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McArdle’s Disease Heterozygotes
Metabolic Adaptation Assessed Using 31P-Nuclear Magnetic Resonance

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Abstract
Two daughters of a propositus with documented McArdle’s disease were shown by enzyme assay, gel electrophoresis, and immunoblotting to be partially deficient in skeletal muscle phosphorylase and, presumably, heterozygous for the trait. Both exhibited only the adult form of the skeletal muscle isozyme. By 31P-nuclear magnetic resonance, both heterozygotes showed a greater production of acid during fully aerobic exercise than when blood flow was occluded in ischemic exercise. This pattern is in contrast to that of control subjects, where there is significantly greater acid production in ischemic versus aerobic exercise, and distinct from that of phosphorylase-negative patients in which no acid is produced in either circumstance. We suggest that these heterozygotes may have adapted to their diminished phosphorylase by enhancing utilization of plasma glucose. If so, this mechanism could account for the observation that most of the symptoms of McArdle’s disease are often manifest only in adulthood. These studies also show that although there are very high concentrations of phosphorylase in skeletal muscle (~2% of the soluble protein), such a high level is essential for normal muscle glycogenolysis.

Introduction
McArdle’s disease is primarily an autosomal recessive disorder causing decreased exercise tolerance due to total absence of skeletal muscle phosphorylase (1–5). The etiology of the disease is clearly complex, as evidenced by variation in age of onset (1–7), levels of skeletal muscle glycogen (4, 8), differences in inheritance patterns (3, 9) and severity of symptoms (1–5, 8), as well as the presence or absence of immunoreactive catalytically inactive protein (10). The patients with a total absence of muscle phosphorylase typically have symptoms that can, but certainly do not always, begin in childhood. More often, they are only detected later in life, worsening progressively until the fourth or fifth decade, when there can be severe weakness and muscle wasting. Since the precise nature of the homozygous enzyme abnormality was clearly established by Schmid and his colleagues (11) in one family and by Pearson, Mommaerts, and their colleagues (12) in another, it is remarkable that the scientific literature contains very little information characterizing the metabolic situation in patients heterozygously deficient for phosphorylase. It would appear that such patients, when carefully studied, might yield useful information concerning the potential metabolic adaptations to phosphorylase deficiency or, possibly, the clinical evolution of this rare genetic disorder that produces a delayed onset of illness (6). We are aware of only a single report in the scientific literature that examines three patients that are heterozygous-deficient in muscle phosphorylase (13). The probable reason for the paucity in number of studies of such patients is that phosphorylase deficiency is an extremely rare condition and patients heterozygous-deficient for phosphorylase may have few or no symptoms that warrant medical attention. A noninvasive method to detect such heterozygotes would clearly be of value.

The present report describes a detailed biochemical analysis of two sisters partially deficient in muscle phosphorylase and illustrates the use of 31P-nuclear magnetic resonance (NMR) as a potential method for detecting such individuals. The results of our study also suggest a mechanism by which a patient heterozygous for phosphorylase could possibly adapt to an enzyme deficiency in order to maintain adequate work levels. Such information might be useful in understanding the nature of the diverse clinical presentations of the McArdle syndrome observed in some patients and the ability of children to play and work actively and yet be deficient in muscle phosphorylase.

Clinical data
Case S.A.W. A 48-yr-old woman was referred for evaluation after an extended experience of muscle pain and cramps associated with physical activity. Although she did not seek medical attention initially, she gave a history of muscle pain with activity since childhood. Since her adulthood, exercise of moderate or even of mild intensity has usually been associated with muscle pain and sometimes cramping. Symptoms have progressed to the point where walking more than a few feet is associated with pain in her leg muscles. The severity is such that the patient has been unable to work for more than 3 yr. In the past her leg pains have been attributed to claudication or, possibly, to lumbar spinal canal stenosis. She underwent a lumbar laminectomy without relief of her symptoms. During her evaluation at the University Medical Center, serum creatine kinase (CK) levels were found to be elevated 24 times above normal. Electromyogram (EMG) confirmed a moderate population of low amplitude brief duration motor unit action potentials, suggesting a myopathic disorder. During ischemic exercise testing the patient contracted the forearm muscles at 2-s intervals for only 45 s before electrically silent (by EMG) muscle contracture occurred. Blood washed out from the ischemic-exercised muscles contained no change in lactate from resting levels. Histochemical analysis of

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1. Abbreviations used in this paper: CK, creatine kinase; EMG, electromyogram; NMR, nuclear magnetic resonance; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.
tissue obtained at a muscle biopsy showed only mildly elevated accumulation of glycogen. Results of biochemical analyses and \(^{31}\)P-NMR studies are presented in the text. This patient has three daughters, each by a different father. Two of the daughters (S.D.R. and D.M.R.) agreed to further study.

