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ORIGINAL COMMUNICATION

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Lactate production upon short-term non-ischemic forearm exercise in mitochondrial disorders and other myopathies

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Abstract *Background* The non-ischemic forearm exercise test (NIFET) has been shown to be as effective as the classic ischemic forearm exercise test (IFET) in the diagnosis of patients with Mc Ardle disease. Recently, the lactate increase normalized to the mechanical energy production in NIFET was suggested to have a intermediate sensitivity and satisfactory specifity for the screening of mitochondrial disorders. *Methods* NIFET at 80 % maximal contraction force (MCF) was performed in normal controls (n = 41), patients with mitochondrial disorders (n = 15) and other myopathies (diseased controls, n = 20). 26 healthy volunteers also underwent IFET at 80 % MCF. The ratio of lactate increase and workload was defined as specific lactate production (mmol x s/N x l). *Results* In normal controls there was no significant different lactate increase during NIFET and IFET. The workload performed showed only a weak significant positive correlation with the lactate increase in the NIFET in normal controls ($r^2 = 0.20$) but not in IFET and NIFET with patients. A moderate negative correlation of specific lactate production and the absolute workload was found in all groups and in both protocols ($r^2 = 0.22$–0.34). The specific lactate production was highest in patients with other myopathies, intermediate in patients with mitochondrial disorders and lowest in normal controls. NIFET showed a sensitivity of only 20 % and a specifity of 95 % for normal controls, but only 75 % for diseased controls. *Conclusion* The specific lactate production during NIFET is neither sufficently specific nor sensitive for the diagnosis of mitochondrial disorders. Increased specific lactate production during rest-to-work transition period might be caused by increased acetyl group deficits.

Key words nonischemic/ischemic forearm exercise test · serum lactate · mitochondrial myopathy

Introduction

Short term, high intensity work of the skeletal muscles is accompanied by an accumulation of lactate derived from pyruvat during the anerobic breakdown of glucose and glycogen. An impaired lactate production has long been established as a diagnostic marker for glycolytic disorders of the skeletal muscles such as Mc Ardle disease [1].

A non-ischemic forearm exercise-test (NIFET) was recently introduced to replace the IFET and to prevent the possible complication of rhabdomyolysis and acute compartement syndrome [2–4]. The monitoring of lac-

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tate during cycle ergometry using constant low-intensity workload are widely-used screening protocols in the diagnosis of mitochondrial disorders [5–8]. Lactate production during short-term exercise is not classically used for the detection of mitochondrial disorders. In patients with mitochondrial disorders the mean lactate curve of 14 patients fell into the normal range [3]. Three other studies using short-term high-intensity protocols which focused on the analysis of oxygen desaturation also reported no different exercise-induced venous peak lactate in patients with mitochondrial disorders, normal and diseased controls [7, 9, 10]. However, if the venous lactate was normalized to the mechanical energy production during exercise the sensitivity of the NIFET for mitochondriopathy was 57 % and the specifity for normal subjects and patients with unclassified exercise-induced complaints was 92 % [3]. Therefore, an increased lactate production normalized to the mechanical energy production (specific lactate production) was suggested as an early and specific indicator of disturbed oxidative phosphorylation in patients with mitochondrial disorders.

The present study compared IFET and NIFET in normal controls and analysed the specific lactate production in patients with mitochondrial disorders, other myopathies, and normal subjects.

Methods

Subjects

15 patients with genetically defined mitochondrial disorder (clinical and molecular data are summarized in Table 1), 41 healthy volunteers as normal controls, and 20 patients with other myopathies as diseased controls were examined. The diagnosis of the diseased controls included myotonic dystrophy type 1 (DM1; n = 6), proximal myotonic muscular dystrophy (DM2; n = 6), polymyositis (n = 2), dermatomyositis (n = 1), muscular dystrophy Becker type (n = 1), facioscapulohumeral muscular dystrophy (FSHD; n = 1), myopathy with tubular aggregates (n = 1), limb girdle muscular dystrophy 2B (LGMD2A; n = 1). The diseased control group had a similar degree of workload and maximal contraction force. The study was approved by the Ethics Committee of the Martin-Luther University. The subjects were fully informed about the nature and the risks of the study before giving written consent to participate.

