Functional and metabolic early changes in calf muscle occurring during nutritional repletion in malnourished elderly patients[1–3]

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ABSTRACT

Background: Metabolic alterations in skeletal muscle associated with malnutrition and the potential reversibility of such alterations during refeeding are not fully understood.

Objective: We characterized early changes in muscle during refeeding in malnourished, hospitalized elderly subjects.

Design: Muscle function, metabolism, and mass were evaluated in 24 clinically stable patients (11 were malnourished) by using isokinetic plantar flexor torque measurements and nuclear magnetic resonance (NMR) imaging for medial gastrocnemius mass assessment and ^{31}P and ^{13}C NMR spectroscopy for inorganic phosphate (Pi), phosphocreatine, and glycogen quantitation.

Results: Malnourished subjects had lower muscle mass ($P < 0.02$) and tended to have lower strength than did control subjects. In malnourished subjects, muscle strength increased after refeeding ($P < 0.01$) whereas muscle mass was unchanged. The ratio of Pi to ATP was lower in malnourished than in control subjects ($P < 0.001$) and increased during refeeding ($P < 0.01$). The mean ratio of phosphocreatine to ATP was lower in malnourished than in control subjects ($P < 0.01$) and increased to control values after refeeding. Muscle glycogen showed a scattered distribution for malnourished subjects; the mean value did not differ significantly from that of control subjects, either at baseline or after refeeding.

Conclusions: The lower ratio of phosphocreatine to ATP in malnourished subjects could have resulted from either lower total muscle creatine or reduced oxidative capacities. High or normal glycogen associated with a low Pi-to-ATP ratio in malnourished subjects suggested preferential use of lipid over carbohydrate for energy supply, which is known to reduce muscle performance. The data suggest that normalization of muscle metabolite content after refeeding improves muscle strength in malnourished subjects. *Am J Clin Nutr* 2001;73:832–8.

KEY WORDS Nuclear magnetic resonance, phosphocreatine, inorganic phosphate, glycogen, isokinetic peak torque, muscle function, muscle mass, muscle metabolism, elderly, hospital patient, malnourished elderly, malnutrition, refeeding, skeletal muscle, protein-energy malnutrition

INTRODUCTION

Protein-energy malnutrition (PEM) is a common problem in elderly patients in acute-care settings (1). Critical illnesses and hospitalization result in catabolic stress, anorexia, and immobilization, which worsen the nutritional status of elderly persons (2). Disuse of muscles because of bed rest or chair rest leads to a loss of muscle mass of ≤1.5%/d in adults (2). In critically ill elderly patients, anorexia and functional dependency resulting in decreased energy and protein intakes (3) could further contribute to decreased muscle mass, as was observed during voluntary food restriction (4). Conversely, adequate muscle mass appears to be the best predictor of good prognosis in critical illness (5). Thus, the reduction of muscle mass during aging (6) could be partially responsible for the frailty of the elderly during critical illnesses.

Muscle function is also impaired in PEM, as was shown by decreased muscle strength (4, 7). Aging itself reduces muscle strength; losses of 0.9–2.4%/y were found between 70 and 90 y, with variations attributed to physical activity, morbidity, and nutritional status (8). Muscle strength is a predictor of survival in the elderly (9). Thus, the potential reversibility of the reductions in muscle mass and function during PEM is of great interest.

In critical illnesses, providing early nutritional support (oral, enteral, or parenteral) does not seem to be efficient at decreasing the likelihood of adverse outcomes or preserving nutritional status in adults or the elderly (3, 10–12). Providing nutritional support during the period of clinical stability could be more beneficial because decreases in the inflammatory process and anorexia (3) typically allow for better use of nutritional supplements, which should result in improved nutritional status (10).