Case S.D.R. This is a 28-yr-old woman with only mild symptoms of muscle pain associated with muscular effort. She recalls having some muscle pains with activity since childhood but has been unconcerned. She has been able to swim, and hike, and even dance for as long as 15–20 min until experiencing occasional muscular discomfort. There have been no symptoms of a nature sufficient to warrant her seeking medical attention. Serum CK levels were within the normal range, and muscle glycogen levels, examined histologically, appeared normal. There were normal EMG responses for two proximal muscles of the lower extremity. Results of biochemical analyses and \(^{31}\)P-NMR studies are presented in the text.

Case D.M.R. This is a 24-yr-old woman with moderate symptoms of muscle pain. Some muscle pain occurs on most days with the daily care of her 7-yr-old son. Muscle cramping occurs if her activities are markedly increased. However, contractures are very rare and no episodes of pigmenturia have been observed. Medical attention had been sought but there appear to have been insufficient indications of significant dysfunction. Laboratory testing revealed normal levels of CK. EMG study demonstrated a few motor unit action potentials with low normal amplitudes and duration, but otherwise showed entirely normal results. Histochemical examination showed normal muscle glycogen levels. Results of biochemical analyses and \(^{31}\)P-NMR studies are presented in the text. Case J.J. The sister 2 yr the junior of S.A.W., she has not been examined at this medical center, but has symptoms that are very similar to S.A.W.’s. A biopsy sample of muscle was made available for these studies courtesy of Dr. B. M. Patten, Baylor College of Medicine, Waco, TX.

Methods

\(^{31}\)P-NMR evaluation

The standard protocol for examination of the metabolism of aerobic and ischemic muscular exercise by \(^{31}\)P-NMR was based on that originally described by Taylor et al. (14). Exercises were performed on each subject in the mornings after breakfast. The muscle belly of the right flexor digitorum superficialis was identified and the overlying skin was marked for positioning of a 2-cm diameter topical coil antenna. The upper arm was wrapped with a grounding strap and a blood pressure cuff. The subject’s arm was then positioned in the 30-cm bore of a 1.89 Tesla superconducting electromagnet (Oxford Research Systems, Oxford, England). Spectra were obtained by pulse-free induction decay and fast Fourier transform using a 30° pulse angle (1.486 ms square wave) for \(^{31}\)P spectra resonating at 32.5 MHz. Uniformity of field was established by adjustment of shimming coils until the water peak from the proton spectrum attained a half-height width < 30 Hz; this was typically 23–25 Hz. Quadrature phase detection and exponential weighting improved the signal/noise ratios. Signal/noise of each \(^{31}\)P spectrum was further enhanced by averaging 32 sequential scans obtained over a total of 64 s. Intracellular pH was calculated from the formula pH = 6.72 + log\(_{10}\) (S − 3.27)/(5.69 − S), where S is the chemical shift in parts per million (15, 16).

A resting phosphorus spectrum was recorded and then the subject began exercising by squeezing a rubber manometer bulb. The squeeze rate was 30/min and timed to the clicks of a metronome. The subjects exercised aerobically for 6 min. During the first 4 min of exercise, the subjects squeezed against 200 mmHg resistance added to the bulb. During the 5th and 6th minutes of exercise the resistance within the bulb was increased to 400 mmHg. At the end of 6 min the subjects stopped exercising and rested with their arm remaining in the magnet until the muscle pH returned to preexercise levels. Subsequently, ischemia of the arm was produced while the subject exercised for 3 min. The blood pressure cuff was inflated to 200 mmHg and maintained there accurately by a servomechanic insufflator, during which time the subject again squeezed the rubber bulb (resting pressure 200 mmHg) to the clicks of a metronome. In a clinical setting it is difficult to ascertain if a person has worked maximally, therefore we sought to achieve uniformity of performance by having each subject squeeze the rubber ball with sufficient force to increase the pressure within the ball by at least 100 mmHg during the first 4 min of aerobic exercise, by 50 mmHg during the final 2 min of aerobic exercise, and by 100 mmHg during the initial 2.5 min of ischemic exercise. Beyond this time of ischemic exercise the work performance of all subjects tended to decrease and most were unable to sustain any further contractions. Exercise of all subjects was monitored by a physiograph. No data is included when the subject did not accomplish the standard protocol. Exercise performance was maintained by verbal feedback. Phosphorus spectra were obtained over serial 64-s intervals, representing rest, the period of exercise, and 15–20 contiguous periods during recovery from exercise. The studies on the patients described were conducted over a 2-mo interval with 16–20 studies performed on each. No more than two exercise protocols were performed on a single day, with an interval of at least 1 h between each exercise protocol.

