AMP deaminase deficiency is associated with lower sprint cycling performance in healthy subjects

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AMP deaminase deficiency (AMPD) deficiency is an inherited disorder of skeletal muscle found in ~2% of the Caucasian population. Although most AMPD-deficient individuals are asymptomatic, a small subset has exercise-related cramping and pain without any other identifiable neuromuscular complications. This heterogeneity has raised doubts about the physiological significance of AMPD in skeletal muscle, despite evidence for disrupted adenine nucleotide catabolism during exercise in deficient individuals. Previous studies have evaluated the effect of AMPD deficiency on exercise performance with mixed results. This study was designed to circumvent the perceived limitations in previous reports by measuring exercise performance during a 30-s Wingate test in 139 healthy, physically active subjects of both sexes, with different AMPD1 genotypes, including 12 AMPD-deficient subjects. Three of the deficient subjects were compound heterozygotes characterized by the common c.34C>T mutation in one allele and a newly discovered AMPD1 mutation, c.404delT, in the other. While there was no significant difference in peak power across AMPD1 genotypes, statistical analysis revealed a faster power decrease in the AMPD-deficient group and a difference in mean power across the genotypes ($P = 0.0035$). This divergence was most striking at 15 s of the 30-s cycling. Assessed by the fatigue index, the decrease in power output at 15 s of exercise was significantly greater in the deficient group compared with the other genotypes ($P = 0.0006$). The approximate 10% lower mean power in healthy AMPD-deficient subjects during a 30-s Wingate cycling test reveals a functional role for the AMPD1 enzyme in sprint exercise.

myoadenylate deaminase deficiency; lactate; exercise; skeletal muscle; fatigue

During short-term, high-intensity exercise, the rate of ATP hydrolysis exceeds the potential of the muscle cell to rephosphorylate ADP and leads to excessive formation of the latter. This displaces the adenylate kinase equilibrium (2ADP ⇌ ATP + AMP) and results in an increase in AMP content. In skeletal muscle, such conditions activate the enzyme adenosine monophosphate deaminase (AMPD), which catalyzes hydrolysis of AMP to inosine monophosphate (IMP) and ammonia. AMPD plays a central role in the regulation of muscle energy metabolism and is believed to be important for normal muscle function (19). For example, the AMPD reaction further displaces the adenylate kinase reaction in the direction of ATP formation during exercise, which simultaneously provides additional energy and prevents a large increase in ADP. This also serves to maintain a high ATP-to-ADP ratio that is advantageous for sustained muscle work.

Approximately 1–2% of the general Caucasian population exhibits a skeletal muscle AMPD deficiency (22, 34). The skeletal muscle-specific isoform of AMPD is encoded by the AMPD1 gene (27), and an overwhelming majority of deficient cases are due to a C to T transition at nucleotide 34 in exon 2 of the gene (c.34C>T; rs17602729), which creates a nonsense codon (p.12Q>X) that prematurely terminates translation (21). However, other rare AMPD1 mutations have also been described (11, 16). CC individuals (homozygotes for the normal allele at nucleotide 34 in exon 2) have high skeletal muscle AMPD activities, TT individuals (homozygotes for the c.34C>T mutation) have extremely low activities, and heterozygotes (CT genotype) have intermediate activities (24).

In accordance with the proposed role of this enzyme in skeletal muscle energy metabolism, exercise intolerance would be an expected consequence of an AMPD deficiency. An inability to deaminate AMP should minimize displacement of the adenylate kinase reaction in the direction of ATP formation during high-intensity exercise and accentuate the accumulation of ADP, which has been shown in rabbit and rodent skeletal muscle to reduce maximal shortening velocity (4, 35) and slow relaxation time (36). Therefore, AMPD deficiency has been proposed to result in earlier inhibition of the muscle contraction process and faster fatigue development. Support for this proposal derives from numerous reports of exercise-induced cramping, pain, and early fatigue in AMPD-deficient individuals (reviewed in Ref. 25). On the other hand, high prevalence of a skeletal muscle enzyme deficiency and the associated AMPD1 c.34C>T mutant allele (24, 34) in the general Caucasian population appears to contradict the proposed causal relationship between this genetic disorder and these myopathic symptoms.

