Defective Adenosine Triphosphate Synthesis

AN EXPLANATION FOR SKELETAL MUSCLE DYSFUNCTION IN PHOSPHATE-DEFICIENT MICE

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ABSTRACT The basis for skeletal muscle dysfunction in phosphate-deficient patients and animals is not known, but it is hypothesized that intracellular phosphate deficiency leads to a defect in ATP synthesis. To test this hypothesis, changes in muscle function and nucleotide metabolism were studied in an animal model of hypophosphatemia. Mice were made hypophosphatemic through restriction of dietary phosphate intake. Gastrocnemius function was assessed in situ by recording isometric tension developed after stimulation of the nerve innervating this muscle. Changes in purine nucleotide, nucleoside, and base content of the muscle were quantitated at several time points during stimulation and recovery.

Serum concentration and skeletal muscle content of phosphorous are reduced by 55 and 45%, respectively, in the dietary restricted animals. The gastrocnemius muscle of the phosphate-deficient mice fatigues more rapidly compared with control mice. ATP and creatine phosphate content fall to a comparable extent during fatigue in the muscle from both groups of animals; AMP, inosine, and hypoxanthine (indices of ATP catabolism) appear in higher concentration in the muscle of phosphate-deficient animals. Since total ATP use in contracting muscle is closely linked to total developed tension, we conclude that the comparable drop in ATP content in association with a more rapid loss of tension is best explained by a slower rate of ATP synthesis in the muscle of phosphate-deficient animals. During the period of recovery after muscle stimulation, ATP use for contraction is minimal, since the muscle is at rest. In the recovery period, ATP content returns to resting levels more slowly in the phosphate-deficient than in the control animals. In association with the slower rate of ATP repletion, the precursors inosine monophosphate and AMP remain elevated for a longer period of time in the muscle of phosphate-deficient animals. The slower rate of ATP repletion correlates with delayed return of normal muscle contractility in the phosphate-deficient mice. These studies suggest that the slower rate of repletion of the ATP pool may be the consequence of a slower rate of ATP synthesis and this is in part responsible for the delayed recovery of normal muscle contractility.

INTRODUCTION

Hypophosphatemia and intracellular deficiency of inorganic phosphate result in dysfunction of a wide range of organ systems in man and experimental animals. Erythrocyte hemolysis (1–3), derangements in the mechanical and phagocytic properties of leukocytes (1, 4), decreased survival and abnormal function of platelets (1, 5), central nervous system abnormalities compatible with metabolic encephalopathy (1, 6, 7), abnormalities in renal function (1, 8), and cardiac (1, 9–11, 13), and skeletal myopathy (1, 12–17) have been described in patients or animals with phosphate deficiency.

A number of hypotheses have been offered to explain the cellular dysfunction resulting from phosphate deficiency. The mechanisms proposed include shifts in the hemoglobin-O_2 dissociation curve leading to tissue hypoxia (6, 18, 19), inhibition of glycolysis and glycogenolysis (3, 6, 20), impaired calcium metabolism (8, 10, 17), alterations in phospholipid content of cellular membranes (17), diminished intracellular ATP

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stores secondary to a defect in ATP synthesis (1, 8, 11–13, 16–18, 21, 22), and disruption of energy transport from the mitochondria to the myofibril via the creatine phosphate (CP)[1] shuttle (11, 13). The latter two mechanisms, i.e., a defect in ATP synthesis and a defect in energy utilization, may be of particular relevance in explaining the muscle dysfunction observed in phosphate deficiency, since both energy production in the form of ATP synthesis in the mitochondria and energy use in the form of CP transfer to ADP at the myofibril increase manyfold during vigorous muscle contraction (23, 24).

