

³¹P-nuclear magnetic resonance studies of bioenergetic changes in skeletal muscle in malnourished human adults¹⁻³

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ABSTRACT In previous studies, both animals and malnourished children receiving 25% of the protein-energy intake of a control group, resulting in a 25% weight loss, had lower ratios of phosphocreatine to β -ATP and of phosphocreatine to inorganic phosphorus, higher free ADP concentrations, and lower free energy of ATP hydrolysis than the control group. Therefore, the effect of malnutrition on muscle energetics in adult humans was examined by using ³¹P-nuclear magnetic resonance spectroscopy in malnourished patients with a mean body mass index (BMI; in kg/m²) of 16.4 compared with healthy control subjects with a significantly higher body mass index of 24.5 ($P < 0.005$). The mean (\pm SEM) ratio of phosphocreatine (PCr) to ATP in the malnourished patients was 2.28 ± 0.27 , which was significantly lower than the ratio of 3.1 ± 0.15 in control subjects ($P < 0.02$). The ratio of inorganic phosphorus (Pi) to ATP in malnourished patients was 0.33 ± 0.04 , which was significantly lower than the ratio of 0.48 ± 0.03 in control subjects ($P < 0.02$), but the ratio of PCr to Pi was not significantly different from that in control subjects. There was a significant correlation between BMI and the ratio of PCr to ATP ($P < 0.01$) and of Pi to ATP ($P < 0.01$). These data suggest that progressive loss of BMI is associated with a relative loss of muscle creatine and phosphorus in relation to ATP. The findings were unlikely to have been due only to atrophy of fast-twitch fibers because such atrophy would have altered the ratio of PCr to Pi. *Am J Clin Nutr* 1998;67:39-43.

KEY WORDS Nuclear magnetic resonance spectroscopy, malnutrition, bioenergetics, ATP, phosphocreatine, inorganic phosphorus, skeletal muscle, adults, humans

INTRODUCTION

Previous studies have shown that hypoenergetically fed growing rats receiving 25% of the protein-energy intake of control animals, resulting in a 25% weight loss, had lower phosphocreatine (PCr) concentrations, a lower ratio of PCr to ATP, lower free energy of ATP hydrolysis (ΔG_{ATP}), but higher free ADP concentrations in the gastrocnemius muscle (1-5) than controls. Stimulation studies showed that rephosphorylation of creatine was slower in the muscles of hypoenergetically fed rats than in those of controls (3). In malnourished children, Gupta et al (5) also found lower PCr-ATP ratios but higher free ADP concentrations than in controls. However, it is not clear whether similar changes occur in malnourished adult humans in whom malnutrition is due

solely to reduced intake, malabsorption, or both, without the effects of a superimposed critical illness. We therefore used nuclear magnetic resonance (NMR) spectroscopy to observe skeletal muscle in malnourished patients and normal control subjects to test the hypothesis that, in adult humans, we would observe changes similar to those observed in animals.

SUBJECTS AND METHODS

Patients and control subjects

Patients were individuals referred by their physicians because of clinical malnutrition. On the basis of a diet history and clinical and biochemical investigations, these patients were judged to have insufficient energy intakes, to be malabsorbing energy, or both; they also had considerable weight loss. In addition, subjective global assessment found that they were all severely malnourished (category C). Three patients were hypoalbuminemic and anemic (patients 1, 2, and 7) and two had lymphocyte counts $< 1.2 \times 10^9/L$ (patients 2 and 8), which indicate moderate malnutrition (6). The characteristics of the seven malnourished patients (aged 25-81 y; two men and five women) are shown in **Table 1**.

These patients were compared with 15 normal control subjects (aged 23-62 y; seven men and eight women) who were volunteers recruited from the staff and student body of the University of Toronto and who were healthy, taking no medications, and not known to be receiving treatment of or to have any disease. The age range of the volunteers included the ages of most of the patients studied. Both groups were studied by using ³¹P-NMR spectroscopic assessment of the right lateral gastrocnemius muscle, according to the protocol described below. The protocol was approved by the University of Toronto Committee for Human Research and informed consent was obtained.