Thus, whether AMPD deficiency alters exercise performance is still unresolved. Previous studies have examined this issue with conflicting results: some reporting diminished performance (5, 26, 28), and others normal performance (6, 25, 29, 33) in AMPD-deficient subjects. There are several factors that may have contributed to variable outcomes across studies, such as a relatively small sample size, the type of exercise performed, and the type of exercise that may have contributed to variable outcomes across studies, such as a relatively small sample size, the type of exercise.during short-term, high-intensity exercise, the rate of ATP hydrolysis exceeds the potential of the muscle cell to rephosphorylate ADP and leads to excessive formation of the latter. This displaces the adenylate kinase equilibrium (2ADP ↔ ATP + AMP) and results in an increase in AMP content. In skeletal muscle, such conditions activate the enzyme adenosine monophosphate deaminase (AMPD), which catalyzes hydrolysis of AMP to inosine monophosphate (IMP) and ammonia. AMPD plays a central role in the regulation of muscle energy metabolism and is believed to be important for normal muscle function (19). For example, the AMPD reaction further displaces the adenylate kinase reaction in the direction of ATP formation during exercise, which simultaneously provides additional energy and prevents a large increase in ADP. This also serves to maintain a high ATP-to-ADP ratio that is advantageous for sustained muscle work.

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employed, and the presence of other neuromuscular complications in deficient subjects. Therefore, the goal of the present study was to examine the effect of AMPD1 genotype on physical performance during short-term, high-intensity exercise in a large group of healthy subjects using a 30-s Wingate cycling test (3). This anaerobic performance test, designed to assess muscle power and fatigueability, has been shown to cause an extreme energy imbalance and activate AMPD, as demonstrated by a profound depletion of ATP and accumulation of IMP (2, 13, 25). Therefore, this protocol should be more suitable than the submaximal exercise protocols used in previous studies to evaluate exercise performance in an AMPD deficiency. Furthermore, our laboratory has previously shown that a 30-s Wingate cycling test results in distinct purine catabolic responses across AMPD1 genotypes (25). For example, AMPD-deficient subjects do not deplete their ATP stores or accumulate IMP, and heterozygotes accumulate less IMP than normal homozygotes. Therefore, we conducted the present study to examine the hypothesis that metabolic differences across AMPD1 genotypes will influence muscle power development and, especially, that AMPD-deficient individuals will have an earlier onset of fatigue during short-term, high-intensity exercise.

METHODS

Subjects

Participants in the present study (n = 139) were healthy, physically active subjects of both sexes, with different AMPD1 genotypes. The genotype and sex distribution of the subjects and their basic anthropometric characteristics are presented in Table 1. The subjects were selected from several studies in our laboratory, in which Wingate cycling tests were performed and ammonia accumulation following exercise was monitored. Data collected from 18 of these individuals, including 4 with AMPD deficiency, were previously reported (25). Blood samples for DNA isolation were collected from all subjects and genotyped for three different AMPD1 mutations: c.34C>T, c.468G>T, and c.404delT. The c.404delT mutation is described for the first time in the present study.

Participants completed a questionnaire regarding their health and physical activity habits. All considered themselves healthy, and none expressed complaints of exercise-induced muscle cramps or myalgias. Physical activity level was assessed by a training index scaled from 1 to 7. This index reflects intensity exercise. Training index 1 indicates sedentary subjects, training 1 h/wk; 2 is moderately trained subjects, training 1–3 h/wk; and 3 is well-trained subjects, training 4–10 h/wk. Percentage of body fat was estimated from skinfold measurements (triceps, biceps, subscapula) and used to calculate fat-free weight (7). All subjects gave their informed consent to participate in the study, which was approved by the Ethics Committee of the Karolinska Institutet.