TABLE 1
Characteristics of the study subjects

	Malnourished subjects (n = 4 M, 7 F)	Control subjects (n = 4 M, 9 F)
Age (y)	87.1 ± 5.7[1]	83.4 ± 6.1
BMI (kg/m^2)	17.63 ± 2.83	23.5 ± 2.4
Energy intake (kJ·kg^{-1}·d^{-1})[2]	63.03 ± 20.65	106.88 ± 22.82
Protein intake (g·kg^{-1}·d^{-1})[2]	0.56 ± 0.22	1.15 ± 0.29
Primary diagnosis		
Depression	3	4
Infectious disease	4	3
Chronic cachexia	4	0
Cardiac arrhythmia	0	3
Femoral neck fissure	0	1
Hypertensive crisis	0	1
Stroke with left hemiparesia	0	1

[1] $\overline{x}$ ± SD.

[2] During acute phase of illness.

For patients subjected to a semistarvation period, the low stores of fat and lean body mass increase the drive to eat and lead to hyperphagia until the initial individual body composition is regained (13). However, refeeding malnourished patients seems to improve their muscle function first, whereas their muscle mass is nearly unchanged (14, 15). It was suggested that improvement of muscle function depends on recovery of energy-producing substrates such as glycogen (15).

^{31}P and ^{13}C nuclear magnetic resonance (NMR) spectroscopy allows the repeated noninvasive quantification of metabolic compounds in vivo. We showed that frail elderly people can tolerate this procedure (16). By using ^{31}P NMR, Thompson et al (17) showed decreases in both inorganic phosphate (Pi) and phosphocreatine in muscle from 8 malnourished individuals. Fasting for 2 d induces a decrease in Pi in humans (18), whereas low-energy feedings for 21 d in rats were followed by decreases in muscle glycogen and phosphocreatine and reductions in mitochondrial respiratory activity (19).

In the present study, we used ^{31}P NMR and ^{13}C NMR to measure the early changes in muscle mass and function in elderly subjects recovering from malnutrition, with special attention to changes in muscle metabolism. After 1 mo of refeeding in malnourished elderly subjects, the changes in muscle contents of phosphocreatine, Pi, and glycogen and in the intracellular pH were compared with changes in muscle mass and muscle strength.

SUBJECTS AND METHODS

Subjects

Elderly malnourished and control patients were recruited during their hospital stays in an acute-care geriatric medicine unit. The patients were recruited when they were clinically stable and able to walk with human help in their rooms. Clinical stability was defined as normalization of vital signs (heart rate, systolic blood pressure, respiratory rate, temperature, oxygenation status, and hydration), return of the ability to eat, and recovery of mental status (20). The inclusion criteria were as follows: not functionally dependent for activities of daily living before hospitalization; not suffering from dementia, pressure ulcers, bilateral neurologic disease, or diseases known to impair muscle function and metabolism, including chronic respiratory impairment (21),

congestive heart failure (22), peripheral arterial disease (23), Parkinson disease (24), and diabetes (25); and no contraindication for NMR investigation. During the study, none of the subjects took any medications known to impair muscle function. The subjects were all hospitalized, allowing us to compare patients with similar mobility restrictions during the first days of hospitalization. Informed, written consent was obtained from all subjects after we explained the nature, purpose, and potential risks and benefits of the study. The study protocol was approved by the ethical committee of the Victor Segalen Bordeaux 2 University.

The subjects were divided into 2 groups according to their nutritional status at study entry. Those who met at least one of the following criteria were classified as malnourished: body mass index (BMI; in kg/m^2) <18, recent weight loss of ≥4% of body weight, and daily energy intake during the acute phase of the disease <83.6 kJ·kg^{-1}·d^{-1} (20 kcal·kg^{-1}·d^{-1}). Dietary intake data were analyzed retrospectively by using routine monitoring records for the patients as available in this unit (1). Patients who did not meet any of these 3 criteria for malnutrition were classified as control subjects. Twenty-six subjects were included; there were 13 in the malnourished group and 13 in the control group. Two patients in the malnourished group were subsequently excluded after ^{1}H NMR imaging of the calf because their subcutaneous adipose tissue was very thick (16.2 and 17.2 mm, respectively) and was associated with small muscle volume (30 and 25 cm^3, respectively). This resulted in unreliable ^{31}P and ^{13}C NMR muscle measurements, because the signal-to-noise ratio was too low. For the 24 remaining subjects, data on age, BMI, primary diagnosis, and energy and protein intakes during the acute phase of illness are shown in **Table 1**. Four patients were diagnosed with chronic cachexia when no other specific diagnoses were found. After careful evaluations, it was determined that these 4 subjects did not have cancer. PEM was attributed to social problems in 2 of these subjects and to voluntary food restriction in the other 2.

