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Hypotonia of Rickets: A Sequential Study by P-31 Magnetic Resonance Spectroscopy

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ABSTRACT. To address the role of high-energy phosphorus compounds in the hypotonia of vitamin D-dependent rickets, nuclear magnetic resonance spectra were obtained sequentially from resting gastrocnemius muscle of a 10-month-old infant with rachitic hypotonia during supplementation with vitamin D, calcium, and phosphorus. During the initial weeks of treatment, the hypotonia resolved before evidence of epiphyseal mineralization. Over the early treatment period, the muscle phosphocreatine/β-adenosine triphosphate [PCr/β-ATP] ratio increased from 2.7–2.8 [wk 1–2] to 3.9–4.5 [wk 7–9]. The PCr/β-ATP ratio for 6-month-old normal infant gastrocnemius and adult forearm were 4.0 and 5.7, respectively. Muscle strength appeared to recover concomitantly with an increase in retained muscle phosphorus and high-energy phosphate compounds, and with relative increase in the muscle phosphocreatine to ATP ratio. The synchrony of clinical recovery may relate to the recovery kinetics of these metabolic changes. (*Pediatr Res* 24:713–716, 1988)

Abbreviations

MRS, magnetic resonance spectroscopy
PCr, phosphocreatine
MDP, methylene disphosphonate
NTP, nucleoside triphosphates
Pi, orthophosphate
ppm, parts per million
BSID, Bayley scales of infant development

The relationship between the clinical features of rickets and the underlying pathogenic mechanisms are unclear, *viz.* the renal mineral losses, the epiphyseal ossification delays, and the muscular hypotonia and weakness in several forms of rickets (1). Vitamin D and mineral supplementation nominally improve intestinal and renal membrane transport of ions and bone deposition (2, 3), but the responsible cellular mechanisms have not been independently determined, and response to treatment as a function of time may be clinically asynchronous. One possibility is that these clinical responses may reflect changes which are modulations of discrete tissue substrate levels, *e.g.* mineral pools, or high-energy metabolite levels. To address the latter, a case study of vitamin D-dependent rachitic hypotonia and its evolution after therapy, that included sequential ^{31}P MRS (4), provided the opportunity to address such inquiry directly *in vivo* in a noninvasive manner. The aim was to make parallel observations of the clinical response to dietary supplementation and the temporal response of the major phosphorus-containing compounds in skeletal muscle.

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CASE REPORT

Patient R. C. was presented at age 9.5 months with the complaint of failing to reach normal developmental milestones. He was born to a 21-year-old G-1, P-0 woman following an uncomplicated pregnancy and delivery; birth weight was 3210 g. He was breast fed to age 5 months, supplemented only with a nonvitamin D-fortified powdered milk. He began feeding poorly at age 6–7 months, taking only 120–240 ml of milk/day (37–75 ml/kg·day) with some blended foods. His motor development had been slow, sitting only with assistance, without rolling over, crawling, or bearing weight. Personal-social and language development were assessed as normal. He had never been hospitalized, and his immunizations were current.

Initial anthropometrics included head circumference 45.5 cm (25th percentile for age); weight and height were 6705 g and 63.5 cm, respectively, (<5th percentile for age and 50th percentile for age 4 months for each parameter by NCHS standards) (5). He had a large anterior fontanelle, split sutures and generalized hypotonia. There was no alopecia. By the BSID, he had a mental development score of 10.5 months and psychomotor development score of 7.1 months.

He was subsequently monitored and treated uneventfully as an outpatient, with the exception of one brief elective hospitalization for metabolic balance studies. Initial data are shown in Table 1. Single-specimen urinary data at presentation [phosphorus 34.90 mmol/liter (108 mg/dl) and creatinine 2625 μmol/liter (29.7 mg/dl)] provided estimation of fractional excretion of phosphorus of 0.21; urinary calcium was undetectable. Chest, wrist, and knee roentgenograms (available on request) showed diffuse osteopenia, with very wide epiphyses and irregular metaphyses; the distal left radius, left ulna, and several metacarpals showed periosteal reaction. The changes were those of severe classical rickets associated with modestly healing fractures. Cranial sonography was completely normal.