The studies cited above document that phosphate deficiency produces cellular dysfunction in a wide range of organ systems, and various mechanisms have been implicated in the production of these abnormalities. The present study was designed to examine one organ system (i.e., skeletal muscle) and to test the hypothesis that mild-to-moderate phosphate deficiency is associated with a demonstrable defect in muscle function, as well as a defect in ATP synthesis. Quantitative determination of muscle function was made in control and hypophosphatemic mice by measuring developed tension in the gastrocnemius muscle of the animals in situ during tetanic stimulation leading to fatigue and during recovery. Biochemical parameters of energy metabolism were assessed in these same animals by quantitating muscle content of ATP and CP, as well as the products of ATP catabolism (25–27). Since ATP use in muscle is proportional to the amount of tension generated (28, 29), the physiological and biochemical parameters taken together provide an index of the relative rates of ATP production and utilization by the gastrocnemius muscle during stimulation and recovery. Results of these studies indicate that in phosphate-deficient animals both the ability of the muscle to generate tension during stimulation and the rate of recovery of muscle function following the production of fatigue are abnormal. These derangements are temporally related to a decrease in the rate of ATP production by phosphate-deficient skeletal muscle.

METHODS

Animal model. 36 8–10-wk-old C57BL6J mice were fed a diet containing 0.09% inorganic phosphorous for 4 wk while 37 age-matched controls were fed a diet containing 0.6% phosphorous. All animals had free access to food and water and were housed in a temperature controlled room (20–21°C) with a 12-h light/dark cycle. Animals were anesthetized with pentobarbital (65 mg/kg i.p.) and the left gastrocnemius muscle (mixed fast-twitch) was carefully dissected free of surrounding tissues with its nerve and blood supply intact. The soleus, plantaris, and rectus femoris muscles were dissected free of the gastrocnemius and removed from the preparation. The leg was secured with a steel pin through the femur and a screw clamp over the hind paw, and the limb was immersed in a 37°C mineral oil bath. After the distal tendon was attached to a Grass FT.03 force transducer with a stainless steel rod (Grass Instrument Co., Quincy, MA), muscle length was adjusted to provide maximal isometric tension. The transected sciatic nerve was repetitively stimulated in situ with trains of tetanizing supramaximal pulses (8 V, 0.5 ms, 125 Hz) lasting 150 ms at a rate of 2 trains/s with a platinum bipolar electrode and a Grass S88 stimulator and SIU 4678 isolation unit. Stimulation was continued for a total of 3 min. Direct muscle stimulation verified the presence of muscle fatigue. The gastrocnemius muscle was frozen in situ with precooled metal tongs immediately after stimulation, or allowed to recover for 2, 5, or 20 min before being frozen and removed. The frozen muscle was first trimmed of any tissue not compressed between the precooled tongs and then stored in liquid nitrogen until it was extracted. Recovery of tetanic tension was assessed by applying the previously described trains of stimuli for 2 s (4 trains) at specific times during the recovery period (0.5, 1, 2, 3, 5, 10, 15, and 20 min). Recovery of function was measured in all animals up to, but not including, the time of biopsy. Resting metabolite content was determined by preparing animals as described and placing them in the oil bath. The muscle remained in the oil bath for 23 min, during which time it was not stimulated. The unstimulated gastrocnemius muscle was then freeze-clamped in situ and removed for analysis.

Analysis of muscle samples. The skeletal muscle (~40 mg) was added directly to a glass tissue grinder containing 0.4 ml of cold (4°C) 12% trichloroacetic acid. Extraction was performed at 4°C for 30 min with periodic grinding of the muscle until a homogeneous slurry was produced. After centrifugation at 4°C for 2 min at 5,000 *g*, the supernate was mixed for 1 min at room temperature with 0.8 ml of 0.5 M tri-*N*-octylamine in freon to remove the acid. After mixing for 1 min, the aqueous and freon-amine layers were separated by centrifugation at 2,000 *g* for 2 min at 4°C, and the aqueous layer removed for analysis. Nucleotide (ATP, ADP, AMP, inosine monophosphate [IMP]); nucleoside (adenosine, inosine); and base (hypoxanthine) analyses were performed with a Waters model 440 High Performance Liquid Chromatograph (Waters Instruments, Inc., Milford, MA), as previously described (30). The various peaks in the extracts were identified by comparison of retention times with known external standards and relative absorbance at 254 nm/280 nm. The results were expressed in either nanomoles or micromoles per gram of protein. (To facilitate comparison of results in the present study with those in the literature, we determined the following ratios of wet weight to protein and wet weight to dry weight for mouse gastrocnemius: wet wt/protein = 6.6/1.0; wet wt/dry wt = 4.3/1.0.) Protein content of the pellet remaining from the acid extract was determined by the method of Lowry et al. (31) after it was solubilized in 0.5 N NaOH.

