Effects of Chronic Renal Failure on Enzymes of Energy Metabolism in Individual Human Muscle Fibers[1]

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ABSTRACT

In order to improve knowledge about the mechanisms underlying the alterations of energy metabolism recently observed in the skeletal muscle of patients suffering from chronic renal failure, this study was designed to test (*1*) whether changes in the activity of key enzymes of energy metabolism occur in the muscle of these patients, and if so (*2*) whether the different muscle fiber types are equally altered in their metabolic machinery. For this, the maximum activities of 14 enzymes were measured in individual muscle fibers microdissected from biopsies of rectus abdominis muscle obtained from seven normal subjects and seven patients with end-stage renal failure before renal replacement therapy. A large decrease in the activities of β-hydroxyacyl-coenzyme A dehydrogenase, a key enzyme of the β-oxidation pathway, of citrate synthase, which initiates the tricarboxylic acid cycle, and of fructose-1,6-bisphosphatase, which contributes to the synthesis of glycogen from lactate, was observed in the three fiber types (slow-twitch oxidative, fast-twitch oxidative-glycolytic, and fast-twitch glycolytic). A smaller reduction of the activities of phosphofructokinase and/or pyruvate kinase, two key enzymes of glycolysis, was also observed in slow-twitch oxidative and/or fast-twitch oxidative-glycolytic fibers. These results demonstrate that the abnormalities of muscle energy metabolism observed in patients with chronic renal failure are due, at least in part, to intrinsic changes in the key enzymes of major energy-providing pathways; they also offer a satisfactory explanation for the defect of oxidative metabolism recently demonstrated in the muscle of these patients.

Key Words: *Uremia, citrate synthase, β-hydroxyacyl-coenzyme A dehydrogenase*

In patients with chronic renal failure, muscular weakness is a well-recognized feature (1), as are other less specific musculoskeletal symptoms such as aches and cramps. Most of the patients complain of easy fatigability and exercise intolerance (2). In these patients, subnormal muscle oxidative energy metabolism has been recently documented by ^{31}P nuclear magnetic resonance (NMR) spectroscopy (3–5). However, very little is known about the cause and mechanism of this impairment of muscle energy production in uremic patients. Many interrelated factors, the relative importance of which remains poorly defined, appear to be potentially involved. Possible pathogenetic causes include altered muscle blood flow (6), anemia (7), defective muscle metabolism of amino acids (8) and lipid (9), hormonal imbalance (10), insulin resistance (11) leading to a reduction of muscle glucose utilization as an energy source, and the inhibitory effects of uremic toxins on muscle metabolism (12).

Human skeletal muscle contains a heterogeneous population of fibers with differing capacities for energy generation. At least three distinct fiber types, which vary in speed of contraction and enzyme composition, can be distinguished (13,14). There are different methods for classifying the different muscle fiber types (15,16). In this study, we used the classification based on the metabolic profile developed by Hintz *et al.* (17,18): fibers with a high potential for aerobic metabolism and low glycolytic capacities have been named slow-twitch oxidative fibers. Fibers with both high glycolytic and oxidative enzymes have been called fast-twitch oxidative-glycolytic. The fast-twitch glycolytic fibers have high glycolytic capacities and a low aerobic potential.

Histologic alterations of fibers have been reported in the uremic state (6,19), but to our knowledge, the measurement of enzyme activities in these specialized muscle fibers has not been performed in these patients as has been done in normal humans (17) and in patients with Duchenne muscular dystrophy (20) or McArdle disease (21). Because various muscle energy metabolism alterations have been demonstrated in the uremic state, it seems important to analyze individual fibers for representative enzymes of different metabolic pathways. The aims of this investigation were therefore (*1*) to test whether changes in maximum activities of key enzymes of the main energy-producing pathways occur in patients suffering from end-stage chronic renal failure and (*2*) to address the issue of whether fibers of different types are equally

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affected in their enzymatic machinery involved in energy production. The results of this study clearly demonstrate that, in all human muscle fiber types, chronic renal failure induces a decrease in the levels of activity of both citrate synthase, the enzyme that initiates the tricarboxylic acid cycle, and β-hydroxyacyl-coenzyme A (CoA) dehydrogenase, a key enzyme of fatty acid β-oxidation, thus offering an enzymatic basis for the recently demonstrated defect in oxidative energy metabolism in uremic muscle.

