Mitochondrial function and oxygen supply in normal and in chronically ischemic muscle: A combined ^{31}P magnetic resonance spectroscopy and near infrared spectroscopy study in vivo

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Purpose: **We used ^{31}P magnetic resonance spectroscopy (MRS) and near-infrared spectroscopy (NIRS) as a means of quantifying abnormalities in calf muscle oxygenation and adenosine triphosphate (ATP) turnover in peripheral vascular disease (PVD).**

Methods: **Eleven male patients with PVD (mean age, 65 years; range, 55-76 years) and nine male control subjects of similar age were observed in a case-control study in vascular outpatients. Inclusion criteria were more than 6 months' calf claudication (median, 1.5 years; range, 0.6-18 years); proven femoropopliteal or iliofemoral occlusive or stenotic disease; maximum treadmill walking distance (2 km/h, 10° gradient) of 50 to 230 m (mean, 112 m); ankle-brachial pressure index of 0.8 or less during exercise (mean, 0.47; range, 0.29-0.60). Exclusion criteria included diabetes mellitus, anemia, and magnet contraindications.**

Simultaneous ^{31}P MRS and NIRS of lateral gastrocnemius was conducted during 2 to 4 minutes of voluntary 0.5 Hz isometric plantarflexion at 50% and 75% maximum voluntary contraction force (MVC), followed by 5 minutes recovery. Each subject was studied three times, and the results were combined.

Results: **Compared with control subjects, patients with PVD showed (1) normal muscle cross-sectional area, MVC, ATP turnover, and contractile efficiency (ATP turnover per force/area); (2) larger phosphocreatine (PCr) changes during exercise (ie, increased shortfall of oxidative ATP synthesis) and slower PCr recovery (47% ± 7% [mean ± SEM] decrease in functional capacity for oxidative ATP synthesis, $P = .001$); (3) faster deoxygenation during exercise and slower postexercise reoxygenation (59% ± 7% decrease in rate constant, $P = .0009$), despite reduced oxidative ATP synthesis; (4) correlation between PCr and NIRS recovery rate constants ($P < .02$); and (5) correlations between smaller walking distance, slower PCr recovery, and reduced MVC ($P < .001$). The precision of the key measurements (rate constants and contractile efficiency) was 12% to 18% interstudy and 30% to 40% intersubject.**

Conclusion: **The primary lesion in oxygen supply dominates muscle metabolism. Reduced force-generation in patients who are affected more may protect muscle from metabolic stress. (J Vasc Surg 2001;34:1103-10.)**

In peripheral vascular disease (PVD), morbidity resulting from claudication is relatively common and not easy to treat.[1] The objective assessment of impaired muscle function is difficult, with the most practical method being the maximum treadmill walking distance (MWD). Although the basic cause is insufficient blood supply to exercising muscle, the correlation between blood flow, symptoms, and exercise ability is poor,[2] which might reflect either helpful metabolic adaptation or an acquired myopathy,[1] perhaps caused by oxidative stress.[3] These questions are difficult, although not impossible,[4-6] to study with invasive methods. Two useful noninvasive methods are ^{31}P magnetic resonance spectroscopy (^{31}P MRS), which is a means of measuring aspects of adenosine triphosphate (ATP) turnover,[7-9] and near-infrared spectroscopy (NIRS), which is a means of measuring muscle oxygenation.[10] Both ^{31}P MRS[8,11-16] and NIRS[17-19] have shown muscle abnormalities in PVD. We set out to make both measurements simultaneously, to clarify the pathophysiology and thereby develop a method for assessing possible treatment mechanisms.

METHODS

Subjects

We observed 11 male patients with PVD and nine male control subjects (Table I). Inclusion criteria included 6 months' or more intermittent calf claudication with proven femoropopliteal or iliofemoral occlusive or stenotic disease; MWD less than 230 m (2 km/h, 10° gradient); and an

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Table I. Characteristics of patients and control subjects

	Control subjects		*Patients with PVD*	
	Mean ± SD	*Range*	*Mean ± SD*	*Range*
Age (y)	58 ± 7	51-71	65 ± 8	55-76
Median duration of symptoms (y)	—	—	1.5	0.6-18.0
Body weight (kg)	81 ± 13	61-96	78 ± 15	57-108
Body mass index (kg/cm^2)	26 ± 5	19-32	16 ± 4	20-31
Systolic blood pressure (mm Hg)	130 ± 16	109-160	154 ± 26	130-190
Diastolic blood pressure (mm Hg)	81 ± 11	60-95	85 ± 18	60-120
MWD (m)	—	—	112 ± 62	53-230
Rest ABPI	—	—	0.73 ± 0.18	0.35-1.00
Exercise ABPI	—	—	0.47 ± 0.10	0.29-0.60

