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# $^{31}$P NMR spectroscopy investigation of muscle metabolism in hemodialysis patients

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**$^{31}$P NMR spectroscopy investigation of muscle metabolism in hemodialysis patients.** Calf muscle metabolism of six patients with end-stage chronic renal failure undergoing maintenance hemodialysis and of six control subjects was studied using $^{31}$P nuclear magnetic resonance spectroscopy at 4.7 Tesla. Spectra were obtained at rest, during exercise and recovery. At rest, the inorganic phosphate, ATP and phosphocreatine concentrations, and the intracellular pH were similar in both groups of subjects. In the patients, the maximum workload achieved at the end of exercise led to a 84% and 46% depletion of phosphocreatine and ATP, respectively; under this condition, the intracellular pH fell to 6.50 ± 0.09. In control subjects, a maximum workload caused no change in ATP concentration at the end of exercise, but a phosphocreatine depletion and an intracellular pH fall similar to those observed in the patients. Although the rate of phosphocreatine depletion during exercise was not different in the two groups of subjects, the decrease in intracellular pH was more rapid in the patients than in control subjects. At the end of maximum exercise, the rates of recovery of both phosphocreatine and intracellular pH were significantly reduced in the muscle of hemodialysis patients when compared to normal subjects. These results suggest that, in the calf muscle of hemodialysis patients, energy production via oxidative metabolism is impaired and compensated for by an increase in anaerobic glycolysis.

Muscle weakness is a common symptom in patients with chronic renal failure; most of these patients complain of easy fatigability and exercise intolerance [1]. These abnormalities of muscle function in chronic renal failure, which are accompanied by atrophy of type I and type II muscle fibers [2–4], have been attributed to various factors such as disturbances of muscle energy metabolism resulting from altered muscle blood flow [4], diminished oxygen supply to muscle due to anemia, defective muscle metabolism of amino acids [5, 6], insulin resistance leading to a reduction of muscle glucose utilization as energy source [7], peripheral neuropathy [1, 3], impaired metabolism of vitamin D and excess of parathyroid hormone [8]. The latter hormone, which has been implicated in the reduction of carnitine palmitoyl transferase activity in muscle of rats in chronic renal failure [9], might be responsible for a diminished supply of energy (in the form of fatty acids) to muscle cells.

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Thus, many interrelated factors whose relative importance remains undefined appear to be potentially involved in the alterations of muscle bioenergetics underlying the exercise intolerance observed in patients with chronic renal failure. To our knowledge, only few data about the characteristics of the alterations of the metabolism of the energy-rich phosphorylated compounds involved in skeletal muscle bioenergetics are available [5, 10]. We have therefore used phosphorus nuclear magnetic resonance spectroscopy ($^{31}$P MRS), a noninvasive tool which has been extensively employed in recent years to investigate metabolic processes related to muscle function in normal [11, 12], and inherited [13] or acquired [14] pathological situations, to examine calf muscle energy metabolism in hemodialysis patients and normal subjects at rest, during exercise as well as during the recovery phase.

The results suggest that, together with the expected decrease in exercising capacity, a deficit of oxidative energy metabolism and a stimulation of anaerobic glycolysis are present in skeletal muscle of patients with chronic renal failure who are undergoing hemodialysis.

## Methods

### *Subjects*

Six patients (3 male and 3 female) with end-stage renal failure (plasma creatinine = 970 ± 78 μmol/liter; mean ± SEM), currently on center hemodialysis, participated in the study upon informed consent. Their original disease diagnoses were chronic glomerulonephritis ($N$ = 2), membranous nephropathy ($N$ = 1), tubular interstitial nephritis ($N$ = 2) and nephroangiosclerosis [1]. Patients with diabetic nephropathy, cardiac arrythmia or failure, emphysema, secondary hyperparathyroidism or with medication of steroids, NSAID were excluded from the study. None had thyroid or hepatic disease. All patients had chronic metabolic acidosis (serum bicarbonate < 21 mmol/liter) documented for at least six months before their inclusion into the study and all had severe anemia (hemoglobin concentration < 10 g/dl). Their mean age was 50.6 ± 14.3 years (range 31 to 77 years). All were receiving hemodialysis three times weekly with a bicarbonate-containing bath during their participation in this study. The mean duration from their onset of dialysis was 65.5 ± 5.0 months (range 22 to 156 months). Each patient was investigated on a day between two dialysis sessions.

Six age and sex matched (3 male and 3 female; mean age 47.8 ± 6.4 years, range 28 to 77 years), healthy sedentary volunteers with normal renal function served as controls.