Study design

At the baseline visit (T0), each subject had a nutritional examination, a measurement of muscle strength made with an isokinetic dynamometer, and an NMR investigation. These tests were completed within a 3-d period. Only malnourished patients had a second visit (T1), during which the same tests were done; this occurred 1 mo after T0. Malnourished subjects were encouraged to recover their appetite and received a 200-mL oral supplement containing 15 g protein and 836 kJ twice daily (Nestle Clinical Nutrition, Sevres, France). The malnourished patients also underwent 20 physiotherapy sessions of 15 min each to reinforce the calf, thigh, and hip muscle groups.

Nutritional examination

BMI was determined for all subjects. After the subjects had fasted overnight, blood samples were drawn between 0800 and 0900 from the cubital vein into evacuated tubes (Becton-Dickinson, Meylan, France). We measured biochemical indexes, including the serum concentrations of visceral proteins such as albumin (normal range: 35–45 g/L) and thyroxine-binding prealbumin (normal range: 1.45–5.8 μmol/L) and acute-phase proteins such as C-reactive protein (normal range: <5 mg/L). The concentrations of these proteins were determined with latex immunonephelometry (BNA; Behring, Rueil-Malmaison, France). A 3-d dietary record was kept prospectively and was analyzed as described previously (3).

Muscle strength measurement

Isokinetic torque was measured with a Cybex Norm dynamometer (Cybex, Division of Lumex Inc, New York). Plantar flexor torque of the right calf (or of the healthy side) was measured with the subjects seated with the knee extended, the lower limb horizontal, and the hip flexed to 45°. The straight knee allowed the gastrocnemius to be stretched throughout plantar flexion (26). The upper body, the pelvis, and the right thigh were secured with clinging straps. The subjects performed 5 repetitions preceded by 3 training exercises for each series. The series were performed twice at an angular velocity of 30°/s and once at 60°/s at the ankle, with a 2-min rest between each pair of series. The highest peak torque obtained during the session was considered the maximal plantar flexor peak torque.

NMR imaging and spectroscopy

A Bruker Biospec 47/50 (Bruker, Wissembourg, France) equipped with a 50-cm bore superconducting magnet operating at 4.7 T was used for imaging and spectroscopy. Studies were begun at 0900 after the patients consumed a breakfast supplying 1045 kJ.

Medial gastrocnemius muscle and subcutaneous tissue volumes

A birdcage resonator tuned at 200.3 MHz was positioned around the subject's right calf. Magnetic resonance imaging was carried out by using a standard spin-echo sequence (18 slices with a slice thickness of 10 mm and no interslice space). Cross-sectional areas of the medial gastrocnemius muscle and subcutaneous tissues were measured in each slice. Volumes were calculated by assuming that the imaged segments were cylindrical in shape. For all subjects, the calculations included 11 slices chosen because they involved as much medial gastrocnemius muscle as possible. In malnourished subjects, bone areas were calculated for comparison with values to be obtained during the second session.

^{31}P NMR

A 50-mm-diameter, triple-tuned, transmit-receive coil (Bruker) was placed on the medial gastrocnemius muscle and was tuned to the ^1H (200.3 MHz) and ^{31}P (81.1 MHz) frequencies. Proton shimming was achieved by using the water proton resonance, with typical widths at half heights of 30–50 Hz. ^{31}P spectra (8 scans, 160 s) were acquired with a 100-μs excitation pulse (90° flip angle in the middle of the coil). Under such conditions, ^{31}P spectra were fully relaxed. The NMR spectra were then routinely deconvolved into Lorentzian lines. The phosphocreatine, Pi, and β-ATP peak areas were calculated and were converted to concentrations with [ATP] = 8.2 mmol/L (27). Intracellular pH was determined by using the chemical shift of Pi relative to phosphocreatine (27).