Identification of Novel AMPD1 Mutation

Sequencing. Flanking primers were used to sequence all AMPD1 exons with BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems). Unincorporated nucleotides and primers were removed from the PCR product by using ExoSAP-IT (Amersham Bioscience). Sequence analyses were performed on an ABI Prisma model 377 DNA sequencer (PE Applied Biosystems) in the KISeq core facility, Center for Genomics and Bioinformatics at Karolinska Institutet, Stockholm.

RNA extraction and cDNA synthesis. A percutaneous muscle sample from vastus lateralis of a compound heterozygote subject harboring the c.404delT mutation was taken using a needle biopsy technique. The biopsy was immediately frozen in isopentane, precooled with liquid nitrogen, and then stored at −80°C. RNA extraction from skeletal muscle was performed using TRIzol Reagents (Invitrogen). Total RNA was quantified spectrophotometrically, and the integrity was determined by 1% agarose gel electrophoresis. Two micrograms of total RNA were used for cDNA synthesis with Superscript II Reverse Transcriptase (Invitrogen) in a 20-μl reaction, according to the manufacturer’s instructions. The cDNA was sequenced to confirm that this newly identified AMPD1 mutation was expressed at the RNA level.

PCR for DNA amplification and allele specificity. Taq DNA polymerase, dNTP mix, PCR buffer, and MgCl2 (Invitrogen) were used for amplification. Reaction mixtures of 25-μl volume contained 10–25 ng DNA and 0.2 μM of each primer. Amplification was run for 30 cycles at 95°C for 40 s, 60°C for 40 s, and 70°C for 40 s, and then the samples were incubated for an additional 5 min at 70°C.

DNA was used as the template for allele-specific PCR, and the annealing temperature was 61°C. Primers used for the allele-specific PCR were 2R: AGCCATGACCTTATGGCTATG, ex2F: AACCATTCGCCAGTGAGAGAAC wild type, and ex2mutF: AAACCTCCAGCTGAAGAGAAAT for mutated allele; 5F: GATAAGCTTACTCACCTTCAG, ex5R: CCGATACAGACCTTGAAGAAAT for mutated allele, and ex5mutR: CGGATACAGACCTTGGCAAAC AAT for wild-type allele; and ex5mutR: CGGATACAGACCTTGGCAAAC AAT for wild-type allele, and ex5mutR: CGGATACAGACCTTGGCAAAC AAT for mutated sites. Location of the used primers is shown in Fig. 1. PCR products were analyzed on a 1.5–2% agarose gel.

Genotyping

Genomic DNA was isolated from peripheral blood using the QIAamp DNA extraction kit (Qiagen). AMPD1 genotyping for the c.34C>T, c.468G>T, and c.404delT mutations was performed by a TaqMan 5’ allelic discrimination assay (Perkin-Elmer ABI Prisma 7700 Sequence Detection system; Applied Biosystems). Primer sequences for the c.34C>T polymorphism were AGTTGGTTCATCAATTTTATCTGGTTTTACCTCAG (forward) and CTTGACAAATGGCGCAAAAAG (reverse), and the TaqMan probe sequences were CAACTACACATTCT and AATACACACACCTTCA (polymorphism in bold). The probes were labeled with fluorescent dyes VIC and FAM, respectively.

Primer sequences for the c.468G>T polymorphism were GGGGACTAGTGCATCGGAGAAAT (forward) and CATCAATGTGCCGCAATTTATTTTC (reverse), and the TaqMan probe sequence was CACCTGAGCTGCAAGAAT (reverse).

Table 1. Basic characteristics of subjects with different AMPD1 genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>FFW, kg</th>
<th>Training Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (men/women)</td>
<td>42/47</td>
<td>24.8 (20–43)</td>
<td>70.6 (51–101)</td>
<td>23.3 (18–30)</td>
<td>55.3 (25–92)</td>
<td>2.7 (1–3)</td>
</tr>
<tr>
<td>CT (men/women)</td>
<td>19/19</td>
<td>25.1 (20–42)</td>
<td>71.4 (48–101)</td>
<td>23.6 (19–30)</td>
<td>56.0 (30–91)</td>
<td>2.6 (2–3)</td>
</tr>
<tr>
<td>TT+ (men/women)</td>
<td>6/6</td>
<td>28.3 (20–40)</td>
<td>72.2 (55–82)</td>
<td>23.5 (19–26)</td>
<td>56.2 (33–74)</td>
<td>2.5 (1–3)</td>
</tr>
</tbody>
</table>

