AMP Deaminase Deficiency Does Not Affect Glycolytic Capacity in Skeletal Muscle during Standardized Ischemic Forearm Exercise Test

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ABSTRACT

Hanisch F, Zierz S. AMP Deaminase Deficiency Does Not Affect Glycolytic Capacity in Skeletal Muscle during Standardized Ischemic Forearm Exercise Test. JEPonline 2011;14(4):59-63. The significance of AMP deaminase (AMPD) deficiency due to the homozygous c.34C>T mutation in the AMPD1 gene is not clear. Products of AMPD as inosine monophosphate (IMP) and ammonia (NH₃) were proposed to be positive effectors of glycolytic enzymes in vitro. Serum lactate and NH₃ production upon standardized ischemic forearm exercise (IEFT) were analyzed in 29 patients stratified for the c.34C>T mutation (n = 6 TT, n = 15 CT, and n = 8 CC). Homozygotes (TT) showed a significantly (P=0.01) lower NH₃ production and NH₃ ratio/lactate ratio than both heterozygotes (CT), and wildtype (CC). The NH₃ production was not different between CT and CC. However, there was no difference in the maximal contraction force, the workload, the lactate production (absolute and normalized workload) between TT, CT, and CC. Although NH₃ production is significant lower in TT this does not result in decreased lactate production during short-term high intensity IFET. Thus, AMPD deficiency does not impair activation of the anaerobic pathway.

Key Words: AMPD1, NH₃, Standardized Forearm Exercise Test, Glycogenolysis
INTRODUCTION

The enzyme AMP deaminase (AMPD) (EC 3.5.4.6.) catalyzes the deamination of AMP to ammonia (NH$_3$) and inosine monophosphate (IMP) in working skeletal muscle. The homozygous nonsense mutation c.34C>T in the $AMPD_1$ gene (OMIM 102770) leads to a catalytically inactive AMPD. The pathological significance of impaired AMPD remains unclear. This deficiency has traditionally been thought to cause exercise intolerance in a subset of individuals; however, the vast majority are asymptomatic. This has raised many doubts about the real pathogenicity of the c.34C>T mutation.

The major regulatory effect of NH$_3$ released by AMPD is its contribution to fumarate to the tricarboxylic acid cycle (TCA) (anaplerotic function). However, a similar production of intermediates of the TCA during incremental cycle ergometry exercise upon exhaustion was found in homozygotes (TT), heterozygotes (CT), and wildtype (CC) for the c.34C>T mutation [6] suggesting that AMPD does not affect TCA anaplerosis. In addition, NH$_3$ has been proposed in in-vitro studies as a positive effector of glycogenolysis [1]. Therefore, lactate and NH$_3$ production during standardized ischemic forearm exercise (IEFT) protocol was analyzed in CC, CT, and TT.

METHODS

Subjects

Included were 29 individuals with known $AMPD1$ genotype: TT (n=6), CT (n=15), CC (n=8). The study was approved by the local Ethics Committee of the Martin-Luther University Halle-Wittenberg, Germany. All subjects gave written consent to participate. There was no difference in maximal contraction force (MCF) and workload in the three groups.

Procedures

All subjects had an overnight fast and a breakfast at least two hours before the experiment. None had performed exercise 24 hrs before. The subjects did not consume alcohol and caffeine 12 hrs before the test. Resting serum and NH$_3$ levels were evaluated after a 30 min rest in sedentary position. Serum lactate and NH$_3$ were collected from the median cubital vein of the exercising arm after a 30 min rest before and immediately after 1 min of exercise. All subjects performed a standardized ischemic forearm exercise (IEFT) using a self-made handgrip dynamometer linked to a computer with a specifically developed software program and a monitor. The grip was adjusted to the hand size. The MCF was determined three times before the insertion of a cubital vein catheter. The highest MCF value was chosen as reference.

The IEFT was performed by 1.0 Hz isometric contractions at 80% of each subject’s MCF for 1 min [2]. The sphygomanometer cuff was inflated to ca. 20 mm Hg above systolic blood pressure. Blood samples were collected from from the median cubital vein of the exercising arm before the exercise and after 1 (i.e., at the end of the exercise), 2, 3 and 5 min to monitor lactate and NH$_3$. No participant interrupted the test. The contraction force was recorded and the workload was determined as area under the curve. The lactate and NH$_3$ production normalized for the workload were termed specific lactate and NH$_3$ production, respectively.

The assay for AMP deaminase activity from skeletal muscle was determined in accordance with the following steps [3]. Frozen muscle was homogenized (1:30 w/v) in Chappel-Perry Medium A buffer (pH 7.5) containing 7.5 mM KCl, 6.0 mM Tris, 1.0 mM MgCl$_2$, 0.4 mM EGTA. The final reaction volume of 1000 µL contained 250 mM imidazole buffer, pH 6.5, 0.1 M KCl, 0.04 M MgCl$_2$, 6 mM α-ketoglutarate, 20 µL of glutamate dehydrogenase (1:5 aqua bidest), 0.15 mM NADPH, and 5, 10 or
20 µL of muscle homogenate. The reaction was started when 7.5 mM AMP was added. Absorbance was monitored at a wavelength of 340 nm.