^{13}C NMR

The same coil was then tuned to the ^{13}C frequency (50.3 MHz). ^1H-decoupled natural abundance ^{13}C NMR spectra were obtained by using a flip angle of 180° at the center of the coil. A total of 4500 scans (75-ms repetition time) were averaged and the raw data were filtered with a 40-Hz Lorentzian line broadening before the Fourier transformation. Proton decoupling was obtained with a WALTZ-16 sequence (15-ms duration, 9 W) (28). Molar concentrations of muscle glycogen were estimated by comparing the signal-to-noise ratio of the C-1 glycogen area of the subjects to the ratio obtained by using a glycogen–potassium chloride (150 mmol/L:50 mmol/L) solution phantom (29). This phantom was built in the shape of a calf. The phantom-to-coil distance was properly calibrated with respect to the subcutaneous fat thickness of each subject, as assessed from magnetic resonance imaging.

Statistical analysis

The statistical analysis was performed with STATWORKS, version 1.2 (Cricket Software Inc, Philadelphia). Multiple regression analyses adjusted for sex and age were used to compare the malnourished and control groups in terms of baseline clinical characteristics, NMR data, and muscle strength at T0. The same method was used to compare NMR data in malnourished subjects after refeeding (T1) with data from controls at T0. We calculated Pearson's product-moment correlation coefficients between muscle strength measurements and NMR data at T0. Only the statistically significant results, defined as $P < 0.05$, are described. We used paired Student's t tests to analyze longitudinal changes (between T0 and T1) in clinical variables, glycogen content, the ratio of phosphocreatine to ATP, the ratio of Pi to ATP, and pH in malnourished patients.

RESULTS

Comparisons between malnourished and control subjects

As shown in **Table 2**, the malnourished group had a lower mean BMI and a lower mean serum albumin concentration than did the control group at T0. However, serum thyroxine-binding prealbumin and C-reactive protein concentrations and dietary intakes at T0 did not differ significantly between the 2 groups.

The medial gastrocnemius muscle volume was significantly lower in malnourished subjects than in control subjects. The subcutaneous tissue volume did not differ significantly between the groups, although values varied widely among the malnourished subjects.

During each exercise session, the maximal isokinetic plantar flexor torque was measured at an angular velocity of 30°/s. For 5 subjects, the average peak torque did not differ significantly between the first and second series at 30°/s, whereas the values obtained during the second series at 30°/s were higher than those in the first series for the other 19 subjects. The peak torque measured at an angular velocity of 60°/s was lower than that recorded at 30°/s for all the subjects. A trend of lower maximal peak torque in malnourished subjects than in control subjects was found (31.1 ± 12.8 compared with 45.9 ± 31.1 N·m, respectively; NS). Maximal plantar flexor torque was low in 2 control subjects (17 and 22 N·m, respectively).

The ^{31}P NMR spectra of a malnourished subject (subject 9) for T0 and T1 are shown in **Figure 1**, A and B, respectively, and the ^{13}C NMR spectrum of another malnourished subject at T0 (subject 3) is shown in **Figure 2**. The Pi:ATP and the phosphocreatine:ATP were significantly lower in malnourished subjects than in control subjects (Table 2), but no significant difference in intracellular pH was observed. No statistical comparison was made for muscle glycogen content because some values observed in malnourished subjects were either <40 mmol/L (20, 31.6, and 36.9 mmol/L) or >100 mmol/L (106.0 and 147.5 mmol/L). However, 3 control subjects had muscle glycogen content <40 mmol/L (33.1, 14.2, and 13.7 mmol/L).

