Muscle function during fatigue in myoadenylate deaminase-deficient Dutch subjects

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ABSTRACT

Myoadenylate deaminase (MAD) is an enzyme active in skeletal muscle, probably during exercise of moderate intensity but certainly during vigorous exercise, when the deamination of AMP leads to increased levels of IMP and ammonia. There is controversy about the clinical significance of MAD deficiency. The main objective of the present study was to investigate the extent to which genetically confirmed MAD deficiency affects muscle function under conditions of maximal short-term electrically induced activation. The left hand was immobilized and adductor pollicis muscle function was investigated. To exclude the influence of central factors, such as the patient’s motivation, the ulnar nerve was maximally electrically activated and force output was measured at the thumb. Sixty rapid shortening contractions resulted in a decrease of maximal power to 34.2±5.4% and 33.3±6.3% (means±S.D.) of the values for unfatigued muscle in the control and MAD-deficient subjects respectively (P<0.05; n=7). Maximal isometric forces and shortening velocities did not differ between groups in unfatigued, fatigued or recovered muscle. None of the subjects experienced exercise-related muscle aches or cramps. In conclusion, MAD deficiency does not appear to affect adductor pollicis muscle force, shortening velocity and relaxation, either during or after maximal short-term activation.

INTRODUCTION

Adenylate deaminase has a muscle-specific isoform, called myoadenylate deaminase (EC 3.5.4.6). The enzyme converts AMP into IMP and ammonia during vigorous muscle exercise. In subjects where there is a deficiency of the enzyme, first described by Fishbein et al. [1], this conversion does not occur. In the vast majority of cases MAD deficiency is caused by a common homozygous point mutation (C34T) in the second exon of the gene. The mutation causes a premature termination codon, resulting in a severely truncated protein [2]. Patients with this defect have been reported to suffer from exercise intolerance, increased fatigability, exercise-induced muscle pains, stiffness, cramping and delayed recovery of muscle strength [1,3–9]. On the other hand, some MAD-deficient subjects do not have any muscle dysfunction [9,10]. A recent study on the frequency of the common C34T mutation in patients with neuromuscular complaints and in healthy volunteers presented evidence that MAD deficiency may be a harmless genetic variant [11]. Nevertheless, many of the reported MAD-deficient subjects seem to experience exercise-related symptoms, which may be of clinical significance [2–8,12], as suggested by Fishbein et al. [1] in 1978.

It has been shown repeatedly that muscle metabolism is different during exercise in MAD-deficient compared with normal muscle [4,8,13–16], and in some studies the deficiency reduced muscle performance during exercise of moderate intensity [4,8,12], whereas others did not

Key words: fatigue, force/velocity, IMP, MAD deficiency, skeletal muscle.
Abbreviations: Fmax, maximal isometric force; MAD, myoadenylate deaminase; Pmax, maximal power; Vmax, maximal shortening velocity; Vopt, optimal shortening velocity.
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find a reduction of performance during high-intensity exercise [14,16]. These previous studies all used some form of voluntary exercise, which has the limitation that the performance to a large extent depends on the subject’s motivation. This is a major point of concern, especially when working with patients who might anticipate not performing very well. In the present study the influence of the subject’s motivation was eliminated by the use of electrical nerve stimulation, which guarantees maximal activation of the entire muscle. In relation to the set-up of the present study, it is important to note that the greatest increases in IMP levels are found during severe metabolic stress when MAD is highly activated [17,18]. Indeed, significant metabolic differences between normal and deficient muscle have been found during high-intensity exercise under ischaemic conditions [14], but it is still unclear whether these metabolic differences are accompanied by differences in muscle output or not. Therefore, in the present study, power production and force relaxation of normal and MAD-deficient human adductor pollicis muscle was assessed before, during and after a series of maximally activated shortening contractions with occluded blood flow.

METHODS

Subjects

Seven healthy (19–57 years of age) and seven MAD-deficient (32–61 years of age) male subjects took part in the experiments. The approval of the Ethical Committee of the Vrije Universiteit was obtained, and all subjects gave their informed consent.

Diagnosis of MAD deficiency was established by an ischaemic forearm test (high lactate in the absence of ammonia) in all seven subjects [19]. Six of these subjects were available for follow up, and MAD deficiency was confirmed in all six both immunohistochemically using muscle material and at the DNA level by demonstration of the C34T mutation in genomic DNA extracted from blood samples [11]. The control and deficient subjects were homozygotes for the normal and mutant alleles respectively.