### $^{31}P$ NMR spectroscopy

$^{31}P$ NMR spectroscopy was performed with a Fourier transform Bruker MSL 200 spectrometer interfaced with a 4.7 Tesla, 40-cm bore superconducting magnet (Oxford Magnet Technology, Oxford, UK) operating at resonance frequencies of 81.04 and 200.2 MHz for $^{31}P$ and $^{1}H$, respectively.

The subjects were studied in the supine position, the calf muscle of the dominant leg laying over a 5-cm home-built double tuned surface coil. A sliding bed was used to move the patient's leg in the center of the magnet. Inside the magnet cavity, the sole of the subject's foot rested on a movable foot plate placed at 90 degrees with respect to the long axis of the leg. The foot plate was itself connected by a lever to an ergometer loaded with a weight of 10 kg, corresponding to a force of 98.1 N. Any pressure exerted by the subject's foot on the foot plate was opposed by the load. Work, expressed in Joules, was calculated as the product of the load by its displacement multiplied by the frequency of pedalling, the pedal movement being linked to a measuring table with a displacement transducer and the pedal frequency being transmitted to a graphic recorder.

Magnetic field homogeneity was optimized while observing the water proton signal, and a proton spectrum was collected (4 transients, repetition time = 5 seconds, pulse width = 200 μsecond); the peak half width at half height never exceeded 35 Hz. Then, a control $^{31}P$ spectrum, which served as the baseline value, was collected for each individual study at rest (64 transients using an excitation pulse of 220 μsecond and a repetition rate of 7 seconds). During the exercise protocol, spectra were recorded with a pulse repetition of 2.2 seconds and a pulse width of 220 μsecond. Eight scans were collected over a total acquisition time of 17.5 seconds yielding a time resolution of approximately 3 spectra per minute.

Signal intensities of phosphocreatine, inorganic phosphate and β-ATP, which were quantitated relative to an external reference signal (methylene diphosphonic acid), were obtained by computer integration after phase and baseline correction of the spectra. The areas were corrected for incomplete saturation at the pulse conditions chosen and, assuming an intracellular water volume content of 0.67 ml per g wet weight of muscular tissue [15], the concentrations of the energy-rich phosphate metabolites were expressed in mM per liter of intracellular water.

Intracellular pH was calculated using the following form of the Henderson-Hasselbalch equation: $pH = 6.75 + \log [(\delta - 3.27)/(5.69 - \delta)]$, where $\delta$ indicates the chemical shift difference (in ppm) between the inorganic phosphate and the phosphocreatine peaks.

### Exercise protocol

After measuring the control spectrum at rest, each control subject and patient was asked to perform a maximum exercise. For this, each control subject and patient had to press on the pedal at a regular frequency (2 Hz) and to maintain the isotonic contractions of his calf muscle as long as possible until exhaustion. Calf muscle discomfort and a drop in rate or displacement were the determinants for terminating the exercise. Each control subject was also ask to mimick the exercise protocol performed by the corresponding patient.

### Statistical analysis

The results are expressed as means ± SEM. The unpaired Student's $t$-test was used to compare values obtained in the patients with those of the controls. A $P$ value $< 0.05$ was considered significant. The least-squares method was used to test the existence of a linear correlation: (i) between the decrease in intracellular pH or in phosphocreatine concentration and time of exercise, (ii) between the increase in intracellular pH and time of recovery after exercise.

## Results

### Spectra of muscle at rest

The $^{31}P$ NMR spectra of the resting calf muscle of one normal subject and one hemodialysis patient are shown in Figures 1 and 2, respectively. It can be seen that, at the high field strength (4.7 T) used, the peaks observed were extremely well separated and that the signal-to-noise ratio was high. This allowed accurate measurements of the different peak areas. From left to right, resonances are assigned to methylene diphosphonic acid, phosphomonoesters, inorganic phosphate (which increased during exercise), phosphodiesters, phosphocreatine (which declined during exercise), and the three ATP resonances of which only the β resonance was used to calculate ATP concentration because it did not contain the ADP and NAD/NADP resonances. In most cases, the phosphomonoester peak was not seen.

Table 1 shows the intracellular pH values and the absolute concentrations of phosphocreatine, inorganic phosphate and ATP as well as the phosphocreatine/inorganic phosphate ratio, which is related to the phosphate potential [16, 17], in calf muscles of the control and hemodialysis subjects at rest. It can be seen that these parameters in hemodialysis subjects were not different from those in the controls.

