High-energy phosphate metabolism during incremental calf exercise in patients with unilaterally symptomatic peripheral arterial disease measured by phosphor 31 magnetic resonance spectroscopy

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Background: **The treadmill exercise test is the most important examination of the functional ability of patients with intermittent claudication or leg pain during exercise, but it does not provide any metabolic information in the calf muscle. The purpose of this study was to investigate the high-energy metabolism in the calf muscle during incremental progressive plantar flexion exercise of a selected peripheral arterial disease (PAD) patient group.**

Methods: **Using a 1.5-T whole-body magnetic resonance scanner, 17 male patients with PAD who had 1 symptomatic and 1 asymptomatic leg and 9 healthy male controls underwent serial phosphor 31 (^{31}P) magnetic resonance spectroscopy during incremental exercise at 2, 3, 4, and 5 W. Furthermore, magnetic resonance angiography was performed, and the ankle-brachial pressure index was determined in the patient group. The runoff resistance (ROR) was separately assessed in each patient's leg.**

Results: The symptomatic legs exhibited significantly increased phosphocreatine (PCr) time constants during the first three workload increments (2-4 W) and the recovery phase compared with the asymptomatic legs and the normal controls. Only two symptomatic legs reached the last increment at 5 W. Compared with the normal controls, the asymptomatic legs showed significantly increased PCr time constants only at 5 W. In the patient group, we detected significant correlations between the PCr time constants and the ROR, as well as the ankle-brachial pressure index. Moreover, the symptomatic legs presented significantly lower PCr levels and pH values at the end of exercise compared with the asymptomatic and control legs.

Conclusions: **Our study shows that muscle function in PAD patients can be objectively quantified with the help of ^{31}P magnetic resonance spectroscopy and correlates significantly with hemodynamic parameters such as ROR and ankle-brachial pressure index. Consequently, ^{31}P magnetic resonance spectroscopy seems to be a useful method to monitor the muscle function of PAD patients for evaluation of established therapies or new therapeutic strategies during research trials. (J Vasc Surg 2006;43:978-86.)**

Intermittent claudication (IC) is the typical symptom of patients with peripheral arterial disease (PAD).[1] However, several epidemiologic studies have shown that the prevalence of PAD ranges up to approximately 20%, depending on the composition of the examined population, whereas most PAD patients do not have classic IC symptoms and often present no symptoms or present atypical leg pain that is difficult to distinguish from other entities.[2-5] The clinical evaluation of PAD patients routinely includes physical examination with peripheral pulse palpation, ankle-brachial pressure index (ABPI), stress tests, several imaging techniques, and the treadmill exercise test.[6] The treadmill test is the gold standard for the assessment of the walking distance, which influences the clinical situation of PAD patients and mainly determines further therapeutic strategies. Nevertheless, several previous studies pointed out that the treadmill test is affected by poor reproducibility,[7] is often not conclusive because of the occurrence of angina pectoris or dyspnea, or cannot be performed by the patients because of comorbidity.[8,9] Consequently, it is difficult to quantify the functional and metabolic impairment in the calf muscle due to atherosclerotic lesions. This would be necessary to evaluate the effect of established or experimental therapeutical interventions on muscle function or metabolism during research trials.

Phosphor 31 (^{31}P) magnetic resonance spectroscopy (MRS) has been established for the noninvasive investigation of mitochondrial capacity in skeletal and cardiac muscle.[10-12] At the onset of skeletal muscle exercise, phosphocreatine (PCr) is hydrolyzed by the creatine kinase in the

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Competition of interest: Drs Schocke and Greiner have a pending patent containing some details of the pedal ergometer and its control system. The other authors do not have any potential conflict of interest that relate to the article.

