Phosphorus 31 nuclear magnetic resonance spectroscopy suggests a mitochondrial defect in claudicating skeletal muscle

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Objective: Decreased oxygen supply is generally accepted as the primary cause of muscle dysfunction in patients with peripheral arterial occlusive disease (PAOD) and intermittent claudication, although reported morphologic changes in the mitochondria of claudicating muscle suggest that impaired energy utilization may also play a role. With the measurement of the phosphate-rich compounds of muscle energy metabolism (adenosinetriphosphate [ATP], adenosinediphosphate [ADP], and phosphocreatine [PCr]) and pH, phosphorus P 31 magnetic resonance spectroscopy (^{31}P MRS) provides a unique, noninvasive method to investigate this hypothesis further.

Methods: Calf muscle bioenergetics were studied in 12 men with moderate claudication (ankle-brachial index ≥0.5 and ≤0.8) and 14 normal control subjects with the use of ^{31}P MRS and standard treadmill testing. Phosphorus MRS evaluation of the superficial posterior calf muscles was carried out with a 90-second submaximal isometric plantar flexion exercise. This mild exercise was chosen to permit in-magnet testing and to allow study of intrinsic mitochondrial efficiency under conditions of unchallenged blood flow. Phosphocreatine and ADP recovery time constants (t.c.), two very sensitive measures of oxidative mitochondrial function, as well as intracellular pH and ATP production via anaerobic glycolysis were determined during three exercise sessions and the results averaged and compared to known values obtained from a control population.

Results: During the ^{31}P MRS protocol, the end exercise intracellular pH (7.11 ± 0.01 vs 7.11 ± 0.01) and ATP production by anaerobic glycolysis (0.13 ± 0.05 vs 0.14 ± 0.03 mmol/L per second) were no different in PAOD patients versus control subjects, confirming that the protocol exercise did not significantly reduce oxygen supply. Phosphocreatine and ADP recovery t.c. (137 ± 41 vs 44 ± 3 seconds and 60 ± 10 vs 29 ± 2 seconds, respectively) were significantly slower than normal ($P < .05$, t test). There was, however, no correlation between these measures of mitochondrial function and any treadmill parameter ($P > .5$, Pearson moment correlation).

Conclusions: Phosphorus 31 MRS provides the first direct evidence of defective energy metabolism in the mitochondria of claudicating calf muscle. This defect appears to be independent of both arterial flow and the severity of occlusive disease in patients with mild to moderate claudication. Coupled with documented ultrastructural and DNA abnormalities in the mitochondria of claudicating skeletal muscle, these data provide evidence for a secondary cause of muscle dysfunction in intermittent claudication. (J Vasc Surg 2000;31:944-52.)

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Muscle bioenergetics in patients with peripheral arterial occlusive disease (PAOD) has not been well characterized. The primary cause of muscle dysfunction in claudication is presumed to be an imbalance between oxygen supply and demand in the symptomatic limb.[1] Arterial occlusive disease restricts the normal increase in blood flow required by skeletal muscle during exercise, inducing muscle ischemia and resulting in symptoms of tightness, cramping, and fatigue. There is, however, increasing evidence that impaired energy utilization may also play a role in the pathophysiology of PAOD. Morphologic defects including hyperplasia, proliferation and ballooning of the cristae, and paracrystalline inclusions have been well described in the mitochondria of claudicating skeletal muscle.[2-4] In addition, recent studies have demonstrated extensive mitochondrial DNA damage in muscle biopsies taken from claudicants.[5,6] These changes in the structure and genetic fabric of mitochondria may result in defects in mitochondrial function and suggest the possibility of impaired energy utilization as a secondary cause of the muscle dysfunction of intermittent claudication.

Recent advances in nuclear magnetic resonance spectroscopy have made it possible to investigate muscle bioenergetics in vivo and noninvasively.[7,8] With the measurement of the concentrations of adenosinetriphosphate (ATP), adenosinediphosphate (ADP), phosphocreatine (PCr), and intracellular pH with a time resolution of 1 second before, during, and after exercise, ^{31}P MRS can determine the rate at which phosphoenergetic reactions occur.[8-10] As the central organelles in cellular energy metabolism, mitochondria are the primary focus of most kinetically resolved ^{31}P MRS exercise studies. We therefore decided to use ^{31}P MRS to study mitochondrial function in patients with claudication. Our hypothesis was that there is a primary mitochondrial defect in claudicating skeletal muscle.