Biochemical analysis

Enzyme assays. Tissue samples (vastus lateralis) were obtained by open-muscle biopsy and immediately frozen by immersion in isopentane at −195°C. Phosphorylase was measured by a modification (17) of the procedure of Cori et al. (18) with the inclusion that phosphate production was measured for the 2–22-min period to control for variability in tissue phosphate content and initial phosphate production from contaminating metabolites. Assays for phosphorylase kinase and glycogen synthase were as previously described (17). 5’-AMP deaminase was measured by a modification of the method of Wheeler and Lowenstein (19). Briefly, muscle powder was homogenized in 15 vol of 50 mM imidazole (pH 6.5), and 20 μl of centrifuged extract was added to 0.5 ml of a solution containing 1 mM 5’-AMP and 150 mM tetrathylammonium chloride in the same buffer. Inosine 5’-monophosphate formation was determined from the difference in absorbance change at 262.5 and 310 nm using a double-beam spectrophotometer (American Instrument Co., Silver Springs, MD) and a 1 mm path-length. Potassium activation and phosphate inhibition were tested at 150 mM and 1.5 mM, respectively, at both 1 mM and 50 μM 5’-AMP.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed essentially as described by Laemmli (20). Aliquots of biopsy extracts containing 120 μg of soluble protein were heated at 65°C for 15 min in a gel sample buffer consisting of 60 mM Tris chloride (pH 6.8), 0.45 mM 2-mercaptoethanol, 2% wt/vol SDS, 6% wt/vol sucrose, 0.01% Bromophenol Blue, and then applied to a 9 × 16 × 0.15 cm slab gel with 5% wt/vol acrylamide in the running gel and 3.5% in the 1-cm long stacking gel. Gels were stained with Coo- massie Brilliant Blue, destained, fixed in 7.5% vol/vol acetic acid, and photographed. Protein in the phosphorylase band was quantitated relative to standards by scanning the photographic negative on a Cary 210 spectrophotometer (500 nm; Varian Associates, Inc., Palo Alto, CA), interfaced with a 9845A computer (Hewlett-Packard, Palo Alto, CA), and integrating peak areas.

Protein immunoblots. Western blots were performed by a modification of the method of Howe and Hershey (21). Biopsy samples of 40 or 80 μg soluble protein and pure phosphorylase b standards in the range of 0.42–6.3 μg, were electrophoresed on a 7.5% Laemmli SDS slab gel (20). Proteins were electrophoretically transferred from the gel to nitrocellulose BA 85 (Schleicher and Schuell, Inc., Keene, NH). After Amido Black protein staining and destaining to test transfer, the nitrocellulose blot was soaked for 1 h in buffer A consisting of 10 mM Tris chloride (pH 7.5), 0.9% NaCl, 0.5% Triton, 0.5% wt/vol bovine serum albumin, 0.01% NaN₃. The blots were then treated sequentially with: (a) goat anti-rabbit...
phosphorylase IgG, 0.19 mg/ml (prepared by DEAE Affi-Gel Blue chromatography; Bio-Rad Laboratories, Richmond, CA); (b) buffer A (5 changes, 30 min each); (c) 125I-rabbit anti-goat IgG; and (d) 5 washes with buffer A. The dried nitrocellulose blot was autoradiographed using X-OMAT film (Eastman Kodak Co., Rochester, NY) and phosphorylase levels quantitated both by scanning the autoradiograph and by cutting out the bands and measuring radioactivity in comparison to the standards.