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TABLE 1

Clinical characteristics of malnourished patients

Subject	Age	Sex	BMI (kg/m ²)	Diagnosis	Medications	Hemoglobin g/L	Lymphocyte count ×10 ⁹ /L	Albumin g/L
1	42	M	10.4	HIV stage IV	3TC, gancyclovir, trimethoprim/sulfamethoxazol-fluoxetine, sodium bicarbonate, clarythromycin, ethambutol, ciprofloxacin, clofazimine, fluconazole	102	1.6	32
2	50	M	15.0	HIV stage IV	Diphenoxylate/atropine, ciprofloxacin, fluconazole, codeine phosphate, acyclovir, trimethoprim/sulfamethoxazole	97	0.3	31
3	25	F	17.7	Anorexia nervosa	None	125	1.82	38
4	35	F	18.4	Anorexia nervosa	Pancreatic enzyme supplementation	140	1.70	37
5	44	F	19.9	Gastroparesis	Ondansetron, docusate sodium, psyllium hydrophilic mucilloid	139	1.71	42
6	77	F	22.2	Self-neglect	Cisapride, ranitidine, timolol eye drops, hypertonic saline eye drops	133	2.24	37
7	81	F	14.1	Gastrectomy	Sotolol, lorazepam, gastrolyte, oral magnesium supplementation, oral citrate supplementation	115	0.912	28

NMR protocol

Spectra were acquired on a 1.5-T Sigma whole-body scanner (General Electric Medical Systems, Waukesha, WI) by using a 20–12.5-cm transmit-receive surface coil tuned to 25.85 MHz and positioned over the lateral head of the right gastrocnemius muscle. Field homogeneity in the sensitive region of the coil was achieved by means of iterative gradient shim adjustments. The pulse sequence used consisted of a single hard pulse with a repetition time of 2 s, a spectral width of 4000 Hz, 2048 data points, and 256 scans. Spectral processing was performed with zero filling to 4096 points, exponential apodization (1 Hz), fast Fourier transformation (7) and phasing, followed by baseline correction by using a polynomial spline function with which to remove the underlying broad resonance originating from bone. All peaks were then identified and fitted by using a Marquardt-Levenberg line-fitting routine (8) from which peak amplitudes, areas, chemical shifts, and widths at half maximum were obtained. Precision and reproducibility were tested on phantoms of known concentrations of inorganic phosphorus (Pi), PCr, and ATP. Spectra were analyzed on a SPARC 20 workstation (Sun Microsystems Inc, Mountain View, CA) by using the computer program SA/GE (SPECTROSCOPY APPLICATIONS GENERAL ELECTRIC (General Electric Medical Systems)).

Correction for partial saturation and pH calculation

The spectra were corrected for partial saturation by taking the ratio of spectra obtained with a repetition time of 2 s to those of fully relaxed spectra in two control and two malnourished individuals and applying them to the data obtained. The correction was not different between control subjects and malnourished patients for two reasons. First, there were no gross differences between the correction factors for both groups. Second, the correction for partial saturation done either by applying the data from control subjects to control subjects and the data from malnourished patients to mal-

nourished patients or the other way around did not influence the significance of the results. Hence, we used a mean value of the four measurements to correct for partial saturation.

pH was calculated indirectly by using the following formula, which was validated previously by using test solutions (1):

$$\text{pH} = 6.75 + \log_{10} \left\{ \frac{[(\text{PCr} - \text{Pi}) - 3.27]}{[5.672 - (\text{PCr} - \text{Pi})]} \right\} \quad (1)$$

Metabolite ratios and estimation of PCr, Pi, and free creatine

To determine metabolite ratios, areas under the PCr, Pi, and ATP peaks were integrated by using a computer program. The areas were corrected for incomplete relaxation as described above and the PCr-ATP, Pi-ATP, and Pi-PCr ratios were calculated from the respective areas.