Pre-experimental preparations

None of the patients was treated with drugs such as valproic acid, acetylsalicylic acid, and biguanids. All subjects had an overnight fast and a breakfast at least two hours before the experiment. The subjects did not consume alcohol and caffeine 12 hours before the test, and had not performed exercise 24 hours before. Resting serum lactate levels were evaluated after a 30 minutes rest in sedentary position.

Experimental protocol

All subjects performed standardized tests using a self-made handgrip dynamometer linked to a computer with a specifically developed software programm and a monitor. The grip was adjusted to the hand size. MCF was determined three times before the insertion of a cubital vein catheter. The highest MCF value was chosen as reference.

The exercise tests consisted of intermittent (1 Hz) isometric contractions at 80 % of MCF for 1 minute ($NIFET_{80\%}$), and 80 % of MCF for 1 minute ($IFET_{80\%}$). In the IFET the sphygmomanometer cuff was inflated to ca. 20 mm Hg above systolic blood pressure. There was a resting period of 30 minutes between the tests. Blood samples were collected from the median cubital vein of the exercising arm before the exercise and after 1, 2, 3, 5, and 10 minutes to monitor lactate. 26 normal controls performed both NIFET and IFET. 41 normal controls

Table 1 Data of 15 patients with mitochondrial disorders

age (years)/ sex	diagnosis/symptoms	mutation size of deletion	level of hetero-plasmy (%)
53/f	CPEO, myopathy, retinopathy	single del 5.0 kB	40
51/m	CPEO, myopathy	single del 5.0 kB	51
46/f	CPEO, myopathy	single del 2.5 kB	78
37/f	CPEO, myopathy, dysphagia	single del 5.0 kB	50
53/m	CPEO	single del 5.0 kB	50
37/m	CPEO	single del 5.0 kB	48
37/f	CPEO	single del 4.5 kB	52
30/f	CPEO, myopathy, retinopathy	single del 6.0 kB	70
44/m	CPEO, myopathy, hypacusis, RBB	single del 5.0 kB	57
13/f	CPEO, diabetes, av-block III°	single del 6.5 kB	43
60/f	CPEO, myopathy	multiple deletions	–
55/m	CPEO, myopathy, hypacusis	multiple deletions	–
58/m	CPEO	multiple deletions	–
55/m	CPEO, diabetes, hypacusis	A3243G	8 (blood)
49/m	MELAS, myopathy, hypacusis, retinopathy	A3243G	32

CFEO chronic progressive external ophthalmoplegia; *MELAS* mitochondrial encephalomyopathy, lactic acidosis and stroke; *RBB* right bundle block

and patients with mitochondrial disorders and other myopathies only performed NIEFT$_{80\,\%}$. No participant interrupted the tests.

Biochemical analysis

Venous blood was sampled in syringes containing 50 µl 0.33 M EDTA and immediately spun at 4°C and analysed for lactate (LX20PRO, Beckman Coulter, USA).

Statistical analysis

Statistical analysis was performed using Student's unpaired t-test or Mann-Whitney Rank Sum test, One-Way ANOVA, simple linear regression, and Pearson Product Moment correlation. Statistical significance was accepted at $p < 0.05$. Values are presented as means + 1 SD (range). The workload was measured as area under the curve. The quotient of Δ lactate/workload was defined specific lactate production (mmol x s/N x l). Data were checked for normal distribution by normality test Kolmogorov-Smirnov.

Results

NIFET$_{80\,\%}$ and IFET$_{80\,\%}$ in normal controls

Resting serum lactate was similar before each test in normal controls in both protocols (Table 2). The absolute workload performed showed a high correlation with the MCF in NIFET$_{80\,\%}$ and in IFET$_{80\,\%}$ ($r^2 = 0.71$ each). There was only a weak correlation between Δ serum lactate and absolute workload respectively in the NIFET$_{80\,\%}$ ($r^2 = 0.20$, $p < 0.05$, power 0.70) but not in the IFET$_{80\,\%}$ ($r^2 = 0.09$). Absolute workload, peak serum lactate, Δ serum lactate, and specific lactate production in NIFET$_{80\,\%}$ and in IFET$_{80\,\%}$ were not significantly different.