The energy state of muscle is the result of a balance between ATP production and utilization. ATP is an energy-rich compound that provides the energy for muscle contraction by dephosphorylation into ADP and inorganic phosphate (Pi):

$$ATP \rightleftharpoons ADP + Pi + Energy$$

During exercise three separate energy pathways blend and with significant overlap provide uninterrupted ATP production. When exercise begins, the most important source of ATP resynthesis is provided by PCr breakdown through the creatine kinase (CK) pathway also known as the "immediate" ATP-generating system:

$$PCr + ADP + H^+ \rightleftharpoons Cr + ATP$$

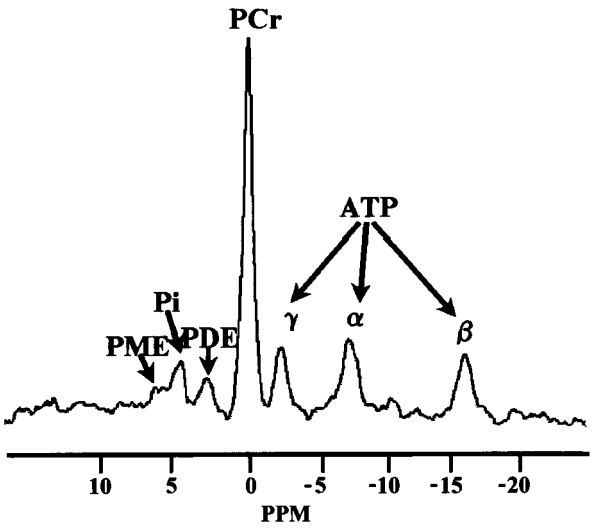

Fig 1. ^{31}P spectrum collected from the calf muscle of a patient with moderate claudication.

Within seconds after the initiation of exercise an increasingly greater proportion of ATP synthesis is generated by anaerobic glycolysis (AnGly). Glycolysis is the "short-term" ATP-generating system of the cell:

$$Glucose + 2ADP + 2Pi \rightarrow 2\ Lactate + 2\ ATP + 2\ H_2O + 2\ H^+$$

This pathway contributes most of the ATP requirements of the muscle in the first few minutes of exercise, until the "long-term" pathway of oxidative phosphorylation (OxPhos) is incrementally activated, becoming the dominant ATP provider for the duration of exercise:

$$3\ ADP + 3\ Pi + NADH + \tfrac{1}{2}\ O_2 + H^+ \rightleftharpoons NAD^+ + 3\ ATP + H_2O$$

With the cessation of exercise, both the CK and AnGly pathways are immediately deactivated. Phosphocreatine levels are then restored via the CK reaction, utilizing ATP produced solely through OxPhos. Similarly, ADP levels that had increased during exercise by dephosphorylation of ATP return back to their low resting levels as OxPhos converts ADP to ATP. Oxidative phosphorylation occurs exclusively in mitochondria, and therefore postexercise PCr and ADP recovery rates are good measures of mitochondrial function.[7,8]

METHODS

This trial was conducted on an outpatient basis at the Henry Ford Hospital, a tertiary care, referral medical center. Twelve patients with mild to moderate intermittent claudication resulting from PAOD