Initial efforts were undertaken to improve general nutrition with adequate calorie and nitrogen intake and to institute therapy for presumptive vitamin D-deficiency rickets with supplemental vitamin D_2 (25.5 μg/kg·day or 1020 IU/kg·day), phosphorus (4.5 mmol/kg·day or 140 mg/kg·day), and calcium (2.5 mmol/kg·day or 100 mg/kg·day). Weight gain after beginning therapy was initially slow, and was interspersed with periods of minimal or no weight increments, associated with short-term episodes of noninfectious diarrhea and/or otitis media with upper respiratory infection. Over the ensuing early weeks when the serum data (Table 2) showed little change, diagnosis of vitamin D-depend-

ency rickets was made (6) and adjustments of these supplements were made progressively to 5.5 mmol (171 mg) phosphorus/kg·day, 5.3 mmol (212 mg) calcium/kg·day, and 75 μg (3000 IU) vitamin D_2/kg·day by 4 wk posttherapy initiation.

Despite the lack of serum ion changes in these first 4 wk posttherapy initiation, and no improvement in bone mineralization by sequential long bone radiologic assessment, R. C. demonstrated a heightened appetite and mood. At this age, it was not possible to quantitate muscle strength or performance; hence three physicians independently judged physical exam for clinical change in motor function and strength. Muscle tone was assessed by physical examination grading based on a linear scale from zero to 4+ with 4+ representing normal tone for age. He clearly showed in this time interval gradual and progressive improvement weekly, from a limb motor score of 0–1+ to 3–3.5+: he began to pass developmental milestones, and sequentially began to sit steadily, roll over, crawl backwards, and pull himself up to a sitting position, while beginning verbalization and showing improved psychosocial interaction. By 8 wk posttherapy, he no longer had clinically detectable hypotonia.

Nonetheless, at 8 wk posttherapy, serum calcium, phosphorus, and alkaline phosphatase aberrations persisted, and a 3-day inhospital balance study was undertaken to evaluate net ion retentions (calculated input minus combined urine-plus-stool losses) (7). The studies showed net retention of calcium (+7.8 mmol/kg·3 day or +313 mg/kg·3 day) and phosphorus (+6.8 mmol/kg·3 day or +211 mg/kg·3 day); the average intake during this time was 3.7 mmol (147 mg) calcium/kg·day and 3.3 mmol (103 mg) phosphorus/kg·day; this calcium/phosphorus intake molar ratio of 1.43 was closely matched by the ratio of the net retention of calcium/phosphorus of 1.48. Total body retention was 69 and 72% of calcium and phosphorus, respectively. The urinary fractional excretion of phosphorus (0.24) was unchanged from that measured earlier; urinary calcium remained very low at 0.30 mmol/liter (1.2 mg/dl). Serum 1,25-dihydroxycholecalciferol was 77 pmol/liter (32 pg/ml) (normal range 80–115 pmol/liter), compatible with vitamin D-dependency type I, in contrast to D-dependency type II in which 1,25-dihydroxycholecalciferol has been generally elevated. Bone roentgenograms still showed minimal healing, and bone age estimated from eight epiphyseal centers in left upper/left lower extremities according to Greulich-Pyle (8) was 4 months.

As a consequence of these observations, vitamin D supplementation was increased to 180 μg (7200 IU)/kg·day at this time, after which serum calcium and phosphorus returned to normal values within 6 wk. Parathyroid hormone blood concentration by 10 weeks had returned to a level of 194 ng/dl. By 15 wk after initial therapy was begun, significant bone mineralization and rachitic healing in upper and lower extremeties were now evident; he had gained 3.8 cm body length and had a head circumference of 47.8 cm. No teeth had erupted, but his cranial sutures fused and the anterior fontanelle was almost closed at this time. A BSID exam at age 21 months was scored at 20 months mental scale and 13 months motor scale.

Table 1. *Initial clinical data for patient R.C. with vitamin D-dependent rickets*

	Value	Normal range
Serum		
Phosphorus	1.15 mmol/liter	1.45–2.16 mmol/liter
Calcium [total]	1.64 mmol/liter	2.50–3.00 mmol/liter
Alkaline phosphatase	36.3 μKat/liter	1.3–5.5 μKat/liter
Parathyroid hormone (C-terminal assay)	2200 ng/dl	10–180 ng/dl
25 OH-cholecalciferol	182 nmol/liter	25–137 nmol/liter
Magnesium	0.7 mmol/liter	0.70–0.95 mmol/liter
Protein (total)	68 g/liter	51–73 g/liter
Urea nitrogen	1.8 mmol/liter	1.8–7.1 mmol/liter
Creatinine	18 μmol/liter	27–53 μmol/liter
Alanine aminotransferase	0.22 μKat/liter	0.22–0.88 μKat/liter
Bilirubin (total)	5 μmol/liter	<25 μmol/liter
Retinol	1.11 μmol/liter	0.35–1.50 μmol/liter
Tocopherol	16 μmol/liter	12–46 μmol/liter
Creatine kinase	2.50 μKat/liter	0.58–3.87 μKat/liter
Sweat chloride	27 mmol/liter	<60 mmol/liter