MATERIALS AND METHODS

Subjects

Seven predialytic patients (four women and three men) suffering from chronic renal failure (plasma creatinine = 862 ± 104 μmol/L; hematocrit = 28 ± 2%; means ± SE) were studied before renal replacement therapy. None of these patients had systemic disease. Their mean age, standing height, and body mass were 45 ± 4 yr, 163 ± 3 cm, and 62 ± 5 kg, respectively.

Seven healthy sedentary subjects (four women and three men; plasma creatinine = 82 ± 7 μmol/L; hematocrit = 43 ± 1%) served as controls. Their mean age, standing height, and body mass were 56 ± 9 yr, 171 ± 3 cm, and 63 ± 5 kg, respectively.

All of the subjects were well informed before the operation and gave their consent to participate. All procedures were approved by the local Medical Ethics Committee.

Muscle Biopsies and Dissection of Muscle Fibers

A biopsy (20 to 50 mg fresh weight) of rectus abdominis muscle was obtained from each patient undergoing catheter implantation for peritoneal dialysis and each control subject undergoing abdominal surgery. Transverse sections of a portion of each biopsy were stained by the ATPase technique of Padykula and Herman (22) to determine the percentage of Type I, IIA, and IIB fibers. Samples were also obtained by the procedure described by Lowry and Passonneau (23). In brief, the muscle was immediately frozen in isopentane cooled with liquid nitrogen. Then, it was freeze-dried under vacuum at −35°C and 50 to 60 individual fibers (from each biopsy), 2 to 5 mm long, were separated at room temperature and stored separately under vacuum at −70°C; when needed for the enzyme assays, the fibers were brought to room temperature and samples were cut off one end of each fiber. The remainder was returned to low-temperature vacuum storage; under the latter conditions of storage, all enzyme activities studied have been shown to remain indefinitely stable (17). Samples were weighed on a homemade quartz fiber balance as described by Lowry and Passonneau (23).

Enzyme Assays

Fiber samples were analyzed in duplicate for the maximum activity of 14 different enzymes: hexokinase (EC 2.7.1.1), glucose-6-phosphate isomerase (EC 5.3.1.9), phosphofructokinase (EC 2.7.1.11), pyruvate kinase (EC 2.7.1.40), lactate dehydrogenase (EC 1.1.1.27), fructose-1,6-bisphosphatase (EC 3.1.3.11), phosphoglucomutase (EC 5.4.2.2), glycogen phosphorylase (EC 2.4.1.1), β-hydroxyacyl-CoA de-