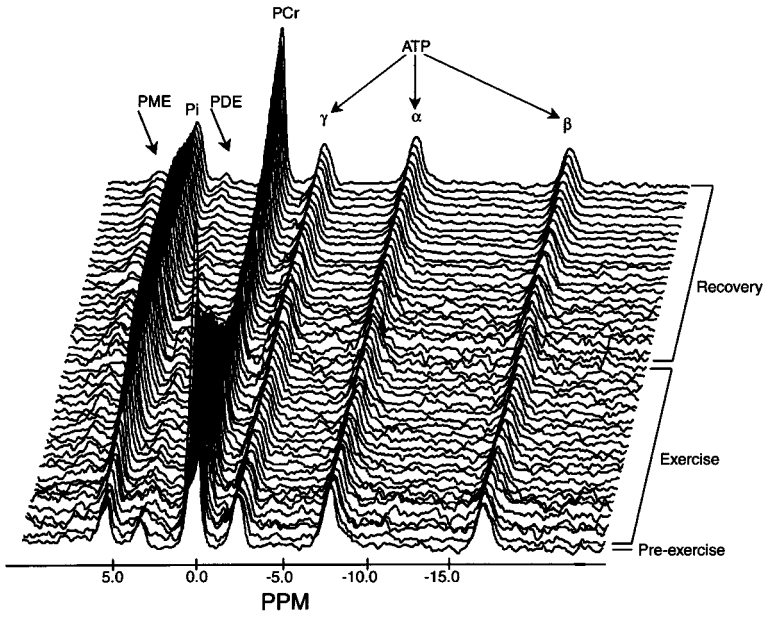

Fig 2. Stacked plot of ^{31}P MRS spectra obtained from the calf muscle of a patient with moderate claudication. The original data set consisted of 400 spectra collected with 1-second time resolution during a single exercise. For clarity, groups of approximately 10 spectra were summed and displayed as a single spectral curve in the figure. During exercise, muscle PCr levels drop and Pi increases, and then they gradually return to baseline during recovery. The ATP levels remained unchanged during the exercise.

and 14 moderately active control subjects were recruited. All claudicants were men and nondiabetic with an ankle brachial index (ABI) of 0.8 or below, but not below 0.5. This protocol was approved by our institutional review board, and informed consent was obtained from all participating patients.

^{31}P MRS measurements

Calf muscle bioenergetics were measured at rest and during and after 90-second isometric submaximal plantar flexion exercise. All PAOD patients underwent ^{31}P MRS evaluations of the calf muscles in the extremity with the lower ABI. All normal control subjects had evaluations of the calf muscles in their right lower extremity. Subjects fasted and abstained from caffeine for 6 hours before being studied.

^{31}P MRS exercise. The main part of the MRS instrument is a 3 Tesla whole-body magnetic resonance imaging and spectroscopy system (Magnex Scientific Ltd, Abington, UK) utilizing an imaging console (Surrey Medical Imaging Systems, Surrey, UK). The ^{31}P MRS protocols used in these series have been previously described in detail.[9,10] In brief, the extremity to be studied is positioned in a laboratory-built exercise device. The apparatus consists of an in-line isometric ergometer, with a force transducer / analyzer (Tedea #1250, 300 kg; Tedea Huntleigh, Inc, Canoga Park, Calif) and a 9-cm inductively coupled surface coil. The coil is tuned to the resonance frequency of phosphorus atoms (51.7 MHz at 3.0 Tesla). The leg is positioned in the device in extension at the level of the knee joint, and the coil is snugly strapped over the posterior calf musculature. The subject's foot is secured to the pedal of the in-line ergometer. Subjects push against the pedal to isometrically exercise their posterior compartment muscles (foot flexors). A standard proton-based magnetic resonance 5-slice image of the calf is first obtained, and the maximum cross-sectional area of the gastrocnemius / soleus muscle group is measured to calculate the theoretical force generating capacity of the muscle group.[11] The subject then plantar flexes his foot to a target force of approximately 60% ± 15% (mean ± SD) of the estimated maximum capacity of his calf muscles. These calculations are based on previous studies performed on normal subjects.[10] While in the magnet the subject performs three isometric plantar flexion exercises. Each in-magnet exercise lasts 90 seconds, and the patient rests for 15 minutes between exercis-

Table I. Comparison of ^{31}P MRS parameters in claudicants and controls

	Claudicants	*Controls*	P *value*
End-exercise values			
PCr (mmol/L)	18.9 ± 1.3	17.4 ± 0.7	.3
Pi (mmol/L)	11.6 ± 1.1	11.3 ± 0.6	.8
ADP(μmol/L)	41 ± 6	42 ± 5	.9
pH	7.11 ± 0.01	7.11 ± 0.01	.9
Exercise metabolism values			
An Gly (mmol/L/s)	0.14 ± 0.01	0.13 ± 0.01	.92
CK (mmol/L/s)	0.07 ± 0.01	0.06 ± 0.01	.6
Ox Phos (mmol/L/s)	0.12 ± 0.02	0.13 ± 0.01	.71
Mean % MVC	56 ± 3%	57 ± 3%	.77
Recovery			
PCr t.c. (s)	137 ± 41	44 ± 3	.02
Pi t.c. (s)	139 ± 42	44 ± 3	.02
ADP t.c. (s)	60 ± 10	29 ± 2	.002

All values are mean ± 1 SE.
% MVC, Percent maximum voluntary capacity of calf flexors maintained during exercise (calculated from muscle cross-sectional area).

es. Phosphorus spectra are collected every 1 second, before and during study exercise and throughout the first 5 minutes after the exercise.

