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Research Article

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Metabolic Abnormalities in Skeletal Muscle of Patients Receiving Zidovudine Therapy Observed by ^{31}P In Vivo Magnetic Resonance Spectroscopy

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Abstract

Patients on long-term zidovudine (AZT) therapy experience muscle fatigue and weakness attributed to AZT-induced mitochondrial toxicity in skeletal muscle. To determine if the clinico–pathological abnormalities in these patients correspond to abnormal muscle energy metabolism, we used ^{31}P in vivo magnetic resonance spectroscopy to follow phosphorylated metabolites during exercise. We studied 19 normal volunteers, 6 HIV-positive patients never treated with AZT, and 9 HIV-positive patients who had been treated with AZT for a mean period of 33 mo (range 12–48 mo) and had muscle biopsy–proven AZT-myopathy with abnormal mitochondria. Changes in phosphocreatine, ATP, and intracellular pH in the gastrocnemius muscle were followed during a graded steady state exercise protocol, and the recovery of phosphocreatine was followed on cessation of exercise. We found that graded steady state exercise produced a greater depletion of muscle phosphocreatine levels in the AZT-treated patients, compared to either HIV-positive patients who were not treated with AZT or normal controls. No differences in the effects of steady state exercise on muscle phosphocreatine levels were observed between the control group and the HIV-positive patients who had not been treated with AZT. The results suggest that the effect of AZT on muscle energy metabolism is significant, and similar to the effect observed in patients with known mitochondrial myopathies. Using a well-known model for control of mitochondrial metabolism, the observed differences in steady state phosphocreatine levels during exercise suggest that AZT treatment decreases the maximal work output and the maximal rate of muscle ATP synthesis. (*J. Clin. Invest.* 1995. 96:126–131.) Key words: HIV infection • mitochondrial myopathy • gastrocnemius muscle • exercise • phosphocreatine

Introduction

Zidovudine (AZT),[1] a nucleoside analogue introduced in 1986 for the treatment of patients infected with the HIV-1 virus, can cause a myopathy (1). Histological, ultrastructural, biochemical, and molecular studies in humans and experimental animals have suggested the myopathy induced by AZT is due to a mitochondrial toxicity (1–8). The myopathic symptoms often begin after the first 6 mo of therapy and include fatigue, muscle weakness, and elevation of muscle enzymes. These symptoms occur in at least 17% of patients and are often reversible when AZT therapy is discontinued. The diagnosis is established by muscle biopsy, which on enzyme histochemistry shows characteristic ragged-red muscle fibers by modified Gomori trichrome staining (1, 2), and accumulation of neutral lipid droplets (4, 5). Ubiquitous abnormalities in muscle mitochondrial structure are always identified by electron microscopy (3).

The ability of working muscle to meet changes in metabolic demand can be evaluated in vivo using phosphorus magnetic resonance spectroscopy (^{31}P MRS) (9–14). For example, ^{31}P MRS can be used to follow changes in phosphorylated metabolites, e.g., ATP, phosphocreatine (PCr) and inorganic phosphate (P_i), as well as intracellular pH, during exercise. A number of studies have used ^{31}P MRS to follow decreases in PCr, or increases in P_i/PCr, during graded steady state exercise (15–20). Other studies have followed the recovery of PCr or P_i/PCr after a sustained exercise period (21–26). These studies have clearly documented metabolic abnormalities in patients with various hereditary mitochondrial myopathies (18, 22, 23, 25–27).

In the present study we used ^{31}P MRS to follow metabolic changes in the gastrocnemius muscle during graded steady state exercise and recovery from sustained exercise. The major goal of this work was to determine if the metabolic changes observed during exercise in patients with biopsy-confirmed AZT-myopathy were similar to the abnormal responses observed in patients with hereditary mitochondriopathies. Our results were compared to those observed in two previous ^{31}P MRS exercise studies of AZT-induced myopathy, which had shown apparently contradictory findings (28, 29).