The subjects in the control group were healthy untrained volunteers. All MAD-deficient subjects except one, who was a symptom-free recreational tennis player, reported muscle aches and early fatigue during and after more intensive use of their muscles in everyday life. The symptom-inducing activities varied among and within patients, e.g. gardening, having a 10 min telephone conversation, doing mechanical work on a motorbike, and using a screwdriver. Three of the MAD-deficient subjects had no (other) known disorders; the other three suffered from atherosclerosis, psoriasis and low-back pain due to a radicular syndrome respectively.

Experimental set-up

The methods for stimulating the adductor pollicis, recording force and imposing length changes are given in detail elsewhere [20]. Briefly, the subject sat in an adjustable chair with the left forearm supinated, the hand held horizontally and the thumb abducted and in contact with a vertical pin attached to a strain gauge mounted below the plane of the hand. The entire hand, with the exception of the thumb, was immobilized with a Perspex plate, which was tightened down on to a mould fitting into the palm. The force transducer was attached, via a lever system, to a linear step motor, such that linear displacement of the motor was converted into rotation around the carpo-metacarpal joint of the thumb.

When the thumb was fully adducted, its length axis was parallel with the length axis of the index finger, and this position was defined as the 0° thumb angle. Because the vertical pin of the force transducer was placed between the thumb and the index finger, the smallest thumb angle at which forces could be measured was 36°. It was possible to increase the thumb angle up to 74° (maximal abduction) before anatomical limits were approached. Thus, during shortening contractions, the maximal angular displacement was 38°.

The timing and duration of stimulation, onset and speed of motor movement and data sampling frequency (1000 Hz) of the force and length signal were computer controlled.

Subjects were first familiarized with the electrical stimulation and other procedures. The adductor pollicis muscle was activated by percutaneous electrical stimulation of the ulnar nerve at the wrist with constant current unidirectional square-wave pulses of 100 μs duration at a frequency of 80 Hz (model DS7; Digitimer Ltd, Welwyn Garden City, U.K.).

To maintain a constant muscle temperature, the subject’s hand and forearm were immersed in a water bath at 45 °C for 30 min prior to the test. During the experiment a lamp was used to maintain skin temperature over the adductor pollicis at 36.0 ± 0.5 °C, measured with a thermocouple.

Experimental protocol

Force/angle curves were obtained for each subject using short isometric contractions. As reported previously [20], force was little affected by the length of the muscle (changes of < 6% within the range studied), with maximal force occurring at a thumb angle of approx. 51°.

Force/velocity curves were constructed using iso-velocity contractions at seven different angular velocities (0, 76, 153, 229, 306, 382 and 458°/s) applied in random order as described in detail elsewhere [21]. The thumb was adducted twice at each imposed velocity: once with, and once without ( = passive shortening), stimulation of the adductor pollicis. At each velocity the passive force
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Figure 1  Force/velocity and power/velocity relationships

Force (left y-axis)/velocity (x-axis) relationships of MAD-deficient (D) and control (+) human adductor pollicis muscle are shown for the unfatigued (A), fatigued (B) and recovered (C) states (means ± S.D.; n = 7). Note that symbols often coincide. The power (right y-axis)/velocity curves of the controls (solid lines) and MAD-deficient subjects (broken lines) were calculated from the fitted force/velocity relationships and normalized to peak power in (A).

was subtracted from the total force trace to provide a measure of the active force. A 2 min rest period was allowed between contractions. The entire relationship was obtained within 20 min.

After the force/velocity measurements had been completed in the unfatigued muscle, blood supply was occluded by a cuff inflated around the subject’s upper arm, and the muscle was fatigued by 60 isovelocity (153°/s) contractions (50 Hz stimulation frequency). The thumb was adducted from 74° to 38° during each contraction, which were of 240 ms duration, with rest periods of 760 ms in between to allow complete force relaxation before the thumb was adducted back to 74° for the start of the next contraction. This 60 s protocol was chosen because it is known that MAD is particularly activated during repetitive shortening contractions without blood flow [17,18]. Moreover, with the shortening speed of 153°/s used during the fatigue protocol, the adductor pollicis muscle would be operating at a speed close to that at which maximal power is produced and, hence, the greatest energy flux was expected. Thus a rather extreme condition was created which was expected to profoundly increase MAD activity in the healthy individuals, and thus potentially would enhance any differences in muscle power output between the groups.