MATERIALS AND METHODS

^{31}P MRS data are reported for skeletal muscle, measured in the patient at 1-, 2-, 3-, 5-, 7-, and 9-week intervals after initiating the therapeutic program. Similar spectra were obtained from a normal well-nourished control infant of age 6 months who closely approximated the patient's weight, muscle mass, and height. Use of the infants' gastrocnemius was optimal to allow positioning of infants of this size within the magnet at the point of greatest field homogeneity. Institutional Review Board and parental approval were obtained for the MRS measurements in control and study infants.

Spectra were measured using a single turn 4-cm diameter surface coil tuned to 32.5 MHz. A 1-cm diameter vial placed directly underneath the center of the surface coil and containing 0.2 M MDP was used as an external standard. The infants under light chloral hydrate-induced sedation were placed inside a 30 cm bore, 1.9 Tesla superconducting magnet prior to optimizing

Table 2. *Clinical data in relation to therapy in patient R.C. with vitamin D-dependent rickets**

	Therapy dosage			Serum values		
Wk of therapy	Vitamin D_2 (μg/kg·d)	Calcium (mmol/kg·d)	Phosphorus (mmol/kg·d)	Alkaline phosphatase (μKat/liter)	Calcium (mmol/liter)	Phosphorus (mmol/liter)
−0.5						
0	25.5	2.5	4.5	36.4	1.65	1.14
1	↓	↓	↓	46.2	1.33	1.20
2	↓	5.3	↓		1.25	1.37
4	75.0	↓	5.5	38.7	1.73	1.11
7	↓	↓	↓	40.6	1.65	1.37
9	180.0	↓	↓	↓	1.60	1.20
10	↓	↓	↓	37.0	1.80	1.27
13	↓	3.2	↓	↓	2.18	1.37
16	↓	↓	↓	29.8	2.35	1.69
18	↓	2.2	2.9	17.7	2.60	1.82

* Previous convention division conversion factors for calcium (mg/dl) 0.250, phosphorus (mg/dl) 0.325, alkaline phosphatase (U/liter) 0.01667, vitamin D (U/kg) 0.025.

the magnetic field homogeneity by shimming on the proton signal from muscle. Data acquisition consisted of a 50-μs excitation pulse, a 2-s interpulse delay, a sweep width of 2500 Hz, 4K data points per free induction decay, and 64 summed transients per spectrum. The results from 2–6 successive data accumulations were summed, and exponential multiplication corresponding to 10 Hz linebroadening applied before Fourier transformation. Baseline correction of spectra was performed using an interpolation routine supplied with the 1280 Software (Nicolet Magnetic Corporation, Fremont, CA). Consistent with the observations of other investigators, infants experienced no overt or adverse effect over an approximate 1-h exposure to the 1.9-T magnetic field.

Previous comparisons have shown that ^{31}P MRS accurately reflects the relative concentrations of PCr, Pi, and ATP in muscle (9). In the present study, the measurement of the absolute concentrations of these metabolites was not feasible, primarily because of uncertainty in the volume of muscle measured by the radiofrequency surface coil (10). We have therefore interpreted our results on the basis of changes in the ratio of ^{31}P MRS peak areas from muscle relative to each other and the external standard.

RESULTS

MRS P-31 data from a 6-month-old control infant's resting gastrocnemius muscle are shown in Figure 1. Evident are peaks for the external standard (MDP), Pi, PCr, and the γ-, α-, and β-peaks of individual nucleoside triphosphates (primarily, ATP). A cumulative integral is superimposed across the ^{31}P MRS spectrum to indicate the summation of signal. For this and all subsequent spectra, computerized integration of areas under the curves was performed using the integration windows listed in the legend to Figure 1. Repeated analysis of select spectra suggest a precision in the area analysis ranging from ±7% for PCr and MDP to ±20% for the three NTP peaks. The individual spectra were compared by analyzing these integrated areas under the respective peaks.

The peak area analysis shows that PCr was maintained at approximately 40 to 45% of the total tissue ^{31}P signal, comparable to that seen in normal infant and adult muscle. The area of β-NTP decreased from 14–15% at wk 1 and 2 to 10% by wk 7 and 9. In comparison, for the normal infant and adult, β-NTP peak areas, respectively, were 10% (our data) and 9% (11). The area of the Pi peak accounted for 6% of the total signal at wk 1; in subsequent weeks this value ranged from 10–15%.