### Exercise studies

Figures 3 and 4 show 28 spectra (out of 40 obtained for each subject) from a typical sequence obtained in one control subject and one hemodialysis patient at rest (spectra 1–4), during exercise until exhaustion (spectra 5–15 in the control subject, and 5–17 in the patient) and subsequent recovery (spectra 16–28 in the control subject, and 18–28 in the patient). As can be seen in these figures, the high field strength of our magnet allowed to obtain high signal-to-noise ratios and an excellent temporal resolution (one spectrum every 17.5 seconds). As expected, there was a net degradation of phosphocreatine to inorganic phosphate during exercise and, at the end of exercise, both inorganic phosphate and phosphocreatine concentrations returned rapidly towards resting values.

Figure 5 shows the changes of workload, phosphocreatine concentration and intracellular pH over eight 17.5-second intervals of maximum exercise for all control subjects and hemodialysis patients. It can be seen that the rate of phosphocreatine depletion observed in the patients was similar to that in the control subjects. When the phosphocreatine concentration (C), expressed in percent of its resting value, was plotted as a

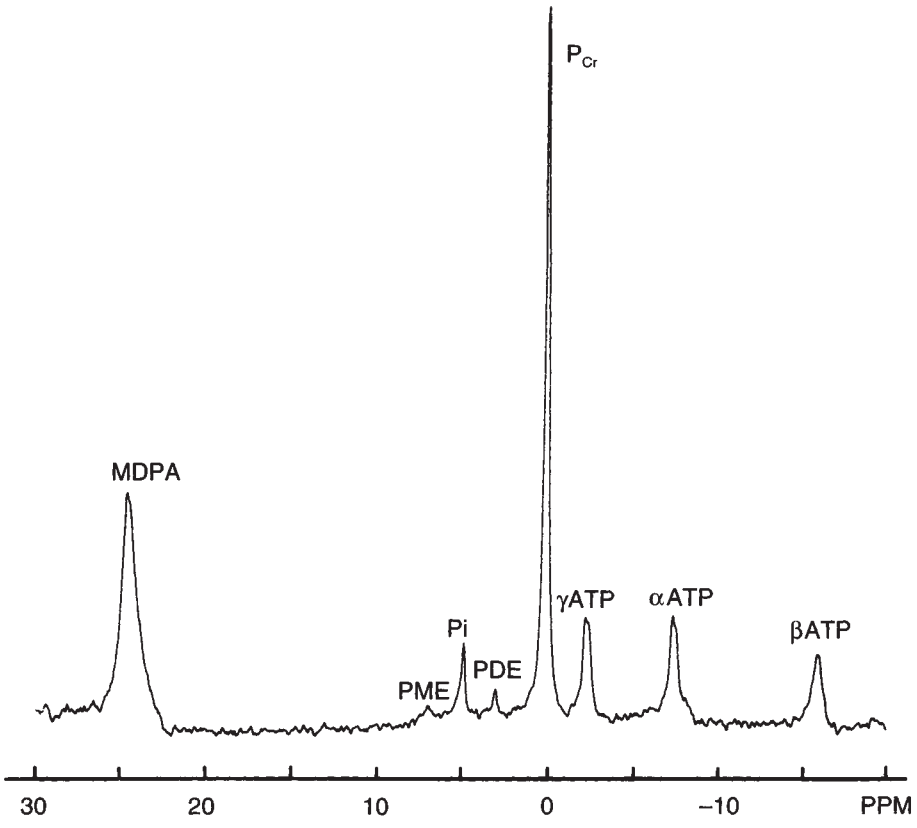


**Fig. 1.** *A $^{31}P$ MRS spectrum from calf muscle of a normal subject at rest.* Abbreviations are: MDPA, methylene diphosphonic acid; PME, phosphomonoesters; Pi, inorganic phosphate; $P_{Cr}$, phosphocreatine; γ-, α- and β-ATP = the γ-, α- and β-phosphate of adenosine triphosphate.

**Table 1.** Concentrations of phosphate metabolites and intracellular pH values in calf muscle of control and hemodialysis subjects at rest

| | Control subjects ($N$ = 6) | Hemodialysis subjects ($N$ = 6) |
|---|---|---|
| pH | 7.02 ± 0.01 | 7.01 ± 0.01 |
| $P_{Cr}$ *mM* | 28.9 ± 3.1 | 31.8 ± 4.8 |
| Pi *mM* | 2.4 ± 0.3 | 2.8 ± 0.7 |
| $P_{Cr}$/Pi | 12.9 ± 1.3 | 12.3 ± 0.7 |
| ATP *mM* | 5.2 ± 1.5 | 4.4 ± 0.9 |

The values, expressed in mM/liter of muscle intracellular water, were obtained using a 7 second pulse interval and corrected for saturation effects. They are given as means ± SEM. No statistical difference was found between the control and hemodialysis subjects. Abbreviations are: $P_{Cr}$, phosphocreatine; Pi, inorganic phosphate.

function of time (t, expressed in 17.5 second intervals), the equation obtained in control subjects (C = − 9.01t + 101.93; $r$ = 0.97) and that obtained in hemodialyzed patients (C = −8.7t + 97.04; $r$ = 0.93) revealed no statistically significant difference.