The results were not modified significantly when we compared the subgroups of subjects with no infections (7 malnourished and 10 control subjects). For these subgroups, the

TABLE 2
Nutritional status, muscle strength, volumes of medial gastrocnemius and subcutaneous tissue of the calf, Pi:ATP, phosphocreatine:ATP, pH, and muscle glycogen content in control subjects at baseline (T0) and in malnourished subjects at T0 and after 1 mo (T1)[1]

	T0		T1	P for control compared with malnourished subjects at T0	P for malnourished subjects at T0 compared with T1
	Control subjects ($n = 13$)	Malnourished subjects ($n = 11$)	Malnourished subjects ($n = 9$)		
BMI (kg/m^2)	23.5 ± 2.40	17.63 ± 2.83	18.60 ± 2.71	<0.001	<0.01
Serum albumin (g/L)	35.48 ± 2.62	28.54 ± 2.47	31.59 ± 3.73	<0.001	<0.05
Thyroxine-binding prealbumin (μmol/L)	3.77 ± 0.73	2.76 ± 0.73	2.90 ± 0.73	NS	NS
Serum C-reactive protein (mg/L)	5.63 ± 7.07	11.91 ± 15.97	5.43 ± 3.06	NS	NS
Energy intake (kJ·kg^{-1}·d^{-1})	98.06 ± 9.45	124.48 ± 52.12	157.63 ± 34.07	NS	NS
Protein intake (g·kg^{-1}·d^{-1})	1.20 ± 0.35	1.22 ± 0.55	1.57 ± 0.42	NS	NS
Medial gastrocnemius volume (cm^3)	75.40 ± 0.50	61.05 ± 17.70	60.15 ± 18.85	<0.02	NS
Calf subcutaneous tissue volume (cm^3)	216.80 ± 16.10	135.64 ± 66.66	145.92 ± 62.21	NS	NS
Maximal plantar flexor isokinetic torque (N·m)	45.9 ± 31.1	31.1 ± 12.8	36.7 ± 14.4	NS	<0.01
Pi:ATP	0.81 ± 0.14	0.53 ± 0.17	0.90 ± 0.29	<0.001	<0.01
Phosphocreatine:ATP	4.75 ± 1.18	3.37 ± 0.89	4.32 ± 0.88	<0.01	NS
Intracellular pH	7.04 ± 0.03	7.06 ± 0.06	7.07 ± 0.05	NS	NS
Muscle glycogen (mmol/L)	57.01 ± 9.53	67.38 ± 35.77	67.12 ± 29.03	NS	Not valid

[1] $\bar{x}$ ± SD. Malnourished subjects were compared with control subjects by using multiple regression analyses adjusted for age and sex; data from malnourished subjects at T0 and T1 were compared by using paired Student's *t* tests.

mean maximal plantar flexor torque tended to be lower, but not significantly so, in the malnourished subjects than in the control subjects (29 ± 12.8 compared with 50.2 ± 25.1 N·m; NS). The Pi:ATP was lower in the 7 malnourished subjects than in the 10 control subjects (0.52 ± 0.20 compared with 0.83 ± 0.15, respectively; *P* < 0.001). For these subgroups, the phosphocreatine:ATP was also lower in the malnourished subjects than in the control subjects (3.33 ± 0.67 compared with 4.76 ± 1.04, respectively; *P* = 0.04). The muscle glycogen content distribution was scattered in the 7 malnourished subjects (65.5 ± 41.1 mmol/L), as it was in the 10 control subjects (54.22 ± 25.04 mmol/L).

In the entire control group (*n* = 13), there was no correlation between maximal plantar flexor torque and either the Pi:ATP, the phosphocreatine:ATP, or glycogen content. In the entire malnourished group (*n* = 11), the Pi:ATP was significantly correlated with the maximal plantar flexor torque (*r* = 0.61, *P* < 0.05). However, no correlation was found between this muscle strength measurement and the phosphocreatine:ATP or muscle glycogen. Glycogen content varied greatly in weak, malnourished subjects (those with maximal plantar flexor torque <20 N·m); it was low in subject 7 (20 mmol/L), normal in subject 6 (58.4 mmol/L), and high in subjects 1 and 8 (106.0 and 147.5 mmol/L, respectively).

