Myoadenylate Deaminase Deficiency

Functional and Metabolic Abnormalities Associated with Disruption of the Purine Nucleotide Cycle

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Abstract. To assess the role of the purine nucleotide cycle in human skeletal muscle function, we evaluated 10 patients with AMP deaminase deficiency (myoadenylate deaminase deficiency; MDD).[1] 4 MDD and 19 non-MDD controls participated in an exercise protocol. The latter group was composed of a patient cohort (n = 8) exhibiting a constellation of symptoms similar to those of the MDD patients, i.e., postexertional aches, cramps, and pains; as well as a cohort of normal, unconditioned volunteers (n = 11). The individuals with MDD fatigued after performing only 28% as much work as their non-MDD counterparts.

Muscle biopsies were obtained from the four MDD patients and the eight non-MDD patients at rest and following exercise to the point of fatigue. Creatine phosphate content fell to a comparable extent in the MDD (69%) and non-MDD (52%) patients at the onset of fatigue. Following exercise the 34% decrease in ATP content of muscle from the non-MDD subjects was significantly greater than the 6% decrease in ATP noted in muscle from the MDD patients (P = 0.048). Only one of four MDD patients had a measurable drop in ATP compared with seven of eight non-MDD patients. At end-exercise the muscle content of inosine 5'-monophosphate (IMP), a product of AMP deaminase, was 13-fold greater in the non-MDD patients than that observed in the MDD group (P = 0.008). Adenosine content of muscle from the MDD patients increased 16-fold following exercise, while there was only a twofold increase in adenosine content of muscle from the non-MDD patients (P = 0.028). Those non-MDD patients in whom the decrease in ATP content following exercise was measurable exhibited a stoichiometric increase in IMP, and total purine content of the muscle did not change significantly. The one MDD patient in whom the decrease in ATP was measurable, did not exhibit a stoichiometric increase in IMP. Although the adenosine content increased 13-fold in this patient, only 48% of the ATP catabolized could be accounted for by the combined increases of adenosine, inosine, hypoxanthine, and IMP.

Studies performed in vitro with muscle samples from seven MDD and seven non-MDD subjects demonstrated that ATP catabolism was associated with a fivefold greater increase in IMP in non-MDD muscle. There were significant increases in AMP and ADP content of the muscle from MDD patients following ATP catabolism in vitro, while there was no detectable increase in AMP or ADP in non-MDD muscle. Adenosine content of MDD muscle increased following ATP catabolism, but there was no detectable increase in adenosine content of non-MDD muscle following ATP catabolism in vitro.

These studies demonstrate that AMP deaminase deficiency leads to reduced entry of adenine nucleotides into the purine nucleotide cycle during exercise. We postulate that the resultant disruption of the purine nucleotide

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1. *Abbreviations used in this paper:* GTP, guanosine 5'-triphosphate; IMP, inosine 5'-monophosphate; Kp, kilopon; MDD, myoadenylate deaminase deficiency; S-AMP, adenylosuccinate.

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cycle accounts for the muscle dysfunction observed in these patients.

Introduction

The purine nucleotide cycle consists of three enzymes, i.e., AMP deaminase, adenylosuccinate (S-AMP) synthetase, and S-AMP lyase, and it is shown diagramatically in Fig. 1. Numerous experimental observations in animal models suggest this series of reactions is especially active and may serve a unique function in skeletal muscle fibers: (*a*) a unique isozyme of AMP deaminase is found only in skeletal muscle (1), and one form of S-AMP synthetase is found predominantly in skeletal muscle (2); (*b*) AMP deaminase activity, the rate-limiting reaction for entry into the purine nucleotide cycle, is an order of magnitude greater in skeletal muscle than in other organs, such as liver, brain, and kidney (3, 4); (*c*) during vigorous muscle contraction ATP catabolism in fast-twitch glycolytic and oxidative fibers is associated with the accumulation of stoichiometric amounts of inosine 5′-monophosphate (IMP), as well as NH_3 production (5); (*d*) repletion of the ATP pool during recovery from muscle contraction is associated with a reduction in the IMP pool to basal levels (5, 6); (*e*) expansion of the pool of citric acid cycle intermediates that occurs during muscle contraction is the result of deamination of aspartate to fumarate via the S-AMP synthetase and lyase reactions (7, 8). Studies in human subjects, although more limited than the animal experiments, have also demonstrated NH_3 and IMP production during strenuous exercise (6, 9) and are thus consistent with the conclusion that flux through the purine nucleotide cycle increases during exercise.

Based on these studies a number of functions have been proposed for the purine nucleotide cycle. It has been suggested that IMP and/or NH_3 production regulate the rate of glycolysis (9–15); in addition, the NH_3 produced in the AMP deaminase reaction may buffer the hydrogen ions produced during ATP hydrolysis (16); deamination of AMP may act in concert with the myokinase reaction to extract more fully energy stored in the adenine nucleotide pool (10), as well as prevent AMP accumulation with its consequent effects on metabolic pathways (10); flux through the purine nucleotide cycle may function in an anaplerotic fashion to increase the production of citric acid cycle intermediates (7, 8, 10), thereby catalytically enhancing mitochondrial respiration fueled by glycogen and lipid substrates; and finally the purine nucleotide cycle may provide a mechanism for storing purine nucleotides that can be used to replete ATP pools following exercise (17). Patients with inherited enzymatic defects, as examples of experiments in nature, often provide insight into the role of metabolic pathways in normal cellular functions. In this report we evaluated 10 patients with AMP deaminase deficiency to elucidate better the role of the purine nucleotide cycle in skeletal muscle function.

More than 35 individuals with AMP deaminase deficiency have been identified and 67% of them exhibit easy fatigability and postexertional muscle cramps and/or myalgias (17–28). However, postexercise symptoms have not been reported in all patients with AMP deaminase deficiency, and this enzyme deficiency has been noted incidentally in some patients with other neurological disorders (18–21, 23–27). As a consequence some investigators have questioned whether AMP deaminase, and indirectly the purine nucleotide cycle, are important in skeletal muscle function (20, 21, 27). Therefore, the objective of this study was to determine whether patients with AMP deaminase deficiency have demonstrable muscle dysfunction, and if so, whether there is biochemical evidence for disruption of the purine nucleotide cycle in these individuals to explain their symptoms.