With the exception of the wk 1 data, the ratio of peak areas for PCr/Pi varied from 2.7–4.3 with no temporal trend; the ratio for the normal infant measured in this study was 1.9, compared to adult muscle of 7.7 (11). The calculated PCr/Pi ratio for wk 1 (7.1) is subject to uncertainty due to potential errors in the integrated area of PCr, because of peak overlap with γ-NTP. However, the PCr/β-NTP ratio for R.C. showed a consistent increase from wk 1–9, reaching a level comparable to the normal infant by 7–9 weeks (Figure 3).

Where there was a clearly discernible Pi peak, muscle pH was estimated to be 7.05 ± 0.10, as calculated from the Pi-PCr chemical shift (for formula to calculate pH, see Ref. 12) and was not different from control muscle pH measurements.

DISCUSSION

On the basis of the clinical presentation, laboratory data, and response to therapy, this infant had a vitamin D-dependent form of rickets (1). Therapy with calcium, phosphorus, and relatively low therapeutic doses of vitamin D allowed the infant to recover in an early phase from debilitating hypotonia. For this patient, resolution of hypotonia was distinct and separate in time from resolution of demineralization and serum ion normalization. The PCr/β-NTP ratio increased to levels measured for a normal infant, concurrent with the recovery of muscle function and independently of the later mineralization healing.

For patient R.C., the first spectrum at week 1 (bottom of Fig. 2) shows that Pi is almost undetectable, PCr appears much lower compared to the normal infant spectrum, and the PCr-γ-NTP resolution is indistinct. Our initial assessment of this spectrum was that patient movement or poor main magnetic field homogeneity was responsible for the lack of resolution. However, repeated shimming did not improve the resolution, and on subsequent analysis we found that the linewidth of the MDP peak (66 Hz) was not substantially larger than that obtained in

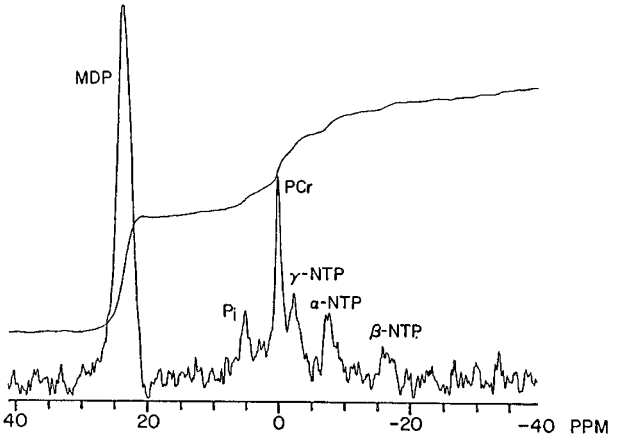

Fig. 1. P-31 MRS spectrum from gastrocnemius muscle of a normal infant (age 6 months). The peak at 24.3 ppm is MDP, the external standard. Identified peaks are Pi, 4.9 ppm; PCr, set to 0.0 ppm; and the respective γ, −2.6 ppm; α, −7.7 ppm; and β-phosphorus, −16.1 ppm of NTP. The cumulative integral of the P-31 signal is shown; the following integration windows were used to calculate percentage total peak area in this and all other spectra: MDP, 30 to 20 ppm; Pi, 6 to 4 ppm; PCr, 1.5 to −1.5 ppm; γ-NTP, −1.5 to −4.0 ppm; α-NTP, −6.0 to −9.0 ppm; β-NTP, −15.0 to −18.0 ppm.

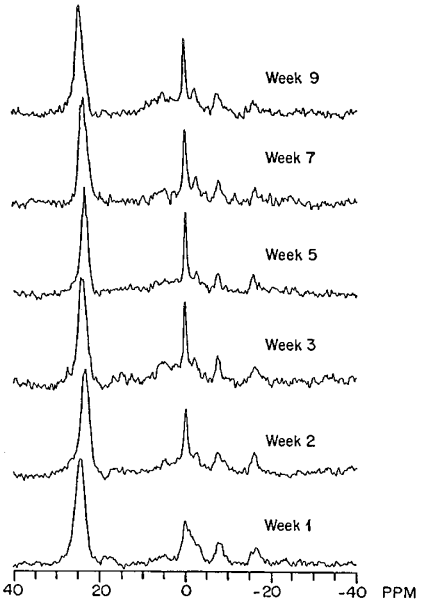

Fig. 2. Sequential P-31 MRS Spectra from gastrocnemius of patient R.C. as a function of time after initiation of therapy. Data acquisition conditions are detailed in the text. The data have been scaled such that the MDP external standard signal is at a constant height for each spectrum. The spectra taken at wk 7 and 9 used 3.5-s interpulse delays rather than 2-s delays, but corrections for differential saturation were not necessary, because the slightly different delays each studied separately in patient R.C. had negligible effect on the relative peak areas.