Values are means with ranges in parentheses; n, no. of subjects. AMPD, adenosine monophosphate deaminase; BMI, body mass index (weight/height²); FFW, fat-free weight. Mutant allele is based on a C→T transition at nucleotide +34 of the AMPD1 gene. CC, normal homozygotes; CT, heterozygotes. The TT+ group consists of nine subjects (four men and five women) who are homozygous for the exon 2 c.34C>T mutation, and three subjects (two men and one woman) who are compound heterozygotes for the exon 2 c.34C>T and the exon 5 c.404delT mutations.
quences were TCGTTTCA/H11022 and TCGTTTCA/H11001. Primer sequences for the c.404delT polymorphism were GGGAT-TGACTCTGATATGCTGACTT (forward) and CACGTATGCATAGTGGCCCGATA (reverse), and the TaqMan probe sequences were TTTGCAAACAATTTCAA and CTTTGCAAACAATTTCAA (polymorphism in bold). The probes were labeled with fluorescent dyes VIC and FAM, respectively.

Prevalences of the newly identified c.404delT mutation and the previously reported c.468G>T mutation (11) were determined in 704 DNA samples from the Swedish population, including all subjects from the present study. AMPD1 genotypes were verified in selected samples by genotyping with a different method (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) (17). The two genotyping methods were in 100% concordance.

AMPD activity was determined in homogenates from skeletal muscle biopsies by HPLC, as previously described (23).

**Experimental Protocol**

Subjects were instructed not to engage in vigorous physical activity for 24 h before the day of the experiment and to refrain from any food intake for 3 h before the test. Each individual performed a 30-s sprint exercise on a mechanically braked cycle ergometer (Cardionics, Sweden) at maximal speed against a resistance of 7.5% of the subject's body weight (Wingate test) (1). Flywheel revolutions were counted with a sensor-microprocessor assembly. Average power output for 5-s periods was automatically registered during the test and related to body mass. Each subject performed two bouts of 30-s Wingate cycling with 20-min rest between the bouts, which is sufficient for full recovery of power output with this exercise regimen (14). Average power outputs for the two bouts were calculated for each subject. Peak power (PP), the highest power output achieved during the test (the average over the first 5-s period), and mean power (MP), the average power output during the 30-s duration of the sprint, were calculated and related to body mass. Fatigue index (FI), the degree of power decrease during the test, was calculated as the difference between PP and power at 15 s as a percentage of PP (FI-15) and the difference between PP and power at 30 s as a percentage of PP (FI-30) (15).

Blood samples for determination of lactate and ammonia were collected from an antecubital vein at rest in the supine position before exercise. Immediately after the first Wingate cycling, the subjects adopted a supine position again, and blood samples were collected for ammonia and lactate measurements at 3, 6, and 9 min after exercise. For the determination of ammonia, blood was centrifuged immediately, and the supernatants were frozen in liquid nitrogen and stored at −70°C until analysis by a flow injection technique (32). Lactate was analyzed in neutralized perchloric acid extracts of whole blood by a fluorometric enzymatic method (20).

**Fiber-Type Composition**

Fiber-type composition was determined in biopsy samples available from 72 subjects (CC, n = 42; CT, n = 22, and TT; n = 8). The TT+ designation is used to indicate the inclusion of compound heterozygotes, as described below. Type I (slow twitch) and type II fibers (fast twitch) were distinguished using a myofibrillar ATPase histochemical stain (8).