The same extract used for nucleotide and nucleoside analysis was used for creatine phosphate determination. 25 μl of extract was mixed with 25 μl of 100 mM Tris HCl buffer (pH 7.4), which contained 0.4 mM [^{14}C]ADP (2.5 μCi/μmol), 10 mM $MgCl_2$, and 1.6 U of creatine phosphokinase (Sigma Chemical Co., St. Louis, MO). After incubation at 37°C for 30 min, a 5-μl aliquot of the reaction mixture was applied to phosphoethyleneimine cellulose F thin-layer chromatography plates and developed in 0.8 M $LiCl_2$ for 80 min. The

[1] *Abbreviations used in this paper:* CP, creatine phosphate; SDH, succinate dehydrogenase.

ATP spot was identified by ultraviolet light, cut out, and counted at 79% efficiency in triton scintillation fluid with a Packard TriCarb Liquid Scintillation Spectrometer (Packard Instrument Co., Downers Grove, IL). CP content was calculated from the amount of ATP produced in this reaction. The results were expressed in micromoles per gram of protein.

Serum and tissue phosphorous measurement. Serum phosphorous was determined in the control and phosphate-deficient group by the method of Dryer et al. (32) with 100 μl of venous blood obtained by retroorbital puncture. To determine tissue phosphorous content, gastrocnemius muscle was obtained from a group of five control animals and nine animals fed a phosphate-deficient diet. The samples were prepared by a modification of the method of Baginski et al. (33) and the content determined colorometrically by a modification of the method of Fiske and Subbarow (34).

Succinate dehydrogenase (SDH) activity. SDH activity was measured by the ferricyanide method of Bonner (35) as modified by Peter et al. (36). The entire gastrocnemius muscle was isolated and removed from five control and five phosphate-deficient mice, weighed, and homogenized in 20 vol of 50 mM *N*-Tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid, 100 mM KCl, 5 mM $MgSO_4$, 1 mM EDTA, and 50 mM potassium phosphate buffer at 25°C, pH 7.4. After gauze filtration, the homogenate was incubated at 37°C for 5 min to deplete endogenous substrate. SDH was assayed at 25°C in 1 ml reaction mixture containing 0.1 M potassium phosphate buffer (pH 7.4), 10 mM sodium azide, 1 mM potassium ferricyanide, 20 mM sodium succinate, 20 μM Rotenone, and 50 μl of homogenate. Enzyme activity was expressed as micromoles of product formed per minute per milligram of protein.

One-way analysis of variance was used for comparison of metabolic data and muscle function between control and phosphate-deficient mice at individual time points. Paired analyses were used to examine changes in function over time within either the control or phosphate-deficient groups. All values were expressed as mean±SEM.

RESULTS

Animal model. Physiological and biochemical measurements in the phosphate-deficient and control group are listed in Table I. Both serum phosphorus concentration and gastrocnemius phosphorous content are significantly lower in the phosphate-deficient group. Body weight is also lower in these animals. There was no apparent difference in physical activity between the phosphate-deficient and control mice and there was no evidence of muscle wasting in the phosphate-deficient mice; i.e., the ratio of gastrocnemius muscle weight to body weight is similar in both groups (Table I). Muscle SDH-activity, an index of oxidative capacity of the muscle (36–38), is not different in the two groups. This indicates that the fast-twitch oxidative vs. glycolytic fiber composition of the gastrocnemius muscle is not altered as a result of the phosphate-deficient diet. Although absolute peak tetanic tension is lower in the phosphate-deficient group compared with the control group, there is no significant difference between the two groups when peak tension is normalized for muscle mass (Table I).