MCF but also absolute workload were significantly higher in men than in women ($p < 0.001$). Peak lactate, Δ lactate, and specific lactate production showed no significant difference between men and women.

NIFET$_{80\,\%}$ in normal controls, patients

The MCF and the workload were significantly higher in normal controls than in both diseased groups (t-test: $p < 0.01$). There was only weak significant correlation between Δ serum lactate and absolute workload in normal controls ($r^2 = 0.14$, $p < 0.05$, power 0.70), but not in patients with mitochondrial disorders ($r^2 = 0.12$, p n. s.) and in patients with other myopathies ($r^2 = 0.13$, p n. s.). All three groups showed a significant moderate negative correlation between specific lactate production and the absolute workload (normal controls: $r^2 = 0.25$, $p < 0.001$, power 0.90; patients with mitochondrial disorders: $r^2 = 0.34$, $p < 0.05$, power 0.63; patients with other myopathies: $r^2 = 0.22$, $p < 0.05$, power 0.55). The specific lactate production was significantly higher in patients with other myopathies compared to normal controls (One-Way-ANOVA and Mann-Whitney-Rank-Sum test: $p < 0.05$, Fig. 2). Patients with mitochondrial disorders showed an intermediate specific lactate production and did not differ significantly from normal controls. The specific lactate production showed a trend towards higher specific lactate production in patients and in those normal controls with low absolute workload and MCF (Fig. 1).

4/15 patients (27 %) with mitochondrial disorders showed elevated serum lactate levels at rest but none of the normal subjects and patients with other myopathies. The specific lactate production was increased in 3/15 patients with mitochondrial disorders (20 %), but also in

Table 2 Venous lactate in normal controls during NIFET$_{80\,\%}$ and IFET$_{80\,\%}$. Data are shown as mean ± SD (ranges given in parentheses). All data showed normal distribution. male/female: 16/10; MCF [N]: 415 ± 156 (118–651)

normal controls[1] (n = 26)	NIFET$_{80\,\%}$	IFET$_{80\,\%}$
workload (kN/s)	6.5 ± 2.5[1] (2.0–11.5)	6.3 ± 2.5[1] (2.5–10.6)
resting serum lactate (mmol/l)	1.2 ± 0.4[1] (0.6–2.1)	1.3 ± 0.4[1] (0.6–2.8)
peak serum lactate (mmol/l)	4.2 ± 1.4[1] (1.3–6.8)	4.8 ± 1.3[1] (1.7–7.1)
Δserum lactate (mmol/l)	3.1 ± 1.4[1] (0.4–5.6)	3.5 ± 0.8[1] (1.2–5.0)
specific lactate production (mmol/l/kN x s)	0.49 ± 0.18[1] (0.13–0.87)	0.57 ± 0.25[1] (0.14–1.20)

[1] differences between the groups with paired t-test: p n. s.