Systolic blood pressure was 24 mm Hg higher, on average, in the patients ($P = .02$). Four patients and 1 control subject were taking antihypertensive medication; 6 control subjects and 10 patients were smokers or ex-smokers.
ABPI, Ankle brachial pressure index; MWD, maximum walking distance; *PVD,* peripheral vascular disease.

ankle-brachial pressure index of 0.8 or less (rest or exercise). Exclusion criteria were diabetes mellitus, anemia, or magnet contraindications. Each subject gave fully informed written consent, and the work was conducted with the approval of the Liverpool Research Ethics Committee, in accordance with the Declaration of Helsinki of the World Medical Association. Subjects were forbidden from smoking for 2 hours before each study. Each subject was studied on three occasions within 1 to 12 weeks (median, 5 weeks).

Magnetic resonance methods

In a Signa Advantage 1.5 tesla whole body MR system (General Electric, Milwaukee, Wis), subjects lay supine on a rig permitting isometric plantarflexion. Force measured by means of a strain gauge was fed back through a light-emitting diode display. Maximum voluntary force (MVC) was measured (in voltage units) three times before each study. An axial T_2-weighted spin echo image was obtained for measuring cross-sectional area. ^{31}P MRS data were acquired with a 10- by 6-cm elliptical RF surface coil over the right lateral belly of the gastrocnemius. NIRS data were acquired simultaneously from the same site with a Runman CES-2000 instrument (NIM, Philadelphia, Pa) with fiberoptic light guides passing through the RF coil. After B_0 field homogeneity was optimized by manually shimming on a water signal, ^{31}P spectra were obtained with 2 second repetition time at rest (128 scans) and then throughout an exercise/recovery protocol (8-scan spectra). Exercise was audibly cued voluntary 0.5 Hz isometric plantarflexion with 50:50 duty cycle. The protocol comprised 3 spectra at rest, 12 spectra during exercise at 50% MVC (3.2 minutes), followed by 20 recovery spectra (5.3 minutes); then 8 spectra during exercise at 75% MVC (2.1 minutes) followed by 20 recovery spectra (5.3 minutes); the second (75% MVC) exercise/recovery was then repeated. If exercise ended early because of the patient's fatigue or pain, recovery collection started early, but the next exercise started at the normal time.

Data acquisition and processing

Force and NIRS data were captured through a PowerLab ADI on a personal computer running Chart software (ADInstruments, Hastings, UK). The NIRS signal (digitized manually in the Chart software) represents the difference in absorption at two wavelengths, proportional to deoxygenated hemoglobin content.[10] Because the scaling factor varies unpredictably between studies, absolute changes were expressed relative to the difference between the extreme signals at the two wavelengths, so that deoxyhemoglobin content is in arbitrary units, but independent of instrument gain. (Scaling to maximum signal change in exhaustive ischemic exercise[10] was not tolerated by many subjects.)

The maximum cross-sectional area of the posterior calf (ie, soleus plus gastrocnemius) was measured by means of point-counting[20] with ANALYZE software (Mayo Foundation, Rochester, Minn). ^{31}P MRS data were analyzed with Magnetic Resonance User Interface software (MRUI 97.1), which implements the AMARES time-domain fitting algorithm. Resting spectra were fitted with 10 Lorentzian peaks: Pi, phosphodiester, phosphocreatine (PCr), 2 peaks (amplitude ratio 1:1) each for γ-ATP and α-ATP and 3 (amplitude ratio 1:2:1) for β-ATP. Exercise spectra were fitted with three Lorentzian peaks: Pi, PCr, and ATP (no significant changes in ATP were observed). Cell pH was obtained from the chemical shift of Pi relative to PCr[21]. Changes in PCr and derived quantities were expressed relative to resting PCr concentration to avoid problems of absolute quantification.