Methods

Subject selection. The following two groups, identified from referrals to the Neuromuscular Diseases Section, National Institute of Health, were studied. (*a*) HIV-infected patients who were taking AZT (HIV/+AZT); and (*b*) HIV-infected patients who had not been exposed to AZT (HIV/−AZT). In addition, healthy, age-matched normal controls were studied concurrently. Patients were excluded if they had symptomatic pulmonary, cardiac, or other systemic illnesses and clinical evidence of neuropathy or peripheral vascular disease. They were also excluded if they had any weakness detectable in the calf muscle which performed the ^{31}P MRS exercise studies, as described later, or any contraindications for being in a strong magnetic field. A total of 34 male subjects enrolled into the study. The protocol was approved by the Institutional Review Board of the National Institute of Neurological Diseases and Stroke, and all subjects signed an informed consent form explaining the details

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1. *Abbreviations used in this paper:* AZT, zidovudine; MR, magnetic resonance; PCr, phosphocreatine; P_i, inorganic phosphate; ^{31}P MRS, phosphorus magnetic resonance spectroscopy.

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of the exercise protocol. None of the subjects had performed fatiguing exercise in the 24 h preceding the ^{31}P MRS procedure.

Myopathic symptoms were rated as mild if the patient had only myalgia and minimal muscle weakness; moderate if proximal muscle strength in the arms or legs were rated 4+/5 on the Medical Research Council (MRC) scale, but the patient was independent in performing daily activities; and severe if the strength in certain proximal muscle groups was rated as 3+ to 4−/5 on the MRC scale and the patient needed assistance with walking or carrying out daily activities. All patients with myopathic symptoms had muscle biopsies taken within a month of the MRS studies.

The 34 subjects enrolled in the study were divided into three groups:

(a) HIV/+AZT. The nine patients with a myopathy which developed while being treated with AZT were identified from a group of patients referred to our institution. Patients with symptoms of myopathy and histological evidence for a metabolic myopathy were included in the study, while patients with predominantly inflammatory myopathy were excluded. The average age and weight of these subjects were 35±6 yr and 72±10 kg, respectively. The average duration of HIV-positive status was 68±38 mo. Four of these subjects had AIDS-defining illnesses, which were well controlled, and these subjects showed no systemic manifestations at the time of the study. The average $CD4^+$ count for the subjects with AIDS-defining illnesses was 120±100 cell/mm^3. The average $CD4^+$ count for the remaining five subjects was 500±170 cells/mm^3.

All subjects in this group were undergoing AZT therapy at the time of the study. The average duration of therapy was 33±13 mo, and the average total AZT dose was 8.2±4.2 gram/kg. All subjects displayed clinical myopathic symptoms: two showed severe symptoms, six showed moderate symptoms, and one showed mild symptoms (see above). Enzyme histochemical studies revealed ragged-red fibers and lipid accumulation (1–5) as the predominant pathological finding in all subjects. Seven subjects had 1.5- to 7-fold elevations of serum creatine kinase (CK) levels, and five had elevated serum lactate levels (4.9±3.5 mM).

(b) HIV/−AZT. The six patients in this group were randomly selected from a group of patients participating in a prospective study to identify neurological complications of HIV infection and from referrals with HIV related myopathy who had not been exposed to AZT. The average age and weight of the subjects was 31±3 yr and 74±8 kg, respectively. None of the subjects had been exposed to AZT. The average duration of HIV-positive status was 55±30 mo, which was comparable to the HIV/+AZT group. None of the subjects had AIDS-defining illnesses, and the average $CD4^+$ count was 730±270 cell/mm^3. Two of the subjects displayed clinical evidence of myopathy: one showed moderate symptoms, and one showed mild symptoms (see above). Muscle biopsies on the two symptomatic subjects showed mild inflammation, but no ragged-red fibers. None of the subjects had elevated creatine kinase or lactate levels.

(c) Controls. This group contained 19 age-matched male normal volunteers. The average age of the subjects was 33±7 yr and their average weight was 78±9 kg.

The maximum calf muscle cross-sectional areas for the three subject groups were 73±19, 75±10 and 76±7 cm^2, respectively. No statistical differences in maximal cross-sectional area were observed between the three subject groups using ANOVA.