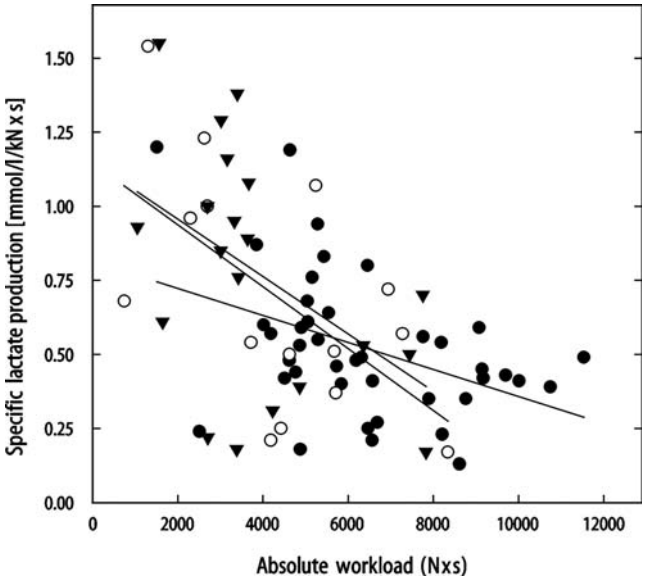

Fig. 1 Correlation of specific lactate production and absolute workload during NIFET$_{80\,\%}$ (normal controls (●): $r^2 = 0.25$, $p < 0.001$; mitochondrial disorders (○): $r^2 = 0.34$, $p < 0.05$; other myopathies (▼): $r^2 = 0.22$, $p < 0.05$)

Table 3 Venous lactate in normal controls, in patients with mitochondrial disorders, and in patients with other myopathies during $NIFET_{80\,\%}$. Data are shown as mean ± SD (ranges in parentheses)

	normal controls (n = 41)	mitochondrial disorders (n = 15)	other myopathies (n = 20)	ANOVA
age (years)	39 ± 13 (20–65)	45 ± 13 (13–60)	44 ± 14 (18–73)	n. s.
male/female ratio	27/14	8/7	12/8	
MCF (N)	478 ± 137 (168–651)	259 ± 113*[2] (41–431)	237 ± 102*[2] (86–434)	$p < 0.01$
workload (kN x s)	6.4 ± 2.1 (1.5–11.5)	4.4 ± 2.2*[2] (0.7–8.3)	4.2 ± 2.2*[2] (1.1–7.8)	$p < 0.01$
Δserum lactate (mmol/l)	3.2 ± 1.2 (0.9–5.6)	2.5 ± 1.5 (0.5–5.6)	2.5 ± 1.4 (0.6–5.4)	n. s.
specific lactate increase (mmol/l/kN x s)	0.53 ± 0.24 (0.13–1.20)	0.69 ± 0.40 (0.17–1.54)	0.75 ± 0.40*[1] (0.17–1.5)	$p < 0.05$

* post-test analysis significantly different from normal controls using unpaired t-test:
[1] $p < 0.05$; [2] $p < 0.01$

5/20 patients with other myopathies (25 %) and 2/41 normal control subjects (5 %). There was a moderate positive correlation between the specific lactate production and the heteroplasmy in patients with mitochondrial disorders and single deletions ($r^2 = 0.42$, Fig. 3). The specific lactate production was not significantly different in patients with mitochondrial disorder with and without paresis (with paresis: 0.74 + 0.38 (0.21–1.54), without paresis: 0.60 + 0.44 (0.17–1.23), unpaired t-test: p n. s.).

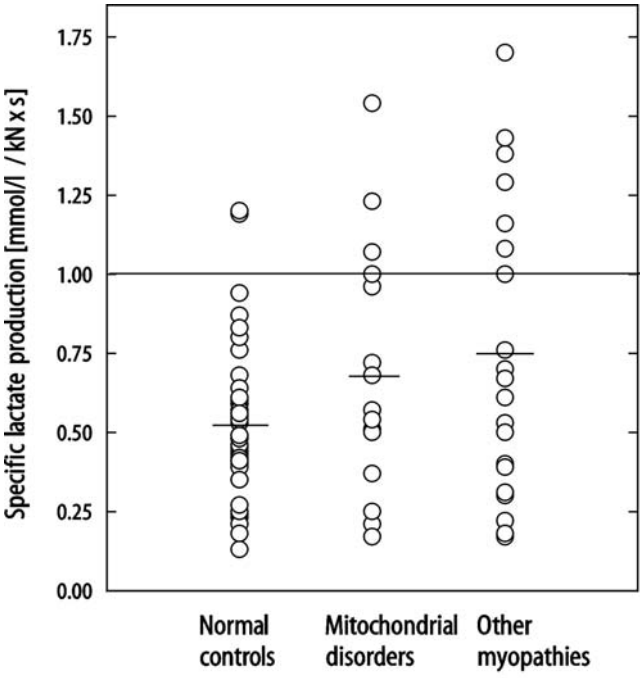

Fig. 2 Specific lactate production in normal controls, patients with mitochondrial disorders and patients with other myopathies in $NIFET_{80\,\%}$. The line indicates the upper normal limit defined as means + 2 SD of the normal controls. Horizontal bars indicate the means

