine to lactate levels in order to investigate the role, if any, of sympathetic system activation during aerobic exercise in MD patients.

In basal conditions plasma lactate levels were increased in 7 out 9 examined MD patients.

The anaerobic lactate threshold was achieved at values ranging from 30 to 50% of the pnPOMax in 8 out 9 of MD patients vs 60-70% in normal controls, this reflecting an early recruitment of glycogenolytic fibres and the activation of skeletal muscle anaerobic metabolism in MD patients. The lactate threshold corresponds to the contractile performance load at which the plasma lactate levels begin to increase exponentially [15] and represents the critical point at which metabolic modifications bring about the transition from aerobic to anaerobic exercise, reflecting the mitochondrial oxidative function. The early activation of anaerobic metabolism in MD patients could then support the hypothesis of a functional mitochondrial damage in this disease. On the other hand we cannot absolutely exclude that the abnormal kinetics of the lactate might be dependent of physical deconditioning of these patients that could determine a oxidative function impairment in type 1 fibers [2, 10]. However this seems inconsistent as our patients showed a mild or moderate muscular involvement, as indicated by MDRS and MRC scores (Table 1).

Basal values of NEP resulted abnormal in 4 patients, while EP concentrations were in the normal range in all the patients.

During exercise plasma catecholamine values were similar to normal controls, except for a slight not significant reduced production of NEP. As expected, NEP and EP thresholds preceded LT in both MD patients and control. However, only in MD patients a second delayed lactate threshold was observed at 60 % of pnPOMax. Heart rate in basal conditions, during exercise and recovery were similar to controls.

These data indicate that in our MD patients there is not an abnormal activation of sympathetic nervous system during incremental workload exercise, excluding a major role for sympathetic activation in muscle lactate over-production. The only difference was the presence of a delayed second LT threshold in MD patients, which could be explained as an extra-activation bout of sympathetic nervous system, may be related to the precocious contractive exhaustion in them. The abnormal kinetics of the lactate observed in our patients could therefore be due to a primary metabolic defect in skeletal muscle.

These observations suggest a probable functional impairment of mitochondria in MD patients, that could contribute to the cell damage in MD and some way be related to the pathophysiology of the disease. The basic mechanism which can relate mitochondrial involvement to the molecular defect responsible for MD has not been understood so far. It seems possible that mitochondrial

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abnormalities found in the muscles of MD subjects are based on mitochondrial DNA mutations and that mitochondrial DNA deletions are dependent on the mutation responsible for MD [20]. In line with this, the gene involved in MD could play an important role in the expression of nuclear* genes essential for the integrity of mitochondrial DNA; alternatively, the mutant gene product could predispose to the damage to mitochondrial DNA, thereby increasing the risk of its mutations. Accumulation of deleted mitochondrial DNAs and consequent mitochondrial dysfunction in skeletal muscle could have unfavourable effects on exercise tolerance, muscle strength and muscular wasting in MD patients.

Address correspondence to:

Gabriele Siciliano, Neurological Clinics, Department of Neuroscience, University of Pisa, Via Roma 67, 56126, Pisa (Italy), phone +39-050-993046/993334, fax +39-050-554808/550563, Email gsicilia@med.unipi.it.

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