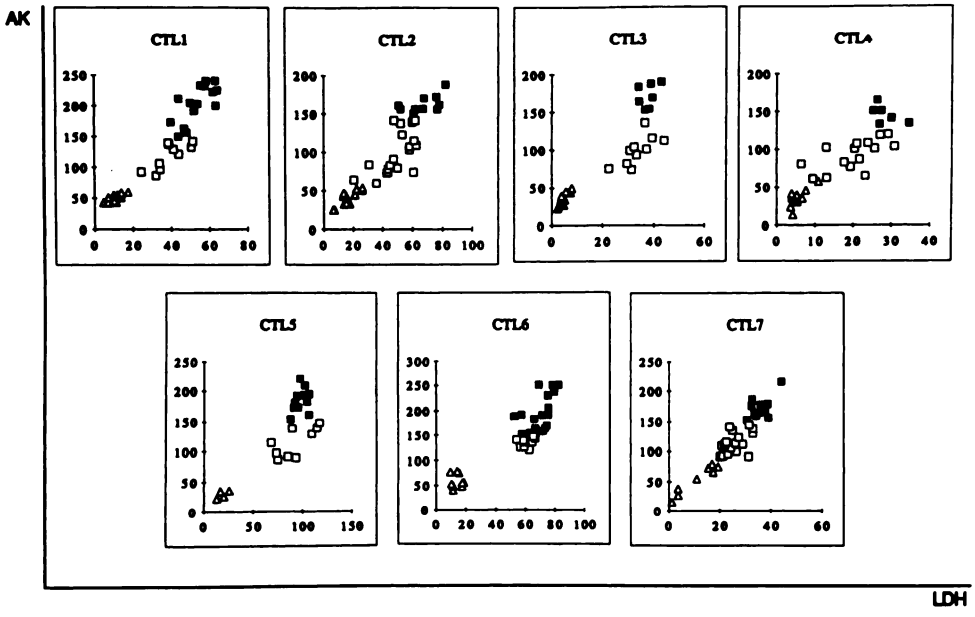

Figure 1. Adenylate kinase (AK) plotted against lactate dehydrogenase (LDH) in single muscle fibers of seven control subjects. Open triangles represent slow-twitch oxidative fibers; open squares represent fast-twitch oxidative-glycolytic fibers, and filled squares are fast-twitch glycolytic fibers. Enzyme activities are expressed in moles per kilogram dry weight per hour at 23°C. Correlations of adenylate kinase with lactate dehydrogenase are 0.95 for CTL1, 0.91 for CTL2, 0.91 for CTL3, 0.90 for CTL4, 0.84 for CTL5, 0.88 for CTL6, and 0.93 for CTL7. All correlations were statistically significant, $P < 0.01$.

hydrogenase (EC 1.1.1.35), citrate synthase (EC 4.1.3.7), creatine kinase (EC 2.7.3.2), adenylate kinase (EC 2.7.4.3), aspartate aminotransferase (EC 2.6.1.1), and alanine aminotransferase (EC 2.6.1.2). All of the assays incorporated a pyridine nucleotide step, which made possible the increase in sensitivity by enzymatic cycling that was needed to measure the activity of all enzymes except lactate dehydrogenase, adenylate kinase, creatine kinase, and phosphofructokinase. Assays were carried out in volumes of 10 μL (or less) that were pipetted under purified oil to avoid evaporation. Starting material was either a small piece of muscle fiber weighing 15 to 50 ng (17) or a small volume (50 to 200 nL) of muscle fiber extracts (24). Technical details of sampling, weighing, preparing the extracts, and conducting the enzyme assays on this scale are those described by Lowry and Passonneau (23).

Expression of Results and Statistical Analysis

The enzyme activities, given as means ± SE, are expressed in moles per kilogram dry weight per hour at 23°C. The one-way analysis of variance was used to compare the data obtained in the chronic renal failure patients with those of the controls, and the least-squares method was used to test in each control subject and patient the existence of a correlation between the activity of adenylate kinase and that of lactate dehydrogenase. For significance, the used level of $P < 0.05$ was retained.

RESULTS

The percentages of muscle fiber types in patients with chronic renal failure were not statistically different from those in the control subjects: these were 49 ± 4 *versus* 54 ± 5% for Type I fibers, 36 ± 4 *versus* 34 ± 4% for Type IIA fibers, and 15 ± 2 *versus* 12 ± 4% for Type IIB fibers, respectively. These values agree with those found in the same muscle and other muscles (25–27) in normal humans by other authors.

Figures 1 and 2 plot the activity of adenylate kinase against that of lactate dehydrogenase in individual fibers of seven control subjects (CTL) and seven chronic renal failure patients (CRF). The use of the latter enzymes, one of high-energy phosphate metabolism and the other of glycolysis, has been shown to be useful for classifying human muscle fibers (17,18). As can be seen in Figures 1 and 2, there was a continuum of activity for both enzymes in most control subjects and patients, whereas in some of them (CTL3, CTL5, CTL6, CRF5, CRF7), there was a clear discontinuity of activity of these enzymes. Good linear correlations were found between the activity of adenylate kinase and that of lactate dehydrogenase in all of the normal and uremic subjects studied (Figures 1 and 2). Similar observations have been reported in normal subjects (17,18,28) and patients suffering from muscular dystrophy or McArdle disease (20,21).