Discussion

The accumulation of lactate in the working muscle during high-intensity exercise of short duration is determined by the degradation of muscle glycogen and glucose, the activation of pyruvate dehydrogenase complex (PDC), and the capacity of the oxidative phosphorylation in the respiratory chain [11, 12]. In the present protocolls work was performed with 80 % MCF. It has been shown that under these conditions all motor units are recruited leading to a maximal energy demand [13]. In the traditional IFET a cuff was applied to exclude entirely the influence of oxidative phosphorylation and to restrict the energy production to oxygen independent routes. However, in the present study both ischemic and nonischemic forearm exercise protocols showed similar increases in serum lactate. This might be explained by the finding that the skeletal muscle is already ischaemic during isometric contraction forces beyond 30 % of MCF [14]. Sinkeler et al. (1985) found a higher lactate accumulation in IFET during high workload levels adopted to MCF (80 %) than lower levels (65 %, 50 %), which might be explained by an increasing blood flow restriction at higher MCF [15]. In IFET no significant correlation was found between the absolute workload and the maximum lactate values [16]. This is consistant with the present study when IFET was performed. However, in NIFET there was only a weak correlation between absolute workload and lactate increase in normal controls but not in the patient groups. In the present study the contraction was performed as intermittent isometric exercise and not as permanent isometric exercise. Thus, exercise might not have been completely ischemic.

During high intensity exercise under aerobic conditions energy production by oxidative phosphorylation accounts for up to 50 % already after 60 seconds [17, 18]. The activation of PDC by skeletal muscle contraction regulates the formation of acetyl-CoA. This can be metabolised in the tricarboxylic acid cycle and the formation of acetylcarnitine. The respiratory chain function during the rest-to-work transition is limited by a lag

in muscle blood flow and oxygen delivery to the contracting muscle but also by an acetyl-group deficit due to PDC activation [11]. It has been shown that the activation of PDC by dichloracetat (DCA), an activator of PDC, decreased glycogenolysis, phosphocreatine utilization, and lactate accumulation under ischemic and nonischemic conditions in canins and humans [11, 19–21].

The lactate production normalized to the mechanical energy production (specific lactate production) provides additional information about the metabolic changes during short-term, high intensity exercise. In the present study there was a significant negative correlation between specific lactate production and absolute workload. Specific lactate production was higher in patients and controls with high MCF and workload than in those with low absolute workload and MCF. However, differences in the specific lactate production can not be explained by impaired oxygen supply alone, because blood flow was restricted in all individuals. Therefore, high specific lactate production might reflect both reduced capacity of oxidative phosphorylation and prolonged activation of PDC due to paresis or low training level. In patients with mitochondrial disorders the degree of heteroplasmy additionally contributed to a higher specific lactate production.

In the present study the sensitivity of specific lactate production in patients with mitochondrial disorders was lower than those of resting lactate alone in the present study. Furthermore, in the group of patients with other myopathies a similar proportion of patients with an increased specific lactate production was found. This is in contrast to Hogrel et al. (2001) who described a higher specifity but also higher sensitivity compared to resting lactate levels [3]. In that study, however, diseased controls included only patients with disorders of the glycogenolysis and patients with isolated intolerance to exercise of unknown etiology, but not patients with manifest paresis and atrophy. This might be important in order to exclude unspecific effects due to workload (paresis) and muscle mass (atrophy)

In conclusion, an increased specific lactate production during short-term, high-intensity exercise seems to be an unspecific phenomenon in muscle disorders independent of its etiology. Furthermore, the specific lactate production during NIFET was neither sufficently specific nor sensitive for the diagnosis of mitochondrial disorders.

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