Because we showed previously that hypoenergetic feeding resulting in a 25% weight loss did not reduce ATP concentrations (1), we calculated PCr and Pi from the ratios reported here by using a previously published value for muscle ATP of 8.2 mmol intracellular water/L (9). PCr and Pi values were calculated from the ratios of PCr to ATP and of Pi to ATP by using the following equations:

$$\text{PCr (mmol/L)} = (\text{PCr:ATP}) \times 8.2 \quad (2)$$

$$\text{Pi (mmol/L)} = (\text{Pi:ATP}) \times 8.2 \quad (3)$$

Free creatine (FCr) was calculated in two ways. On the assumption that 1) PCr + FCr = 42.5 mmol intracellular water/L (8) in both control subjects and malnourished patients, and that 2) PCr + FCr was reduced by 15%, to 36.1 mmol/L in malnourished patients. This assumption was made because in surgical patients, Smyreng et al (10) showed that PCr + FCr was 15% lower in those who were severely malnourished.

Free magnesium

Because the main effect of free Mg^{2+} concentrations is the alteration of the equilibrium constant of the creatine kinase reaction (K_{CK}) and also because free Mg^{2+} concentrations have been reported to vary [0.2–0.4 mmol/L (11, 12), 1 mmol/L (13), and 2.5 mmol/L (14)], we used a range of values to calculate free ADP concentrations in two previous studies in animals (1, 3). Although concentrations of free Mg^{2+} altered the K_{CK} , the relative differences between control rats and hypoenergetically fed rats remained constant (1, 3) because Mg^{2+} was not significantly different between the two groups of rats. Gupta et al (15, 16) showed that the relative chemical shift between the $\alpha\beta$ and $\gamma\beta$ resonances of ATP can be used to estimate free Mg^{2+} in muscle. Because the relative chemical shifts between $\alpha\beta$ and $\gamma\beta$ resonances of ATP were not significantly different between the control subjects and malnourished patients in this study (data not shown), the free Mg^{2+} content in muscle was not different between the two groups. These data were consistent with our previous observations in rats (1). Therefore, free ADP was calculated by using the value 1.0 mmol Mg^{2+} /L.

Calculation of free ADP

Free ADP concentrations were calculated by using the following equation:

$$[ATP] = [ATP][Cr]/[PCr][H^+]K_{CK} \quad (4)$$

Concentrations of ATP, PCr, and H^+ were determined by using NMR spectroscopy. The constant K_{CK} was calculated by using the equation of Lawson and Veech (13). In this equation K_{CK} depends on pH and free Mg^{2+} . For the reasons given above, the concentration of Mg^{2+} used was 1.0 mmol/L. Because the pH was not significantly different between control and malnourished patients, the average observed pH for the group derived from the ^{31}P -NMR spectra was used to calculate K_{CK} . K_{CK} and FCr values derived on the basis of the assumptions given earlier were used to calculate free ADP.

Calculation of ΔG_{ATP}

ΔG_{ATP} was calculated as follows:

$$\Delta G_{ATP} = \Delta G^0_{ATP} + \ln 9[FCr][Pi]/([PCr][H^+])K_{CK} \quad (5)$$

FCr was calculated by assuming the two different values of PCr plus FCr referred to earlier. The ΔG^0_{ATP} value depends on Mg^{2+} and pH and was calculated from Alberthy's tables as described previously (1).

Statistical methods

Data are reported as means \pm SEMs. Differences between control subjects and malnourished patients were tested with unpaired t tests (17). The relations of BMI to PCr:ATP, Pi:ATP, and Pi:PCr were analyzed by linear regression (17). All hypotheses testing was two-sided; the level of significance was set at $P \leq 0.05$.

RESULTS

Patients and control subjects

The patients had a variety of diseases associated with malnutrition, which patient histories indicated had been present for at least

6 mo (Table 1). Three patients (patients 1, 2, and 7) had malabsorption and chronic diarrhea. Four patients (patients 3–6) were not eating sufficient food as determined by diet history and observations in the hospital. There was no evidence of neuromuscular disease and none of the patients or control subjects had sustained any major orthopedic injuries to their lower limbs. The mean BMI was 16.4 in the patients and 24.5 in the control subjects.

Metabolite ratios

Mean PCr-ATP and Pi-ATP ratios were significantly lower in patients than in control subjects (Table 2). Linear regression showed a significant positive correlation ($P < 0.05$) between these ratios and BMI (Figures 1 and 2). In contrast, there was no significant correlation between BMI and Pi:PCr (Figure 3). There was also no significant difference in pH between the two groups.