Recovery of malnourished subjects between T0 and T1

Clinical characteristics

Two malnourished subjects completed the first visit only; subject 6 (a woman) developed pneumonia and subject 10 (a man) needed a cardiac pacemaker after T0. During the recovery period (T0 to T1), all subjects were compliant with the nutritional intervention as assessed by the high average intakes of energy and protein at T0 and T1. The 20 physiotherapy sessions were completed by all malnourished subjects except one (subject 11). Only one subject was not able to walk without help at T1 (subject 5).

Muscle and subcutaneous tissue volume and maximal plantar flexor torque

During the observation period, no significant changes in the volumes of medial gastrocnemius muscle or local subcutaneous tissues were noted (Table 2). Muscle strength increased significantly between T0 and T1 (*P* < 0.01). However, one-third of the subjects (the weakest at T0: subjects 1, 5, and 7) did not improve their performance.

Pi:ATP, phosphocreatine:ATP, and intracellular pH

Shown in Figure 1 are the typical changes in ^{31}P spectra seen in a malnourished subject between T0 (part A) and T1 (part B). The
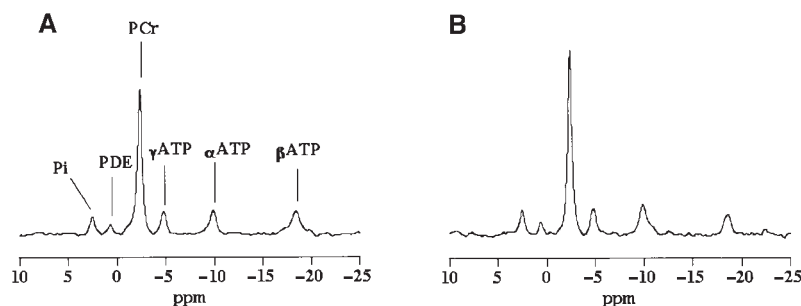
FIGURE 1. Typical ^{31}P nuclear magnetic resonance spectra of a malnourished subject recorded at the baseline visit (A) and 1 mo later (B). PCr, phosphocreatine; Pi, inorganic phosphate; PDE, phosphodiesters.
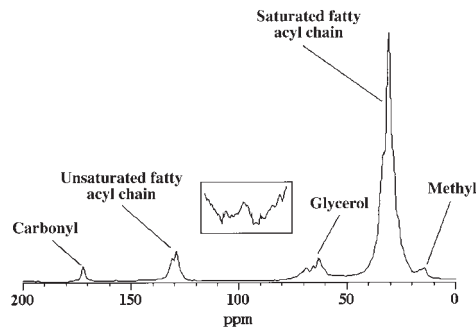
FIGURE 2. Typical ^{13}C nuclear magnetic resonance spectrum of a malnourished subject at the baseline visit. The C-1 glycogen resonance is shown with vertical expansion of the 80–120-ppm region.

Pi:ATP increased markedly in all subjects except one (subject 11; Table 2). The evolution of the phosphocreatine:ATP varied; in some subjects (1, 2, 4, 5, 7, and 9) it increased and in others (3, 8, and 11) it decreased (NS). No significant variations in pH were observed. At T1, ^{31}P NMR data of malnourished subjects no longer differed significantly from data of control subjects at T0.

Muscle glycogen content

The 2 subjects with high glycogen content at T0 had normal values at T1 (subjects 1 and 8). For the other subjects, the values were either unchanged (subjects 5 and 9), increased (subjects 2, 3, 7 and 11), or decreased (subject 4).

DISCUSSION

In this study, we measured changes in skeletal muscle function and metabolism occurring in elderly malnourished patients during the early phase of recovery. We used isokinetic muscle strength measurements and NMR spectroscopy and imaging for noninvasive functional and metabolic investigations and muscle mass assessment.