Methods

Patients. Four patients with AMP deaminase deficiency (myoadenylate deaminase deficiency; MDD) documented on muscle biopsy were evaluated by both bicycle ergometer testing and percutaneous muscle biopsies as described below. Each of these individuals sought medical attention because of easy fatigability and postexertional onset of muscle aches, cramps, or pains. Ischemic lactate production was normal in each of these individuals, while NH_3 production during ischemic exercise was blunted. Two of these four patients had mild elevations of serum creatine kinase at rest, but electromyograms were normal in all four patients.

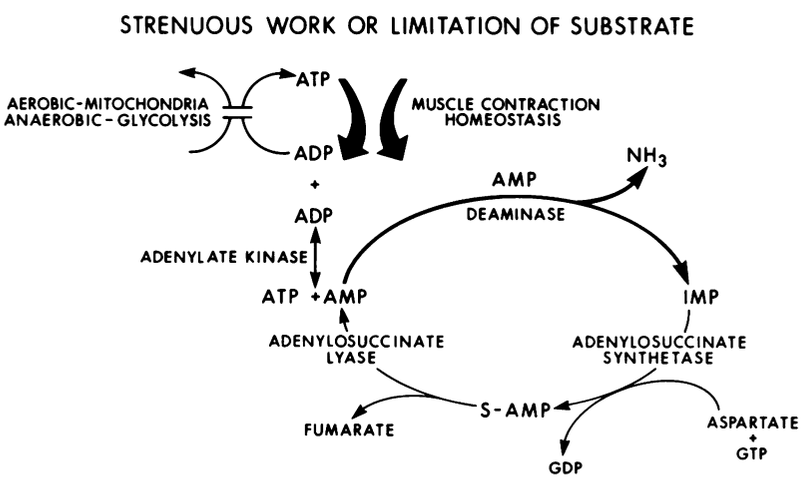

Figure 1. Reactions of adenylate metabolism in skeletal muscle.

Three of the patients were female (ages 39, 49, and 58 yr) and one was male (56 yr).

Eight patients with normal AMP deaminase activity in muscle biopsy specimens (non-MDD) were selected for study based on their presentation to a neurologist with the constellation of symptoms referred to as aches, cramps, and pains. Muscle biopsies from six of these subjects were normal when examined by routine light microscopy and histochemical studies for glycogen and lipid storage myopathies. One patient, a 20-yr-old male with documented myophosphorylase deficiency, was also included in the non-MDD patient control group. This individual was included, not only because he had normal AMP deaminase activity and similar symptoms to the other patients, but also because he provided an example of a well-characterized clinical syndrome to which the MDD subjects could be compared physiologically and biochemically. There were seven males (age 20–56 yr) and one female (33 yr) in this group. All eight of these non-MDD patients underwent bicycle ergometer testing and percutaneous muscle biopsy.

11 normal, unconditioned volunteers participated in the bicycle ergometer testing described below, but did not undergo muscle biopsy. This control group consisted of five males (age 21–41 yr) and six females (24–47 yr).

Skeletal muscle (vastus lateralis, soleus, or gastrocnemius) obtained at open-biopsy from seven individuals with normal AMP deaminase activity was frozen immediately on dry ice and stored in liquid nitrogen until used in the in vitro catabolism study described below. None of the controls in this group participated in the exercise studies described in this report. Skeletal muscle obtained in a similar manner and from the same muscle groups from seven patients with AMP deaminase deficiency were frozen immediately and stored in liquid nitrogen until used in the in vitro catabolism study. One of these patients also participated in the exercise studies.

Exercise protocol. Subjects were exercised on a bicycle ergometer (Tunturi, Inc., Finland), with a wheel diameter of 40 cm, at a constant pedal rate of 80 rpm. Beginning at 1 kilopon (Kp) of braking power, the work load was increased by 0.5 Kp every minute for males and 0.5 Kp every 2 min for females until the subject stopped exercising because of exhaustion and/or muscle discomfort. Total work loads were calculated from the following equation: total work = (Kp × D × T), where Kp = kilopons of braking power; D = distance (270 m/min at a constant pedal rate of 80 rpm); T = elapsed time in minutes.

Muscle sampling. Needle biopsy samples were taken from the vastus lateralis muscle and immediately frozen in liquid nitrogen as previously described (17). Samples were stored in liquid nitrogen for no more than 24 h and were extracted in 12% trichloroacetic acid (TCA) at 4°C. The samples were neutralized with 2 vol of 0.5 M tri-*N*-octylamine in freon as previously reported from this laboratory (29).

Samples were obtained from all patients at rest and again within 90 s after the termination of exercise (end-exercise). In the patients with AMP deaminase deficiency, additional muscle samples were taken during the 1.5–3 min following exercise. Multiple samples were obtained in these patients to assure that muscle tissue from these experimental subjects would be available for analysis. Although creatine phosphate content tended to increase and adenosine content tended to decrease during the 1.5–3-min period, the changes were relatively small, and consequently no definitive conclusions could be reached regarding the rate at which these pools returned to resting values following exercise. Nucleotide pools did not change detectably during this time. For statistical comparison between the MDD and non-MDD groups only the 90-s samples were used, since both groups were biopsied at this point. In situations where individual MDD patients are considered, the results from all end-exercise samples are given. The text specifies which biopsy samples were used for the data presented.

In vitro catabolism protocol. The frozen muscle obtained at open biopsy was divided under liquid nitrogen into three parts. One part was extracted into cold acid as described above, without permitting the tissue to thaw before extraction. Another part was immersed in 300 μl of distilled water, while the third part was immersed in 300 μl of water containing 200 μM 2′-deoxycoformycin, an inhibitor of adenosine deaminase. After incubation of both these samples at 4°C for 60 min to permit diffusion of the 2′-deoxycoformycin throughout the tissue, the samples were incubated at 37°C for 30 min. At the conclusion of this incubation, 40 μl of cold 100% TCA (vol/vol) was added to make a final concentration of ~12% TCA (vol/vol), and the extraction of nucleotides, nucleosides, and bases carried out.