Muscle function. Fig. 1 *A* depicts the decline in tetanic tension during 3 min of sciatic nerve stimulation in the control and phosphate-deficient groups. The decline in developed tension is significantly more rapid in the phosphate-deficient animals compared with the control group. The decline in tension to 8% of initial values within the first 30 s of stimulation is characteristic of the fatigue response reported by others for fast-twitch muscle subjected to vigorous stimulation (25, 26). By 30 s, tetanic tension falls to near minimum values in both groups, and the difference between groups is no longer significant ($P = 0.07$). During the second and third minutes of stimulation, very little additional loss of tetanic tension occurs.

With termination of the fatiguing stimulus, tetanic tension recovers in a biphasic pattern in both groups of animals (Fig. 1 *B*). Previous studies in rats have also shown a biphasic return of tetanic tension (25, 26). A rapid period of recovery of tetanic tension is noted within the first 2 min after cessation of the fatiguing

TABLE I
Biochemical and Physiologic Parameters in Control and Phosphate-deficient Mice

	Control animals	Phosphate-deficient animals	*P* value
Serum phosphorous (*mg/dl*)	6.76±0.24	3.07±0.18	0.0001
Tissue phosphorous (*μmol/g protein*)	162±16	89±8	0.001
Mouse weight, (*g*)	25±1	18±1	0.0001
$\frac{\text{Mouse weight } (g)}{\text{Gastrocnemius weight } (g)}$	210±9	230±23	0.386
SDH activity (*μmol/min/mg protein*)	0.13±0.01	0.13±0.01	—
Peak tetanic tension (*g*)	142±4	110±4	0.0001
$\frac{\text{Peak tetanic tension } (g)}{\text{Gastrocnemius weight } (g)}$	1,200±91	1,400±140	0.320

All results are mean±SEM.