**Statistics**

Factorial ANOVA was performed with AMPD1 genotype as an independent variable, and age, height, weight, body mass index, percent body fat, fat-free weight, and training index as dependent variables to verify matching of the three genotype groups. Repeated-measures ANOVA, with AMPD1 genotype as an independent variable and time (power output after 5, 10, 15, 20, 25, and 30 s of cycling) as a dependent variable, was used to analyze differences between genotypes in power development during the Wingate test. To evaluate the differences in the power output profiles across genotypes, factorial ANOVA was performed with AMPD1 genotype as an independent variable, and PP, MP, FI-15, and FI-30 as dependent variables. Repeated-measures ANOVA, with AMPD1 genotype as an independent variable and time (lactate and ammonia levels at rest and at 3, 6, and 9 min following Wingate) as a dependent variable was used to analyze differences between genotypes in lactate and ammonia accumulation after the Wingate cycling. Correlation between variables was tested by linear regression analysis (single and multiple). Statistical significance was accepted at P < 0.05.

**RESULTS**

**Mutation Analysis**

A novel AMPD1 mutation was investigated in three siblings (family 1 in Fig. 2), one of who had been previously characterized as a c.34C>T heterozygote devoid of skeletal muscle AMPD activity (24, 25). Genomic DNA was used to amplify and sequence all exons in the AMPD1 gene in these three individuals. Two of the three siblings, including the c.34C>T heterozygote, showed a single nucleotide deletion in exon 5, c.404delT (Fig. 3). The resulting frame shift mutation in AMPD1 mRNA is predicted to result in amino acid substitutions from residues 135–174 and premature termination of translation at codon 175. Sequencing of cDNA demonstrated that both mutations were expressed on the RNA level. Results from allele-specific PCR performed on DNA from the three siblings provide strong circumstantial evidence that the c.34C>T and c.404delT mutations are located within separate alleles in this compound heterozygote, which would be consistent with the previously observed skeletal muscle enzyme deficiency.

![Fig. 2. Pedigree showing the three compound heterozygotes for the c.34C>T and the c.404delT mutations in the AMPD1 gene (subjects 3, 4, and 5) identified in two families. Proposed haplotypes of the mutant alleles are also shown. wt, Wild type; del, deletion.](http://jap.physiology.org/)
The newly identified c.404delT mutation and a previously described c.468G>T mutation (11) were analyzed in 704 DNA samples from the Swedish population, including all subjects participating in the present study. Although no c.468G>T mutations were identified, one c.404delT allele was found (individual 4 in Fig. 2), indicating that this frame shift mutation is rare in the Swedish population. Notably, the individual in this sample carrying the c.404delT mutation was also a compound heterozygote with the common c.34C>T AMPD1 mutation. Furthermore, additional genotyping revealed a sibling who was also a compound heterozygote subject carrying the c.404delT and the c.34C>T mutations (individual 5 in Fig. 2). AMPD assays performed using skeletal muscle biopsy samples showed that both compound heterozygous siblings in family 2 lacked enzyme activity, further supporting the notion that the c.404delT mutation results in a dysfunctional AMPD enzyme.

Subjects

Table 1 shows sex and AMPD1 genotype distribution across all subjects and their basic anthropometric characteristics. Of the 139 subjects participating in the present study, 89 were homozygotes for the normal C34 allele (CC), 38 were heterozygotes (CT), and 9 were homozygotes for the mutant c.34C>T allele (TT). Based on their lack of AMPD activity, the three compound heterozygotes harboring the c.34C>T and c.404delT mutations were included in the latter group, and these 12 individuals are referred to collectively as TT+.

The three groups were well matched, and no significant differences across genotypes were observed in height, weight, body mass index, fat free weight, or training index. The TT+ group was older than the CC group (mean ± SD; 28 ± 5 vs. 25 ± 4, Scheffé’s post hoc test, \( P = 0.037 \)). However, multiple-regression analysis with age and genotype as independent variables, and PP, MP, FI-15, and FI-30 as dependent variables, showed that age did not contribute significantly to any of the measured power variables.