Metabolite analyses. Nucleotides, nucleosides, and bases were separated and quantitated by high-performance liquid chromatography as previously described (29). The presence of adenosine in the muscle extract, which coeluted with authentic adenosine and exhibited the appropriate 254/280 absorption ratio, was confirmed by demonstrating that this peak on the chromatogram shifted to inosine following exposure of the extract to commercially prepared adenosine deaminase (Sigma Chemical Co., St. Louis, MO). Creatine and creatine phosphate were identified and quantitated by high-performance liquid chromatography as previously described (30). All metabolite data in the exercise studies are expressed as nanomoles per micromole total creatine (i.e., creatine plus creatine phosphate). This is a reliable reference base on which to normalize metabolite data in skeletal muscle since total creatine content does not change significantly during strenuous exercise in human vastus lateralis muscle (31, 32). (For purposes of comparison, metabolite data expressed in nanomoles per micromole of total creatine can be converted to micromoles per gram of muscle wet weight by dividing by a factor of 28 [33].)

In the in vitro catabolism experiments prolonged storage of some samples precluded normalizing the data to total creatine content. For these studies individual nucleotide, nucleoside, and base values are expressed as nanomoles per 100 nmol of purine in the biopsy. This method of data normalization was selected since no purine is lost during in vitro catabolism. The following compounds were quantitated and included in the calculations of purine content: ATP, ADP, AMP, IMP, inosine, adenosine, and hypoxanthine. Guanosine 5′-triphosphate (GTP) content of human skeletal muscle is only 2–3% of the ATP content (32), and guanosine 5′-diphosphate and guanosine 5′-monophosphate represent <0.5% of the purine in human muscle. Since no guanosine was detected in the catabolism experiments, the small contribution of guanine nucleotides was ignored in the calculation of purine content. No xanthine or uric acid was detected in any biopsy as might be expected in a tissue that contains little xanthine oxidase activity. (Failure to detect urate in the biopsies was not explained by oxidation of urate to allantoin, since human tissues do not contain uricase activity.)

Informed consent was obtained from all patients before performing the exercise studies and obtaining the muscle biopsies. The studies described in this report were approved by the Committees on Experimentation in Human Subjects at Duke University Medical Center, Tufts-New England Medical Center, and College of Physicians & Surgeons of Columbia University.

Data management and statistical analyses. All data were managed and analyzed using the CLINFO Data Analysis System of Duke University Medical Center (GCRC-RR-30) supported by the National Institutes of Health Division of Research Resources. Comparisons of metabolite values in resting and end-exercise muscle samples within a patient

group were performed using the Wilcoxon signed rank test for paired analyses; comparisons between patient groups were made using the Wilcoxon rank sum test for two groups. Due to the small number of MDD patients participating in the exercise protocol (n = 4), it was not possible to achieve a P value <0.1 for comparisons within this group using the Wilcoxon signed rank test, no matter what t value was obtained. Therefore, statistical comparisons of metabolite values in resting and end-exercise biopsies within the MDD group have little meaning. The non-MDD group was large enough (n = 8) to permit valid comparisons within this group. Comparisons of metabolite content in the end-exercise biopsies between the MDD and non-MDD groups was valid and results of these statistical tests are included in the text. Comparisons of changes in metabolite values in the in vitro catabolism study were performed with the Wilcoxon rank sum test for two groups. All comparisons were performed using two-tailed tests.

Results

Exercise tolerance. Unconditioned, normal volunteers (control; n = 11), patient controls (non-MDD; n = 8), and AMP deaminase-deficient subjects (MDD; n = 4) were exercised to the onset of fatigue while pedaling a stationary bicycle at a rapid, fixed rate (80 rpm) with gradually increasing work loads (Fig. 2). Males (Fig. 2 *A*) and females (Fig. 2 *B*) were tested using separate progressions of workloads because of the known differences in exercise tolerance between the sexes (34, 35).

In the male population, the six patients with aches, cramps, and pains and normal AMP deaminase activity (non-MDD) were comparable to the five untrained normal volunteers (control) with regard to both total work performed (P = 0.536) and duration of exercise (P = 0.178). For the single male patient with AMP deaminase deficiency, total work performed and duration of exercise were both less than the smallest amount of work and shortest duration of exercise performed by any member of the group of untrained, normal volunteers or of the group of patients with aches, cramps, and pains. The non-MDD male patient (No. 8), who was the same age as the male MDD patient (No. 3), performed three times more work during the exercise protocol (Table I). Although we were not able to compare the exercise performance of only one MDD patient to that of the non-MDD patients and normal controls on a statistical basis, another patient with a well-documented metabolic myopathy, i.e., myophosphorylase deficiency, was included in the exercise study for purposes of clinical comparison. The exercise performance of this individual was similar to that of the MDD subject and fell below the minimum total work and duration of exercise performed by any member of the control or non-MDD patient groups.

Although only one female patient with aches, cramps, and pains (non-MDD) was available for study, she was comparable to the six untrained, normal female volunteers (control) with regard to total work performed and endurance. Three female patients with AMP deaminase deficiency participated in the study. There was no overlap in the amount of work performed by any of the three MDD female patients and any of the six untrained, normal female volunteers; both total work (P = 0.016) and endurance (P = 0.048) were significantly lower in the group of female patients with AMP deaminase deficiency when compared with the group of normal volunteers.