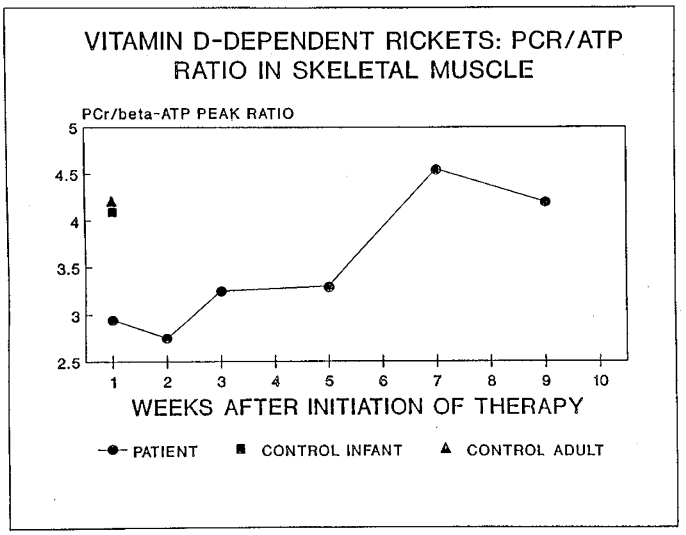

Fig. 3. Ratio of peak areas of phosphocreatine to β-NTP in gastrocnemius of patient R.C. (●) as a function of time after initiation of therapy. Control infant (age 6 months) and adult forearm peak ratios are indicated as ■ and ▲, respectively.

later studies of patient R.C., the normal adult, and the normal infant (linewidths range: 67–48 Hz). This suggests that the magnetic field homogeneity was not significantly different than in other studies. We also ruled out patient movement as a likely explanation in view of the similarity of the linewidth (59 Hz) of the α-NTP peak compared to later studies on this patient and the controls (69–31 Hz). At present, we do not have an explanation for this observation; in any event our conclusions are not dependent on this single time point. By 7 wk posttherapy, the P-31 MRS spectrum showed no effective differences from control infant muscle, and subsequent spectra thereafter showed no further significant changes over the succeeding 7 wk.

In contrast, P-31 MRS observations made by us (Mize CE, Nunnally R, unpublished data) and by Smith *et al.* (13) of muscle of patients with vitamin D-resistant rickets who do not manifest hypotonia do not differ from those of normal muscle. While the vitamin D-resistant rickets studies differ in that they were performed on teenage or adult forearm flexor digitorum, not gastrocnemius muscle, these differences do not address the underlying mechanism that would explain the clinical hypotonia in acquired deficiency and vitamin D-dependent rickets, in contrast to vitamin D-resistant rickets (X-linked hypophosphatemia), the explanation for which is presently unknown.

The significance of the muscle pH in our patient is that it is in the normal range for normal muscle as measured here and reported previously (12). Previous studies of adult muscle have shown that pH and the ratio PCr/β-ATP decrease during intense exercise; the ratio is low for certain muscle pathologies compared to normal resting muscle (11, 14). The decrease in this ratio is thought primarily to reflect low muscle PCr levels, in an effort to maintain ATP at normal levels.

On the basis of these preliminary results, we hypothesize that the PCr/β-ATP ratio may serve as an indicator for relative cellular deficits in high energy phosphorylated metabolites in skeletal muscle responsible for the functional hypotonia in this infant. One confounding variable in proving this hypothesis will be to account for age-related changes in phosphorylated metabolites. Recently it was demonstrated that both PCr/Pi and PCr/β-ATP ratios increase substantially in normally developing mouse skeletal muscle, changing from values of 2 and 1.5, respectively, at 7 days of age, to 7 and 3 by 1 months of age (15). In the present study, both R.C. and the normal infant had much lower PCr/Pi and PCr/β-ATP ratios, compared to the adult (see "Results" and Ref. 9). It is clear that P-31 MRS results obtained from infant patients such as R.C. need to be interpreted against baseline control data defining normal development.

The metabolite recovery during early low vitamin D dosage treatment suggests a priority-based selectivity of retention in intracellular phosphorus pools of skeletal muscle. The current data are not sufficiently sensitive to provide information about the mechanism of this effect, but open a technology base to investigate differing clinical expressions of hypotonic disorders related to muscular bioenergetics.

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