The mean FCr calculated under the assumption that the sum of PCr and FCr was identical in control subjects and in malnourished patients (42.5 mmol intracellular water/L) was significantly higher ($P < 0.02$) in malnourished patients (24.2 ± 2.2 mmol/L) than in control subjects (17.6 ± 1.2 mmol/L). In contrast, when the sum of PCr and FCr was assumed to be reduced to 36.1 mmol/L in the malnourished patients, FCr was not significantly different between control subjects (17.6 ± 1.2 mmol/L) and malnourished patients (17.8 ± 2.2 mmol/L).

When PCr + FCr was assumed to be identical in control subjects and malnourished patients (42.5 mmol/L), calculated free ADP concentrations were numerically but not significantly higher in malnourished patients (9.2 ± 4.4 μ mol/L) than in control subjects (5.6 ± 0.6 μ mol/L). The mean difference in free ADP concentrations between the two groups was only 1.2 μ mol/L when PCr + FCr was assumed to be reduced to 36.1 mmol/L in the malnourished patients. ΔG_{ATP} was not significantly different between control subjects and malnourished patients, irrespective of the assumed PCr + FCr value.

DISCUSSION

Growing rats fed a hypoenergetic diet providing 25% of the protein-energy intake of controls and studied after a 25% loss in body weight had lower PCr-ATP and Pi-PCr ratios, higher calculated free ADP concentrations, and lower (G_{ATP} in the gastrocnemius muscle (1–3) than controls. Similar findings were noted in malnourished children by Gupta et al (5), who used ^{31}P -NMR spectroscopy. However, rats deprived of food for 2 d (1) had a lower PCr-ATP ratio than hypoenergetically fed animals but free ADP concentrations that were not significantly higher. In these animals, the reason for the lower PCr-ATP ratio was related to a

TABLE 2
Metabolite ratios in muscle

	Control subjects	Malnourished patients
Ratio		
PCr:ATP	3.11 ± 0.15^1	2.28 ± 0.27^2
Pi:ATP	0.48 ± 0.03	0.33 ± 0.04^2
Pi:PCr	6.67	7.39
pH	7.18 ± 0.01	7.14 ± 0.01

¹ $\bar{x} \pm$ SEM.

² Significantly different from control subjects, $P < 0.02$.

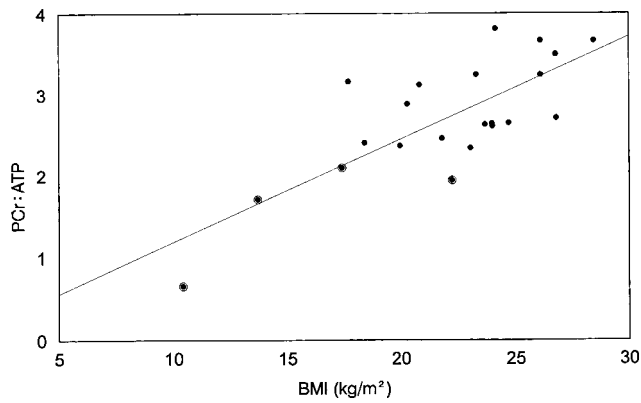


FIGURE 1. Positive correlation ($r = 0.76$) between the ratio of phosphocreatine (PCr) to ATP and BMI. The circled data points represent patients who were either the oldest or who had AIDS.

lower total creatine (PCr + FCr) content in muscle (1). In adults with a low BMI, findings were similar to those in rats deprived of food, with no significant differences in pH and Pi-PCr ratios but a significantly lower PCr-ATP ratio compared with control subjects. Although we did not measure muscle creatine directly in this study, a likely explanation for our findings is that in malnourished adults there is a fall in total creatine and phosphorus without a significant rise in free ADP. The finding that free ADP concentrations were not significantly different between control subjects and malnourished patients in our study supports this conclusion. Further support for a reduction in muscle creatine is the finding by Smyreng et al (10) of a reduction in total creatine in the muscles of malnourished surgical patients.