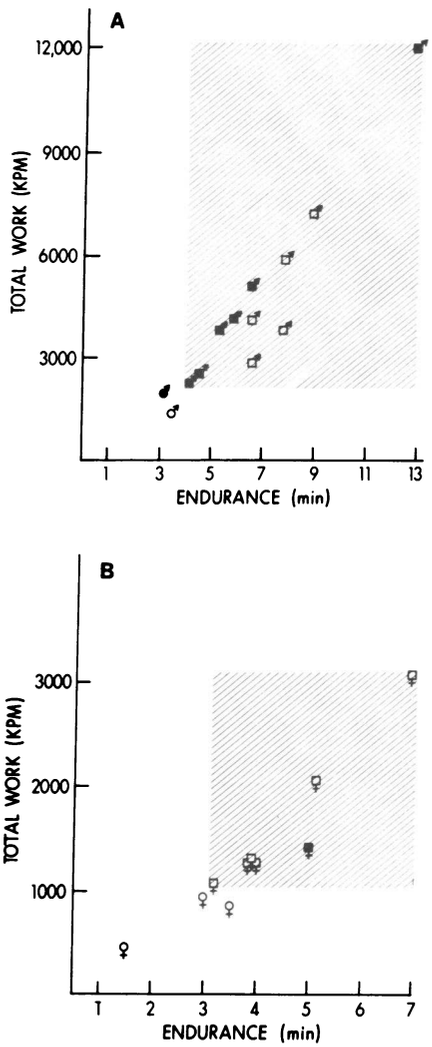

Figure 2. Exercise performance in males (panel *A*) and females (panel *B*). All individuals exercised on a bicycle ergometer and total work (KPM, kilopon · meters) is plotted as a function of time, i.e., endurance. The exercise performance in the five healthy male volunteers (□) and six of the non-MDD patients (■) is illustrated in panel *A;* the exercise performance of the one male MDD patient is indicated by the open circle (○). The filled circle (●) indicates the exercise performance of the male McArdle's patient. The exercise performance in the six healthy female volunteers (□) is illustrated in panel *B*. The closed square (■) indicates the performance of the one non-MDD female patient with aches, cramps, and pains. The open circles (○) indicate the exercise performance of the three female MDD patients. The shaded area in both panels indicates the range of exercise performance and endurance in the combined healthy volunteer and non-MDD patient groups.

Table I. Nucleotide Content of Percutaneous Muscle Samples Taken at Rest and After Exercise

Rest

	Age	Sex	Ado	Ino	Hx	AMP	ADP	ATP	IMP	TAD	CP
Non-MDD											
Patient 1	24	M	0.07	0.06	0.04	0.25	12.8	98.9	0.19	112.2	573
2	47	M	0.13	0.23	0.26	0.62	18.6	150.0	0.39	169.7	463
3	20	M	0.10	0.05	0.13	0.18	13.7	106.9	0.20	121.3	560
4	45	M	0.30	0.24	0.42	0.35	22.8	154.9	0.67	179.4	446
5	34	M	0.04	0.15	0.13	0.53	25.5	163.0	0.36	189.7	520
6	33	F	0.21	0.18	0.37	0.56	19.5	129.9	0.10	150.6	533
7	51	M	0.22	0.28	0.80	4.60	29.5	134.0	3.40	172.6	540
8	56	M	0.21	0.39	0.03	0.45	25.0	160.1	2.08	188.2	442
Median	40		0.17	0.21	0.20	0.49	21.2	142.0	0.38	171.2	527
MDD											
Patient 1	49	F	0.18	0.15	0.42	1.06	19.4	121.6	0.70	143.2	546
2	39	F	0.06	0.22	0.28	0.72	19.0	103.3	0.06	123.7	472
3	56	M	0.04	0.13	0.18	0.38	20.1	133.1	0.07	154.0	569
4	58	F	0.54	1.23	1.23	1.73	25.0	149.0	0.04	178.8	434
Median	53		0.12	0.19	0.35	0.89	19.8	127.4	0.07	148.6	509

End-Exercise*

	Total‡ work	Ado	Ino	Hx	AMP	ADP	ATP	IMP	TAD	CP
Non-MDD										
Patient 1	1.958	0.43	3.43	0.10	0.45	10.9	41.5	64.3	120.7	255
2	2,430	0.34	3.05	0.24	0.52	21.8	122.5	35.6	183.7	303
3	5,265	0.21	0.60	0.34	0.39	18.3	111.8	5.4	136.9	351
4	12,218	0.21	1.36	0.45	0.62	25.0	120.0	30.6	178.0	289
5	3,879	0.08	1.97	0.48	1.16	26.5	107.0	49.6	186.7	198
6	1,413	0.52	2.83	1.60	0.89	19.3	72.1	53.6	150.3	145
7	2,583	0.25	6.41	1.31	1.69	27.3	76.6	73.9	187.2	169
8	3,962	0.17	3.29	1.17	0.43	26.3	107.8	59.0	198.1	225
Median	3,231	0.23	2.94§	0.47§	0.57	23.4	107.4§	51.6§	180.9	240§
MDD										
Patient 1	945	3.53	3.54	2.42	0.69	21.6	103.4	6.3	141.5	188
2	855	0.83	0.89	0.30	1.49	26.0	96.6	3.5	129.6	77
3	1,350	0.75	0.94	0.67	0.56	23.3	130.9	2.3	159.4	167
4	450	0.33	0.72	1.35	2.70	27.4	139.9	4.7	177.1	182
Median	900	0.79‖	0.92	1.01	1.09	24.7	117.2	4.1‖	150.5	175

Data represent nanomoles per micromole total creatine. Ado, adenosine; Ino, inosine; Hx, hypoxanthine; TAD, total adenine derivatives, i.e., ATP, ADP, AMP, IMP, Ado, Ino, and Hx; CP, creatine phosphate. * Data indicate metabolite contents in the first muscle sample obtained following exercise (i.e., within 90 s). Data from subsequent samples (MDD patients) are not included in this table due to the tendency for creatine phosphate to increase and adenosine to decrease as a function of time postexercise. For purposes of comparison, metabolite values in this table can be converted to micromoles per gram wet muscle weight by dividing by a factor of 28 (33). ‡ Expressed as kilopon · meters. § Indicates a $P < 0.05$ for paired analyses of rest vs. end-exercise samples from non-MDD group of patients. ‖ Indicates a $P < 0.05$ for comparison of end-exercise biopsies in MDD vs. non-MDD groups.

Biochemical changes in muscle following vigorous exercise. At rest there were no detectable differences in creatine phosphate, ATP, ADP, AMP, IMP, adenosine, inosine, or hypoxanthine content of muscle obtained from AMP deaminase-deficient patients when compared to muscle obtained from those subjects with aches, cramps, and pains with normal AMP deaminase activity (Table I; all $P > 0.16$ when MDD group is compared to non-MDD group). GTP, uridine triphosphate, and cytidine triphosphate content of human skeletal muscle is quite low relative to that of ATP (32), and the content of these nucleotides was not significantly different in muscle of MDD compared to non-MDD patients, irrespective of whether the samples were obtained at rest or following exercise (data not shown). In contrast, muscle content of creatine phosphate, ATP, IMP, adenosine, inosine, and hypoxanthine changed in patients from one or both groups following exercise, and comparison of the changes observed in muscle from the MDD patients to the changes noted in the muscle of the non-MDD group illustrates the derangements in nucleotide metabolism that result from AMP deaminase deficiency.