On the basis, on the one hand, of the results obtained in the latter studies for human muscle, which showed that lactate dehydrogenase activity rather sharply separates slow-twitch oxidative from fast-twitch fibers and that adenylate kinase activity permits a better definition between fast-twitch oxidative-glycolytic and fast-twitch glycolytic fibers (17) and on the other hand of the levels of activity of adenylate kinase and lactate dehydrogenase found in our study, we used arbitrary limits to distinguish in each control subject and patient between slow-twitch oxidative fibers (low activity of both enzymes), fast-twitch glyco-

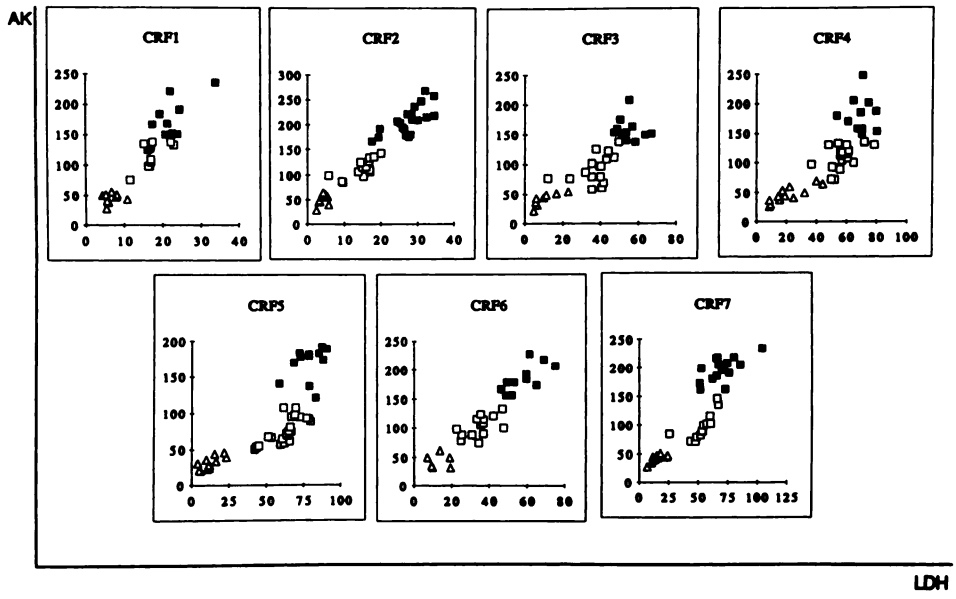

Figure 2. Adenylate kinase (AK) plotted against lactate dehydrogenase (LDH) in single muscle fibers of seven patients with chronic renal failure. All details are as in Figure 1. Enzyme activities are expressed as in Figure 1. Correlations are 0.92 for CRF1, 0.91 for CRF2, 0.76 for CRF3, 0.84 for CRF4, 0.81 for CRF5, 0.94 for CRF6, and 0.86 for CRF7. All correlations were statistically significant, $P < 0.01$.

lytic fibers (high activity of both enzymes), and fast-twitch oxidative-glycolytic fibers (intermediate activity of these enzymes).

Fibers with adenylate kinase activity higher than 150 mol/kg dry wt per hour (or 125 mol/kg dry wt per hour for CTL4 and CRF5) and the highest activities of lactate dehydrogenase were selected as fast-twitch glycolytic fibers. Fibers of the slow-twitch oxidative type were also selected according to the pattern of the adenylate kinase-lactate dehydrogenase activity values; this group contained fibers with adenylate kinase activities lower than 75 mol/kg dry wt per hour and lactate dehydrogenase maximal activities between 5 and 40 mol/kg dry wt per hour. The remaining fibers were considered to be fast-twitch oxidative-glycolytic. The fact that the above separation of fibers into three groups was justified was subsequently confirmed by measurements of the activity of oxidative enzymes such as β-hydroxyacyl-CoA dehydrogenase, which is higher in fast-twitch oxidative-glycolytic than in fast-twitch glycolytic fibers.

In order to allow comparisons among enzyme levels in the same fiber, the activities of the 12 other enzymes studied were measured in a major fraction of the fibers dissected in each control subject and patient shown in Figures 1 and 2. The mean values obtained from the mean activities measured in 4 to 21 fibers from the same fiber type in each control subject and patient are presented in Tables 1 and 2.