Our results potentially could have been confounded by the fact that we had elderly subjects and AIDS patients in this study who may have had altered muscle metabolism because of age or disease. However, it is unlikely that AIDS or age altered our results because the Pi-ATP, PCr-ATP, and Pi-PCr ratios in our two oldest patients and the two AIDS patients (Figures 1 and 2) were close to the regression line and are not the outliers. Hence, they had ratios related to the degree of wasting, a finding that is no different from observations in younger patients and patients without AIDS. These observations show that neither AIDS nor age influenced resting NMR substrate ratios when expressed in relation to BMI. The conclusions in relation to age are support-

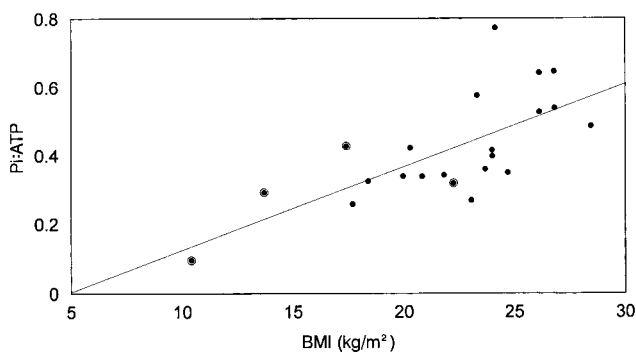


FIGURE 2. Positive correlation ($r = 0.70$) between the ratio of inorganic phosphorus (Pi) to ATP and BMI. The circled data points represent patients who were either the oldest or who had AIDS.

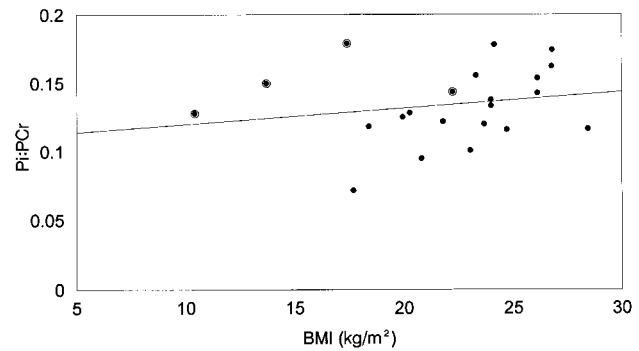



FIGURE 3. Absence of correlation ($r = 0.21$) between the ratio of inorganic phosphorus (Pi) to phosphocreatine (PCr) and BMI. The circled data points represent patients who were either the oldest or who have AIDS.

ed by other studies that showed that resting ratios were not influenced by age but that the response to exercise was reduced with aging (18, 19).

The reduction in the PCr-ATP ratio in malnourished patients could not have been due to acidosis or anoxia because there was no spectroscopic evidence of a change in muscle pH, and experimental malnutrition has not been associated with changes in blood pH, partial pressure of carbon dioxide, or HCO_3^- (1). Previously, malnutrition was shown to cause atrophy of fast-twitch fibers (20) but it is unlikely that our findings resulted from fast-twitch fiber atrophy because such atrophy is typically associated with a decrease in the Pi-PCr ratio, which was not observed in this study (21).

The data presented here are different from those obtained by Gupta et al (5) in malnourished children. Gupta et al's data are similar to the data from our previous work in hypoenergetically fed rats, possibly because of one important fact: the studies in rats and children were both conducted in growing organisms whereas the data in this study were obtained in adult individuals.

The finding of a reduced PCr-ATP ratio indicating a reduction in PCr and perhaps in total creatine in muscle likely indicates functional consequences. In other studies, increasing muscle creatine was shown to enhance performance (22); therefore, it is possible that creatine depletion may impair performance. However, to confirm a reduction in muscle creatine, it will be necessary to measure it directly by using $^1\text{H-NMR}$ together with $^{31}\text{P-NMR}$ and to determine whether creatine supplementation or overall nutritional rehabilitation will improve muscle function as well as other nutritional and biochemical outcome variables, such as immune function, plasma protein status, and cytokine metabolism. 