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cytosol, which transfers a high-energy phosphate from PCr to adenosine diphosphate.[13] The remaining creatine is rephosphorylated at the mitochondrial site and is therefore a shuttle of high-energy phosphates.[13-15] The PCr concentration decreases during muscle exercise until the oxygen supply is adapted to meet the increased metabolic demand of the mitochondria. In case of sufficient oxygen supply, PCr hydrolysis passes into a steady state with a typical monoexponential curve progression.[16] Previous experimental ^{31}P MRS studies investigating the effect of cuff stenosis or occlusion on intramuscular high-energy metabolism have shown that PCr hydrolysis progressively decreases under ischemic conditions.[17,18] Furthermore, the time constants of PCr rephosphorylation are prolonged after plantar flexion exercises and cuff stenosis.[19] Phosphor 31 MRS studies of PAD patients are rare. Two previous studies investigated the high-energy phosphate metabolism in the calf muscle of PAD patients during isometric exercises.[20,21] Both studies detected prolonged time constants of PCr rephosphorylation after exercise, as already indicated by several previous studies that calculated the PCr recovery rate via the quotients of PCr and inorganic phosphate (Pi).[22-25] However, the cause for this prolongation was controversially discussed, and it was attributed to a reduced oxygen supply due to atherosclerotic lesions[20] or mitochondrial impairment due to chronic ischemia.[21]

Because the previous ^{31}P MRS studies were focused on PCr recovery rather than the PCr on-kinetics during exercise, the aim of this study was to investigate the high-energy metabolism of the calf muscle during incremental plantar flexion exercise of a selected PAD patient group that presented one symptomatic and one asymptomatic leg in comparison to a healthy control group.

METHODS

Informed consent was obtained from all patients and volunteers before they participated in the study. All experiments were completed in accordance with the institutional review board's requirements.

Patient and control group. We enrolled 17 patients with PAD Fontaine stage II onto this study. The median age of the patients was 62.5 years (range, 53-74 years). All patients were nondiabetic and had a symptomatic and an asymptomatic leg. The symptomatic legs presented a typical walking distance limited by IC of the calf. The patients underwent routine evaluation, including a history and physical examination, measurement of the walking distance with a treadmill test (3 km/h and 12% gradient angle), and determination of the ABPI at rest. Furthermore, each patient received magnetic resonance angiography (MRA) of both legs. In addition, nine healthy men agreed to participate in this study and underwent the exercise protocol. The median age of the volunteers was 67 years (range, 53-79 years). None of them was specifically trained in sports. All of them were nonsmokers and nondiabetics and did not have any history of PAD symptoms or coronary artery disease. All volunteers had a palpable extremity pulse and ABPIs of 1.0 or greater at rest and after active pedal plantar flexion.

Fig 1. The self-built exercise bench permits isotonic stress exercises of the calf muscle with a defined workload, which is measured via force at the piston and via distance and is adjusted by a feedback control system. The volunteer lying on the exercise bench indicates the correct positioning for the phosphor 31 magnetic resonance spectroscopy examination.

Equipment. The exercise bench (Fig 1) used for the experiments was a modification of the pedal ergometer that was recently described.[10,11] The pedal ergometer consisted of one pedal that was conjoint to a piston and a cylinder and mounted on the middle of a wooden bench. The cylinder was connected to an air pressure system for the exercise protocol. The force, produced in the cylinder of the pedal system by the air pressure, and the distance of pedal movement were continuously recorded at the piston and the suspension of the pedal. The force and distance parameters during exercise were transmitted to a computer system that controlled the air pressure in the pedal system to maintain a constant power output during one workload level. One of the subject's feet was fixed on the foot pedal at 0° of plantar flexion and restrained at the heel and at the dorsum of the foot by 3-cm-wide straps. Two additional 3-cm-wide straps were placed above the knee and on the lower leg to maintain the position of the calf muscle groups on the surface coil that was integrated into the exercise bench. The foot pedal permitted a plantar movement of 7 cm, and this resulted in an end angle of 21°. The subject's upper body was locked in position by two shoulder belts. During the exercise protocol, a metronome gave the frequency of plantar flexions, and the subject was requested to perform full flexions over the distance of 7 cm. Muscle exercises were performed at a constant frequency of 30 plantar flexions per minute. The power output during the different increments was controlled by self-written software.

Exercise protocol. Each patient and volunteer performed the same exercise protocol during ^{31}P MRS measurement: after 1 minute at rest, the exercise protocol consisted of four consecutive exercise increments with 2, 3, 4, and 5 W, whereby each exercise increment lasted 4 minutes. The recovery time was 5 minutes. In both the PAD patients and the volunteer, both legs were separately measured, and the two exercise sessions were separated by a rest interval of 1 hour at minimum.