The short-duration isometric workout of the foot flexors performed during ^{31}P MRS was specifically chosen in contrast to the longer isotonic protocol of the entire leg used during treadmill evaluation because (1) it enables in-magnet testing, (2) it isolates work to a single muscle group, and (3) it is a mild exercise that does not challenge the diseased arterial inflow of PAOD patients enough to produce exercise-induced muscle ischemia. Muscle bioenergetics can thus be tested under conditions of normal oxygen supply, which allows for direct study of intrinsic mitochondrial efficiency.

Phosphorus MRS spectra analysis. Fig 1 depicts a typical in vivo ^{31}P MRS spectrum of the posterior calf musculature obtained at rest. The phosphorus atom has an odd number of neutrons and protons in its nucleus and is therefore detectable with magnetic resonance techniques. Phosphorus is the central element of phosphate, which in turn is an essential component of many important biomolecules. The phosphorous nuclei of DNA, RNA, most phospholipids, and phosphoproteins are immobile and do not yield an easily measurable signal in a magnetic field. ATP, ADP, Pi, and PCr are the principal mobile phosphorus-rich compounds present in sufficient concentrations in tissue to yield a sharp, easily measurable phosphorus spectroscopic signal. Each one of the three phosphate atoms of ATP have different chemical shifts and clearly resolved peaks. In the ^{31}P MRS spectrum the most prominent peak is PCr, which lies just to the left of γ-ATP. The spectra of the two ADP phosphates cannot be seen because their natural concentrations in the cell are very low (50-1000 times lower than those of ATP) and do not produce clearly resolved peaks. ADP concentrations, however, can be readily calculated from the pH and the measured concentrations of ATP, PCr, and free creatine. The three smaller peaks to the left of PCr are phosphodiesters (PDE), Pi, and phosphomonoesters (PME). The Pi peak is of particular importance because it represents the sum of visible mono and dibasic inorganic phosphates. These two phosphate species participate in the intracellular phosphate buffer system and are in rapid exchange, depending on the cellular pH. The chemical shift of the Pi peak is the weighted mean of these two species; changes in the pH alter the balance between these two phosphate species and thus produce a change in the chemical shift of the Pi peak. Acidosis shifts the peak closer and alkalosis shifts it farther away from the PCr peak. These migrations of the Pi peak in relation to the stable position of the PCr peak can be used as a very sensitive in vivo measure of intracellular pH.[12,13]

Fig 2 depicts a series, or stackplot, of spectra obtained during exercise in a patient with moderate claudication. Phosphocreatine concentration drops and Pi increases during exercise, and then these quickly return to normal values during recovery. ATP levels remain unchanged during the exercise. Maintenance of stable ATP levels is of critical importance to normal cellular function; ATP concentrations drop only under extreme circumstances (eg, profound ischemia or direct cellular damage).

Important information regarding cellular energy metabolism can be obtained from these spectra. By using the bioenergetic stoichiometric equations, the

Table II. Correlation between treadmill stress testing parameters and functional ^{31}P MRS mitochondrial indices

	Baseline ABI	*% ABI drop*	*ABI at 1 minute*	*ABI recovery time*	*ICD*	*MWC*
PCr t.c.	$r = -0.114$	$r = -0.159$	$r = 0.131$	$r = 0.064$	$r = -0.121$	$r = -0.039$
	$P = .725$	$P = .641$	$P = .691$	$P = .850$	$P = .708$	$P = .905$
ADP t.c.	$r = -0.128$	$r = -0.309$	$r = 0.276$	$r = -0.268$	$r = -0.206$	$r = -0.012$
	$P = .692$	$P = .355$	$P = .411$	$P = .426$	$P = .521$	$P = .969$

% ABI, Maximum drop in the ABI; *ABI at 1 minute,* ABI at 1 minute postexercise; *ABI recovery time,* time required for the ABI to return to baseline; *ICD,* initial claudication distance; *MWC,* maximum walking capacity.

relative contributions of the OxPhos, AnGly, and CK pathways to ATP production during exercise can be derived. Intracellular pH can be used as a marker for the presence of anaerobic metabolism. Finally, the functional status of the muscle cell mitochondria can be determined by measuring the postexercise recovery of PCr and ADP. The recovery rates of these compounds are governed solely by mitochondrial OxPhos and constitute a sensitive measure of mitochondrial efficiency.