Data analysis

Background. In exercising muscle, the myosin adenosine triphosphatase (ATPase) does mechanical work with ATP generated either by glycolysis to lactate or (more efficiently) by full oxidation to CO_2; the creatine kinase equilibrium ensures that any ATP shortfall is met by net "splitting" of PCr[22]. In the current experiments, ATP

synthesis is largely oxidative (see the "Results" section), which simplifies the following explanation. At the start of exercise, ATP is supplied only by PCr splitting; then as oxidative ATP production increases, PCr concentration stabilizes.[7] At the same time, deoxyhemoglobin increases as oxygen consumption exceeds vascular oxygen supply and may also reach steady state. When exercise ceases, PCr recovers at the expense of oxidative ATP synthesis; because mitochondrial oxygen use declines faster than vascular oxygen supply, deoxyhemoglobin decreases.

Measuring overall shortfall in ATP production. The average rate of PCr change during exercise, the total PCr change ($-\Delta[PCr]$) divided by exercise duration (Δt), is a measure of the all-exercise average failure of oxidative ATP synthesis (strictly, oxidative plus glycolytic) to meet ATP demand.[23] In pathophysiologic terms, $-\Delta[PCr]/\Delta t$ will increase (ie, PCr change will increase, duration will decrease, or both) either because ATP demand is increased or because oxidative ATP supply is impaired.

Measuring ATP demand. The initial rate of fall of PCr in exercise ($-d[PCr]/dt$) is a measure of ATP turnover rate.[9,24] This measure divided by force output per unit cross-sectional area is a measure of contractile efficiency (reciprocal of contractile cost[9]), which measures how well ATP is used to generate force; this is a function of fiber type[25] and exercise mechanism.[26-28] $-d[PCr]/dt$ was calculated from a least-squares monoexponential fit of PCr against time. When fitted without constraints, the rates at 75% MVC and 50% MVC were linear with power, as expected[29-31]; so to improve precision, we refitted the data with this as a constraint and reported the rate for 50% MVC.

Measuring oxidative ATP synthesis. The initial-recovery rate of PCr resynthesis ($d[PCr]/dt$, calculated from a monoexponential fit) is an estimate of the end-exercise rate of oxidative ATP synthesis.[32,33] Dividing this by the ATP turnover rate in preceding exercise (as aforementioned) gives the end-exercise "fractional oxidation rate" (the fraction of ATP produced oxidatively). It will be seen (see "Results" section) that this is near unity and also that pH changes (and, therefore, lactate accumulation) are small, which independently suggest[34] that the exercise is largely oxidative.[7] Oxidative ATP synthesis is a function of three factors: mitochondrial volume and competence, oxygen supply, and the metabolic "driving force" (the change in, eg, ADP concentration[33,35]). The first two factors can be lumped as the "mitochondrial capacity," the notional maximum oxidative ATP synthesis rate; in the absence of large pH changes in exercise,[36,37] this is conveniently reflected by the postexercise PCr recovery rate constant (0.693/halftime).[7,35,38]

Measuring oxygenation kinetics. The average rate of NIRS fall in exercise (total NIRS change divided by exercise duration, $-\Delta$**NIRS**$/\Delta t$) is an overall measure of the rate at which oxygen supply falls short of oxygen demand. We also calculate the maximum rate of deoxygenation with sequential linear regression of time-binned NIRS data throughout exercise and express this relative to the rate of ATP turnover. Postexercise NIRS recovery reflects the rate at which oxygen supply exceeds the oxygen demand of recovering muscle; although unlike PCr, NIRS recovery is not necessarily truly monoexponential, the simplest measure is the halftime,[10,18,19] conveniently expressed as the notional rate constant (0.693/halftime).

Combining results from different exercise periods. Results from the two 75% MVC exercise periods were not significantly different and were combined. For brevity and clarity, we also combined data from the 50% and 75% MVC exercise periods wherever the physiology permits: ATP turnover rates are derived from constrained fits of exercise at both powers (as aforementioned). For average rates of change during exercise and for rates of deoxygenation, the appropriate combination is the weighted mean (ie, multiplying the 75% value by 50/75); for rate constants of NIRS recovery[39] and PCr recovery,[36,39] which are independent of power, results at 50% and 75% MVC are combined as a simple mean.