Phosphor 31 MRS protocol. The spectroscopic measurements were performed on a 1.5-T whole-body magnetic resonance scanner (Magnetom VisionSymphony; Siemens, Erlangen, Germany) by using a circular polarized double resonator surface coil that permits the receipt of ^{1}H resonances at 63.5 MHz and ^{31}P resonances at 25.8 MHz. The transmitter coil had a diameter of 21 cm, and the

receiver coil had a diameter of 14 cm. A free induction of decay sequence with a repetition time of 1000 milliseconds, an echo time of 0.13 milliseconds, a flip angle of 90°, 15 averages, and an acquisition time of 15 seconds was used. The nuclear Overhauser enhancement was applied to all spectroscopic measurements.[10,11]

MRA protocol. MRA was performed in all patients by using a 1.5-T whole-body magnetic resonance scanner (Magnetom VisionSymphony) and a dedicated MRA coil covering the pelvis and both legs (Siemens). The MRA images were acquired in three levels by using fast low angle shot (FLASH) three-dimensional sequences and the moving bed technique. A three-phase gadobenate dimeglumine (20 mL; Multihance; Bracco, Italy) was injected into the cubital vein with the help of a commercially available magnetic resonance injector (Spectris; Medrad, Pittsburgh, Pa) and monitored by the care bolus feature of our magnetic resonance system. The FLASH three-dimensional sequences had a field of view of 420 mm, a matrix of 384 to 448, a repetition time of 6.2 milliseconds, an echo time of 2.3, a slice thickness of 0.8 to 1.2 mm, a slab thickness of 96 to 128 mm, and an acceleration factor of 2 derived from the integrated parallel imaging technique (iPAT) with a generalized autocalibrating partially parallel acquisitions (GRAPPA) algorithm. All FLASH three-dimensional sequences were acquired before and after three-phase gadolinium administration (8 mL at 1.2 mL/s; 12 mL at 0.8 mL/s; and 30 mL of saline at 0.8 mL/s).

MRA analysis. The subtracted, contrast-enhanced MRA volumes were processed with the help of maximum-intensity projection and formed the three-dimensional MRAs (Fig 2). An experienced radiologist determined the runoff resistance (ROR) by using the angiographic scoring system proposed by the Society for Vascular Surgery[26] and modified by Peterkin et al[27] and Williams et al,[24] whereby Williams et al extended the score to suit the purpose of ^{31}P MRS studies in the calf muscle.

Outflow was divided into abdominal, pelvic, thigh, and calf segments; the contribution of each segment to gastrocnemius and soleus perfusion was weighted equally (arbitrarily chosen to be 3). Individual vessels were weighted as follows: (abdomen) common iliac artery, 3; (pelvis) internal iliac artery, 1, and external iliac artery, 2; (thigh) deep femoral artery, 1, and superficial femoral artery, 2; and (calf) anterior tibial artery, 1, posterior tibial artery, 1, peroneal artery, 0, and pedal arch, 1. The degree of occlusion was classified as follows: 0, 0% to 19% stenosis; 1, 20% to 49% diameter stenosis; 2, 50% to 99% stenosis; 2.5, occlusion for less than half the length of the vessel; and 3, occlusion for more than half of the length. The pedal arch was graded as follows: 0, completely patent; 1.5, partially occluded; and 3, little or no arch visualized. The resistance across each segment was calculated by multiplying the vessel weight by the degree of occlusion for each vessel in the segment and adding 1 to allow for the intrinsic resistance of the normal vessel. Total resistance was calculated by summing the resistance of the four segments.