Treadmill evaluation

Treadmill stress testing. Patients with PAOD walked on an inclined (gradient, 12.5 degrees) treadmill at a speed of 1.5 mph for a total of 5 minutes (or less if claudication forced them to stop). Standard technique was used for the calculation of ABIs. More specifically, brachial and ankle (dorsalis pedis and posterior tibial arteries) systolic blood pressures were measured with use of a suitably sized blood-pressure cuff and a handheld, pencil-sized continuous wave Doppler flow probe. For each extremity the ratio of the higher of the two ankle pressures over the higher of the two brachial pressures was calculated as the baseline ABI. The same pedal artery was used for calculation of the postexercise ABIs. Treadmill stress testing produces maximum flow demands to the most symptomatic leg, its parameters correlate well with calf blood flow measurements, and it is a highly accurate and readily available method for assessing the severity of PAOD.[14] Measured parameters included (1) baseline ABIs (2) ABIs at 1 minute postexercise, (3) maximum drop in the ABIs, and (4) time required for the ABIs to return to baseline.

Walking distances. After completion of the initial treadmill test and a rest period of 30 minutes, patients walked again on the treadmill, and this time the initial claudication distance and the maximum walking capacity were determined. *Initial claudication distance* is the distance the patient can walk under the above standardized circumstances before experiencing the onset of pain; *maximum walking capacity* is the maximum distance walked before discomfort forces the patient to stop.

Four selected patients also underwent ABI measurements in the most affected limb before and at 1 minute after performance of a submaximal 90-second plantar flexion exercise. The exercise consisted of standing on the toes and raising the heels off the ground for 90 seconds and was intended to simulate the in-magnet exercise to see if this exercise changed arterial flow.

Statistics

MRS parameters from control subjects and patients with PAOD were compared by means of a *t* test. Correlations between treadmill and MRS data in the PAOD population were performed by using the Pearson correlation. A *P* value less than .05 was considered indicative of a significant difference. Results are expressed as means ± SE unless otherwise stated. Sigmastat (SPSS, Inc, Chicago, Ill) and Sigmaplot (Jandel) statistics software was used for statistical analysis and graph generation.

RESULTS

NMR data

Exercise spectra. All PAOD patients and normal control subjects completed the MRS exercise protocol without having any calf symptoms resembling claudication pain. As expected, during exercise PCr decreased and Pi and ADP increased, while ATP levels remained stable. There was no significant difference in the relative change of any of these individual phosphorus metabolites between the PAOD and normal groups.

Table I displays the end exercise intracellular pH in the PAOD group compared with the control subjects. In the claudicants the end exercise muscle cell

pH remained in the normal range and was the same as in normals (7.11 ± 0.01 vs 7.11 ± 0.01). A comparison between the activation pattern of anaerobic pathways during exercise in claudicants and in the control subjects is also presented in Table I. Specifically, in the claudicating muscle ATP production by AnGly remained in a normal range and was essentially the same as in normal muscle (0.14 ± 0.01 vs 0.13 ± 0.01 mmol/L per second). The ATP consumed during exercise in both PAOD and normal muscle was regenerated by using virtually the same combination and ratios of the OxPhos, AnGly, and CK pathways (Table I).

Recovery spectra. Phosphocreatine recovery was significantly slower in claudicating muscle than in normal muscle (137 ± 41 vs 44 ± 3 seconds). In addition, ADP recovery was significantly slower in claudicating muscle (60 ± 10 vs 29 ± 2 seconds). Interestingly, however, not all PAOD patients demonstrated such delayed PCr and ADP recovery. Three of the 12 (25%) claudicants had mitochondrial indices (PCr recovery t.c. of 39 ± 8 seconds and an ADP recovery of 26 ± 7 seconds), that fell within the normal limits. In this case, normal limits were defined as the mean ± 2 SD of the corresponding value in the control group (ie, for PCr t.c. = 22-66 seconds and for ADP t.c. = 17-41 seconds). The only noticeable, although not statistically significant ($P > .05$), difference between the group of claudicants with "normal" mitochondrial function and the group with "abnormal" function was the duration of claudication symptoms. In the subgroup with normal mitochondrial parameters, claudication symptoms had been present an average of 1.3 ± 0.3 years; in the subgroup with abnormal mitochondrial parameters symptoms had been present a mean of 4.4 ± 1.6 years.