Exercise protocol. Subjects lay in a supine position in the magnet bore, with the right leg resting on an elevated platform containing the radio-frequency "probe;" described later. The leg was positioned so that the belly of the gastrocnemius muscle, i.e., the region of the leg that contained the maximum cross-sectional area, lay directly on the probe. In this position, any contribution from the smaller soleus muscle, although unlikely, cannot be excluded. The leg was loosely strapped in place, and the foot was secured to a pedal. A free-standing weight outside of the magnet was attached, through a system of pulleys, to the heel of the pedal.

Exercise consisted of plantar flexions against the free-standing weight at a repetition time of one flexion per second. During the plantar flexions the weight was raised by a distance of 3.2 cm. The initial weight load was 2.3 kg, and was incremented by 2.3 kg every 5 min. Subjects were asked to continue through four weight loads, or to the point of fatigue. At this point exercise was terminated, and the PCr recovery monitored.

MRS protocol. All MRS studies were performed with a magnetic resonance (MR) system (1.5 T Signa; General Electric Co., Wilmington, MA). ^{1}H MR images (TE = 12 ms, TR = 500 ms) were acquired to confirm the placement of the muscle and to determine the maximum cross-sectional area of the muscle.

The radio frequency "probe" was composed of three single-turn surface coils: a 12-cm diameter "proton-receive" coil, a 4.5-cm diameter phosphorus-transmit coil, and a 3.8-cm diameter phosphorus-receive coil. Shimming was performed on the water signal acquired from the proton-receive coil. During the exercise protocol ^{31}P MR spectra were acquired from the phosphorus-receive coil using a repetition time of 1 s. The pulse width was adjusted to optimize the phosphocreatine signal observed under these conditions. 30 free induction decays were collected in 30 s. The total time for the acquisition and storage of each 30-s block of free induction decays was 40 s. Each block of 30 free induction decays was transformed to produce a single ^{31}P MR spectra. Seven "resting" ^{31}P MR spectra were acquired before exercise began, seven ^{31}P MR spectra were acquired during each exercise level, and at least nine ^{31}P MR spectra were acquired during recovery.

Data analysis. An exponential filter (10 Hz) was applied to all free induction decays. A commercial software package that calculated the amplitude of the PCr and ATP signals, and the chemical shift of the inorganic phosphate signal (New Methods Research, Inc., Syracuse, NY) was used for spectral analysis. For the graded exercise protocol, the last four spectra taken at each weight load were summed to provide a composite steady state spectrum. The data from the highest weight load was not included in the steady state analysis if the subject did not exercise the full 5 min at that weight load.

The relative PCr was estimated from the ratio of the amplitude of the phosphocreatine signal in the steady state composite ^{31}P MR spectra acquired at different weight loads, to the amplitude of the phosphocreatine signal in the resting composite ^{31}P MR spectrum. A similar normalization procedure was followed to estimate the relative ATP. This procedure was justified by the observation that the linewidths of the phosphocreatine and the ATP signals did not change significantly during the graded exercise protocol (data not shown). Intracellular pH was estimated from the difference in the chemical shifts of the inorganic phosphate and phosphocreatine signals in the composite spectra (30).

The rate of PCr decrease as a function of weight load during steady state exercise, k, was estimated using a standard least-squares linear fit to the equation:

$$[\mathrm{PCr}\,(w)] = 100\% - k\,w, \tag{1}$$

where w is the steady state weight load. k was not calculated for subjects that did not complete at least two exercise levels.

Physiological interpretation of k. The average values of k for the different subject groups can be interpreted using a well-known model for the control of oxidative phosphorylation in isolated mitochondria (11, 12, 31). In this model, the rate of mitochondrial ATP production, v, is controlled by the free ADP concentration through a simple Michaelis–Menton relationship

$$v = \frac{V_{max}}{1 + K_{ADP}/[\mathrm{ADP}]} \tag{2}$$

where V_{max} is the maximal rate of ATP production, and K_{ADP} is the Michaelis constant for ADP. If the creatine kinase reaction is near equilibrium in the cytoplasm, [ADP] can be expressed as a function of the phosphocreatine concentration, [PCr], and Eq. 2 can be written as

$$v = \frac{V_{max}}{1 + K_1\,[\mathrm{PCr}]/([\mathrm{PCr}_{tot}] - [\mathrm{PCr}])}. \tag{3}$$

where $[\mathrm{PCr}_{tot}]$ is the sum of the creatine and phosphocreatine concentrations, $K_1 = K_{ADP}\,K_{ck}\,[\mathrm{H}]/[\mathrm{ATP}]$, and K_{ck} is the creatine kinase equilib-