Determinant of phosphorylase isozyme type by nondenaturing gel electrophoresis. The procedure used was adapted from Sato et al. (22). Tissue powders were homogenized at 0°C in 63 mM Tris chloride (pH 7.5), 6.3 mM EDTA, 25 mM 2-mercaptoethanol, 1 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, and 0.1 mM tosylamide-2-phenylethyl chloromethyl ketone. The extracts were centrifuged and the supernatants incubated for 30 min at 30°C with 10 U/ml α-amylase (Bacterial Type IIA; Sigma Chemical Co., St. Louis, MO). After α-amylase digestion each extract was mixed with 0.5 vol of 188 mM Tris chloride (pH 6.8), 10% wt/vol 2-mercaptoethanol plus 60% wt/vol sucrose. The samples (containing 90–165 μg soluble protein) were electrophoresed according to Orstein and Davis (23) using 0.3-cm slab gels of 6% wt/vol acrylamide containing 0.005% wt/vol glycerol, and a 1-cm long 3.5% wt/vol acrylamide stacking gel. Electrophoresis was performed at 5°C for 30 min at 30°C. After electrophoresis, the gels were stained with Coomassie Brilliant Blue (Pierce) and destained in 10% ethanol, 6% acetic acid, 10% acetic acid, 10% acetic acid, 10% acetic acid.

Results

Biochemical analysis of muscle enzymes. Analyses of the level of phosphorylase in the biopsy specimens of the vastus lateralis muscles of the three subjects, plus the proband’s sister (J.J.), were performed by three procedures, namely, determination of enzyme activity (Table I), SDS-PAGE (Figure 1 A), and immunoblotting (Figure 1 B). By each procedure, no significant level of phosphorylase (or immunoreactive protein) was detectable in either the proband (S.A.W.) or her sister (J.J.), confirming in each the diagnosis of McArdle’s disease. In both daughters (D.M.R. and S.D.R.), phosphorylase was detectable by all three procedures, with excellent consistency between the measurements, indicating the level in each to be in the range of 25–45% of normal (Table I). These values are compatible with each being heterozygous for McArdle’s syndrome, albeit there may be a tendency for the amounts to be somewhat less than 50%. The normal values of phosphorylase observed in this study are consistent with previously reported values (10, 25–27). All four subjects had levels within the normal range for phosphorylase kinase, glycogen synthase, and adenylyl deaminase (Table I). The activity states of phosphorylase kinase (pH 6.8.8.2, activity ratio) and glycogen synthase (percent glucose-6-phosphate stimulation), and the allosteric regulation pattern of adenylyl deaminase (K+ activation, HPO42− inhibition) were also normal (data not shown).

Isozyme evaluation. It has previously been shown (24, 28) that there are clearly demonstrable levels of phosphorylase in muscle cells from McArdle’s patients, when grown in culture, that are attributable to the reappearance of the fetal isozyme as the myocytes grow. Hence, we tested the possibility that the two daughters (D.M.R. and S.D.R.) might be deficient in the adult form of phosphorylase but exhibit phosphorylase activity because of an elevated level of the fetal isozyme. Nondenaturing gel electrophoresis, with phosphorylase detected by enzyme activity stain (Fig. 1 C), demonstrated that both daughters contained only the adult form. (The efficacy of the system in separating both forms was shown by the use of rat brain extracts which contain both fetal and adult forms.) The only isozyme detectable in both daughters conigrated with enzyme from control (human) muscle.

31P-NMR studies of exercising forearm. The metabolism during exercise of the two subjects with diminished levels of phosphorylase (McArdle heterozygotes) was examined by the use of the noninvasive technique of 31P-NMR, pioneered for human studies by the groups of Radda (29) and Chance (30). Resting pH of flexor digitorum superficialis, as observed by this technique in normal healthy volunteers, was 7.03±0.01 (±SEM, n = 22). For normal subjects the profile of pH changes which occur during the standard protocols for aerobic and ischemic exercise (see Methods) are depicted in Figure 2. During 6 min of aerobic exercise, with the protocol as described in the Methods section, the pH in normal subjects fell to 6.60±0.05 (n = 22). Muscle pH fell even further to 6.31±0.05 (n = 22) during 3 min of ischemic exercise. Aerobic exercise of the proband McArdle patient (S.A.W.) failed to lower the pH, as has been reported by Ross et al. (29); if anything, there was a tendency for it to be elevated (Fig. 3). To avoid inappropriate stress on this patient, the ischemic protocol was not performed, but Ross et al. (29) have reported on a similar patient, using similar exercise protocols, that ischemic exercise also does not result in a depression.
of muscle pH. The exercise protocols used in this study are similar to those used by that group (29).