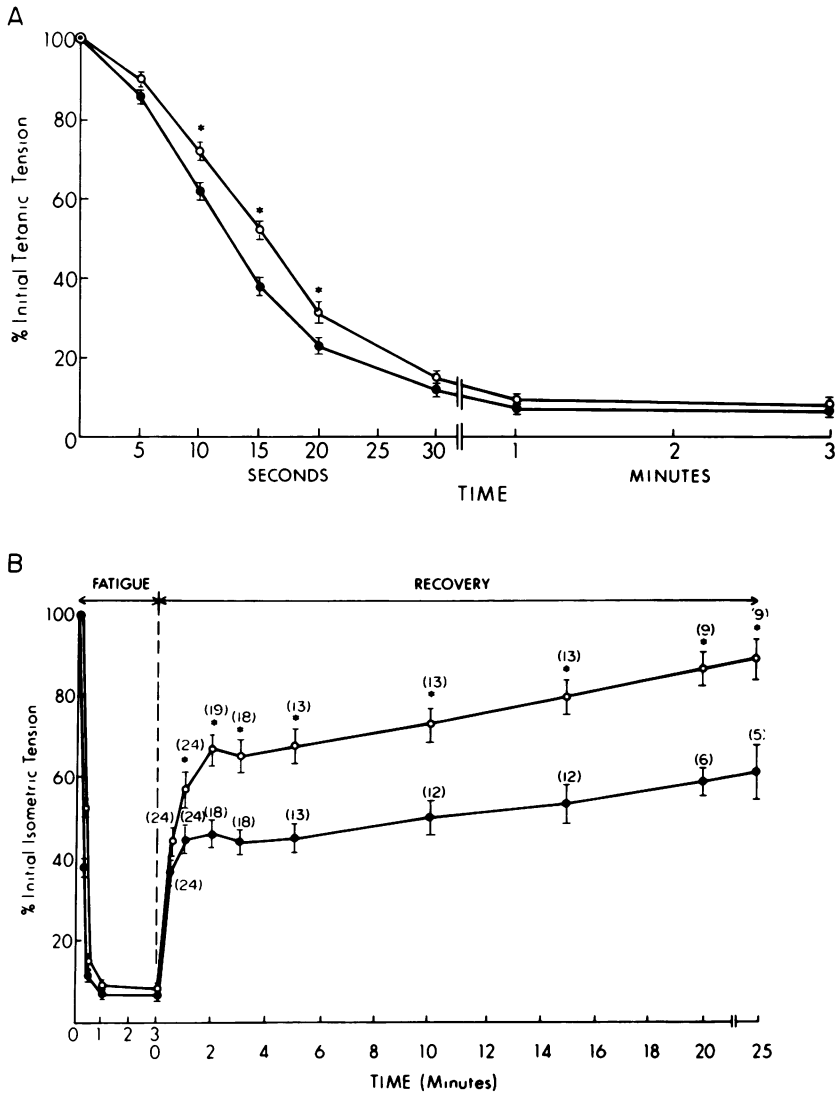

FIGURE 1 (*A*) Percentage of initial tetanic tension during 3 min of stimulation of the gastrocnemius muscle with trains of tetanizing supramaximal pulses (8 V, 0.5 ms, 125 Hz) 150 ms in duration at a rate of 2 trains/s. O, control animals (n = 31); ●, phosphate-deficient animals (n = 32). (*B*) Percentage of initial tetanic tension of the gastrocnemius muscle during recovery from 3 min of stimulation leading to fatigue. The value in parentheses indicates number of animals in each group. All values are mean±SEM. *, $P < 0.05$, phosphate-deficient vs. control.

stimulus. There is a significantly greater return of function during the first 2 min of recovery in the control group (66±4% of initial tension) compared with the phosphate-deficient group (46±3% of initial tension, P = 0.003). The difference in tetanic tension between the phosphate-deficient and control groups is statistically significant by 30 s of recovery (P = 0.02) and persists throughout the remainder of the study. The period of rapid recovery is followed by a more gradual increase in tetanic tension in both groups. By 20 min, the control group has recovered to 89±5% of initial tetanic tension, whereas the phosphate-deficient group has recovered to only 60±7% of the initial value (P = 0.003).

Changes in purine nucleotide content. Changes in ATP and IMP content of gastrocnemius muscle from the control and phosphate-deficient groups during fatigue and recovery are shown in Fig. 2. Values for ATP content of resting muscle are not significantly different in the control and phosphate-deficient mice

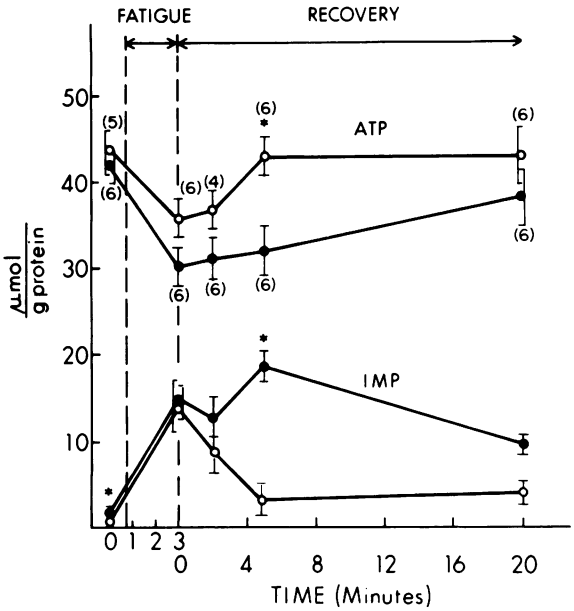

FIGURE 2 ATP and IMP content of the gastrocnemius muscle during tetanizing pulses leading to fatigue, and during the recovery period. O, control; ●, phosphate-deficient animals. The value in parentheses indicates number of animals in each group. All values are mean±SEM. *, $P < 0.05$, phosphate-deficient vs. control.