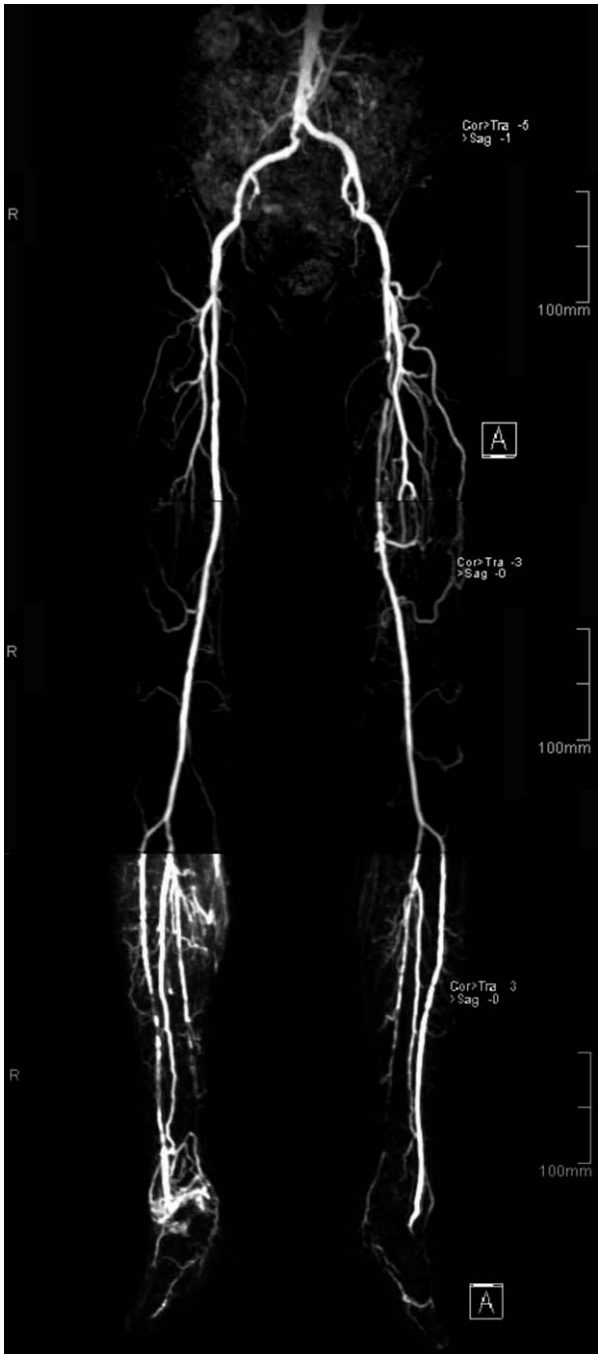

Fig 2. Magnetic resonance angiogram of a 64-year-old patient of the patient group who presented with a left symptomatic leg and a right asymptomatic leg.

Phosphor 31 MRS data analysis. The spectral data were processed by using the commercial software package provided by the manufacturer (Siemens). The peak areas of PCr and Pi were fitted in the frequency domain. In addition, the positions of the peaks of PCr and Pi were determined. Because absolute concentrations were not calcu-

lated, the integrals of the metabolite peaks were not corrected for partial saturation and the nuclear Overhauser enhancement effects. Moreover, we assumed that the T1 relaxation times of the metabolites were constant throughout each ^{31}P MRS session.[29] The changes in the metabolites were determined as percentage changes in relation to the rest integral of each metabolite. The intracellular pH was calculated from the chemical shift of Pi according to the equation[11]

$$pH = 6.75 + \log(\delta - 3.27)/(5.69 - \delta), \quad (1)$$

where δ is the chemical shift of the Pi peak in parts per million relative to PCr.

The time constants and ΔPCr_{ss} values, meaning the differences between baseline and steady-state PCr levels, were calculated for each workload increment by using a nonlinear regression analysis in SPSS 11.0 for Windows (SPSS Inc, Chicago, Ill), as previously reported.[28] For that purpose, the last PCr integral of the preceding increment was regarded as the baseline PCr value. Then the following PCr integrals of the increment were normalized to the averaged baseline PCr integral and expressed as percentage changes. The six rest PCr values at the beginning of the exercise protocol were averaged as baseline for the first 4.5-W increment. The time constant τ and the ΔPCr_{ss} values were iteratively calculated by using the equation[29]

$$\Delta PCr_t = PCr_0 - \Delta PCr_{ss} \times (1 - e^{-t/\tau}) \quad (2)$$

for the increase in workload and PCr hydrolysis and the equation

$$\Delta PCr_t = PCr_0 + \Delta PCr_{ss}(1 - e^{-t/\tau}) \quad (3)$$

for the decrease in workload and PCr recovery, where PCr_0 is the baseline value, ΔPCr_t is the PCr value at the time t, and ΔPCr_{ss} is the difference between the baseline PCr value and the estimated steady-state level. The coherence between the monoexponential model and the data of each subject was described[28] by a coefficient of determination r^2:

$$r^2 = 1 - \text{residual sum of squares}/\text{corrected sum of squares} \quad (4)$$

A coefficient r^2 greater than 0.6 was considered an acceptable fit, whereas a coefficient r^2 less than 0.6 indicated a weak agreement with a monoexponential model. The PCr time courses of these increments were visually evaluated with respect to a progressive PCr decrease without any steady state. When a progressive PCr breakdown was detected within an increment, this progressive decrease was considered a sign of exercise-induced ischemia. Because progressive PCr decreases do not follow a monoexponential course, PCr time constants were not calculated for those increments.