The fatigue protocol was immediately followed by a series of isovelocity contractions (seven different imposed velocities in random order) to obtain a force/velocity relationship in the fatigued muscle. The entire series of isovelocity contractions in the fatigued state took less than 20 s. Thereafter the cuff was deflated and the muscle allowed to recover. The recovery process was studied in detail. One isometric and one isovelocity contraction (153°/s) were, in alternating order, applied at 45 s and 1.5, 3 and 6 min following restoration of blood flow. After a 12 min recovery period a complete force/velocity relationship was obtained.

During pilot experiments, the first contraction in the fatigued state was repeated at the end (eight contractions in total). The force during this last contraction was approx. 10–15% lower than that during the first contraction in the fatigued state. This extra fatigue was inevitable, as blood flow was occluded and hence there was no recovery. However, this did not affect our results, because the contractions at the seven different speeds were applied in random order to the control and MAD-deficient subjects.

Data analysis

Forces measured at the 51° thumb angle were used to construct the force/velocity relationship of the muscle in the unfatigued, fatigued and recovered states. Data points were fitted (least squares) to a hyperbola described by the Hill equation [22]. Force values from these curves were multiplied by the velocity to obtain power/velocity curves. Optimal shortening velocity (\(V_{\text{opt}}\)) was defined as the velocity of shortening giving the highest power output on the velocity/power curve. Maximal shortening velocity (\(V_{\text{max}}\)) was determined as the intercept of the Hill curve with the velocity axis. The half-times of relaxation from the isometric contractions were determined as the time taken for force to fall to 50% of its maximum value at the end of stimulation. During the fatigue protocol, work per contraction was calculated by integrating force over adduction angle; consequently work output is presented in N°.

Statistics

All results are presented as means ± S.D. Analysis of variance (ANOVA) for repeated measures was used for
determination of statistical significance ($P < 0.05$) with respect to the within-subjects factor ‘time’ (this is the effect caused by the fatiguing exercise), the between-subjects factor ‘deficiency’ (the effect of the MAD deficiency) and the interaction of these two main effects. Simple difference contrasts were used for further analysis where statistical differences were located over time.

**RESULTS**

The force/velocity relationships of the fresh adductor pollicis muscle of control and MAD-deficient subjects were not different (Figure 1A). $V_{\text{max}}$, $V_{\text{opt}}$ and maximal isometric force ($F_{\text{max}}$) in control and MAD-deficient subjects respectively were $736 \pm 100^\circ/s$ and $735 \pm 124^\circ/s$, $222 \pm 20^\circ/s$ and $224 \pm 36^\circ/s$, and $77.0 \pm 9.8$ N and $78.7 \pm 11.7$ N.

Power decreased significantly during the 60 repetitive shortening contractions (Figure 2). For the 60th contraction, the power output of the control subjects ranged from 35.1 to 46.8% of that for the first contraction (= 100%). For five of the MAD-deficient subjects, including the symptom-free tennis player, power during the 60th contraction decreased to $27.2 \pm 0.9\%$ (range $25.6–27.8\%$), whereas the values for the other two deficient subjects ($40.7$ and $39.2\%$) fell clearly within the range of values for the control subjects. However, the decrease in power during the 60 contractions was not significantly different between the groups (Figure 2). The total work output for the control and MAD-deficient subjects was $46913 \pm 2255$ and $43995 \pm 7746$ N° respectively ($P > 0.05$).

Following the fatiguing exercise, the force/velocity relationships were shifted to the left in both groups (Figure 1B). $F_{\text{max}}$ decreased to $47.9 \pm 5.2$ N and $47.1 \pm 5.8$ N in the control and MAD-deficient groups respectively ($P > 0.05$). $V_{\text{max}}$ also declined to similar values in the control ($492 \pm 47^\circ/s$) and the deficient ($460 \pm 52^\circ/s$) group. Consequently, there was a significant decrease in maximal power production, to $34.2 \pm 5.4\%$ (controls) and $33.3 \pm 6.3\%$ (MAD-deficient) of the values for fresh muscle (Figure 1B). There was an approx. 3-fold slowing of muscle relaxation, which was similar ($P > 0.05$) in the two groups (Figure 3B). Together, these findings illustrate that there was a significant degree of muscle fatigue, which was not statistically different between the two groups.
Figure 1(C) shows the force/velocity and power/velocity data after a 12 min recovery period. Maximal power ($P_{\text{max}}$) had returned to $99.0 \pm 3.4\%$ and $90.0 \pm 7.1\%$ of baseline values in muscles from MAD-deficient and control subjects respectively. However, when $P_{\text{max}}$ values were tested statistically between the two groups in the fresh, fatigued and recovered muscles, there were no significant differences. Similar statistical results were obtained with respect to $F_{\text{max}}$, $V_{\text{opt}}$ and $V_{\text{max}}$, indicating that there were no differences between the two groups.