Treadmill data

Treadmill evaluation was performed only on the claudicants. Subjects at rest had an average ABI of 0.65 ± 0.03 (range, 0.5-0.8). The ABI at 1 minute after exercise dropped to 0.3 ± 0.05, constituting a maximum reduction of 56% ± 7%. Posttreadmill ABIs required an average of 13 ± 3 minutes to return to baseline. The initial claudication distance was 98 ± 32 meters, and the maximum walking capacity was 354 ± 64 meters. There was no correlation between any of these treadmill parameters, which primarily reflect the severity of the arterial occlusive process and any of the ^{31}P MRS parameters, which primarily assess end organ metabolic function (Table II). More specifically, individuals with the worst treadmill parameters did not necessarily have the worst ^{31}P MRS indices of mitochondrial function.

For the four claudicants who performed the 90-second plantar flexion exercise while standing on their toes, the ABI of the tested extremity at rest averaged 0.675 ± 0.05 (range, 0.6-0.8), and at 1 minute postexercise changed insignificantly, to 0.65 ± 0.1 ($P > .05$).

DISCUSSION

Intermittent claudication is a debilitating condition affecting more than 4 million people in the United States.[15-17] Although the main cause of claudication is insufficient oxygen supply resulting from arterial occlusive disease, the pathobiology of the muscle dysfunction in claudication cannot be fully explained on the basis of reduced blood flow alone. Several studies have shown that measurements of calf blood flow and ABIs do not correlate with treadmill walking distances and that revascularization of a diseased arterial segment does not necessarily normalize exercise performance.[18-20] It seems likely that other factors contribute to the muscle dysfunction seen in PAOD. Mitochondrial morphologic defects have been well described in pathologic studies of claudicating muscle.[2-4] In addition, recent studies have demonstrated extensive damage in the mitochondrial DNA of skeletal muscle biopsy specimens taken from claudicants.[5,6] These changes in the structure and genetic content of mitochondria suggest the possibility that impaired energy metabolism may play a role in claudication.

The objective of this study was to test the hypothesis that there is a functional defect in these abnormal claudicating mitochondria. A protocol was designed to test skeletal muscle bioenergetics under conditions of minimally perturbed blood flow, which would allow for direct study of mitochondrial function without the confounding effects of ischemia. We employed MRS methodology and designed a specific exercise that enables in-magnet testing at a level mild enough that muscle oxygen demand does not appear to exceed oxygen supply. A number of modifications were used to produce a much more complete and accurate picture of muscle biochemical function.[21-28] These included (1) an in-magnet exercise bench that isolates work to a single muscle group, (2) total ergometric standardization of exercise protocols, (3) use of an isometric exercise to minimize motion artifacts, (4) testing only nondiabetic persons, to exclude possible interference of abnormal glucose metabolism with measurements of

muscle energy states, and (5) performance of high temporal resolution studies.

Exercise spectra showed that the ATP consumed during exercise in both claudicating and normal muscle was generated through activation of the same combination of OxPhos, AnGly, and CK pathways. No additional activation of anaerobic pathways was observed in the PAOD patients compared with the control subjects. At the end of the exercise, the intracellular pH and the concentrations of ATP, ADP, PCr, and Pi were no different in healthy versus claudicating muscle. These results strongly suggest that the ^{31}P MRS exercise used did not significantly reduce the oxygen supply to the exercising muscles in the PAOD group. The minimal drop in the ABIs of the 4 patients tested after the performance of the 90-second plantar flexion exercise further supports our conclusion that the short, submaximal isometric exercise did not demand more blood flow than can be supplied by the diseased arterial circulation in patients with mild to moderate claudication. Both normal and PAOD subjects had comparable ATP generation and utilization during exercise. All subjects therefore began the exercise recovery phase having similar energy debt to replenish through mitochondrial OxPhos.

During the recovery phase, PCr and ADP recovery time constants were significantly slower in the PAOD group than in the normal subjects. Since the exercise protocol did not appear to significantly stress arterial inflow / oxygen supply to the exercising muscle, this difference is likely the result of altered mitochondrial function. These data strongly suggest that the mitochondria in claudicating muscle do not function normally.