Table I. Phosphocreatine Depletion during Steady State Exercise

	Level 1 (2.3 kg)	Level 2 (4.5 kg)	Level 3 (6.8 kg)	Level 4 (9.1 kg)	⟨k⟩ (PCr/kg)
					%
Controls	93.0±3.6 (n = 19)	86.7±4.4 (n = 19)	77.9±8.3 (n = 19)	62.4±12.9 (n = 12)	3.5±1.0 (n = 19)
HIV/−AZT	92.1±6.4 (n = 6)	87.6±6.4 (n = 6)	78.6±12.4 (n = 6)	67.8±15.8 (n = 5)	3.3±1.5 (n = 6)
HIV/+AZT	82.5±4.9*‡ (n = 9)	76.4±3.5*‡ (n = 5)	65.7±5.1* (n = 4)	54.6±7.3 (n = 4)	5.2±0.5*‡ (n = 5)

Values are means±SD. Relative PCr values (%) were determined from steady state ^{31}P MRS spectra at each exercise level, normalized to resting PCr values (100%). ⟨k⟩ is the average slope of the linear relationship between normalized steady state PCr values and exercise weight load. * Significant difference between HIV/+AZT group and control group ($P = 0.05$). ‡ Significant difference between HIV/+AZT group and HIV/−AZT group ($P = 0.05$).

rium constant (11, 12, 32). K_1 has been estimated to be ~ 0.6 (11, 12). Eq. 3 can be rearranged to give [PCr] as a function of v, and the initial slope of a plot of [PCr] as a function of v is given by the expression

$$\left[\frac{d[\mathrm{PCr}]}{dv}\right]_{v\to 0} = -\,[\mathrm{PCr_{tot}}]\,\frac{K_1}{V_{\max}} \tag{4}$$

We assume that the rate of ATP production is linearly dependent on the rate of external work performed by the muscle, w. This assumption is consistent with our observation that intracellular ATP levels are essentially unchanged during exercise (see Results). Under these conditions, k is given by the expression

$$k = \left[\frac{d[\mathrm{PCr}]}{dw}\right]_{w\to 0} = -\,[\mathrm{PCr_{tot}}]\,\frac{K_1}{W_{\max}}. \tag{5}$$

where W_{max} is the maximal work output of the muscle. Thus, the initial slope of a plot of [PCr] as a function of w is inversely proportional to the maximum work output, W_{max}.

Statistical methods. Average values of the normalized steady state PCr values for the three subject groups were compared at each weight load using ANOVA. The average values of k for the three subject groups were also compared using ANOVA. In both cases, the Games Howell "post hoc" analysis (33) was used to control for the effects of multiple comparisons.

At each weight load, relative ATP values and intracellular pH values for the three subject groups were compared using ANOVA and the Games Howell post hoc analysis. Within a specific subject group, relative [ATP] values and intracellular pH values at different weight loads were compared to the values observed in the resting state using ANOVA and Dunnett's post hoc procedure (34).

Single ^{31}P MR spectra acquired and stored in 40 s were used to analyze the [PCr] recovery data, using a monoexponential function. The time constants for the three subject groups were compared using ANOVA. The time constants for the HIV/+AZT and the HIV/−AZT groups were also compared using a one-tailed Students t test.

All statistical tests were performed using $P = 0.05$. Unless otherwise noted, results are given as mean ±SD.

Results

All of the HIV/+AZT patients completed the first exercise level, five completed the second level, and four completed the third and fourth levels. All of the HIV/−AZT patients and all of the controls completed the first three exercise levels and a large fraction completed the fourth level (Table I). Three control subjects and one HIV/−AZT patient completed a fifth exercise level, but the data for this level were not included in the analysis of the steady state PCr data.