Power Output Profiles

Figure 4 shows power output profiles during 30-s Wingate cycling normalized for body weight for the different AMPD1 genotypes, which are presented for both sexes together and also separately for men and women due to the general sex-related differences in power output. The power output profiles were different across AMPD1 genotypes (ANOVA interaction term; genotype × time; \( P = 0.0003 \) for men and women together, \( P = 0.009 \) for men separately, and \( P = 0.025 \) for women separately). However, there were no significant differences in the effect of AMPD1 genotype between sexes (ANOVA interaction term; genotype × sex; \( P = 0.346 \)), and further statistical evaluations of genotype effect on power output variables were performed on men and women together (Table 2). While there was no significant difference in PP across AMPD1 genotypes, a faster power decrease in the TT+
group resulted in a difference in MP across the genotypes ($P = 0.020$). This divergence was most striking at 15 s of the 30-s cycling and somewhat less at 30 s. Assessed by the FI, the decrease in power output at 15 s of exercise was significantly greater in the TT+ group than in the CC and CT groups ($P = 0.0006$). This difference remained at 30 s of exercise ($P = 0.046$), but was slightly smaller.

Fiber-type composition

The mean (range) percentage of type I fibers was 52% (25–73%) in CC, 53% (28–72%) in CT, and somewhat higher at 57% (43–69%) in TT+, but no statistical difference in the relative proportion of type I fibers between the three genotypes was found ($P = 0.547$). There was a significant inverse relation between the percentage of type I fibers and FI-30 ($r = 0.308$, $P = 0.009$), whereas no correlation was found between the percentage of type I fibers and MP ($r = 0.082$, $P = 0.493$), PP ($r = 0.033$, $P = 0.782$), or FI-15 ($r = 0.089$, $P = 0.450$). Moreover, correlations between the percentage of type I fibers and FI-30, as well as between the percentage of type I fibers and FI-15, demonstrate that the TT+ group develops higher FI than the CC and CT groups at the same percentage of type I fibers. Furthermore, a multiple-regression analysis was applied to investigate the correlation between the fiber-type composition and genotype on the one hand and the measures of fatigue FI-30 and FI-15 on the other. While the fiber-type composition ($P = 0.005$) but not the genotype ($P = 0.100$) made a significant contribution to FI-30, the opposite was observed for the FI-15, where genotype ($P = 0.028$), but not the fiber-type composition ($P = 0.298$), made a significant contribution. This demonstrates that fatigue development is related to fiber-type composition during the 30-s Wingate cycling, but also points to an important contribution of the genotype to the observed earlier power decrease.

Metabolic Responses

The TT+ group showed almost no increase in plasma ammonia after the Wingate cycling, which is consistent with a lack of skeletal muscle AMPD enzyme activity. Their plasma ammonia levels were (means ± SD) 15.9 ± 8.0 μmol/l at rest and increased to the highest level of 21.7 ± 9.0 μmol/l at 6 min after Wingate cycling. There was no difference in plasma ammonia accumulation between the CC and CT groups at any time point. Regardless of AMPD1 genotype, plasma ammonia accumulation was different between men and women (ANOVA interaction term; sex × time; $P < 0.001$). Plasma ammonia levels following exercise were lower at every time point in women compared with men and leveled off earlier in women. The highest values (excluding TT+) of 102 ± 46 and 164 ± 43 μmol/l were observed at 9 min after exercise in women and men, respectively.

The levels of venous blood lactate at rest and following exercise are presented in Fig. 5. There was no difference in blood lactate accumulation across AMPD1 genotypes (ANOVA interaction term; genotype × time; $P = 0.851$), but, like plasma ammonia levels, a significant difference was observed between the sexes (ANOVA interaction term; sex × time; $P < 0.001$). Blood lactate levels following exercise were lower in women than in men at every time point, and peak values of 10.0 ± 2.3 and 12.2 ± 2.6 mmol/l were observed at 9 min after exercise in both women and men, respectively.