Following exercise the creatine phosphate content of vastus lateralis muscle of the MDD and non-MDD patient groups fell by 69 and 52%, respectively ($P = 0.111$, MDD vs. non-MDD, end-exercise). In contrast, ATP content of muscle from the non-MDD patients fell by 34% ($P = 0.018$, rest vs. end-exercise), while ATP content of muscle from the MDD group fell by only 6%. The decrease in ATP content of muscle from the non-MDD group was significantly greater than that of the MDD group following exercise ($P = 0.048$, non-MDD vs. MDD). Examination of individual patients in the two groups also demonstrated a difference in ATP catabolism between MDD and non-MDD subjects; a measurable, i.e., >20%, reduction in ATP content of muscle following exercise was noted in seven of eight non-MDD vs. one of four MDD patients.

Comparison of the change in creatine phosphate or total phosphagen (creatine phosphate plus ATP) to the amount of work performed in the MDD vs. the non-MDD patients demonstrated a greater extent of creatine phosphate or total phosphagen depletion per unit of work in the MDD group. The median decrease in creatine phosphate plus ATP (nanomoles per micromole of total creatine) per unit of work ([kilopon · meters] $\times 10^{-3}$) in the MDD group was 435 (range of 299 to 580) vs. 87 (range of 16 to 315) in the non-MDD group ($P = 0.008$).

The patients with AMP deaminase deficiency produced less IMP in their muscle following exercise when compared with the non-MDD patients (Table I; $P = 0.008$, MDD vs. non-MDD, end-exercise). However, this type of group comparison is somewhat misleading since the magnitude of the decrease in ATP was different in the two groups of patients. Prior studies in human and rodent models have demonstrated that the amount of IMP produced in skeletal muscle following exercise is almost stoichiometrically related to the decrease in ATP (5, 6). Therefore, a more valid method for assessing IMP production is to compare the amount of IMP produced relative to the amount

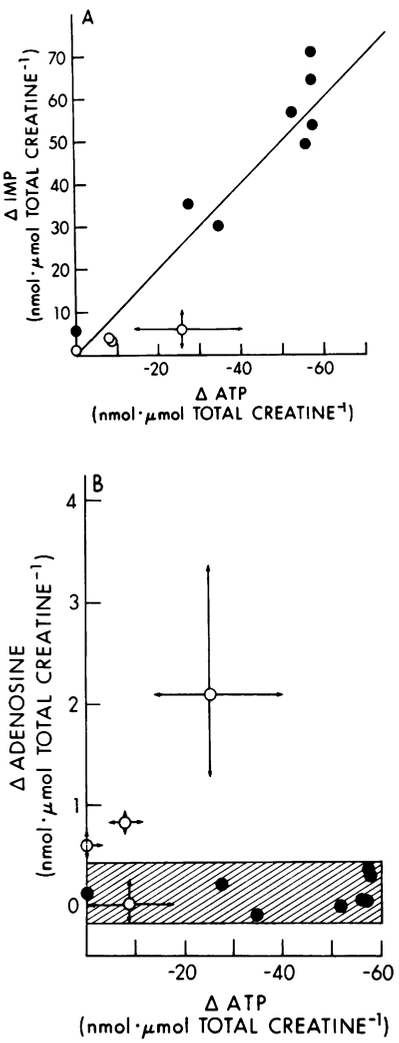

Figure 3. Change in IMP and adenosine content of muscle of MDD (○) and non-MDD (●) patients. The changes in IMP (*A*) and adenosine (*B*) are plotted as a function of the change in ATP content. For these studies, the content of the different metabolites was quantitated in resting muscle and in all the biopsies obtained following exercise. In cases where multiple resting samples were available from a single patient the median value was used to estimate metabolite content. For the MDD patients the vectors indicate the range of the change in metabolites in the multiple biopsies obtained following exercise. Multiple end-exercise biopsies were not obtained in the non-MDD group. The tangent in *A* indicates stoichiometry between ATP depletion and IMP accumulation. The shaded area in *B* indicates the range of values obtained for adenosine production in the non-MDD patients.

of ATP catabolized (Fig. 3 *A*). In the seven non-MDD patients in whom the decrease in ATP content was measurable there was a stoichiometric accumulation of IMP. As pointed out above, only one of the four MDD patients exhibited a measurable decrease in ATP content of muscle following exercise. In this individual the increase in IMP content of the muscle was not stoichiometric with the decrease in ATP. A similar observation

was noted in the muscle obtained from all seven AMP deaminase-deficient patients in the in vitro studies described below.

Analysis of changes in purine nucleoside and base content of muscle following exercise indicated other differences in metabolism between the MDD and non-MDD patients. Based on intrapatient comparisons there was a 16-fold increase in adenosine content of muscle in the MDD group following exercise. Adenosine content of muscle from the non-MDD patients did not change significantly following exercise ($P = 0.109$, rest vs. end-exercise) even though the reduction in the ATP pool was greater in the muscle of these patients (see above). Median adenosine content of the end-exercise muscle samples from the MDD patients was threefold greater than that of the non-MDD group ($P = 0.028$; MDD vs. non-MDD, end-exercise). The adenosine content of the muscle increased in three of four MDD patients following exercise, and the greatest increase in adenosine content was noted in the MDD patient with the largest decrease in ATP (Fig. 3 *B*). Three of the four patients exhibited a >13-fold increase in adenosine content over that observed in resting muscle. This increase in adenosine production suggests there was some ATP catabolism in three of four MDD patients during exercise even though the decrease in ATP content was not detectable. This apparent discrepancy may be accounted for by recognizing that the adenosine pool of resting muscle is quite small while the ATP pool is quite large.