After an initial increase due to the consumption of protons by the creatine kinase reaction operating in the direction of phosphocreatine utilization, the intracellular pH fell in both groups of subjects (Fig. 5). When the pH (P), expressed in pH units, was plotted as a function of time (t) from the second to the eighth 17.5 second intervals of intense exercise, the equations obtained in the hemodialysis patients (P = −0.08t + 7.26; $r$ = 0.97) and in the control subjects (P = −0.06t + 7.31; $r$ = 0.96)

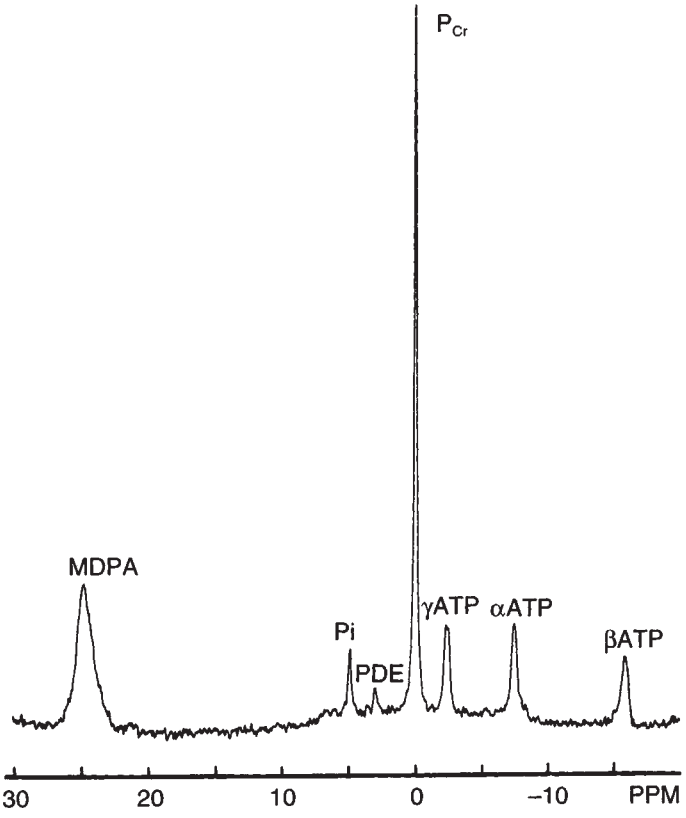


**Fig. 2.** *A $^{31}P$ MRS spectrum from calf muscle of a hemodialysis patient at rest.* For peak assignments, see Fig. 1.

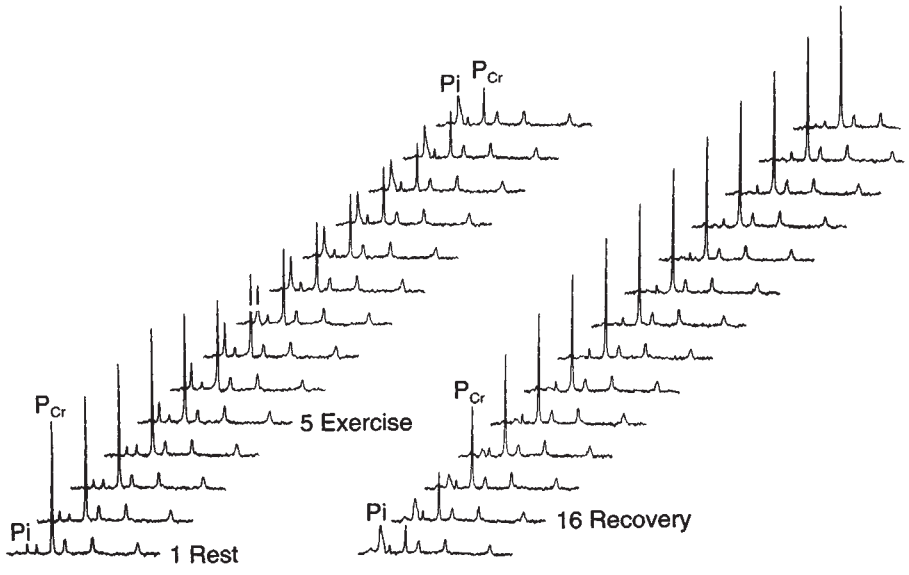


**Fig. 3.** *Stacked plot from a maximum exercise in a representative control subject.* Spectra 1–4 (rest), 5–15 (exercise until exhaustion) and 16–28 (recovery). For peak assignments, see Fig. 1.