The main difficulty in this study was determining whether we could attribute specifically to malnutrition the abnormalities revealed by skeletal muscle exploration. The weakness of hospitalized patients may result from malnutrition, immobility, or various diseases. The subjects in this study were hospitalized, so they had all been restricted to their rooms or beds for at least several days. We did not include patients with chronic functional impairment or those with diseases known to affect skeletal muscle function (21–25). The control group was not representative of free-living elderly persons, but instead was experiencing living conditions similar to those of the malnourished group during the investigations. The severity of the underlying disease may have been milder in the control group than in the malnourished group. However, the proportions of subjects with infectious diseases and depression were similar in both groups. Chronic cachexia was diagnosed in malnourished subjects without dementia, chronic functional impairment, or cancer. Moreover, after exclusion of the subjects who had an infectious disease during the hospitalization, the results were not significantly modified. Thus, we considered the differences between the control and malnourished groups to be the result of malnutrition. Another rationale for attributing the alterations in skeletal muscle to malnutrition is their potential reversibility during nutritional repletion.

Muscle strength was assessed isokinetically in this study because this technique was shown to be reliable for measuring ankle plantar flexor peak torque (30). As described previously, the maximal plantar flexor peak torque was recorded by using a velocity of $30\,°/s$ (31). The mean strength of the control subjects was in the same range as that of subjects in a representative cohort of elderly subjects (31), but individual values varied considerably. Indeed, restricted mobility resulting from hospitalization (32) probably caused the marked weakness in 2 control subjects in this study.

The nutritional support provided to malnourished subjects was accompanied by muscle reinforcement therapy, which could itself increase muscle strength in the elderly (33). Muscle weakness induces activity restriction, as described in anorexia nervosa patients (15). During nutritional repletion, spontaneous recovery of activity without specific physiotherapy interventions can lead to an increase in muscle strength, as we observed in subject 11. Furthermore, in adult malnourished subjects who returned to normal nutritional status but not to their usual activity levels, muscle strength remained lower than in free-living control subjects (32). Fiatarone et al (34) showed that supplementation with multiple nutrients was not efficient at improving muscle strength in frail elderly subjects without concomitant exercise. Thus, in the present study, the increase in muscle strength during the 1-mo observation period could be a result of either the nutritional repletion, the physical activity, or, most likely, the combination of both.

Despite the increase in muscle strength observed during this period, no significant change in medial gastrocnemius volume was found. Indeed, the 4% increase in mean BMI was much lower than the mean increase in strength of ≈25% in malnourished subjects after 1 mo. This confirms previous studies that showed that muscle function improves sooner than does muscle mass during nutritional repletion (15, 35).

^{31}P NMR was shown to be a reliable technique for in vivo determination of ATP, Pi, and phosphocreatine concentrations and cytosolic pH in human muscle. Lower phosphocreatine:ATP in malnourished subjects than in control subjects were found in the present study, as in other studies in adults (17, 36), children (37), and animals (19, 38). In the last 3 studies, the authors concluded that ATP was maintained during starvation or fasting at the expense of phosphocreatine. The other hypothesis is that total creatine is lower in malnourished muscles, and that subsequently both free creatine and phosphocreatine decrease (17, 36). During nutritional repletion, no significant increase in the phosphocreatine:ATP occurred. In 6 subjects this ratio did increase, but in 3 others no change or a decrease was observed; these latter 3 subjects had phosphocreatine:ATP values in the range measured in control subjects at baseline. This suggests that a decreased muscle phosphocreatine concentration is not a constant feature during malnutrition.