**DISCUSSION**

The aim of the present study was to examine the effect of AMPD1 genotype on physical performance during short-term, high-intensity exercise in a relative large group of healthy, moderately trained subjects. In total, 139 individuals with different AMPD1 genotypes were compared. Eighty-nine CC subjects with high AMPD enzyme activities, and 38 CT and 12 TT+ individuals with intermediate and low-enzyme activities, respectively, performed 30-s Wingate cycling. Although no differences in PP outputs were observed across different AMPD1 genotypes, the TT+ group exhibited a more rapid power decrease and, consequently, lower MP output compared with the CC and CT genotypes. Conversely, no difference in power output was observed between CC and CT subjects.

Previous studies have also attempted to confirm the original suggestion of Fishbein et al. (9), i.e., that skeletal muscle AMPD deficiency is associated with reduced exercise performance. Some have reported diminished performance in TT vs. CT and/or CC subjects (5, 26, 28), whereas others observed no differences across AMPD1 genotypes (6, 25, 29, 33). One potential reason for these conflicting results may have been a limitation in the number of study participants, particularly AMPD-deficient subjects. AMPD deficiency occurs in 1–2% of Caucasians and is even less frequent in other populations (10, 22, 26). It is, therefore, difficult to find and recruit large numbers of AMPD-deficient subjects. Given this limitation, it is notable that studies reporting differences in exercise performance across AMPD1 genotypes are generally those that have involved relatively larger numbers of total participants or the highest number of AMPD-deficient individuals. For example,
Another potential reason for conflicting results in previous comparative exercise performance studies may have been variation in the type and intensity of exercise, which determines substrate utilization and can result in different metabolic responses with variable effects on AMPD activity. Also, the diminished exercise performance of TT individuals observed in some studies may have been related to factors other than an AMPD deficiency per se. This abnormality in purine catabolism was initially identified among patients with various neuromuscular disorders, and some of the earlier studies evaluated exercise performance in patient cohorts exhibiting diverse clinical presentations that likely had an influence on the observed results (6, 21, 22). Furthermore, it was subsequently discovered that 2% of the entire Caucasian population harbored an inherited AMPD deficiency and that most of these individuals were asymptomatic (22, 34). This strongly suggests that there are other contributing factors to the myopathy in symptomatic patients with an apparent AMPD1 deficiency.

The present study attempted to circumvent these limitations. A relatively large volunteer group was employed comprised solely of healthy, physically active volunteers with similar training background, yet different AMPD1 genotypes. In addition, more asymptomatic AMPD-deficient subjects were examined than in any previous study. Also, the 30-s Wingate cycling test was used to assess exercise performance, which, in normal subjects, has been shown to produce a pronounced depletion of ATP and accumulation of IMP that indicates a powerful activation of AMPD (13, 25, 31). Furthermore, AMPD1 genotypes determine skeletal muscle AMPD enzyme activity, which correlates with the amount of ATP depletion and IMP accumulation (13, 25, 31). Thus the 30-s Wingate cycling test is suited to an assessment of potential differences in performance between AMPD1 genotypes.

Results of the present study indicate that AMPD deficiency is associated with diminished exercise capacity in healthy individuals during the demanding conditions of a Wingate cycling test, although the magnitude of this effect was relatively small in TT+ subjects. Nevertheless, the approximate 10% lower MP during the very demanding exercise underscores a functional role for AMPD in skeletal muscle. How this might relate to exercise-related cramping and pain in a small subset of the AMPD-deficient population is unclear. De Ruiter et al. (5) examined eight individuals with AMPD deficiency, one of whom was asymptomatic and the only one among this group able to complete the protocol without difficulty. The mechanism by which an AMPD deficiency leads to earlier fatigue also remains to be elucidated. Although skeletal muscle lactate accumulation was not measured in the present study, the lack of observed differences in plasma lactate accumulation across genotypes indicates that anaerobic energy utilization is not impaired in AMPD1 deficiency. This conclusion is also supported by earlier studies using other exercise conditions that also reported no differences in lactate accumulation across AMPD1 genotypes (28, 29, 33).