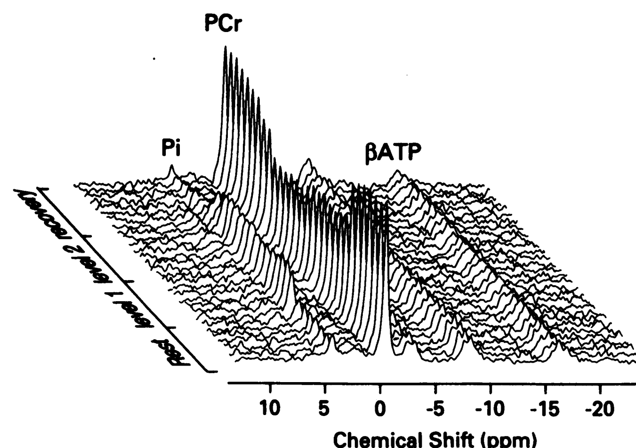

Figure 1. ^{31}P MR spectra from gastrocnemius muscle before, during, and after graded steady state exercise protocol (see text). Each spectrum was acquired in 30 s. This data was obtained from an HIV-positive patient undergoing AZT treatment, who was able to complete only two levels of exercise.

The major signals in the ^{31}P MR spectrum arise from phosphocreatine, inorganic phosphate, and the three (α, β and γ) phosphate groups in ATP. The typical response to the graded exercise protocol used is a drop in PCr, a rise in P_i, and no change in ATP. ^{31}P MR spectra obtained during the exercise protocol from an HIV-positive patient undergoing AZT treatment is shown as a stacked plot in Fig. 1.

A plot of the steady state relative PCr values (Table I) as a function of weight load is displayed in Fig. 2. The slope of a plot of PCr as a function of weight load is defined as k (see Eq. 1). ⟨k⟩ was significantly larger in the HIV/+AZT patient group, compared to the HIV/−AZT group and the control group. The ⟨k⟩ values of the HIV/−AZT group were not significantly different from that of the normal controls. Table I shows the values of ⟨k⟩, i.e., the average values of k, for the three subject groups.

The steady state intracellular pH values observed under resting conditions, and during each exercise level are shown in Table II. No significant differences in intracellular pH were observed among the three subject groups at rest, or at any of the exercise levels. Within each subject group, the intracellular pH observed during the first three exercise levels was not sig-

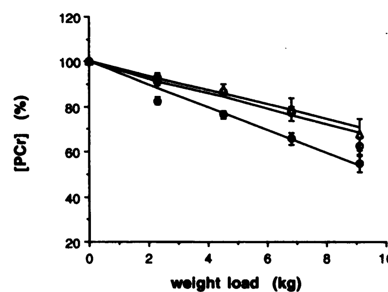

Figure 2. Relative phosphocreatine concentration, [*PCr*], in gastrocnemius muscle as a function of weight load during the graded steady state exercise protocol. Values are mean ±SEM. for the control group (*triangles*), the HIV/−AZT group (*white circles*), and the HIV/+AZT group (*black circles*). The solid lines are calculated from Eq. 1, using the values of ⟨k⟩ shown in Table I.

Table II. Intracellular pH and ATP during Steady State Exercise

	Rest	Level 1 (2.3 kg)	Level 2 (4.5 kg)	Level 3 (6.8 kg)	Level 4 (9.1 kg)
(*a*) pH					
Controls	7.04±0.02	7.04±0.02	7.03±0.03	7.01±0.04	6.94±0.10*
HIV/−AZT	7.06±0.02	7.08±0.06	7.07±0.04	7.02±0.06	6.96±0.17
HIV/+AZT	7.03±0.03	7.06±0.03	7.04±0.03	7.05±0.02	6.98±0.02*
(*b*) ATP					
Controls	100	99.8±6.9	98.4±7.5	95.7±6.2	93.3±10.9
HIV/−AZT	100	98.9±8.3	99.5±13.0	98.7±10.7	94.5±10.5
HIV/+AZT	100	96.2±7.4	97.3±2.7	85.7±9.3*	92.7±7.7

Values are means±SD. Intracellular pH values were determined from steady state ^{31}P MRS spectra. Relative ATP values (%) were determined from steady state ^{31}P MRS spectra, and normalized to resting ATP values (100%). * A statistically significant difference ($P = 0.05$) compared to the value obtained during the rest period.