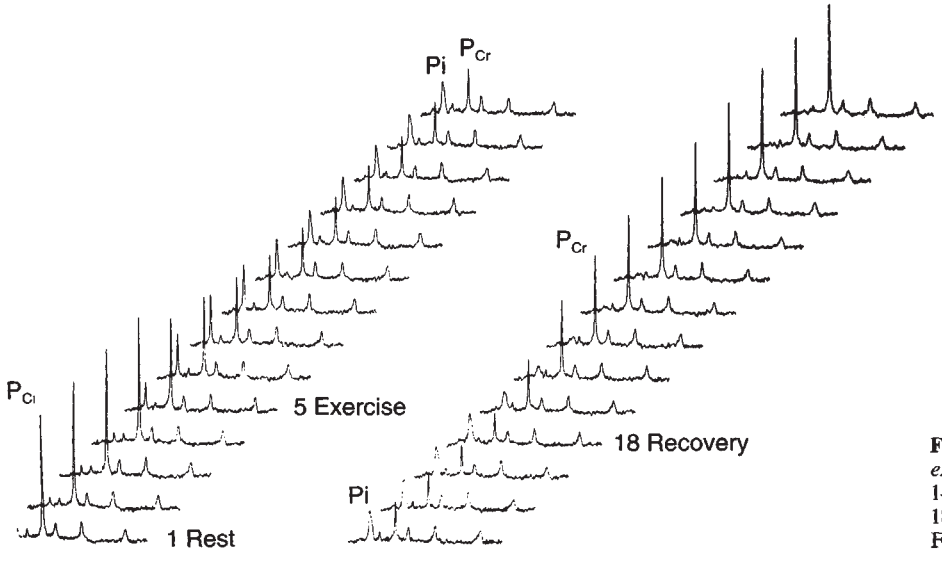


**Fig. 4.** *Stacked plot from a maximum exercise in a hemodialysis patient.* Spectra 1–4 (rest), 5–17 (exercise until exhaustion) and 18–28 (recovery). For peak assignments, see Fig. 1.

indicate that the intracellular pH declined more rapidly ($P < 0.001$) in the patients than in the normal subjects.

Table 2 shows the metabolic changes observed at the end of the maximum exercise carried out by the hemodialysis and the control subjects. It also shows the changes observed at the end of the exercise performed by the control subjects to mimick the maximum exercise of the hemodialysis patients.

The workload needed to reach muscle exhaustion in patients corresponded to a moderate exercise in the control subjects who did not experience fatigue. This workload did not alter the intracellular pH and led to only a 39% depletion of phosphocreatine in healthy subjects.

On the average, the workload needed to produce muscle exhaustion in the control subjects was 7.4 times greater than in the patients. At the end of maximum exercise, such a workload led in the control subjects to a large phosphocreatine depletion similar to that observed in hemodialysis subjects (Table 2). The significant intracellular acidification due to accumulation of lactic acid resulting from anaerobic glycolysis was of similar magnitude in both groups of subjects at the end of maximum exercise. Muscle ATP concentration did not change in the control subjects indicating that the ATP degraded during muscle contraction was resynthesized thanks to the stimulation of oxidative phosphorylation and to ATP synthesis by the creatine kinase reaction ($H^+$ + ADP + phosphocreatine → ATP + creatine) as well as by the stimulation of anaerobic glycolysis revealed by intracellular acidification when the concentration of inorganic phosphate exceeded that of phosphocreatine. By contrast, muscle ATP concentration was markedly diminished at the end of the exercise in the patients (Table 2). However, resting muscle ATP levels were found to be restored by the first 17.5 second interval of recovery from exercise in the patients.

Figure 6 shows the phosphocreatine concentration and intracellular pH recovery after exercise. It can be seen that, despite