Statistical analysis. The data were tabulated in SPSS 11.0 for Windows. Because most of the data were not normally distributed, as revealed by the Kolmogorov-Smirnov test, nonparametric tests were used for further statistical evaluation. The significance level was set at $<.05$. The Kruskal-Wallis test was used to evaluate significant effects between the symptomatic and asymptomatic as well as normal controls in the PCr time constants and ΔPCr_{ss} values during the different increments and recovery, as well as in the ABPIs, ROR, and PCr levels at the end of exercise. For post hoc testing, the Mann-Whitney *U* test was used with a corrected significance level ($<.01$). Regarding the intramuscular pH, we statistically evaluated the values at the end of exercise and during the recovery phase, because we obtained the lowest pH values at these time points. For that, we averaged the serial pH values to a time resolution of 30 seconds and performed the Kruskal-Wallis test at each time point, followed by the Mann-Whitney *U* test (corrected significance level $<.01$). Consequently, the PCr time constants, the ΔPCr_{ss} values, the ABPIs, the ROR, the PCr levels at the end of exercise, and the pH values were expressed as medians with description of the range. Furthermore, the PCr time constants, the ΔPCr_{ss} values, the ABPIs, the RORs, and the pH values that showed significant differences were correlated by using the Spearman rank correlation coefficient *r*. A correlation coefficient *r* of 0.35 to 0.49 was interpreted empirically as low; 0.5 to 0.79, as moderate; and 0.8 or greater, as high. Each coefficient *r* was tested for significance by SPSS.

RESULTS

Clinical data. Each of the 17 patients with PAD who were included into the exercise protocol had a symptomatic and an asymptomatic leg. Between the patient group and the healthy controls, we did not observe any significant difference in age ($P = .276$). The ROR was significantly higher in the symptomatic legs (median, 11.0; range, 5.0-21.5) compared with the asymptomatic legs (median, 6.0; range, 4.0-12.0; $P < .001$). Furthermore, the symptomatic legs (median, 0.72; range, 0.44-1.18) showed significantly lower ABPI values compared with the asymptomatic legs (median, 1.05; range, 0.77-1.21; $P < .001$). Four symptomatic legs stopped the exercise protocol during the third increment, and 11 legs did so during the fourth increment. Two of the symptomatic legs succeeded at all increments. Except for the last two legs, all symptomatic legs showed a progressive PCr decrease during the increment of exercise stop, as shown in Fig 3. For these increments of exercise stop, we did not calculate a PCr time constant. All asymptomatic legs and the legs of the healthy controls fulfilled the entire exercise protocol.

Changes in PCr. We detected significant differences in the PCr end-of-exercise level (Fig 3) between the symptomatic (median, 52.17%; range, 22.47%-70.27%) and the asymptomatic (median, 61.93%; range, 35.88%-73.90%; $P = .009$) legs, as well as the normal controls (median, 66.98%; range, 52.41%-80.94%; $P < .001$), but not between the asymptomatic legs and the normal controls. The calculation of the PCr time constants revealed a median coefficient of determination r^2 of 0.94 (range, 0.68-0.99) for the symptomatic legs, of 0.92 (range, 0.64-0.99) for the asymptomatic legs, and of 0.96 (range, 0.70-0.99) for the healthy controls. We observed significant differences in PCr time constants between the symptomatic and the asymptomatic legs during the second and third increment and