Enzymes of Glycolysis

As shown in Table 1, the mean levels of hexokinase and glucose-6-phosphate isomerase activity were in the same range in the different fiber types, both in control subjects and in patients. Chronic renal failure did not alter the activity of these two enzymes. By contrast, the activity of phosphofructokinase, pyruvate kinase, and lactate dehydrogenase was higher in fast-twitch than in slow-twitch fibers in both the control subjects and the patients. In patients with chronic renal failure as compared with control subjects, lactate dehydrogenase activity remained unchanged in all fiber types, whereas a modest but statistically significant decrease in pyruvate kinase activity was found in slow-twitch oxidative and fast-

TABLE 1. Activities of enzymes of glycolysis and glycogen metabolism in single muscle fibers of normal subjects and patients with chronic renal failure[a]

Enzyme	Single Fibers		
	Slow-Twitch Oxidative	Fast-Twitch Oxidative-Glycolytic	Fast-Twitch Glycolytic
Hexokinase			
CTL	0.418 ± 0.031	0.429 ± 0.031	0.395 ± 0.032
CRF	0.424 ± 0.094	0.408 ± 0.087	0.386 ± 0.094
Glucose-6-Phosphate Isomerase			
CTL	25.6 ± 2.7	31.9 ± 3.7	31.0 ± 3.5
CRF	24.1 ± 2.3	30.2 ± 2.3	31.4 ± 2.1
Phosphofructokinase			
CTL	3.39 ± 0.24	5.15 ± 0.43	6.10 ± 0.30
CRF	2.78 ± 0.34[b]	4.78 ± 0.44	5.97 ± 0.62
Pyruvate Kinase			
CTL	22.2 ± 3.9	37.9 ± 6.6	40.5 ± 6.6
CRF	18.9 ± 1.8[b]	34.2 ± 3.2[b]	38.9 ± 3.5
Lactate Dehydrogenase			
CTL	11.5 ± 1.9	45.7 ± 8.5	55.9 ± 8.3
CRF	11.9 ± 1.9	39.8 ± 6.7	54.4 ± 7.5
Fructose-1,6-Bisphosphatase			
CTL	0.065 ± 0.014	0.139 ± 0.021	0.173 ± 0.021
CRF	0.051 ± 0.005[b]	0.077 ± 0.009[b]	0.105 ± 0.018[b]
Phosphoglucomutase			
CTL	15.8 ± 1.9	23.4 ± 2.7	27.2 ± 3.3
CRF	16.1 ± 1.7	23.3 ± 2.7	27.6 ± 3.0
Glycogen Phosphorylase			
CTL	2.55 ± 0.50	4.12 ± 0.83	4.68 ± 0.93
CRF	2.41 ± 0.18	4.15 ± 0.24	4.76 ± 0.47

[a] Mean ± SE. CTL, control subjects; CRF, patients with chronic renal failure. Activities are expressed in moles per kilogram dry weight per hour at 23°C.
[b] CRF significantly different from CTL; $P < 0.05$.

TABLE 2. Activities of enzymes of high-energy phosphate, oxidative, and amino acid metabolism in single muscle fibers of normal subjects and patients with chronic renal failure[a]

Enzyme	Single Fibers		
	Slow-Twitch Oxidative	Fast-Twitch Oxidative-Glycolytic	Fast-Twitch Glycolytic
Adenylate Kinase			
CTL	43.6 ± 3.8	110 ± 5	176 ± 7
CRF	43.3 ± 2.1	102 ± 5	183 ± 6
Creatine Kinase			
CTL	494 ± 35	563 ± 34	620 ± 41
CRF	520 ± 27	604 ± 27	666 ± 37
β-Hydroxyacyl-CoA Dehydrogenase			
CTL	7.89 ± 0.79	5.94 ± 0.70	4.09 ± 0.38
CRF	5.08 ± 0.45[b]	3.82 ± 0.33[b]	2.78 ± 0.21[b]
Citrate Synthase			
CTL	3.24 ± 0.36	2.95 ± 0.42	2.10 ± 0.34
CRF	2.14 ± 0.17[b]	1.67 ± 0.17[b]	1.24 ± 0.09[b]
Aspartate Aminotransferase			
CTL	10.3 ± 1.2	8.4 ± 1.3	6.1 ± 1.4
CRF	8.8 ± 1.2[b]	6.8 ± 0.8[b]	5.2 ± 0.7[b]
Alanine Aminotransferase			
CTL	0.344 ± 0.095	0.265 ± 0.059	0.187 ± 0.043
CRF	0.204 ± 0.045[b]	0.170 ± 0.030[b]	0.155 ± 0.033[b]