**Statistical Analyses**

Statistical analysis was performed using one-way ANOVA. Post hoc analysis was done using Tukey test (SigmaStat, Switzerland). Statistical significance was accepted at P=0.05. Values are presented as means ± 1SD (range). Data were checked for normal distribution by normality test Kolmogorov-Smirnov.

**RESULTS**

No significant differences in the NH₃ ratio<sub>peak/rest</sub> and the NH₃ ratio/lactate ratio between CT and CC were found. Both parameters were significantly lower in TT than CT and CC. However, lactate at rest, the peak lactate, the lactate ratio<sub>peak/rest</sub> and the specific lactate production upon standardized exercise did not differ among the three groups (Table 1).

Table1. Results of IEFT in individuals according to the c.34C>T genotype. Data are given as means±SD (range).

<table>
<thead>
<tr>
<th></th>
<th>TT</th>
<th>CT</th>
<th>CC</th>
<th>One-Way ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>6</td>
<td>15</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td><strong>Sex (male/female)</strong></td>
<td>2/4</td>
<td>5/10</td>
<td>4/4</td>
<td></td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>41±14 (21-62)</td>
<td>43±13 (19-65)</td>
<td>40±6 (32-50)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>AMP deaminase activity [U/g protein]</strong></td>
<td>2.1±1.8 (0.04-4.6)</td>
<td>14.9±4.6 (10-24)</td>
<td>40.9±19.8 (14-80)</td>
<td>P=0.01²</td>
</tr>
<tr>
<td><strong>MCF&lt;sup&gt;1&lt;/sup&gt; [N]</strong></td>
<td>320±151 (118-561)</td>
<td>312±129 (109-577)</td>
<td>431±134 (269-642)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Workload [kN x s]</strong></td>
<td>5.2±2.3 (2.2-8.5)</td>
<td>5.8±2.3 (1.2-9.6)</td>
<td>7.0±2.5 (3.2-10.6)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Lactate ratio&lt;sub&gt;peak/resting&lt;/sub&gt;</strong></td>
<td>3.3±1.4 (1.7-5.7)</td>
<td>4.1±1.6 (1.5-6.8)</td>
<td>4.6±1.7 (2.7-7.4)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>NH₃ ratio&lt;sub&gt;peak/resting&lt;/sub&gt;</strong></td>
<td>1.1±0.2 (0.8-1.3)</td>
<td>3.5±2.1 (1.8-8.3)</td>
<td>4.1±1.4 (2.1-5.6)</td>
<td>P=0.01²</td>
</tr>
<tr>
<td><strong>Lactate ratio/NH₃ ratio</strong></td>
<td>3.32±1.30 (1.30-5.50)</td>
<td>1.90±0.72 (1.8-8.3)</td>
<td>1.04±0.54 (1.49-2.20)</td>
<td>P=0.01²</td>
</tr>
<tr>
<td><strong>Specific lactate production [mmol x s / L x kN]</strong></td>
<td>0.61±0.32 (0.39-1.20)</td>
<td>0.67±0.34 (0.20-1.70)</td>
<td>0.63±0.26 (0.39-1.08)</td>
<td>ns</td>
</tr>
<tr>
<td><strong>Specific NH₃ production [mmol x s / L x kN]</strong></td>
<td>0.21±0.10 (0.11-0.39)</td>
<td>0.60±0.29 (0.21-1.90)</td>
<td>0.56±0.26 (0.26-1.8)</td>
<td>P=0.01²</td>
</tr>
</tbody>
</table>

<sup>1</sup> MCF mean contraction force

<sup>2</sup> post hoc analysis: P≤0.01 TT vs. CT and TT vs. CC
DISCUSSION

If NH₃ acts as an effector of glycolysis in-vivo, the lactate production upon short-term high intensity exercise should be lower in individuals with AMPD deficiency than in wildtype. However, the lactate production related to the workload did not show significant differences upon ischemic short-term high intensity exercise in CC, CT, and TT in the present study. These results are in accordance with previous studies which found no different lactate accumulation upon different exercise protocols in CC and TT [5,7]. In these studies, however, the lactate production was not related to the workload. Lactate and NH₃ concentrations showed no correlation within the 3 genotype groups.

CONCLUSIONS

AMPD deficiency does not seem to affect either TCA or glycolysis by anaplerosis upon exercise in-vivo. The findings of this study are consistent with the notion that AMPD deficiency represents a harmless variant rather than a muscular disorder [4].

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