The most dramatic difference in power output profiles across AMPD1 genotypes in the present study was the faster power decrease in TT+ compared with CT and CC subjects. Moreover, the power output profile in these healthy AMPD-deficient individuals assumed a concave shape, while those of the CC and CT groups were nearly linear and more characteristic for this type of exercise. The atypical power development profile

![Fig. 5. Venous blood lactate (means ± SE) at rest and after 30-s Wingate cycling in different AMPD1 genotypes: CC, CT, and TT+. A: men and women; B: men; C: women. P value for the interaction term (genotype × time) in the ANOVA analysis indicates level of significance for the comparison of plasma lactate levels between genotypes.](http://jap.physiology.org/)

Rico-Sanz et al. (26) studied a group of 503 individuals and reported a higher rating of perceived exertion, as well as lower maximal values for power output in six TT subjects during submaximal exercise progressing to exhaustion. In addition, De Ruiter et al. (5) used voluntary and electrically stimulated repetitive submaximal isometric contractions and observed earlier fatigue in eight AMPD-deficient (TT) patients compared with a similar number of controls. Conversely, two other studies employing smaller numbers of total individuals, i.e., 15–19, and/or smaller numbers of TT subjects, i.e., 3–5, were unable to detect any difference in exercise performance across AMPD1 genotypes that was statistically significant (22, 25), although notable trends were reported. For example, the results of Tarnopolsky et al. (33) indicated lower PP and shorter time to fatigue for TT subjects compared with the two other genotype groups during incremental cycle exercise to exhaustion. Similarly, data reported in Sinkeler et al. (29) suggest lower maximal voluntary contraction force and shorter endurance times in AMPD-deficient patients compared with controls.
in an AMPD deficiency could be related to an earlier and accentuated ADP accumulation during conditions of high ATP turnover. Transient increases of ADP likely occur in contracting muscle, particularly at fatigue when the capacity to repolymerize ADP is decreased. The transient accumulation of ADP displaces adenylate kinase equilibrium and results in an increase in the production of AMP. In normal muscle, AMP is rapidly deaminated to IMP by an activated AMPD, which limits the accumulation of ADP. Increases in ADP have been shown to reduce maximal shortening velocity of contracting skeletal muscle (4, 35) and slow relaxation time (36). The inability to deaminate AMP in AMPD-deficient individuals could, therefore, lead to earlier inhibition of the muscle contraction process and faster fatigue development. Studies in adenylate kinase knockout mice have demonstrated the importance of AMP accumulation and subsequent deamination in limiting the skeletal muscle accumulation of ADP and showed that delayed relaxation kinetics was a functional consequence of AMPD deficiency in these animals, although the contractile performance was relatively unaffected by supraphysiological levels of this metabolite (12). However, the slowing of the relaxation kinetics due to a longer cross-bridge attachment at elevated ADP concentrations involves a reduced shortening velocity and may explain the faster decrease in power output during Wingate cycling in TT+ individuals.

On the other hand, the augmented ADP accumulation could lead to a more pronounced activation of oxidative phosphorylation and thereby to a more rapid onset of oxidative metabolism in TT+ individuals. During the later phase of a 30-s Wingate test, there is an increasing contribution of oxidative metabolism to energy generation (18, 30), and the observed smaller difference in power output at 30 s compared with 15 s between TT+ and other individuals may indicate a greater dependence on oxidative metabolism that could act as a compensatory mechanism in AMPD deficiency.

Although fiber-type composition seemed to influence power decrease in the present study, the earlier fatigue development in TT+ subjects was shown to be more dependent on the AMPD1 genotype. This supports the conclusion that the atypical power development profile in these subjects may be related to an earlier and accentuated ADP accumulation. Moreover, the larger power decrease observed in the TT+ group than in CC and CT groups at a certain fiber-type composition may indicate a greater dependence on oxidative metabolism that could act as a compensatory mechanism in AMPD deficiency.

In conclusion, this study was designed to overcome limitations inherent to previous functional examinations of skeletal muscle AMPD deficiency and has generated data that show a significant effect on exercise performance in otherwise healthy individuals harboring this abnormality in adenine nucleotide catabolism. Although quantitatively small, diminished exercise performance in an inherited deficiency of AMPD lends support to the idea that this enzyme plays a functional role in healthy skeletal muscle.

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