[a] Mean ± SE. Abbreviations are the same as in Table 1. Activities are expressed in the same units as in Table 1.
[b] CRF significantly different from CTL; $P < 0.05$.

twitch oxidative-glycolytic fibers and in phosphofructokinase activity in slow-twitch oxidative fibers.

Enzymes of Glycogen Metabolism

The data presented in Table 1 also show that the activity of the three enzymes of glycogen metabolism studied was higher in fast-twitch than in slow-twitch fibers. Neither the activity of glycogen phosphorylase nor that of phosphoglucomutase in fibers of patients with chronic renal failure was different from those in fibers of normal subjects. By contrast, when compared with that in the corresponding fibers of control subjects, the mean activity of fructose-1,6-bisphosphatase, an enzyme considered to contribute to the synthesis of glycogen from lactate, fell by 21% in slow-twitch oxidative, 45% in fast-twitch oxidative-glycolytic, and 39% in fast-twitch glycolytic fibers of the patients.

Enzymes of High-Energy Phosphate, Oxidative, and Amino Acid Metabolism

As can be seen in Table 2, the activities of adenylate kinase and of creatine kinase were altered in none of the fiber types by chronic renal failure. The most striking changes caused by chronic renal failure in all fiber types were the 32 to 43% decreases in the activities of citrate synthase, the enzyme which initiates the tricarboxylic acid cycle, and of β-hydroxyacyl-CoA dehydrogenase, a key enzyme of β-oxidation of fatty acids. Depending on the fiber types, chronic renal failure also induced a 15 to 40% reduction of the activities of aspartate and alanine aminotransferases.

DISCUSSION

The fact that the enzymatic activities measured in this study in individual fibers of normal subjects (Tables 1 and 2) are in the same ranges and display the same distribution between different fiber groups as those found by other authors (17,18) who developed and used the analytical techniques used in our laboratory indicates the reliability of our results. It should be emphasized that, in this study, the measurement of enzyme activities in individual human muscle fibers appears fully justified because this allowed us to identify changes in certain enzyme activities of specific fiber types that would have been blunted or masked in muscle homogenates of uremic patients. Another important point to consider is whether or not the observations obtained from the rectus abdominis muscle also apply to other muscles. We feel that this is indeed the case for several reasons: (*1*) the percentages of fibers of different types found in the rectus abdominis muscle are in the same ranges as those in other skeletal muscles (17); (*2*) as mentioned above, the enzyme activities are also very similar to those found in other muscles such as the biceps or vastus lateralis (17,18); (*3*) the rectus abdominis muscle contributes not only to maintain the abdomi-

nal pressure and the posture but also, like other muscles, to maintain the locomotion (25).

The data obtained clearly indicate that chronic renal failure induces changes in the maximum activities of a number of key enzymes of energy metabolism. Therefore, the impairment of energy metabolism in muscle of patients suffering from chronic renal failure is due, at least in part, to intrinsic muscular disturbances and not solely to potentially important factors such as insufficient blood flow (6) or diminished oxygen supply secondary to anemia (7,29).

One of the most important findings of this study is that the maximum activities of citrate synthase and β-hydroxyacyl-CoA dehydrogenase are drastically reduced in all fiber types in patients with chronic renal failure (Table 2). Because these enzymes catalyze key steps in the tricarboxylic acid cycle and the β-oxidation of fatty acids, two major mitochondrial energy-providing pathways in muscle, our results are compatible with and offer, at least in part, a satisfactory explanation for the observation made by NMR spectroscopy in our and two other laboratories that, in patients with chronic renal failure, muscle oxidative metabolism is impaired (3–5). However, our results are in disagreement with the findings of Bradley *et al.* (6) and Barany *et al.* (30), who found an increased mitochondrial oxidative capacity; this discrepancy may be because our patients were in a predialytic state, whereas the patients studied by the latter authors had undergone hemodialysis and adaptation to chronic anemia for long periods (6,30).