The difference in adenosine production was also illustrated by comparing the ratio of inosine to adenosine content of the muscle samples taken at end-exercise in the two groups. The ratio of inosine to adenosine in MDD muscle was 1.2 (range 1.0–2.2) compared with 8.5 (range 2.9–25.6) for the non-MDD individuals ($P = 0.004$).

Having quantitated ATP, ADP, AMP, IMP, adenosine, inosine, and hypoxanthine in muscle obtained at rest and following exercise, a balance sheet was constructed to determine whether the sum of these purine compounds changed during exercise. This type of balance sheet has little meaning, however, unless the ATP content of the muscle decreased to a measurable extent during exercise, since ATP accounts for ≃85% of the purine content of resting muscle. Therefore, we developed a balance sheet only for those patients in both groups in whom the ATP content of the muscle fell by 20% or more during exercise (Table II). There was only one MDD patient who could be used for this comparison, while there were seven non-MDD subjects in whom we could construct a meaningful balance sheet. In the seven patients with normal AMP deaminase activity there was no evidence for net purine loss from the muscle following exercise. Instead, there was a redistribution of adenine nucleotides into other nucleotide pools, predominantly IMP. In contrast, in the one MDD individual in whom there was a measurable decrease in ATP there was only a minimal increase in IMP. Although there was a large increase in purine nucleoside and base content of the muscle obtained from this patient following exercise, the sum of the increase in adenosine, inosine, hypoxanthine, and IMP accounted for only 48% of the ATP catabolized.

*Table II. Metabolic Consequences of ATP Catabolism in Those Patients Exhibiting a Greater Than 20% Reduction in ATP following Exercise**

Metabolite	MDD‡ ($n = 1$)	Non-MDD ($n = 7$)
	Δ following exercise	*median Δ following exercise*
ATP	−25.2	−56.0 (−27.5 to −57.8§)
ADP plus AMP	−0.1	+1.3 (−5.1 to 3.1)
IMP	+5.5	+51.4 (29.9 to 70.5)
Adenosine	+2.1	+0.1 (−0.1 to 0.4)
Inosine plus hypoxanthine	+4.7	+3.4 (1.2 to 6.6)
Net Δ	−13.0	+8.8 (−3.0 to 14.6)

* Only those patients from both groups with measurable, i.e., >20%, reductions in ATP content were included in these analyses.
‡ Data represent the average of four biopsies from this patient.
§ Numbers in parentheses indicate range of changes in metabolites.

These findings suggest that AMP deaminase deficiency results in an alternate route of AMP metabolism during vigorous muscle contraction. In the MDD patient AMP is hydrolyzed to adenosine instead of being preferentially deaminated to IMP and entering the purine nucleotide cycle. Adenosine may then diffuse out of the myocyte in vivo and be lost from the perfused muscle. This may lead to a reduction in total purine content of the muscle in MDD patients, if sufficient amounts of ATP are catabolized during exercise.

Biochemical changes in muscle following ATP catabolism in vitro. Additional insight into the metabolic consequences of AMP deaminase deficiency was obtained from studying catabolism of endogenous ATP in biopsy specimens where no metabolites were lost into the blood stream. In addition, greater ATP depletion was achieved with in vitro catabolism making it easier to compare biochemical differences in the AMP deaminase-deficient and control groups.

In the frozen muscle samples from the AMP deaminase-deficient group ($n = 7$) and the control group ($n = 7$) used in this study the ATP content was 85 (range, 68–87) and 83 (range, 76–87) nmol/100 nmol of purine, respectively ($P = 0.804$; MDD vs. non-MDD). These results are in good agreement with the values obtained for the percutaneous muscle biopsy specimens for resting muscle reported in Table I; 84 (range, 83–86) and 86 (range, 78–88) nmol/100 nmol of purine for the MDD and non-MDD groups, respectively ($P = 0.21$, MDD vs. non-MDD).

The results shown in Fig. 4 illustrate the changes observed in purine metabolism following extensive catabolism of the endogenous ATP pools in the muscle biopsies obtained from the MDD and non-MDD patients. After incubation at 37°C for 30 min, the ATP content of muscle biopsy specimens fell by 44 (range, 14–56) and 48 (range, 34–58) nmol/100 nmol of purine in the MDD and non-MDD groups, respectively ($P = 0.26$; MDD vs. non-MDD). As noted in the patients during the exercise

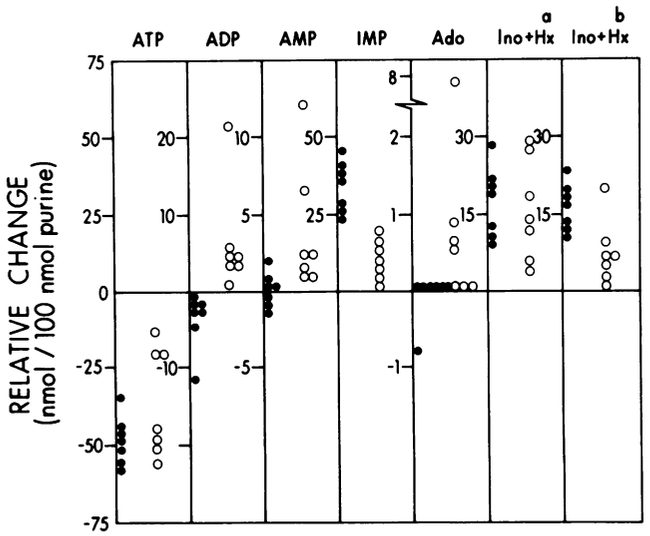

Figure 4. Adenine nucleotide catabolism in vitro in muscle samples from MDD (○) and non-MDD (●) patients. Metabolite content was determined in fresh-frozen muscle and after the muscle had been incubated in vitro at 37°C for 30 min; the change in metabolite content during this period of in vitro catabolism is illustrated here. Inosine (Ino) and hypoxanthine (Hx) were determined in muscle samples incubated without (*a*) and with (*b*) 200 μM 2′-deoxycoformycin.

study, ATP catabolism in muscle from AMP deaminase-deficient individuals was not associated with as great an increase in IMP production as observed in the muscle from subjects with normal activity of this enzyme. The increase in IMP was 8 (range, 4–18) nmol/100 nmol of purine in MDD muscle vs. 38 (range, 25–46) nmol/100 nmol of purine in non-MDD muscle ($P < 0.001$). Some of the IMP formed in the muscle from the AMP deaminase-deficient subjects may have been an artifact of the in vitro preparation, since lysis of cells upon freeze-thawing provided the accumulating AMP access to the AMP deaminase in erythrocytes and other blood cells mixed with the myocytes in these surgical specimens. The amount of IMP accumulated in the muscle from non-MDD patients ranged from 98 to 53% (median 72%) of the catabolized ATP. In contrast, only 11–40% (median 27%) of the ATP catabolized in muscle from MDD patients was recovered as IMP.