The patterns of pH changes occurring with exercise obtained with D.M.R. and S.D.R. (McArdle heterozygotes) were strikingly different from those observed either with the normal controls or with the subject totally deficient in phosphorylase. The changes in muscle pH are indicated in Fig. 4, A and B. These two subjects with partial deficiencies of phosphorylase invariably formed less acid with the ischemic exercise protocol than with aerobic exercise (P < 0.05). This finding is the reverse of the expected pattern observed in healthy volunteers (P < 0.001), but was a consistent finding on multiple testing on different days and at any level of workload performed (not shown). It is interesting to note that S.D.R. always tended to form more acid than D.M.R., but had less measurable phosphorylase activity. On one occasion, while performing the same amount of work as healthy controls, S.D.R. demonstrated a total inability to form acid (i.e., similar to what was normally observed with her mother, S.A.W., who is completely phosphorylase-deficient), although all other components of the 31P-NMR spectrum were normal (Fig. 5). After a brief rest and a meal, S.D.R. was again able to form acid during exercise with the same pattern as shown in Fig. 4 B.

**Discussion**

Individuals with McArdle’s disease cannot utilize their stores of muscle glycogen, thus lactate is not formed during exercise or hypoxia. The most common symptoms in these subjects are a low exercise tolerance, muscle fatigue, and pains and cramps during physical activity. These symptoms may disappear if the subject is able to sustain the exercise via the second wind phe-

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2. Eight of the controls were matched in age and sex with McArdle heterozygote subjects; this control subset showed no difference from the entire control group. The data for this subset was as follows: resting pH, 7.01±0.02; 6-min aerobic exercise pH, 6.61±0.08; 3-min ischemic exercise pH, 6.32±0.10.

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3. The precise timing of the switch-over from the fetal to the adult form of phosphorylase has not been reported for humans. In rats this occurs within the first 5 d after birth (39). McArdle’s disease appears to be due to an absence of the adult form only because myocytes cultured from the muscle of McArdle’s patients have been shown to express the fetal isozyme (24, 28).
similar to the second wind phenomenon (12, 31), provides oxidizable substrate, even from the onset of exercise, to keep children with this defect relatively symptom free, even with high activity. If such an adaptive mechanism were to be subsequently diminished with age it could account for the observed pattern of severe symptoms appearing only in the fourth or fifth decade of life.

The patterns of acid production obtained with the two McArdle heterozygotes in the present study are clearly distinct from those obtained with normal subjects. With normal subjects, initial exercise is supported almost entirely by glycolysis (32, 33), which results in extensive lactic acid production when

The exercise is performed anaerobically, and less lactic acid production but more complete tricarboxylic acid cycle activity when oxygen is available. In contrast to this, more acid was produced in the phosphorylase heterozygous subjects with aerobic exercise than with the ischemic protocol. The diminished anaerobic acid production in heterozygotes compared with controls indicates

**Figure 2.** Change in forearm muscle pH measured by $^{31}$P-NMR during exercise of normal healthy volunteers. Aerobic exercise (---) continued for 6 min. Ischemic exercise (--o--) continued for 3 min. The vertical line indicates beginning of recovery for both exercises. Conditions of exercise and NMR measurements are given in Methods. The values are for 22 sets of exercise, the bars indicate the standard errors.

**Figure 3.** $^{31}$P-NMR spectra of the exercising flexor muscles of the proband S.A.W. Before exercise, a spectrum was obtained at rest. The spectra marked "Exercise" identify the 0–1st, 2nd–3rd, and 5th–6th minutes of aerobic exercise as described in the Methods section. The spectrum marked "Recovery" was obtained during the 1st minute of recovery after the aerobic exercise. The resonances observed in the spectra are identified in the top right of the figure. P is inorganic phosphate, PCr is phosphocreatine, and ATP identifies the $\alpha$, $\beta$, and $\gamma$ phosphates of adenine nucleotides. The numbers over the inorganic phosphate resonances are the calculated pH's of muscle obtained from each of the spectra shown.