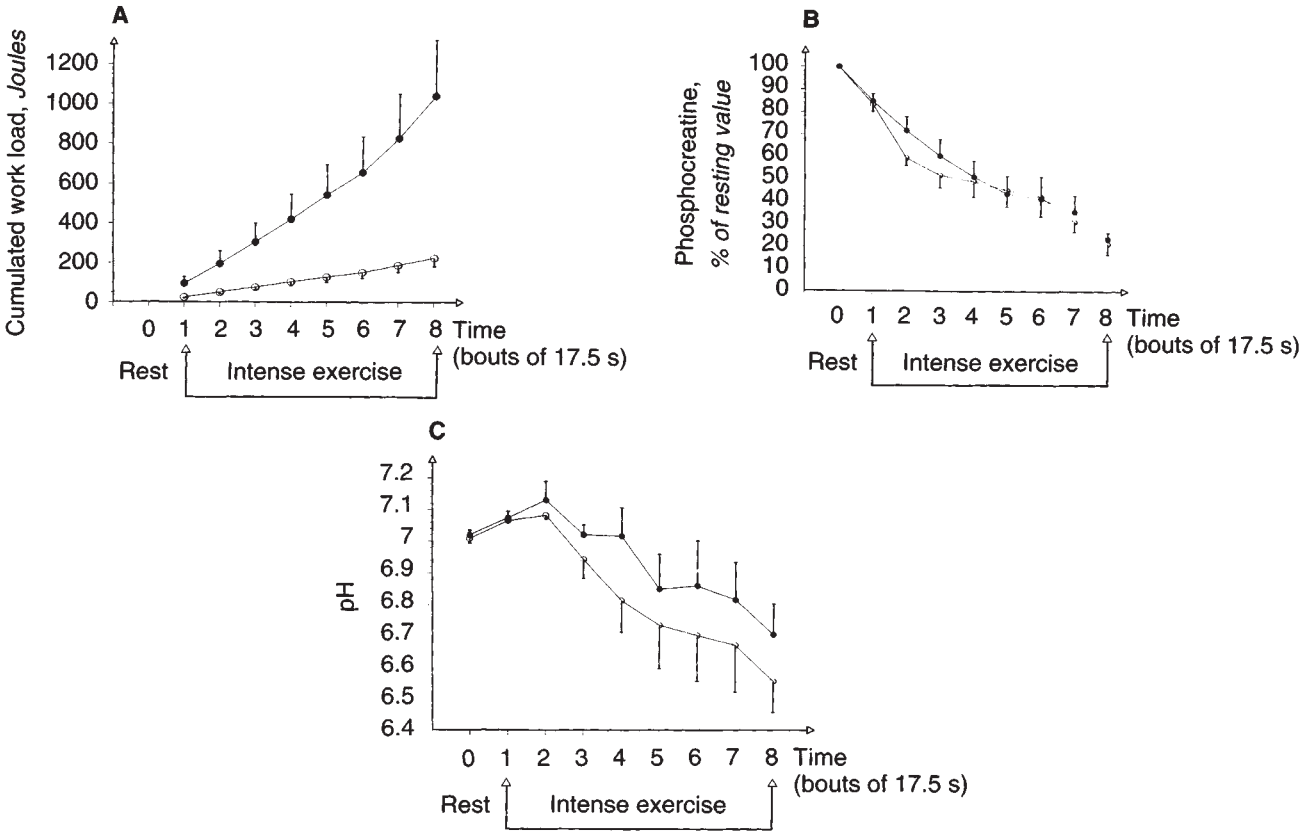


**Fig. 5.** *Workload, phosphocreatine concentration and intracellular pH changes during eight 17.5-second intervals of maximum exercise in 6 control subjects (●) and 6 hemodialysis patients (○).* Values are means ± SEM.

**Table 2.** Effect of exercise on intracellular pH, phosphocreatine depletion and resynthesis, and ATP depletion in calf muscle of control and hemodialysis subjects

| | Exercise duration *17.6 second intervals* | Total workload *Joules* | Intracellular pH | | Phosphocreatine depletion[a] | Phosphocreatine resynthesis | ATP depletion[a] |
|---|---|---|---|---|---|---|---|
| | | | At rest | At the end of exercise | *% of resting value* | $T_{1/2}$ *in seconds* | *% of resting value* |
| Control subjects (moderate exercise) | 12.0 ± 1.8 | 357 ± 40 | 7.02 ± 0.01 | 7.00 ± 0.03 | 39.2 ± 9.0 | 22.9 ± 2.3 | ND |
| Hemodialysis subjects (maximum exercise) | 12.0 ± 1.8 | 321 ± 45[b] | 7.01 ± 0.01 | 6.50 ± 0.09 | 84.7 ± 2.0[b] | 58.4 ± 10.9[b] | 46.5 ± 9.8[b] |
| Control subjects (maximum exercise) | 13.6 ± 1.2 | 2380 ± 465 | 7.02 ± 0.01 | 6.56 ± 0.04 | 84.5 ± 3.4 | 30.5 ± 2.4 | 2.8 ± 2.8 |

Abbreviations are: ND, not determined; $T_{1/2}$, time for recovery of 50% of the phosphocreatine depleted at the end of exercise. The values obtained in 6 control and 6 hemodialysis subjects are given as means ± SEM. Statistical difference was measured by the unpaired Student's *t*-test against the hemodialysis subjects.