Adenosine production was not evident in the muscle from subjects with normal activity of AMP deaminase. However, in the samples from four of seven AMP deaminase-deficient patients adenosine content increased in association with ATP catabolism, and this resulted in a significant increase in the median adenosine content for this group (increase of 0.4 [range, 0–7.3] nmol/100 mol of purine for MDD vs. no increase [range, −1.5 to 0] for non-MDD, $P = 0.038$). Inosine plus hypoxanthine increased similarly in both groups (14 [range, 5–29] and 20 [range, 9–28] nmol/100 nmol of purine for MDD and non-MDD, respectively, $P = 0.71$) in association with ATP catabolism, but a different route of metabolism accounted for the formation of these purine compounds in the two patient groups. The difference in metabolism was brought out by including 2′-deoxycoformycin, an inhibitor of adenosine deaminase in the in vitro catabolism experiment. In the AMP deaminase-deficient subjects inosine and hypoxanthine production was substantially reduced in all cases in the presence of 2′-deoxycoformycin, suggesting that a major portion of the inosine and hypoxanthine produced in these individuals was derived from deamination of adenosine following dephosphorylation of AMP. In contrast, control subjects exhibited similar increases in inosine and hypoxanthine in the presence and absence of 2′-deoxycoformycin, suggesting that hydrolysis of IMP was the likely source of these compounds. Although the increase in inosine plus hypoxanthine was comparable in the muscle from the two groups of patients in the absence of 2′-deoxycoformycin, the inosine plus hypoxanthine produced in MDD samples in the presence of 2′-deoxycoformycin was significantly less than that produced in the non-MDD samples under the same conditions (6 [range, 2–20] vs. 17 [range, 12–23] nmol/100 nmol of purine, respectively, $P = 0.018$). These results are consistent with the interpretation that inosine and hypoxanthine production in muscle with normal activity of AMP deaminase is derived primarily from IMP; in muscle from AMP deaminase-deficient subjects most of the inosine and hypoxanthine is derived from adenosine produced from AMP hydrolysis.

The in vitro studies also revealed a further derangement in nucleotide metabolism secondary to AMP deaminase deficiency, which was not appreciated from the in vivo studies. In association with ATP catabolism in the muscle from AMP deaminase-deficient patients there was an increase in both AMP (2.4 [range, 0.9–12] nmol/100 nmol of purine) and ADP (4.2 [range, 0.9–22] nmol/100 nmol of purine). In muscle from control patients there was essentially no change in AMP (0.1 [range, −0.8 to 1.9] nmol/100 nmol of purine), while there was a decrease in ADP (−2.4 [range, −0.9 to −11 nmol/100 nmol of purine). The increase in AMP in MDD muscle was significantly greater than the increase in non-MDD muscle ($P = 0.004$), and the increase in ADP in MDD samples was also significantly greater ($P < 0.001$). The fact that we were not able to detect increases in AMP and ADP in vivo may be explained by the less pronounced drop in ATP during exercise, and the time that elapsed between the end of exercise and the sampling of the muscle. During this time the small amount of free (not bound to actin) ADP and free AMP that accumulated could have been rephosphorylated to ATP.

Discussion

In the present study we have evaluated exercise performance in MDD patients and correlated these measurements with biochemical changes in the muscle that result from this enzyme deficiency. We have found that patients with AMP deaminase deficiency fatigue more quickly and perform less work than do normal controls or patients with similar symptoms of aches,

cramps, and pains. Although there is no uniformly accepted biochemical marker of fatigue, most investigators would agree that fatigue results when energy demand exceeds energy production. One consequence of this imbalance in energy production and utilization is a decrease in muscle stores of high energy phosphates, i.e., creatine phosphate and ATP (36, 37). The decrease in total phosphagen (creatine phosphate plus ATP) per unit of work provides an index of energy production by the muscle, since the amount of phosphagen utilized is proportional to work performed by the muscle (37). In the patients with AMP deaminase deficiency the median decrease in total phosphagen per unit of work was fivefold greater than in the non-MDD patient group. This observation suggests that skeletal muscle of AMP deaminase-deficient patients has a lower capacity for energy production than does the muscle from the non-MDD patients.

A number of factors affect energy production in the myocyte, and one of the most important is the state of conditioning of the individual (37–39). During exercise training a number of changes take place in skeletal muscle that enhance the capacity of the myocyte to produce energy (37, 39). However, we do not believe differences in conditioning account for the difference in exercise performance noted between the MDD and non-MDD patients or controls for the following reasons. Normal volunteers were not accepted in the study if they routinely participated in sports or other physical activities. This group was composed of individuals who were employed in laboratory or secretarial positions. We also selected a patient group with symptoms, life-styles, and histories of physical activity similar to those reported by the AMP deaminase-deficient patients.

Age is another factor which affects exercise performance (34, 40). It was more difficult to age-match the groups, however. As stated above, the patient control group was limited to individuals with similar symptoms and life-styles. The median age of this patient group (40 yr) was lower than that of the myoadenylate deaminase-deficient group (53 yr), as was the median age of the normal volunteer group (32 yr). However, in the three cases where we were able to age-match MDD patients with either patient controls or normal volunteers, the latter individuals outperformed their MDD counterparts.

Although we have not completely excluded an age effect as a contributory factor in the reduced exercise tolerance noted in the myoadenylate deaminase-deficient patients, we believe that disruption of the purine nucleotide cycle as a result of AMP deaminase deficiency plays a primary role in the muscle dysfunction noted in these patients for the following reason. In an animal model in which another enzyme of the purine nucleotide cycle is inhibited, i.e., S-AMP lyase (Fig. 1), skeletal muscle fatigues more rapidly and performs less work before the onset of fatigue (41). Since the effects of age, sex, conditioning, and effort were controlled in this animal model, we conclude that flux through the purine nucleotide cycle plays a role in muscle function.