Assessing and reducing variability. All means of scaling exercise intensity have only limited ability to reduce intersubject variability in ATP turnover.[40] The effects of this are reduced by means of appropriate choice of data analysis methods: exercise changes will be expressed in terms of deviations from the resting state, corrected for ATP turnover; recovery rate constants are, to a first approximation, independent of end-exercise state.[36,39]

For variability to be measured, a coefficient of variation (CV = SD/mean%) was calculated for each quantity measured in the three studies in each subject, and its mean value across the group was taken as a measure of interstudy (intrasubject) variability. The CV of mean-of-three-study values across the group is a function of intersubject variability, but contains a contribution from purely interstudy (intrasubject) variability. True intersubject variability was, therefore, obtained by partioning the relative variances: $CV^2_{INTERSUBJECT} = CV^2_{OBSERVED} - (\frac{1}{3})CV^2_{INTERSTUDY}$, in which the factor ⅓ allows for the effect of the triplicate measurement in reducing the pure interstudy contribution. We also calculate the expected intersubject variability for a single measurement as: $CV^2_{EFFECTIVE} = CV^2_{INTERSUBJECT} + CV^2_{INTERSTUDY}$.

Other statistical methods. Results are presented as the mean ± SEM unless stated. Differences were assessed with the Student unpaired *t* test, and correlations were assessed with the Pearson correlation coefficient.

RESULTS

Resting muscle, force, and area. There were no significant differences between patients and control subjects in resting pH. Resting PCr/ATP and Pi/ATP were slightly increased in patients (Table II); the simplest interpretation,[8] consistent with biopsy evidence,[5,6] is that ATP concentration is decreased (by 12% ± 5%), with no change in PCr concentration.[8] There were no significant differences in MVC force or muscle area or the force per unit area (Table II).

Table II. Results

	Control subjects	*Patients with PVD*	P *value*	*Ratio (< 1)*
Resting MRS				
PCr/ATP	3.14 ± 0.09	3.38 ± 0.06	.03	0.92 ± 0.03
Pi/ATP	0.42 ± 0.03	0.50 ± 0.02	.04	0.83 ± 0.07
pH	7.027 ± 0.006	7.046 ± 0.007	.07	—
Force and area				
MVC force (arbitrary)	14 ± 2	11 ± 1	.2	—
Cross-sectional area (cm^2)	47 ± 2	45 ± 3	.6	—
Exercise duration (Δt)				
At 50% MVC (min)	3.01 ± 0.06	2.55 ± 0.15	.01	0.83 ± 0.06
At 75% MVC (min)	2.21 ± 0.09	1.72 ± 0.11	.5	0.76 ± 0.03
Rates and fluxes in exercise				
Initial ATP turnover (1/min)	0.59 ± 0.08	0.70 ± 0.11	.9	—
Contractile efficiency (1/[min.cm^2])	0.13 ± 0.01	0.11 ± 0.01	.6	—
Mean maximum NIRS rate/ATP turnover*	12 ± 2	24 ± 3	.0003	0.46 ± 0.07
End-exercise "fractional oxidation rate"				
At 50% MVC	0.90 ± 0.06	0.84 ± 0.07	.2	—
At 75% MVC	1.04 ± 0.09	0.72 ± 0.07	.003	0.69 ± 0.09
Total changes in exercise				
ΔpH–50% MVC	0.03 ± 0.03	0.14 ± 0.04	.03	—
ΔpH–75% MVC	–0.05 ± 0.01	–0.11 ± 0.03	.08	—
Mean Δ NIRS*	–7 ± 1	–14 ± 2	.01	0.48 ± 0.12
Mean ΔNIRS/Δt* (1/min)	–3.5 ± 0.7	–8.1 ± 1.2	.004	0.43 ± 0.11
Mean Δ[PCr]/Δt* (1/min)	–0.20 ± 0.04	–0.30 ± 0.05	.2	—
Mean ΔNIRS/Δ[PCr]	19 ± 2	30 ± 2	.003	0.65 ± 0.08
Recovery				
Mean NIRS rate constant (1/min)	1.02 ± 0.12	0.42 ± 0.06	.001	0.41 ± 0.07
Mean PCr rate constant (1/min)	2.08 ± 0.20	1.09 ± 0.09	.001	0.53 ± 0.07

Values are presented as mean ± SEM. Resting MRS ratios not corrected for saturation. "Mean" data are the mean of 50% MVC and 75% MVC exercise: for quantities that are turnover- or force-dependent (*), the mean is weighted for force ratio (ie, 75:50); otherwise, they are unweighted. ATP turnover is calculated simultaneously for both exercise intensities (see the "Methods" section). The *P* value equals the statistical significance (*t* test) of difference between patients and control subjects. If $P < .05$, the last column shows the size of the defect as the ratio of the result in patients to that in control subjects (mean ± SEM, with standard error-propagation analysis), or vice versa, whichever gives a value less than unity (to facilitate comparison of abnormalities in variables that increase or decrease in disease).