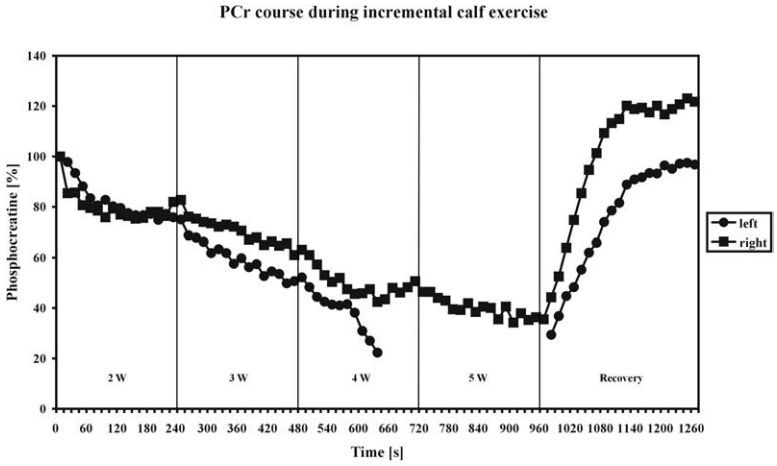

Fig 3. Time course of phosphocreatine *(PCr)* during incremental calf exercise at 2, 3, 4, and 5 W in the peripheral arterial disease patient whose magnetic resonance angiogram is presented in Fig 2. Please note the lower PCr level and the progressive PCr decrease shortly before exercise stop in the symptomatic left leg.

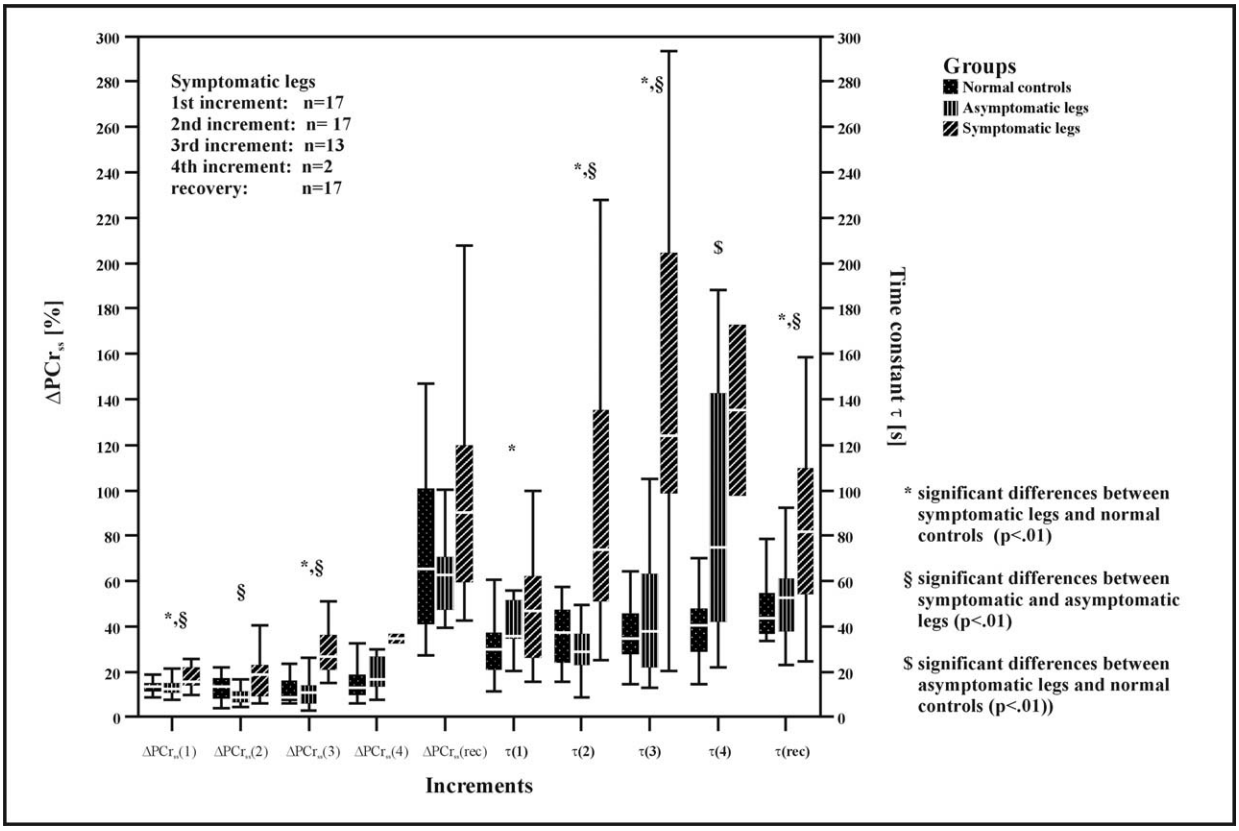

Fig 4. The boxplots present the ΔPCr_{ss} values, meaning the phosphocreatine *(PCr)* difference between the baseline and steady-state level, and the PCr time constants τ for each workload increment (1-4) and the recovery phase *(rec)*. Significant effects are marked.