REFERENCES

1. Pichard C, Vaughan C, Struk R, Armstrong RL, Jeejeebhoy KN. The effect of dietary manipulations (fasting, hypocaloric feeding and subsequent refeeding) on rat muscle energetics as assessed by nuclear magnetic resonance spectroscopy. *J Clin Invest* 1988; 82:895-901.
2. Argov Z, Chance B. Phosphorus magnetic resonance spectroscopy in nutritional research. *Annu Rev Nutr* 1991;11:449-64.
3. Mijan de la Torre A, Madapallimattam A, Cross A, Armstrong RL, Jeejeebhoy KN. Effect of fasting, hypocaloric feeding, and refeed-



- ing on the energetics of stimulated rat muscle as assessed by nuclear magnetic resonance spectroscopy. *J Clin Invest* 1993;92:114–21.
4. Mizobata Y, Rounds JD, Prechek D, DeRosa E, Wilmore DW, Jacobs DO. ^{31}P magnetic resonance spectroscopy demonstrates expansion of the extracellular space in the skeletal muscle of starved rats. *J Surg Res* 1994;56:491–9.
 5. Gupta RK, Mittal RD, Agarwal KN, Agarwal DK. Muscular sufficiency, serum protein, enzymes and bioenergetic studies (31-phosphorus magnetic resonance spectroscopy) in chronic malnutrition. *Acta Paediatr* 1994;83:327–31.
 6. Blackburn GL, Bell SJ, Mullen JL. *Nutritional medicine*. Philadelphia: WB Saunders, 1989.
 7. Arfken GB, ed. *Mathematical methods for physicists*. 3rd ed. Orlando, FL: Academic Press, 1985.
 8. Marquardt DW. An algorithm for least squares estimation of non-linear parameters. *J Soc Ind Appl Math* 1962;11:431–41.
 9. Harris RC, Hultman E, Nordesjo L-O. Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. *Methods and variance of values*. *Scand J Clin Lab Invest* 1974;33:109–20.
 10. Smyreng T, Larsson J, Schildt B, Wetterfors J. Nutritional assessment reflects muscle energy metabolism in gastric carcinoma. *Ann Surg* 1983;198:146–50.
 11. Maughan D. Diffusible magnesium in frog skeletal muscle cells. *Biophys J* 1983;43:75–80.
 12. Gupta RK, Gupta P, Yushok WD, Rose ZB. Measurement of the dissociation constant of MgATP at physiological nucleotide levels by a combination of ^{31}P NMR and optical absorbance spectroscopy. *Biochem Biophys Res Commun* 1983;117:210–6.
 13. Lawson JWR, Veech RL. Effects of pH and free Mg^{2+} on the K_{eq} of the creatine kinase reaction and other phosphate hydrolases and phosphate transfer reactions. *J Biol Chem* 1979;254:6528–37.
 14. Wu ST, Pieper GM, Salhany JM, Eliot RS. Measurement of free magnesium in perfused ischemic arrested heart muscle. A quantitative phosphorus-31 nuclear magnetic resonance and multiequilibria analysis. *Biochemistry* 1981;20:7399–403.
 15. Gupta RK, Benivic JL, Rose ZB. The determination of the free magnesium level in the human red blood cell by ^{31}P NMR. *J Biol Chem* 1978;253:6172–6.
 16. Gupta RK, Moore RD. ^{31}P NMR studies in intracellular free Mg^{2+} in intact frog skeletal muscle. *J Biol Chem* 1980;255:3987–93.
 17. Sokal RR, Rohlf FJ. *Biometry: the principles and practice of statistics in biological research*. San Francisco: Freeman, 1981.
 18. Zanonato S, Buchthal S, Barstow TJ, Cooper DM. ^{31}P -magnetic resonance spectroscopy of leg muscle metabolism during exercise in children and adults. *J Appl Physiol* 1993;74:2214–8.
 19. Coggan AR, Abduljalil AM, Swanson SC, et al. Muscle metabolism during exercise in young and older untrained and endurance-trained men. *J Appl Physiol* 1993;75:2125–33.
 20. Goldspink G, Ward PS. Changes in rodent muscle fibre types during postnatal growth, undernutrition and exercise. *J Physiol* 1979;296:453–69.
 21. Kushmerick MJ, Moerland TS, Wiseman RW. Mammalian skeletal muscle fibers distinguished by contents of phosphocreatine, ATP, and Pi. *Proc Natl Acad Sci U S A* 1992;89:7521–5.
 22. Balsom PD, Soderlund K, Sjodin B, Ekblom B. Skeletal muscle metabolism during short duration high-intensity exercise: influence of creatine supplementation. *Acta Physiol Scand* 1995;154:303–10.