Exercise response. Control subjects finished early in 19% of exercise periods at 50% MVC and 39% of those at 75% MVC; corresponding figures for patients were 56% and 81%. Thus, exercise duration was significantly reduced at both 50% and 75% MVC (Table II). Control subjects falling short reported fatigue; patients generally reported typical claudication. Cell pH changes during exercise were small, being significantly nonzero only at 75% MVC (control subjects, $P = .008$; patients, $P = .001$) and significantly different between patients and control subjects only at 50% MVC (Table II).

Both NIRS signal (Fig 1, *A*) and PCr (Fig 1, *B*) fell during exercise, more at 75% than 50% MVC, and then recovered. Changes were larger and recovery was slower in patients than in control subjects, especially for NIRS. However, the initial rate of PCr depletion (ATP turnover rate) did not differ (Table II). Variability in force/area and ATP turnover was quite high, but these were correlated (control subjects, $r = 0.82$, $P = .007$; patients, $r = 0.80$, $P = .003$), and their ratio, the contractile efficiency, was not significantly different (Table II). Nevertheless, the overall rate of PCr fall was larger in patients (Table II) and correlated with ATP turnover (Fig 1, *C*); for a given ATP turnover rate, PCr changes were larger in patients. Thus, although ATP demand is the same, oxidative ATP production is reduced in patients, requiring more splitting of PCr. Consistent with this, the end-exercise fractional oxidation rate (Table II) at 75% MVC was lower in patients and significantly less than unity ($P = .003$).

Both the overall NIRS change and its overall rate were increased in patients (Table II). Both correlate with ATP turnover rate, but values are higher at given ATP turnover in patients (shown for overall rate in Fig 1, *D*). Similarly, the maximum rate of NIRS fall correlates with ATP turnover (control subjects, $r = 0.95$, $P = .0002$; patients, $r = 0.78$, $P = .004$), but is greater for a given ATP turnover in patients (Table II). NIRS changes are also larger compared with PCr changes in patients (ΔNIRS/Δ[PCr] in Table II). Thus, measured in several ways, NIRS changes in patients exceed what can be explained by ATP turnover.

Recovery kinetics. The recovery of PCr (Fig 2, *A*) and NIRS (Fig 2, *B*) was slower in patients after both 50% and 75% MVC exercise; the rate constants were reduced (Table II). Furthermore the PCr and NIRS rate constants correlate (Fig 2, *C*). Finally, the NIRS rate constant correlates with exercise duration ($r = 0.84$, $P = .001$); for PCr, the association falls short of statistical significance ($r = 0.44$).

Relationships to maximum walking distance. Fig 3 shows some relationships to MWD, expressed as a reciprocal to fit control subjects (with "infinite" MWD) on the