**Figure 4.** Changes in flexor muscle pH during exercise and recovery from exercise for D.M.R. (panel A) and S.D.R. (panel B). Each data point is the mean (±SEM) of the pH values obtained for six sets of exercise for D.M.R. (panel A) and seven sets of exercise for S.D.R. (panel B). Aerobic exercise (---) continued for 6 min. Ischemic exercise (--o--) continued for 3 min. The vertical line indicates the beginning of recovery for both types of exercises. Paired t test for these sets of exercise indicated the aerobic exercise depression of pH was significantly greater ($P < 0.05$) than that produced by the ischemic protocol. In contrast, a similar comparison with either age-matched controls ($P < 0.05, n = 8$) or other control subjects ($P < 0.001, n = 22$) showed that the ischemic protocol produced a greater decrease in pH than that observed with aerobic exercise.
that their capacity for glycolysis is impaired. A possible explanation for our observations is that these heterozygotes have compensated for their impairment with an enhanced ability to take up and utilize plasma glucose as an energy source to support the initial phase of exercise. In normal subjects, plasma glucose only becomes an important fuel to support skeletal muscle activity after an initial diminishment of skeletal muscle glycogen (34, 35). In the phosphorylase heterozygotes, under ischemic conditions, the only source of lactic acid is the diminished glycolytic capacity, whereas under nonischemic conditions, this may well be supplemented by glycolysis using plasma glucose, hence accounting for the higher quantity of acid production. To prove this hypothesis definitively, however, would require arterial and deep venous blood sampling to directly measure glucose uptake by muscle during aerobic and anaerobic exercise, which the subjects in this study were adverse to having done. 31P-NMR studies of more than two subjects heterozygous-deficient in phosphorylase are needed to confirm this hypothesis, but with the rarity of this disease and the even more limited number of heterozygotes available to examine, additional studies have not yet been possible. Enhanced skeletal muscle glucose utilization as a consequence of phosphorylase deficiency has, however, been suggested by three other studies. In one circumstance (8), where the McArdle's disease was associated with a grand mal seizure, ischemic exercise induced subsequent hypoglycemia. In a second report (34), glucagon administration before exercise markedly enhanced the exercise performance of a McArdle patient, most likely as a consequence of an increased hepatic delivery of plasma glucose. Lastly, in a third study of a McArdle patient (6), when blood sugar was raised by the intravenous administration of epinephrine, work performance appeared to improve. Each of these observations is compatible with an increase in muscle glucose utilization when muscle glycogen cannot be mobilized.

The recognition of an adaptation to support skeletal muscle activity in the initial phase of exercise through the utilization of plasma glucose can provide an explanation of why the onset of severe symptoms in McArdle patients most frequently does not occur until adulthood (1–6). It is well documented in several species (35–38) that with age there is a decreased capacity for glucose uptake by muscle. Thus, it may be that the onset of symptoms in McArdle patients coincides with their diminished ability to utilize plasma glucose as an energy source for muscular activity rather than the absence of phosphorylase per se.

Both heterozygous subjects showed a diminished production of acid in the 31P-NMR study during ischemic exercise, indicating that glycogenolytic potential was suppressed. A possible explanation of these metabolic patterns could be that glycogenolysis was not suppressed, but that the diminished acid production was due to less glycogen being present in muscle for ischemic lactate formation. We consider this an unlikely explanation. Serial muscle biopsies of the flexor digitorum superficialis muscle performed before and after each exercise to measure muscle glycogen content would be necessary to test this possibility, and such a study is unfeasible. The histological stains of vastus lateralis muscle biopsy specimens obtained from our heterozygous subjects demonstrated abundant glycogen stores.

Despite the fact that the most reasonable conclusion from these studies is that the two daughters (S.D.R. and D.M.R.) are heterozygotes, there is a striking similarity between their clinical presentation and those symptoms frequently reported for the early stages of classical McArdle’s disease (1–5). Both, and in particular D.M.R., have begun to show some evidence of exercise intolerance, while S.D.R. on one occasion (Fig. 5) failed to form any acid during exercise, similar to her homozygous-deficient mother (S.A.W.). It is of interest that the measurement of phosphorylase in both S.D.R. and D.M.R. by several methods gave values that were at the lower end of the range that might have been anticipated for a heterozygote. These observations lead to the speculation that possibly one or both daughters are heading towards a McArdle crisis. An alternative explanation of the typical late onset of symptoms may be that in some McArdle patients the defect is not an absence of phosphorylase per se but an abnormal regulation of its expression. Thus, symptoms might arise in adulthood coincident with a depression of the synthesis of phosphorylase; no prospective study has been reported that would eliminate such a possibility. Possibly, we have serendipitously initiated the needed prospective study. It is of interest that phosphorylase in normal muscle is a highly abundant protein (~2% of total cytosolic protein) yet when this level was diminished to approximately half in either heterozygote subject, glycolysis (during ischemia) was markedly impaired. Thus, this study provides the first evidence that the high level of phosphorylase in normal muscle is essential for normal function.

Whichever may be the correct conclusion about the two subjects of this study (S.D.R. and D.M.R.), i.e., that they are heterozygotes or that their level of phosphorylase is decreasing with age, the study presented here has documented the characteristics of subjects with diminished levels of phosphorylase. The noninvasive technique of 31P-NMR provides a ready mechanism for follow-up in these subjects and potentially for screening others for a McArdle trait.

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