(44±3 vs. 42±2 μmol/g, $P = 0.50$). During the 3 min of stimulation that leads to fatigue, ATP content of the gastrocnemius falls to 36±2 μmol/g in the control group ($P = 0.02$ compared with resting), and 31±2 μmol/g in the phosphate-deficient group ($P = 0.002$ compared with resting). IMP content of resting muscle is slightly higher in the phosphate-deficient group (1.0±0.2 μmol/g) compared with the control group (0.4±0.1 μmol/g, $P = 0.05$). Coincident with the decrease in ATP during vigorous muscle contraction, there is an increase in IMP content of the muscle in both groups. IMP increases to 14±3 μmol/g ($P = 0.003$ compared with resting) in the control group, and 15±2 μmol/g ($P = 0.001$ compared with resting) in the phosphate-deficient group.

During the period of recovery from fatigue, ATP content of the muscle in control animals increases rapidly and is restored to resting levels between 2 and 5 min of recovery (43±2 μmol/g, $P = 0.30$). In contrast, in the phosphate-deficient mice, ATP content remains depressed (32±2 μmol/g, $P = 0.002$) during the first 5 min of recovery. After 20 min of recovery, however, ATP content is not significantly different from resting levels in the phosphate-deficient group (38±3 μmol/g, $P = 0.14$). Thus, ATP repletion occurred between 5 and 20 min of recovery in the phosphate-deficient animals. The exact time cannot be determined from the time-points chosen in this study. At all time-points sampled during recovery, the ATP content of gastrocnemius muscle from control animals is higher than that of the phosphate-deficient animals, with statistical significance attained at the 5-min time-point ($P = 0.006$).

The response of the IMP pool during recovery is also different in the two groups. In the control group, IMP decreases rapidly during recovery, with a statistically significant drop occurring in the first 5 min ($P = 0.01$). In contrast, IMP remains elevated during the first 5 min of recovery in the phosphate-deficient group. Even after 5 min of recovery, IMP content is still elevated ninefold above resting levels (9.2±1.5 μmol/g, $P = 0.001$) in the phosphate-deficient group. IMP content is greater in phosphate-deficient animals than in the control animals at all time points during recovery, with statistical significance reached at the 5-min time-point ($P = 0.001$).

AMP content is higher in the phosphate-deficient than in the control group before stimulation (0.15±0.02 vs. 0.09±0.01 μmol/g, $P = 0.03$), and at the end of stimulation (0.17±0.03 vs. 0.10±0.01 μmol/g, $P = 0.05$). During recovery, AMP content tended to be higher in the phosphate-deficient group compared with the control group, but the differences did not reach statistical significance. ADP content is similar in the control (4.9±0.4 μmol/g) and phosphate-deficient groups (5.1±0.4 μmol/g, $P = 0.33$) before stimulation, and does not change significantly during stimulation and recovery. The inability to detect changes in ADP content during stimulation or to detect differences between the two groups is not surprising, since >90% of the ADP measured in acid extracts of muscle is bound to actin (39). Consequently, it would be difficult to detect a change in the very small pool of free or unbound ADP.

CP content. CP content of unstimulated muscle is 116±4 μmol/g in the control group, and 113±2 μmol/g in the phosphate-deficient group ($P = 0.5$) (Fig. 3). With stimulation, CP content decreases by ~40% in both groups (control, 73±7 μmol/g; phosphate-deficient, 67±2 μmol/g; $P = 0.001$ for each group when compared with the resting value for the respective group). During recovery, CP content returns to resting levels in both groups between 5 and 20 min of recovery. This rate of return of CP is similar to that reported by others (25, 26) in rat muscle. No differences in CP content were observed between the two groups at the specific time-points evaluated in this study.