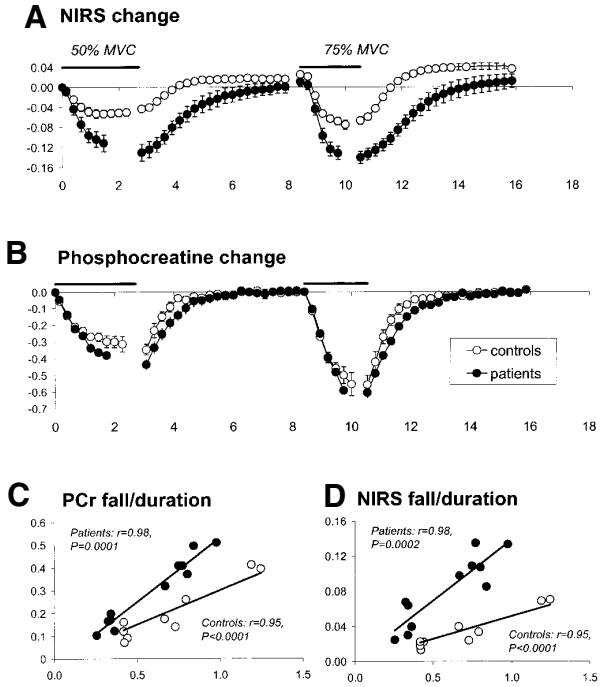

Fig 1. MRS and NIRS changes during exercise and recovery. **A,** Mean change in NIRS signal and **B,** mean change in PCr concentration, during periods of exercise at 50% MVC and 75% MVC (*horizontal lines*) and subsequent recovery are shown. Intersubject variation has been reduced by correcting for ATP turnover (see "Methods" section). These panels are a composite presentation of studies in which, especially for patients, exercise duration was often reduced; this is indicated by breaks in graphs. **C,** Average rate of PCr fall and **D,** average rate of NIRS fall are shown in individual subjects as function of ATP turnover rate. Results are the mean of three different studies, and results for 75% MVC combine two repetitions. In **C** and **D**, results are weighted means of 50% and 75% MVC exercise (see "Methods" section). Results are for patients (*solid circles*) and control subjects (*open circles*). Error bars show SEM.

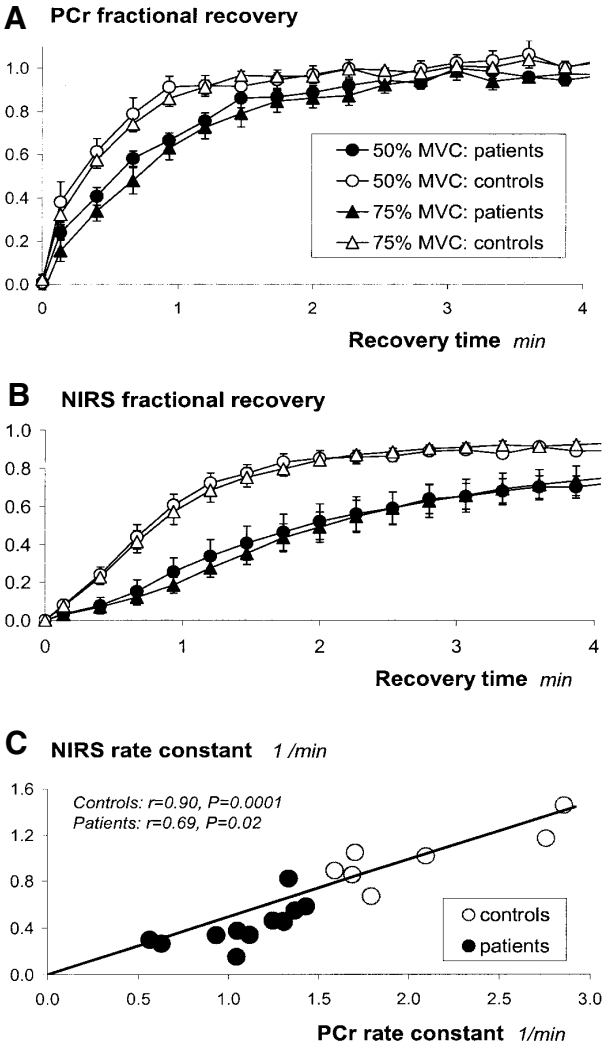

Fig 2. NIRS and MRS postexercise recovery kinetics. **A,** Fractional recovery of phosphocreatine and **B,** fractional recovery of NIRS signal, as a function of recovery time after 50% MVC and 75% MVC exercise (see key), are shown. **C,** PCr rate is shown constant as function of NIRS rate constant in individual subjects. Panel also shows regression line for control subjects only (for clarity), constrained through the origin. Results are the mean of three different studies, and results for 75% MVC combine two repetitions. In **C,** results are unweighted means of 50% and 75% MVC exercise (see "Methods" section). Results are for patients (*solid circles and triangles*) and control subjects (*open circles and triangles*). Error bars show SEM.

graph. Although there is no overall abnormality in force or contractile efficiency (Table II), the patients who were the clinically worst affected were the least effective at generating force (Fig 3, *A*) and, consequently, had lower ATP turnover rates and PCr changes. They also had less severely impaired PCr recovery rate constants (Fig 3, *B*), but there is no significant relationship to NIRS rate constant.

Variability. The three main "outputs" of this analysis are the contractile efficiency, the PCr recovery rate constant (measures mitochondrial function), and the NIRS recovery rate constant (measures "vascular function"). Their interstudy CV, which measures the precision of repeated measurements, was 12%, 17%, and 18%, respectively. Population variability is conveniently measured by means of the single-study intersubject CV (corrected for interstudy CV), which was 30%, 37%, and 40%, respectively (all CVs are expressed as the mean value for patients and control subjects).