during the recovery phase, as shown in Fig 4. Because only two symptomatic legs succeeded at the fourth increment, we abstained from a statistical evaluation of this increment. Compared with the normal controls, the PCr time constants of the symptomatic groups were significantly increased during the first three increments and the recovery phase (Fig 4). The asymptomatic legs and the normal controls showed significant differences only during the

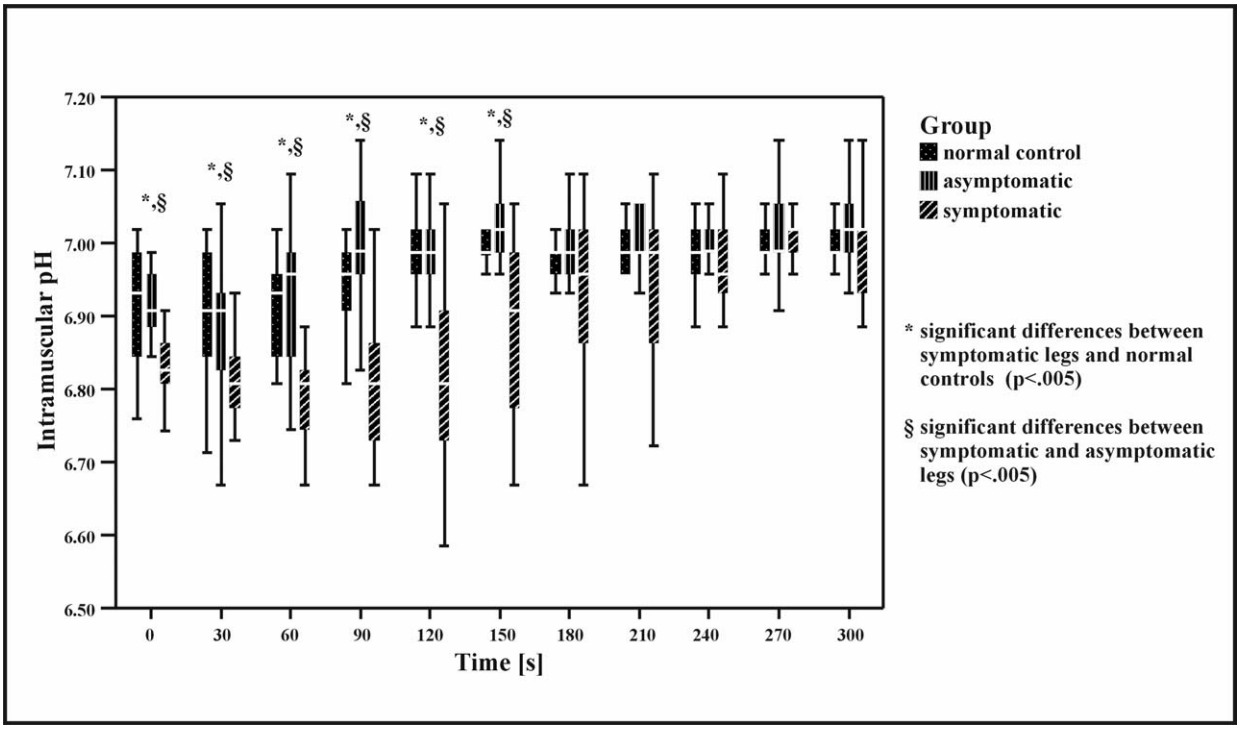

Fig 5. The boxplots show the pH changes over time during the recovery phase, separately for each group (symptomatic, asymptomatic, and control legs). Significant differences are marked.

fourth increment. The ΔPCr_{ss} values of the symptomatic legs were significantly increased during the first three increments, but not during the recovery phase, compared with the asymptomatic legs. Moreover, we detected significant differences in ΔPCr_{ss} between the symptomatic legs and the normal controls during the first and third increments. We did not observe any significant differences between the asymptomatic legs and normal controls.