Table III. Postexercise Recovery Times for Phosphocreatine

	Time constant (s)
Controls	45±15 (n = 17)
HIV/−AZT	40±12 (n = 6)
HIV/+AZT	60±19 (n = 7)

Values are means±SD.

nificantly different from the intracellular pH observed during resting conditions. However, for the control group and the HIV/+AZT group, the intracellular pH observed during the fourth exercise level was significantly lower (~ 0.08 pH U) than the intracellular pH observed during resting conditions.

No significant differences in relative ATP values were observed among the three subject groups at rest, or at any of the exercise levels. Within each subject group, the relative ATP values observed during the first two exercise levels were not significantly different from the relative ATP values observed during resting conditions. For the HIV/+AZT group, the relative ATP value observed during the third exercise level was significantly lower than the relative ATP value observed during resting conditions, but this change was not significant at the fourth exercise level. Table II shows the steady state relative ATP values as a function of weight load in the three groups studied.

The average time constants for the postexercise recovery of PCr for the three subject groups are shown in Table III. No significant differences were observed in the average time constants for the three groups using ANOVA. However, if only the HIV/+AZT and HIV/−AZT groups are compared (see Methods), the time constant for the HIV/+AZT group was significantly larger than the time constant for the HIV/−AZT group.

Discussion

The first aim of this study was to determine if AZT-induced myopathy is associated with abnormal muscle metabolism. Table I shows that, for the same steady state weight load, the decrease in muscle PCr is larger for the HIV/+AZT group, compared to either the HIV/−AZT group or the control group. Given the central role of phosphocreatine in muscle energy metabolism (11, 12, 14, 16, 24), these results demonstrate that long-term AZT therapy has a significant effect on muscle energy metabolism.

The simplest explanation for the observed effects of AZT on muscle energy metabolism is that AZT directly perturbs mitochondrial function. The differences in steady state PCr values at identical work loads can be interpreted in terms of a well-known model for control of mitochondrial metabolism (11, 12, 14). The model assumes that the dominant factor controlling mitochondrial ATP production is the free cytoplasmic [ADP], i.e.,

$$v = \frac{V_{max}}{1 + K_{ADP}/[ADP]}$$

where v is the rate of ATP production, V_{max} is the maximal rate of ATP production, and K_{ADP} is the effective Michaelis constant for ADP (11, 31). The free cytoplasmic ADP can be estimated from the cytoplasmic PCr (11, 12, 32), and the model can be used to relate the slope of plots of PCr against steady state muscle work to the maximal work output of the muscle, W_{max}. The model predicts that k, the initial slope of a plot of PCr against the steady state weight load, should be inversely proportional to the maximum work output, W_{max} (see Eq. 5). Within the context of this model, the results shown in the last column of Table I indicate that the maximum muscle work output is significantly reduced for HIV patients that have been treated with AZT, compared to HIV patients that have not been treated with AZT or controls.

One possible explanation for a reduced maximum work output for HIV-positive patients treated with AZT could be a smaller muscle mass. This explanation is not supported by the observation that the maximal leg muscle cross-sectional areas are not significantly different for the HIV/+AZT group, the HIV/−AZT group, or the control group. Another explanation for a reduced maximum work output could be an indirect effect of AZT on muscle metabolism, e.g., through reduced blood flow or reduced neuromuscular activation during exercise. However, this explanation is not consistent with the observation that AZT treatment increased the PCr recovery time in HIV patients (see below).

The maximum work output, W_{max}, and the maximal rate of ATP production, V_{max}, are closely related. For the low-level, graded exercise protocols used in the present study, intracellular ATP levels are essentially unchanged (Table II), and the steady state work performed by the muscle should be proportional to the rate of ATP production. Under these conditions, W_{max} should be proportional to V_{max}.

The treatment of the experimental data in a linear analysis of PCr against weight load, presents a realistic approach for comparing steady state ^{31}P MR exercise data taken with control subjects and different patient groups. This has been a widely used approach by investigators in this field who have studied the responses of PCr (or P_i/PCr) to a graded work load protocol for controls and patients (18, 27, 35). As observed previously, although direct verification is not possible, the Michaelis constants are expected to remain the same for controls and patients (25) in the use of the model discussed above (see Methods).