DISCUSSION

Exercise protocol. This protocol has a number of advantages. Exercise at two powers improves the chance that changes will be large enough for precise measurement of recovery kinetics, and the multiple-exercise element improves precision. The dominant oxidative contribution suits it to assessment of mitochondrial ATP synthesis in

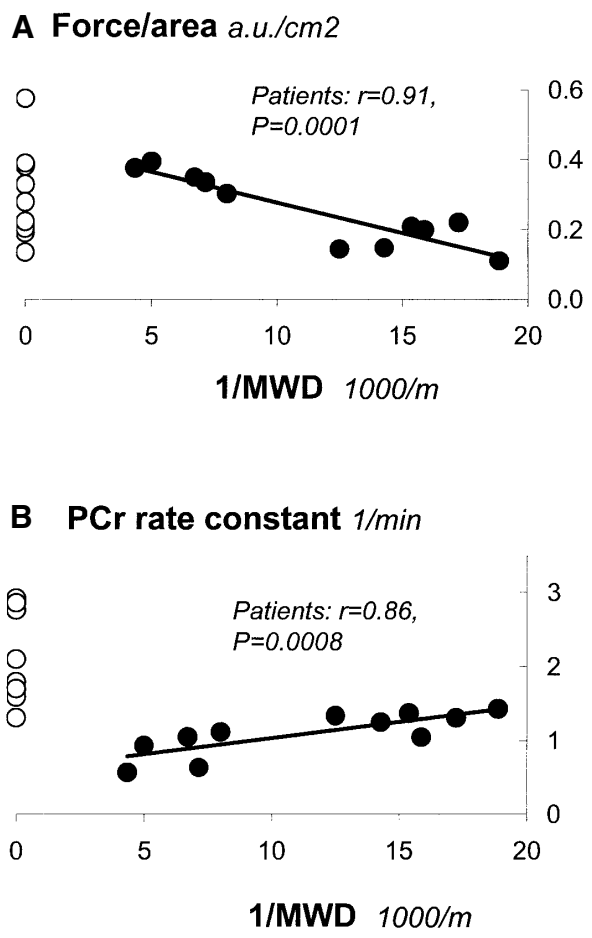

Fig 3. Relationships to maximum treadmill walking distance. This figure plots values individual subjects as a function of MWD, expressed here as a reciprocal so that control subjects (notionally infinite MWD) can be included on same graphs. **A,** MVC force (arbitrary units) expressed relative to posterior compartment cross-sectional area. Similar relationships are seen when force is replaced by ATP turnover, NIRS rate, or average rates of fall of PCr and NIRS (not shown). **B,** Rate constant of PCr recovery (unweighted mean of 50% MVC and 75% MVC exercise; mean of three studies).

exercise,[7] and the small pH changes simplify the analysis of PCr recovery,[36,37] although at the cost of making it unsuitable for assessing acid efflux.[8] Force is here scaled to MVC.[15,40] Other approaches are to scale to muscle volume[40] or lean body mass[8], but no approach is perfect.[40] Residual force-dependence is minimized by means of appropriate analysis.

NIRS and ^{31}P MRS have been combined either in separate experiments[10] or simultaneously with a large surface coil with light-guides mounted laterally.[39] Although both ^{31}P MRS[8,11-14,41] and NIRS[17-19] have been applied in PVD, this is the first time they have been used simultaneously in the same, or at least concentric, regions of interest. This is especially desirable for PVD, in which muscle blood supply may be unpredictably inhomogeneous, depending on the vascular lesion and the development of collaterals.

Exercise and recovery abnormalities. We found no decrease in MVC force, in contrast to an earlier report.[42] An earlier study of more severely affected patients found an increase in ATP turnover for dynamic work, suggesting a possible decrease in muscle mass or efficiency.[23] However, with direct measurement, neither was abnormal here. Thus, ATP demand was normal, and so the exercise abnormalities must result from the effect of impaired oxygen delivery on ATP supply. As judged by the end-exercise fractional oxidation rate, oxidative ATP synthesis was slightly reduced, implying a decreased functional "capacity" for mitochondrial ATP synthesis, best quantified by means of the postexercise PCr recovery rate constant.[7,35,37] This depends on mitochondrial volume fraction,[38] intrinsic mitochondrial function, and vascular oxygen delivery. We, like others,[8,14,15] found that PCr recovery is impaired; and the simplest interpretation is that this results directly from impaired oxygen supply. Although the authors of a recent report[15] argue that impaired PCr recovery after 90 seconds of submaximal isometric plantarflexion is evidence of an intrinsic mitochondrial defect in PVD, separable from reduced oxygen delivery, making this inference from this study would be unwise, in view of the substantial changes in oxygenation.