[a] at the end of exercise

[b] $P < 0.05$ vs. control subjects (maximum exercise)

a phosphocreatine depletion of similar magnitude in the control and hemodialysis subjects at the end of exercise, the rate of phosphocreatine concentration recovery was lower in the patients than in the control subjects. The time for recovery of half the phosphocreatine broken down, which reflects the mitochondrial oxidative metabolism [18], was considerably increased in the hemodialysis patients indicating a deficient oxidative phosphorylation in these patients (Table 2). Another important biochemical difference observed is that after an initial further decrease in pH after the end of exercise (due to the accumulation of part of the protons synthesized by the creatine kinase reaction operating in the direction of phosphocreatine resynthesis), the pH returned towards the resting value at a rate smaller in the patients than in the normal subjects ($P < 0.001$). When the pH (P), expressed in pH units, was plotted as a function of time (t) from the second to the 15th 17.5 second intervals of recovery in the control subjects and from the third to the 15th 17.5 second intervals of recovery in the patients, the equations obtained were $P = 0.05t + 6.27$ ($r = 0.94$) and $P = 0.04t + 6.11$ ($r = 0.93$), respectively.

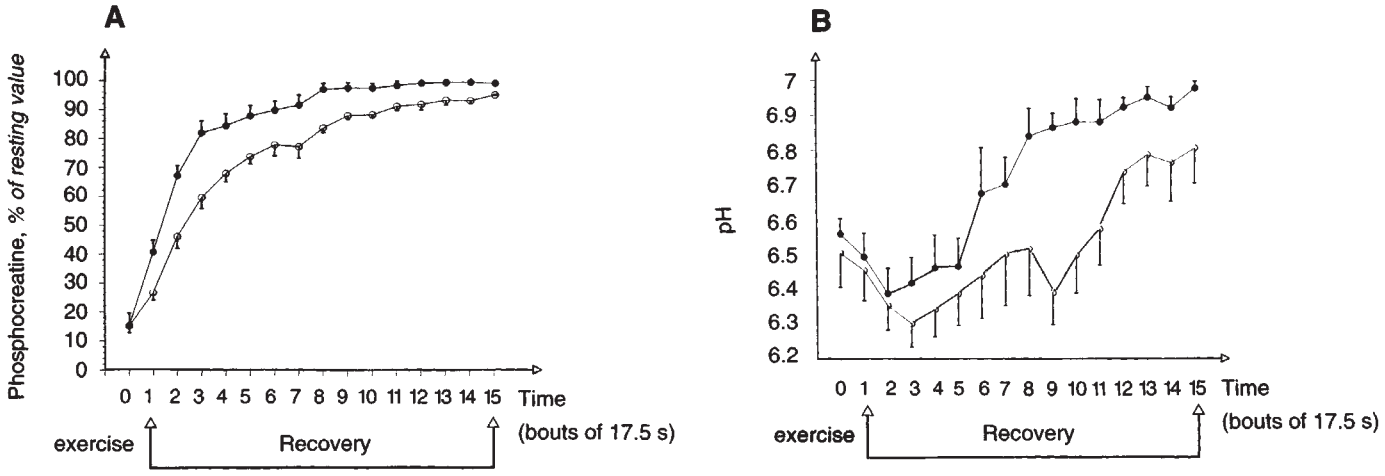


**Fig. 6.** *Phosphocreatine concentration and intracellular pH recovery after exercise.* Results are ± SEM for 6 control subjects (●) and 6 hemodialysis patients (○).

## Discussion

In the present study, we used $^{31}P$ MRS to determine if the fatigability and exercise intolerance experienced by patients with end-stage renal failure under maintenance hemodialysis is associated with altered skeletal muscle metabolism. We evaluated several variables central to muscle metabolism: the concentration of ATP, $P_{Cr}$ and Pi, the $P_{Cr}$/Pi ratio, which is related to the phosphorylation potential [ATP/(ADP+Pi)] [16, 17], and intracellular pH which falls during exercise secondary to lactic acid accumulation resulting from increased glycolysis when the $P_{Cr}$/Pi ratio decreases approximately below 1 [14].

The results obtained show that at rest, the concentrations of the energy-rich phosphate metabolites and of Pi as well as the intracellular pH in hemodialysis patients were not different from those in normal subjects. Although our method of estimation of the concentrations of the major phosphorus containing compounds is not the most commonly one used [19], we deliberately chose to estimate the absolute concentrations of ATP, $P_{Cr}$ and Pi by using methylene diphosphonic acid as the external standard to be able to compare their concentrations in patients with those in control subjects. This seemed more appropriate than to use, as is most commonly done [19], muscle ATP concentration as the standard without being certain that this concentration in calf muscle of hemodialysis patients was normal. An important point to consider is whether or not the surface coil examined the same muscle mass in patients and normal subjects. That this was indeed the case is suggested by the absence of difference between the normal and hemodialysis subjects in the water-to-fat ratio estimated from the signals obtained in the $^{1}H$ spectra. This ratio was equal to 2.9 ± 0.7 in the control subjects versus 3.0 ± 0.8 (NS) in the patients. It should be mentioned that the increase in extracellular water content observed in muscle of hemodialysis patients [20] cannot have significantly diminished muscle intracellular water mass that has been examined in our patients compared to control subjects, because extracellular water represents only a small fraction (<10%) of total muscle water content [15].