Changes in purine nucleoside and base content. Although cytosol 5'-nucleotidase activity is low in skeletal muscle (40) and IMP accumulates in amounts that are almost stoichiometric with the decrease in adenine nucleotides (Fig. 2), some IMP is hydrolyzed to nu-

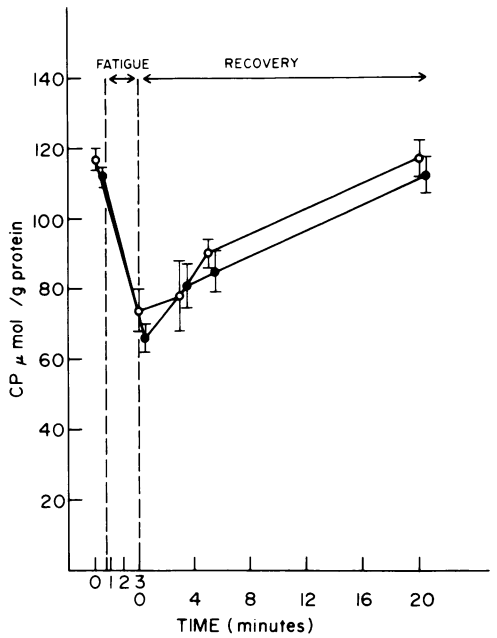

FIGURE 3 CP content (μmol/g) of the gastrocnemius muscle during tetanizing pulses leading to fatigue, and during the recovery period. O, control; ●, phosphate-deficient animals. All values are mean±SEM.

cleosides and bases. Inosine and hypoxanthine both increase in the samples taken after vigorous muscle contraction. Adenosine was not detectable (<5 nmol/g) in any muscle sample. In the control group, the resting level of inosine plus hypoxanthine is 167±54 nmol/g, and this sum increases to 374±32 nmol/g after stimulation. During recovery, the inosine plus hypoxanthine content remains elevated at both 5 (352±74 nmol/g) and 20 (601±81 nmol/g) min. In the phosphate-deficient group, the resting level of inosine plus hypoxanthine is 256±68 nmol/g, increases to 520±49 nmol/g with stimulation, and remains elevated at 5 (605±112 nmol/g) and 20 (522±82 nmol/g) min of recovery. The inosine plus hypoxanthine content of the muscle from phosphate-deficient animals is significantly greater than that of the control group at end stimulation ($P = 0.05$).

DISCUSSION

A comparison of the present study with prior reports suggests that the myopathy associated with phosphate deficiency is a continuum of abnormalities. Models that produce the greatest reductions in serum phosphorous concentration are associated with a more severe myopathy, which is characterized by muscle wasting, weakness with even modest activity, and diminished ATP levels in resting cardiac and skeletal muscle (10, 11, 13, 16, 17, 22). Even in these severely phosphate-deficient animals, the myopathy is reversible following phosphate supplementation (10, 16). The model used in this study leads to a more modest reduction in serum and tissue phosphorous concentration. There is no evidence of muscle wasting (body weight/gastrocnemius ratio) or alteration in fiber type (SDH activity of the gastrocnemius) in these animals. No myopathic findings are demonstrable in resting muscle of these mice, as evidenced by the normal initial peak tetanic tension. However, when maximally stimulated, the gastrocnemius of these phosphate-deficient mice fatigues more rapidly and regains normal contractility more slowly after fatigue. On the basis of these comparisons, we conclude that easy fatigability and delayed recovery of muscle strength following fatigue are early manifestations of the myopathy produced by phosphate deficiency.