NIRS is a means of monitoring the amount of deoxygenated hemoglobin, which is the result of vascular oxygen supply and metabolic oxygen usage. We, like others,[17-19] found that NIRS recovery is slowed in PVD, so oxygen use outpaces delivery. Thus, oxidative metabolism is impaired by reduced oxygen delivery. In support of this, the rate constants of PCr and NIRS recovery are correlated (Fig 2, *C*), and the fractional abnormality in NIRS recovery was larger than in PCr (Table II). (This contrasts with chronic heart failure, in which PCr recovery is more abnormal, because impaired oxygen use is the dominant defect.[10]) During exercise, patients show greater and faster deoxygenation during exercise, despite reduced oxygen consumption (ie, oxidative ATP synthesis). The increase in the extent of deoxygenation[6] probably reflects increased oxygen extraction, although this is inferential.

Many,[8,11-14] although not all,[15] MRS studies have shown increased changes in pH and PCr during exercise in PVD, as occurs in experimental ischemia.[12,43,44] This would be consistent with reports of increased lactate production.[4,5] The importance of this depends on the nature of the exercise. In the substantially oxidative, isometric exercise used here and elsewhere,[15] it is at most a small effect.

Biochemical and histologic abnormalities. Could the defect in oxygen delivery be partially mitigated by adaptations such as mitochondrial proliferation? The literature on this is confusing. There are reports of increased mitochondrial enzymes and protein in an experimental model[45] and increased mitochondrial content[46,47] and aerobic

enzymes[47-50] in patients. However, the activity of mitochondrial succinate oxidation is variously reported as increased[51] or decreased,[52] and there are other reports of decrease[53] or no change[42] in various aerobic enzymes. Recent studies find increased mitochondrial DNA[54] or no change in mitochondrial DNA but a reduction in cytochrome c oxidase (mitochondrially coded) relative to citrate synthase (nuclear coded),[3] with increased mitochondrial DNA damage, perhaps caused by oxidative stress.[3]

The metabolic properties of muscle are related to fiber type, but again the literature on this is confusing. There are reports of normal[52] or increased[55,56] relative abundance of type I fibers; of reduced type IIa and increased type IIb numbers[52] or vice versa[57]; of reductions[53] in both Type I and II fiber area; and of reduced diameter,[58] relative area,[42] and numbers[55,58] of type II fibers. Arguably the best guide to functionally significant changes is the myosin heavy chain (MHC) type: there is a relative increase in MHC I with increasing clinical severity.[2] Other things being equal, this would increase functional mitochondrial capacity, a potentially useful adaptation. However, even if this occurs, the relationship between the NIRS measures of "vascular function" and "mitochondrial capacity" (Fig 2, *C*) suggests that it can only be a small effect.

Clinical and research implications. Judged by MWD, the worse affected muscles generate less force (Fig 3, *A*), with consequently smaller ATP turnover and NIRS changes during exercise; in effect, the reduced force protects them against metabolic disturbance. This may have implications for therapy: a rehabilitation regimen that increased force alone might worsen the consequences of impaired oxygen delivery. Furthermore, the worse-affected muscles have the less severe oxidative defects (Fig 3, *C*), perhaps because mitochondrial compensation is more effective. Slower reoxygenation is associated with reduced exercise duration in the scanner (see the "Results" section). Thus, exercise on the treadmill and in the scanner is not limited by the same factors, although the muscle sensations are reported to be similar.

^{31}P MRS is a more specialized and expensive technique than NIRS, but offers a more direct view of muscle metabolism. Fig 2, *C* shows that the physiologic muscle defect in PVD is well-defined by means of NIRS recovery measurements alone,[17-19] which could not necessarily have been assumed (because of potential alterations in contractile efficiency, and compensating effects of mitochondrial proliferation). However, a hypothetical treatment that stimulated mitochondrial function without improving vascular oxygen supply might further increase muscle deoxygenation, and both methods would be required for assessment.

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