The recovery of PCr after cessation of exercise is believed to occur through oxidative pathways (36), and the exponential

time constant for PCr recovery has been used to estimate the maximal rate of mitochondrial ATP synthesis, V_{max} (25, 37, 38). While the average time constant for PCr recovery was larger for HIV/+AZT patients compared to HIV/−AZT patients or controls (Table III), this difference was not statistically significant using an ANOVA analysis of all three groups. However, if only the HIV/+AZT group and the HIV/−AZT groups are compared, the time constant for the HIV/+AZT group was significantly larger than the time constant for the HIV/−AZT group (see Results). This analysis suggests that AZT has a significant effect on the PCr recovery time compared to non-AZT-treated HIV-positive patients, but it does not allow a comparison of the PCr recovery times for the HIV/−AZT group and the control group.

The second aim of this study was to ascertain if AZT-induced abnormalities in muscle metabolism observed with ^{31}P MR spectroscopy were consistent with abnormalities observed with known mitochondrial myopathies. ^{31}P MRS exercise studies have shown that, on average, patients with known mitochondrial myopathies have a larger decrease in PCr (or increase in P_i/PCr) than control subjects for a given steady state exercise level (18, 22, 26, 27). Also, postexercise recovery times of PCr tend to be longer for patients with known mitochondrial myopathy than control subjects (22, 23, 25–27). The changes in the steady state PCr levels, and the PCr recovery times observed with AZT-treated HIV patients are thus consistent with observations on patients with known mitochondrial myopathies.

^{31}P MRS studies have also shown that intracellular pH values of patients with known mitochondrial myopathies are not significantly different from those observed for normal controls (39). Furthermore, after vigorous exercise, intracellular pH values for mitochondrial myopathies are either not significantly different (26) or slightly larger (27) than those observed for normal controls. The lack of significant differences in intracellular pH observed for the HIV/+AZT, HIV/−AZT, and control groups, either during rest or during moderate steady state exercise, are thus consistent with observations on patients with known mitochondrial myopathies.

The ^{31}P MRS changes observed in our patients is the direct effect of AZT on muscle, as documented by the histological findings on the muscle biopsies performed on the studied patients. These changes were consistent with mitochondrial abnormalities and were noted only in patients treated with AZT and not in the HIV-infected patients, with or without myopathy, who have never been exposed to AZT. Ubiquitous mitochondrial abnormalities, as severe as those noted in the present patients, are seen in the muscle biopsies of AZT-treated patients who experience fatigue but have normal muscle strength, regardless of CD4 counts (40). These patients have similar abnormalities by ^{31}P MRS (41), indicating that ^{31}P MRS can identify changes in muscle energy metabolism early and before the development of overt clinical signs of muscle weakness. This is also in accord with the experience of Weissman et al. (28) who demonstrated ^{31}P MRS changes consistent with a mitochondriopathy in patients treated with AZT, even though only one of their studied patients had clinical signs of myopathy. Miller et al. (29) also used ^{31}P MRS of skeletal muscles to study a group of HIV-infected patients. Although they found no differences between HIV-infected patients and normal controls the design of their study did not permit the evaluation of the effects of AZT on the muscle.

In conclusion, our ^{31}P MRS exercise studies demonstrate that AZT treatment alters normal muscle energy metabolism in HIV-positive patients with a myopathy. The observed alterations in muscle metabolism correlate with pathological findings on muscle biopsy that revealed histological abnormalities of mitochondria. They are also consistent with the known effects of AZT to cause mitochondrial toxicity in vivo and in vitro, mitochondrial uncoupling, reduction in cytochrome activity, depletion of mitochondrial DNA (1–8), and impaired oxidative phosphorylation (OXPHOS) (42), the main source of mitochondrial energy. Our results are also consistent with previous ^{31}P MRS exercise studies of patients with known mitochondrial myopathies and suggest that AZT treatment decreases the maximal work output, and thus the maximal rate of mitochondrial ATP synthesis, in human gastrocnemius muscle.

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