The changes over time, particularly during the recovery process, were studied in more detail for $F_{\text{max}}$ (Figure 3A), half-relaxation time (Figure 3B) and dynamic force (force during shortening at $153^\circ /s$) (Figure 3C). For these three parameters there were significant changes over time (unfatigued, fatigued, and after 0.45, 1.5, 3, 6 and 12 min of recovery), but again there were no differences between groups. $F_{\text{max}}$ and half-relaxation time had returned to baseline values after 6 min of recovery (at $t = 7$ min in Figures 3A and 3B). Dynamic force, however, did not recover completely in the control subjects (Figure 3C). There was a significant interaction effect ($P = 0.03$) of the factors ‘deficiency’ and ‘time’, indicating that the recovery of dynamic force was influenced by the MAD deficiency. This interaction was significant after 6 and 12 min of recovery, which means that, similar to the recovery of $P_{\text{max}}$, there was a tendency for more complete recovery of dynamic force in MAD-deficient muscle (see also Figure 1C).

**DISCUSSION**

The present investigation gives the first detailed account of exercise-induced changes in muscle function of MAD-deficient muscle. The main conclusion of the present work is that there were no significant differences in adductor pollicis muscle power output between the MAD-deficient and the control subjects before, during or after short-term maximal dynamic exercise. Some previous studies showed an effect of MAD deficiency on performance during exercise of rather moderate intensity [4,8,15], whereas others failed to show such an effect during more intensive exercise [14,16]. There are indications that MAD deficiency is a harmless polymorphism and not a disease in itself [11,23].

It has to be noted that, in contrast with the voluntary exercise protocols used thus far [4,8,14,15], only a small muscle was activated in the present study. The adductor pollicis muscle was chosen for practical reasons: it can easily be maximally activated by electrical stimulation of the nerve, and its force can be measured without interference of other muscles. Moreover, it is easy to make this muscle ischaemic. The adductor pollicis is a relatively small muscle, but muscle size seems insignificant, because in MAD deficiency there is a lack of functional enzyme with potential local (metabolic) effects, and therefore the quality rather than the quantity of the exercised muscle seems of importance. Moreover, our patients indicated that problems occurred locally in specific (temporarily overloaded) muscles. Therefore it seems that it is not the size of the muscles involved during the exercise, but rather the load placed upon them, that induces the clinical symptoms. Furthermore, it was shown recently that performance during 30 s of maximal sprinting exercise on a cycle ergometer, which involves large muscles, was also not affected in MAD-deficient subjects [16].

The tendency for a more complete recovery in the MAD-deficient subjects (Figures 1C and 3C) was unexpected, and does not fit into the general clinical profile of MAD-deficient patients reported thus far. None of the subjects experienced any abnormal sensations, such as cramps, pain, etc., during or in the hours and days following the test.