The biochemical changes produced in skeletal muscle by phosphate deficiency in this study are consistent with the findings noted in cardiac muscle by others (11, 13) and support the hypothesis of a defect in energy metabolism of the myocyte in this condition. Prior studies (11, 13) suggest that energy metabolism of the myocyte may be disrupted at several different stages in phosphate deficiency. Mitochondrial high energy phosphate (ATP) synthesis via oxidative phosphorylation may be defective; transformation of energy generated in the mitochondria into CP for transport to the myofibril may be abnormal; and resynthesis of ATP from CP at the myofibril to supply the energy needed for muscle contraction may be impaired. This chain of events, i.e., the transport of energy from the mitochondria to the myofibril via CP, has been termed the CP shuttle (41, 42). Results of the present study are most relevant to the first stage, i.e., ATP production in the mitochondria, but the findings do not exclude an abnormality in energy transduction at the myofibril as well.

Evidence from this study that supports a defect in energy production can be summarized as follows: (*a*) during muscle stimulation, ATP and CP content fall to an equal extent in control and phosphate-deficient animals, whereas ATP catabolites (AMP, IMP, inosine, and hypoxanthine) increase to a greater extent in the phosphate-deficient group. Since energy consumption is less in the phosphate-deficient group, as evidenced by the more rapid onset of fatigue, we conclude that ATP production is slower in the phosphate-deficient group during active muscle contraction. (*b*) During the recovery phase, restoration of ATP content from the precursors AMP and IMP takes place at a slower rate in the phosphate-deficient group. Since energy consumption in the resting muscle is minimal and probably not different in the two groups of ani-

mals, we conclude that the slower rate of repletion of the ATP pool in the phosphate-deficient group is the result of a decreased rate of ATP synthesis. These conclusions are supported by studies with mitochondria isolated from cardiac myocytes of phosphate-deficient animals that demonstrate a defect in ATP synthesis in vitro (11, 13). The defect in ATP synthesis may be a direct consequence of a deficit in intracellular phosphate, since recent data suggest that intracellular phosphate concentration in normal skeletal muscle is not sufficient to provide maximal rates of oxidative phosphorylation (43).

The times at which muscle samples were taken in the present study were selected with the primary purpose of detecting differences in nucleotide content. Consequently, potential differences in creatine phosphate content of the muscle of the two groups of animals at other time points may have been overlooked. If we assume, however, that the data presented here are representative and that there was no difference in creatine phosphate content between the two groups at any time point, the similar time-courses for depletion and repletion of the creatine phosphate pools in the two groups may be explained by the following postulate. Dislocation of creatine phosphokinase from the mitochondria and myofibrils in skeletal muscle of phosphate-deficient animals, similar to the changes described in cardiac muscle of phosphate-deficient animals (11, 13), could lead to a defect in both the production (mitochondria) and utilization (myofibril) of creatine phosphate. If the reduction in creatine phosphate production were balanced by a reduction in creatine phosphate utilization, this could explain the apparently comparable rates of creatine phosphate depletion during vigorous muscle contraction and repletion during recovery in control and phosphate-deficient animals. This postulate does not exclude differing rates of depletion and repletion of the ATP pools in these two groups of animals, since ATP production by the mitochondria and ATP use by the myofibril need not be the same as that for creatine phosphate production and use at these two sites. This hypothesis, however, needs to be tested in more direct experiments before concluding that the creatine phosphate shuttle is disrupted in the skeletal muscle of phosphate-deficient animals.

In summary, the following conclusions can be drawn from the present study. The temporal correlations between increased susceptibility to muscle fatigue and evidence of decreased ATP synthesis and between slower recovery of normal contractility and evidence for a slower rate of repletion of ATP pools suggests that the physiological abnormalities noted in muscle of hypophosphatemic animals are causally related to the derangements in nucleotide metabolism. Although this study does not rule out other mechanisms, these results do provide support for the hypothesis that a defect in ATP synthesis is in part responsible for the myopathy seen in phosphate deficiency syndromes.

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