To understand how AMP deaminase deficiency and disruption of the purine nucleotide cycle might lead to muscle dysfunction, one must consider the mechanisms that control energy production and utilization in the normal myocyte during exercise. Energy production in the mitochondria is transformed to creatine phosphate and transported to the myofibril in the form of this high-energy phosphate. At the myofibril creatine kinase bound to the contractile proteins uses the creatine phosphate to synthesize ATP, which is in turn used by actomyosin adenosine triphosphatase (ATPase) for muscle contraction (42). As long as creatine phosphate production keeps pace with demand, ADP and H^+ (products of ATP hydrolysis) do not accumulate to an appreciable extent, because both ADP and H^+ are used in the creatine kinase reaction to resynthesize ATP. However, when creatine phosphate production can no longer keep pace with utilization, as during intense exercise, creatine phosphate content of the muscle decreases (36). When the decrease in creatine phosphate reaches a critical level, $\leq$50% of control, ATP content also begins to decrease (36). In normal skeletal muscle the decrease in ATP content is not associated with an increase in ADP or AMP because AMP deaminase activity increases when the energy charge in the cell falls (43) and this prevents AMP accumulation; AMP deamination pulls the myokinase reaction toward ATP formation thereby preventing ADP accumulation (Fig. 1). The net effect of these two reactions is the maximal extraction of energy from the adenylate pool without a build-up in ADP or AMP in the cell. The IMP produced in the AMP deaminase reaction provides substrate for S-AMP synthetase and the resultant increase in the activity of this enzyme leads to increased substrate availability for S-AMP lyase (Fig. 1). Some studies suggest both limbs of the purine nucleotide cycle operate simultaneously during muscle stimulation (7, 8), while others suggest the two limbs operate in series, i.e., AMP $\rightarrow$ IMP during muscle contraction and IMP $\rightarrow$ S-AMP $\rightarrow$ AMP during the relaxation period following muscle contraction (5, 44). With each complete turn of the cycle one molecule of fumarate and NH_3 are produced and one molecule of aspartate and GTP consumed (Fig. 1). The fumarate can be used to replete or expand the pool of citric acid cycle intermediates in skeletal muscle (7, 10), potentially enhancing energy production (8), and the NH_3 can be used to buffer H^+ produced during ATP hydrolysis (16).

We have presented biochemical evidence in this study that demonstrates disruption of the purine nucleotide cycle in the skeletal muscle of AMP deaminase-deficient patients during exercise. In vitro studies with skeletal muscle obtained from the MDD patients corroborate and extend the in vivo findings. We conclude from these biochemical analyses that less of the AMP formed from ATP enters the purine nucleotide cycle in MDD subjects during vigorous exercise and more is hydrolyzed to adenosine. The in vitro results indicate that AMP and ADP accumulate during ATP hydrolysis in myocytes that have reduced AMP deaminase activity, and we presume this can also occur in vivo during intense muscle contraction.

There are a number of mechanisms by which AMP deaminase deficiency and the consequent disruption of the purine nucleotide cycle could lead to the muscle dysfunction observed

in these patients. Loss of the anaplerotic role, which has been documented for the purine nucleotide cycle in skeletal muscle (8, 10), could lead to an impairment in energy production in the myocyte. Szent-Gyorgi initially, and others subsequently (45), have shown that the addition of fumarate, as well as other citric acid cycle intermediates, to suspensions of skeletal muscle leads to a six- to eightfold increase in ATP production over that which could be accounted for simply by oxidation of fumarate. This result has been interpreted to indicate that expansion of the pool of citric acid cycle intermediates in skeletal muscle catalytically enhances the use of endogenous substrates, such as glycogen and/or lipids, for energy production in the mitochondria (45). Our findings are consistent with the conclusion that loss of the anaplerotic role of the purine nucleotide cycle, which results from AMP deaminase deficiency, leads to a decrease in the efficiency or capacity for energy production and this in turn leads to the more rapid onset of fatigue. The greater decrease in total phosphogen per unit of work in the MDD patients supports this hypothesis.

Another mechanism by which AMP deaminase deficiency could lead to an abnormality in skeletal muscle function relates to the role that this enzyme plays, in concert with adenylate kinase, to maintain the adenylate energy charge in the cell (43). The net effect of these two reactions is the complete utilization of energy stored in the adenylate pool. In the skeletal muscle deficient in AMP deaminase activity AMP, ADP, and adenosine accumulate in vitro when ATP use exceeds production. Since adenosine accumulates in vivo in the muscle of MDD patients during exercise, we suspect that local or transient accumulation of AMP and ADP may also occur in vivo. Accumulation of ADP and AMP could result in inhibition of actomyosin ATPase (46), and this in turn could account for the smaller drop in ATP content of skeletal muscle of the AMP deaminase-deficient patients following a maximal exercise stimulus. Failure to extract all of the energy stored in the adenylate pool could contribute to a more rapid onset of fatigue in the MDD patients.

Reduction in the amount of NH_3 produced in AMP deaminase-deficient muscle could also slow the rate of ATP hydrolysis. It has been proposed by others that NH_3 produced in the AMP deaminase reaction serves as a buffer for H^+ produced during ATP hydrolysis and loss of this buffering capacity could inhibit the actomyosin ATPase reaction (16). Since ATP is the immediate source of energy for muscle contraction, inhibition of ATP hydrolysis through the accumulation of either H^+ or ADP could lead to the earlier onset of fatigue in MDD patients.

These proposed explanations for the more rapid onset of fatigue in AMP deaminase-deficient patients are not mutually exclusive. For example, a given mechanism could be more important in one stage of muscle contraction and another at a different stage of contraction, or several mechanisms could summate under appropriate conditions to produce a more severe degree of muscle dysfunction than could be accounted for by a single abnormality. Additional mechanisms can also be envisioned, but we have focused on those derangements in metabolism which we believe to be the most promising to examine experimentally in future studies with patients and animal models.

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