The fact that 25% of the claudicants demonstrated "normal" mitochondrial function indices suggests that the etiology of this defect is multifactorial and cannot be attributed solely to the presence of occlusive disease in the arterial tree supplying the tested extremity. A combination of factors related to the overall magnitude of the "ischemic insult" to which the claudicating muscle is exposed may be producing these defective mitochondria. Possible factors include the location (eg, aortoiliac vs infrainguinal) of the occlusive process; the duration of claudication symptoms; subjects' normal exercise levels; the presence, severity, and duration of associated comorbidities (eg, smoking, diabetes, hypertension, dyslipidemias, congestive heart failure); and even the subjects' underlying nutritional status. When we analyzed the characteristics of these patients, the only noticeable difference identified was a shorter duration of claudication symptoms in the subgroup of claudicants with "normal" mitochondrial function. This finding, although not statistically significant, may indicate a possible cumulative insult to mitochondrial function in claudicating muscle resulting from repetitive episodes of ischemia / reperfusion as encountered during routine walking. Furthermore, the fact that severity of the arterial occlusive process does not necessarily equate with the gravity of the ischemic insult it produces may be the reason why the postexercise treadmill parameters did not correlate with the ^{31}P MRS indices. Treadmill stress testing, while being a very accurate method for quantifying the severity of occlusive disease,[14] does not address any other components related to the overall magnitude of the "ischemic insult" produced by the occlusive lesions it quantifies.

Previous investigations of claudicating muscle that have used ^{31}P MRS have suggested significantly prolonged recovery rates for PCr and ADP.[21-28] In most of these studies, patients were exercised to muscle exhaustion with isotonic exercise protocols that introduce exercise-induced muscle ischemia as a significant confounding variable. Under such circumstances, investigators did not attempt to extract specific conclusions about mitochondrial function. The work of Zatina et al,[21] however, is an interesting exception. They performed ^{31}P MRS on claudicants before, and soon after, lower extremity revascularization. Although postoperatively the hemodynamics of the limb were immediately restored to normal, there was no corresponding improvement in the measured ^{31}P MRS indices of oxidative function.[21] Based on these findings, Zatina's group concluded that "there is an inadequacy of oxidative metabolism in the limb after revascularization that is not secondary to ischemia." At that time, there was uncertainty about the exact meaning of the discrepancy, but based on our current findings it seems clear that this "inadequacy" is probably the result of a primary mitochondrial defect in claudicating skeletal muscle. This defect can be unmasked when "claudicating" mitochondria are studied under conditions of either a very mild exercise, such as in this study, or soon after revascularization, as in Zatina's work.[21] Under either circumstance, mitochondrial function can be measured in a state of minimally perturbed blood flow. Zatina's results also suggest that this mitochondrial defect is not rapidly reversible with revascularization

Findings from other laboratories suggest that skeletal muscle in extremities of patients with PAOD undergoes significant morphologic and metabolic

alterations. Mitochondrial ultrastructure is abnormal, DNA and enzyme expression is altered, intermediates of oxidative metabolism accumulate, and enzyme activity may not increase normally in response to exercise stimulation.[2-6,29-34] These results, combined with our findings, strongly suggest an intrinsic mitochondrial defect in claudicating muscle that is very similar to those seen in mitochondrial myopathies.[7,34]

The significance of this defect is not entirely clear at this point, but it seems likely that mitochondrial dysfunction is at least a contributing factor to the abnormal muscle physiology seen in claudication. This reduction in metabolic efficiency may also be responsible for the inadequacy of revascularization to completely reverse exercise performance in patients with PAOD.[20,21] Additionally, amelioration of this defect may be the reason why exercise rehabilitation and certain medications, like pentoxifylline, cilostazol, and carnitines, improve the walking performance of claudicants without modifying large vessel hemodynamics.[35-37]

This study represents an initial attempt to investigate mitochondrial function in claudicating skeletal muscle, while minimizing the confounding effects of reduced oxygen supply. Our data strongly suggest the presence of an intrinsic mitochondrial defect that results in impaired oxidative metabolism in patients with mild to moderate claudication. Studies are currently underway in our laboratory to assess the degree to which this defect is reversible with exercise training, pharmacologic therapy, and revascularization. Information from these studies will be important to defining the true nature of this defect and its contribution to the pathophysiology of intermittent claudication.

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