By contrast, our study clearly establishes that muscle metabolism in hemodialysis subjects was markedly different from that in control subjects during both exercise and recovery from exercise. Although the maximal workload achieved by the patients, which represented a very moderate workload for the control subjects (Table 2), led to a depletion of phosphocreatine of similar magnitude in both groups of subjects, a significant decrease in ATP was found at the end of the maximum exercise in patients but not in the control subjects. Such an ATP depletion is known to occur in healthy subjects only during extremely vigourous exercises leading to more than 90% $P_{Cr}$ depletion [21]. In addition, the rate of intracellular acidification was found to be higher in the control subjects than in the hemodialysis patients (Fig. 5, text). This suggests that the stimulation of anaerobic glycolysis due to the recruitement of muscle glycolytic fibers, which occurs predominantly when the workload is sufficiently raised [22] and leads to the production of large amounts of lactic acid, was greater in the patients than in the normal subjects. All these abnormalities together with the delayed recovery of $P_{Cr}$ at the end of exercise, which has also been observed in various other pathological states such as mitochondrial myopathies [23], heart failure [14], peripheral vascular insufficiency [24] and chronic respiratory failure [25] are consistent with an impaired muscle resynthesis, by the mitochondrial oxidative phosphorylation, of the ATP used for muscular contraction. Such a reduced resynthesis of ATP would in turn lead to a delayed resynthesis of $P_{Cr}$ by creatine kinase catalyzing the following reaction:

$$ATP + Cr \rightarrow P_{Cr} + ADP + H^{+}.$$

It is difficult from our results to identify the precise mechanisms responsible for a diminished muscular resynthesis of ATP in our patients. On the basis of data available in the literature, a reduced mitochondrial population or efficiency can be ruled out since the number of mitochondria in muscle of patients suffering from chronic renal failure has been found normal [10] and an increased oxidative capacity of these isolated mitochondria has also been observed [4, 10]. Therefore, it is more likely that the abnormalities observed resulted from a diminished substrate supply or to a reduced oxygen delivery secondary to

anemia and/or to insufficient blood flow to the leg during exercise.

It should be emphasized that no correlation was found in our study between the degree of anemia and the time for recovery of half the $P_{Cr}$ broken down at the end of exercise, or between the degree of anemia and the fall of ATP concentration at the end of exercise. These results, however, do not rule out a role of anemia *per se* in the muscle metabolism alterations observed because the involvement of this factor may be obscured by that of other factors, such as reduced substrate supply and insufficient blood flow.

It should be stressed that a significant reduction of muscle blood flow has been demonstrated in patients with chronic renal failure when compared to normal subjects [4]. The fact that the deficient skeletal muscle vascularization found in peripheral vascular disease has been shown to be associated with an unchanged mitochondrial population and an increased oxidative capacity [4, 10] is in favor of a major role of chronic underperfusion of working muscle in the metabolic disturbances found in our hemodialysis patients.

The role of hormonal and nutritional factors contributing to a reduced substrate supply to muscle cells in chronic renal failure cannot be evaluated from the data of the present study. Similarly, our results do not permit any conclusion about the possible inhibition of muscle mitochondrial respiration by the so-called uremic toxins to be drawn [26].

The reason why the intracellular pH returned towards resting values much more slowly in the patients cannot be related to the resynthesis of phosphocreatine (which is accompanied by the synthesis of protons), because this resynthesis was faster in the control than in the hemodialysis subjects. It is conceivable that it was due to continued anaerobic glycolysis or to a decrease in the muscle buffering capacity, or in the rate of export of protons from muscle cells of hemodialysis patients. Finally, since muscular fatigue has been related to the accumulation of protons [27, 28], the large and early pH fall and the delayed recovery of pH might be responsible, at least partially, for the exercise intolerance in our patients.

In conclusion, our ability to demonstrate clear disturbances of muscle metabolism in hemodialysis subjects due to $^{31}P$ MRS is clinically important. In addition to improving our understanding of the biochemical basis of exercise intolerance in these patients, this noninvasive tool offers the possibility to evaluate various nutritional and therapeutic approaches liable to diminish their fatigability.

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