Mitochondrial dysfunction with ADP accumulation during malnutrition is one possible mechanism (39) for the diminished muscle performance observed in this study. Concentrations of phosphorylated metabolites were calculated on the basis of the assumption that concentrations of ATP and total creatine are constant, at 8.2 mmol/L and 42.5 mmol/L, respectively. However, Symreng et al (36) used biochemical techniques in muscle biopsies to show that malnutrition was associated with lower ATP and creatine concentrations (16% and 29% lower than control values, respectively), whereas another study in malnourished

surgical patients found no change (40). We calculated ADP concentrations (data not shown) by using the equation

$$[ADP] = \{([total\ creatine]/[phosphocreatine]) - 1\}[ATP]/(K[H^+])\qquad(1)$$

where total creatine is taken as 42.5 mmol/L cell water and K is the equilibrium constant (1.66×10^9 L/mol) (41). By using this value for creatine concentration (which is uncertain), we found that the mean ADP concentration was higher in malnourished subjects than in control subjects. However, the ΔGp (or phosphate potential) was not significantly different between the 2 groups. Furthermore, there were no significant changes in this ΔGp during nutritional repletion, whereas ADP values became no longer different from control values. This is probably because the Pi:ATP was low in malnourished subjects and increased during nutritional repletion. In the article by Thompson et al (17) in which the ΔG_{ATP} was calculated, no differences were found between malnourished and control subjects. Thus, even though this possibility cannot be ruled out, there was no evidence of a defect in mitochondrial oxidative capacities in malnourished human subjects.

The Pi:ATP was lower in malnourished subjects than in control subjects, as observed in previous studies (17, 18). Furthermore, after the 1-mo recovery period, this ratio increased in 8 of 9 malnourished subjects. During starvation, lipids are the preferred fuels for energy supply (42). Fatty acid oxidation results in decreased Pi concentrations, whereas glucose oxidation is associated with increased Pi concentrations (18). It was shown that during nutritional repletion, the oxidation of lipids decreases (42). Thus, the early increase in Pi could reflect the shift from lipids to carbohydrates as the main source of energy during refeeding.

The muscle glycogen content was determined by natural abundance ^{13}C NMR, as proposed by Taylor et al (29). In subjects with thick subcutaneous adipose tissue, the muscle in the field of view of the surface coil was not sufficient to record enough signal for reliable calculations. One of the malnourished subjects excluded from this study after calf imaging had a small muscle volume (23 cm^3) and the ^{31}P NMR spectra were also of poor quality, indicating that local body composition should be known when studying muscle metabolism in frail elderly persons. In the other subjects, the muscle glycogen contents varied greatly. Furthermore, low glycogen concentrations were not specific to malnutrition, because they were observed in the control subjects as well.

Low muscle glycogen was described as a feature of malnourished humans (36, 40) and rats (19, 43), with possible increases after refeeding (40). In the present study, refeeding of elderly malnourished subjects produced inconsistent effects on muscle glycogen. One of the most intriguing observations was the high glycogen content measured in 2 patients that normalized after refeeding. This could be an artifact of measurement resulting from low lipid contents in the analyzed muscle of malnourished subjects. However, because proton imaging of the calf did not show contrast abnormalities, one can hypothesize that the muscle of these particular subjects was not composed of leaner tissue than that of control subjects (data not shown). McLoughlin et al (4) described glycogen granule accumulation in muscle from anorexia nervosa patients. A similar description was found for muscle from malnourished adults with chronic alcoholic myopathy (44). Despite the high muscle glycogen contents, the 2 subjects in our study and the other subjects with normal glycogen contents had a low Pi:ATP, suggesting preferential lipid oxidation, as discussed above. Furthermore, the muscle glycogen contents of these 2 subjects returned to normal during refeeding and their Pi:ATP increased. Again, this suggests that lipids are used preferentially during malnutrition.

For the purpose of muscle performance, the efficiency of lipid oxidation seems lower than the efficiency of carbohydrate oxidation (45, 46). One study showed that the glycogenolysis rate was lower in underfed rats than in control rats (43). This was associated with a decrease in muscle performance as evaluated with a fatigue test. In untrained adult subjects, carbohydrates are spared during low-intensity exercise and are used for strenuous exercise. Performance of strenuous exercise is limited by the amount of stored carbohydrates (47, 48).

In conclusion, we found an early increase in muscle strength and normalization of the metabolic profile of muscle during nutritional repletion of malnourished subjects. The results suggest either alterations in the oxidative capacities of muscle cells or a preferential use of lipids over carbohydrates during nutritional deprivation.

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