MAD is certainly activated under conditions where the splitting of phosphocreatine, glycolysis and oxidative metabolism cannot (temporarily) keep up with ATP demand. This is expected, for example, when a screwdriver is used during mechanical work on a bike, a situation in which one of the subjects reported muscle pain. Therefore, in the present study we deliberately created extreme conditions for the muscle in order to up-regulate MAD activity as much as possible. With our electrical stimulation protocol the muscle was very heavily loaded, and fatigued to an extent that is difficult to achieve during voluntary exercise in most subjects. The underlying reasoning was that, if the deficiency of the enzyme did not affect muscle function under our extreme conditions, it would be unlikely that MAD dysfunction by itself was the direct cause of the clinical symptoms of the MAD-deficient subjects. However, there is evidence that, at least in rat muscle, the purine nucleotide cycle is also active during moderate-intensity exercise, when it may play an anapleurotic role in providing citric acid cycle intermediates, and in that situation IMP accumulation would not occur because IMP is re-aminated to AMP [24,25]. Such a metabolic function of MAD during moderate-intensity exercise may explain why some of our MAD-deficient subjects also reported problems during basically aerobic activities such as gardening and having a 10 min telephone conversation. On the other hand, the similar frequencies of MAD deficiency in patients with exercise intolerance, in patients with other neuromuscular complaints and in healthy volunteers suggests that MAD deficiency may be a harmless polymorphism and not a disease in itself [11,23]. In addition, as suggested by others [10], there may be other (as yet undiscovered) defects which could, possibly in interaction with the MAD deficiency, cause the physical problems reported by six of the seven MAD-deficient and control subjects respectively. However, when $P_{\text{max}}$ values were tested statistically between the two groups in the fresh, fatigued and recovered muscles, there were no significant differences. Similar statistical results were obtained with respect to $F_{\text{max}}$, $V_{\text{opt}}$ and $V_{\text{max}}$, indicating that there were no differences between the two groups.
deficient subjects in the present study. In fact, as was described in the Methods section, there were alternative explanations for the physical discomfort in three of them. Furthermore, the possibility cannot be excluded that adaptations have occurred in MAD-deficient muscles which can somehow compensate for potential negative consequences of the deficiency during short-term maximal exercise.

It is unlikely that differences between the two groups remained undetected due to a lack of specificity of the test used. As stated above, the exercise protocol used in the present study was designed to considerably up-regulate the deamination of AMP to IMP. Although we did not measure any metabolites, the large decreases in force and velocity (and hence power) indicate that the metabolic stress upon the muscle must have been substantial. In human skeletal muscle, high levels of IMP have been reported following 25 s [26] and 30 s [27] of voluntary sprinting exercise, when power output was reduced to about 50%. In the present study the decrease in power was even greater (to about 33%). Therefore significant amounts of IMP were probably produced in the adductor pollicis muscles of the control subjects. However, it may be argued that the adductor pollicis muscle, which was chosen for practical reasons (see above) but consists of approx. 80% slow-twitch fibres [28], is not the best choice for studying the effects of MAD deficiency on muscle function, because the activity of the enzyme is known to be higher in fast muscle fibres. For example, in human muscle biopsies of normal homozygotes, a significant (but weak) negative correlation (−0.34) has been found between the percentage of slow muscle fibres and MAD activity [23]. Nevertheless, even in biopsy material that consisted of 80% slow fibres, MAD activity was still approx. 70% of the maximal value (Figure 3a in [23]). Moreover, it has been demonstrated that MAD is functionally active in human slow-twitch fibres, since significant increases in IMP levels have been found following a 25 s sprint on a cycle ergometer, not only in fast but also in slow muscle fibres [26]. It is likely that, in the present study, IMP production in the slow-twitch fibres was even greater than during a 25 s sprint, because the exercise duration was longer (60 s) and the production of significant amounts of ATP by oxidative phosphorylation was prevented by ischaemia. In addition, the ratio of power production in fast compared with slow human muscle fibres is approx. 8:1 [29]. This means that, although only 20% of the adductor pollicis consists of fast fibres, around two-thirds of the total power production [(20% × 8)/(20% × 8 + 80 × 1%) = 0.67] of adductor pollicis muscle will be delivered by the fast fibres. Therefore, even in the highly unlikely situation that during repetitive contractions only power production by the fast fibres would be affected by MAD deficiency, we believe that this would have been detectable in the present study.

In rat muscle stimulated in situ, a high positive correlation between muscle ATP degradation (IMP formation) and fatigue has been reported following vigorous short-term exercise of various durations [18]. Similar results have been found for isolated frog fibres [30], and it has been suggested that IMP could be an important cause of muscle fatigue [30–32]. This suggestion is not supported by the present findings, since the decrease in power in the MAD-deficient muscle, which did not produce IMP, was similar to that of the control muscles. It is more likely that accumulation of hydrogen ions and inorganic phosphate is the underlying cause of the decreases in velocity and force in both normal and MAD-deficient muscle [33].

In conclusion, the changes in contractile properties of the human adductor pollicis muscle in response to 60 electrically induced shortening contractions were similar in MAD-deficient and control muscles. Consequently, the absence of MAD activity does not appear to have important functional consequences during or following maximal short-term muscle activity in humans. However, additional studies are required to investigate the possible effects of MAD deficiency on impairment of physical performance during moderate-intensity activities.

REFERENCES

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