Another important observation made in this study is that, in all fiber groups, chronic renal failure did not alter the activity of creatine kinase (Table 2); thus, the delayed postexercise recovery of phosphocreatine observed in the muscle of these patients by NMR spectroscopy (4,5) cannot be ascribed to a reduced activity of creatine kinase but is rather due to an insufficient provision of ATP at the level of this enzyme.

Our data show that two key enzymes of glycolysis, phosphofructokinase and pyruvate kinase, are less active mainly in the slow-twitch oxidative fibers of the patients with chronic renal failure when compared with the control subjects (Table 1). From these results, one could be tempted to conclude that, during exercise, glycolysis is diminished in the muscle of patients with chronic renal failure; such a conclusion would contradict our own results based on NMR spectroscopy measurements, which suggested that anaerobic glycolysis is stimulated in the muscle of such patients (4). Further studies are clearly needed to establish whether or not, in the muscle of patients with chronic renal failure, the moderate reduction of the activity of phosphofructokinase and/or pyruvate kinase is accompanied by a diminished production and accumulation of lactic acid. If one accepts that glycolysis is reduced in the muscle of patients with chronic renal failure, our observation that aspartate aminotransferase activity, which plays an important role in the transport of reducing equivalents into the mitochondrial compartment, is also diminished in all fiber groups (Table 2) is consistent with a reduction of the need to transport glycolysis-derived reducing equivalents, at least in slow-twitch oxidative and fast-twitch oxidative-glycolytic fibers, in which the activity of one or two key enzymes of glycolysis was found to be decreased.

Another interesting observation made in this study is that the activity of fructose-1,6-bisphosphatase, which allows the synthesis of glycogen from lactate in muscle, was diminished in patients with chronic renal failure. Because it has been demonstrated that the muscular content of glycogen is unchanged in this pathologic state (31,32) and because we found in this study no change in the activity of muscular glycogen phosphorylase, the enzyme initiating the degradation of glycogen, this suggests that the decrease in glycogen synthesis from lactate was compensated by an increased conversion of glucose into glycogen; consistent with this view are (*1*) the finding that the activity of glycogen synthase is stimulated in the muscle of patients with chronic renal failure (32) and (*2*) our tentative suggestion, on the basis of enzymatic measurements, (see above) that, in uremic patients, the glucose-6-phosphate synthesized from glucose by an unchanged activity of hexokinase might be directed in increased amounts toward the synthesis of glycogen as a result of an inhibition of key glycolytic enzymes such as phosphofructokinase and pyruvate kinase.

Finally, the diminished activity of alanine aminotransferase found in all fiber types of patients with chronic renal failure is somewhat surprising because this enzyme is considered to play a central role in the synthesis of the alanine released by muscle; because, besides glutamine, alanine is one of the major amino acids exported by muscle (33) and because chronic renal failure is known to stimulate proteolysis, which leads to an increased release of amino acids that are in part converted into alanine (33), one would have expected an increased activity of alanine aminotransferase. Thus, the functional significance of the diminished activity of alanine aminotransferase in the muscle of uremic patients remains to be established.

In conclusion, this study clearly demonstrates that, in all fiber types of human muscle, chronic renal failure leads to a large reduction of the activities of two key enzymes of oxidative metabolism, an observation that helps to explain why, at the whole-organ level, the latter metabolism appears to be impaired when explored by NMR spectroscopy in this pathologic state. Other enzyme activity measurements suggest that, in certain muscular fiber types of uremic patients, the glucose-6-phosphate synthesized by hexokinase might be used in increased amounts for glycogen synthesis at the expense of glycolysis. Whether the latter changes in glucose metabolism indeed occur and, if so, the extent to which they occur and their pathophysiologic significance should be experimentally tested by other methods in patients with chronic renal failure.

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