Changes in pH. We did not detect any significant differences in intramuscular pH between the asymptomatic legs and the normal controls during the entire exercise protocol. Furthermore, the intramuscular pH of the symptomatic legs did not significantly differ from that of the asymptomatic legs and normal controls during first two increments; the statistical evaluation of differences in pH between the symptomatic legs and the remaining groups was hampered by an increasing number of symptomatic legs that stopped the exercise protocol. However, the intramuscular pH at the end of exercise was significantly lower in the symptomatic legs (median, 6.83; range, 6.67-6.99) compared with the asymptomatic legs (6.91; range, 6.73-6.99; $P = .004$) and the normal controls (6.93; range, 6.76-7.02; $P = .003$). As shown in Fig 5, these significant differences were also detected during the first 150 seconds of the recovery time.

Correlation of the parameter. In the patient group, we detected a significant correlation between the RORs and ABPIs ($r = 0.603$; $P < .001$) (Fig 6). In addition, the RORs and ABPIs correlated significantly and moderately with the PCr time constants and the ΔPCr_{ss} values of different increments and the recovery, as demonstrated in the Table. Furthermore, we obtained significant correlations between the PCr levels at the end of exercise, the pH at the end of exercise and during the first 150 s of recovery, the RORs, and ABPIs, as also shown in the Table.

DISCUSSION

As already addressed in the introduction, a substantial number of PAD patients exhibit atypical symptoms or no symptoms.[2-5] This range of atypical leg symptoms beyond the classic IC symptoms is often difficult to distinguish from other differential diagnoses, such as degenerative spine diseases or neuropathies, whereby PAD patients have significantly lower muscle functioning compared with patients with leg pain and without PAD.[5] Most of the methods used in clinical routine for the evaluation of PAD patients, such as peripheral pulse palpation, ABPI, stress tests, or several imaging techniques, reflect and quantify the hemodynamic impairment due to artherosclerotic lesions.[6] Only the treadmill exercise test evaluates the muscle function of PAD patients. However, this test is based on the patients' pain sensation during walking but does not give any information about the metabolic changes in the muscle groups that are involved during walking—mainly the calf muscle. Therefore, an objective method for quantitative determintation of muscle function is required for the evaluation of improvements due to established or experimental therapeutic interventions. In this study we correlated the metabolic changes with the ROR, which was established and

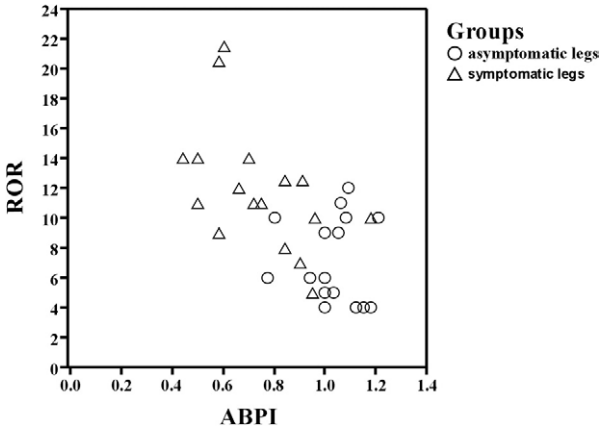

Fig 6. The scatterplot shows the relationship between runoff resistance *(ROR)* and ankle-brachial pressure index *(ABPI)* separated for the symptomatic and asymptomatic legs.

used for the quantification of artherosclerotic lesion load on conventional angiograms.[24,26,27,30] Because the diagnostic accuracy of MRAs is comparable to that of conventional angiograms with respect to the evaluation of PAD,[31,32] we applied the ROR score to MRAs of our patient group and detected a significant correlation between RORs and ABPIs.

Previous experimental ^{31}P MRS studies detected progressive breakdowns of PCr during exercise under cuff occlusion or stenosis in healthy volunteers. This was interpreted as a sign of ischemia within the muscle.[17,18] Several previous studies investigated muscle metabolism in PAD patients and were focused on the PCr recovery after exhaustive exercise rather than on PCr changes during a sophisticated exercise protocol.[20-25] Only one previous study used two different workload levels that were separated by a recovery phase.[20] Two of these previous studies examined the on-kinetics of PCr hydrolysis during isometric exercise.[20,21] However, isometric exercise of the calf muscle is not a physiological condition and is not comparable to isotonic exercise, such as walking.[33,34] In contrast, we performed an incremental, isotonic exercise protocol of the calf muscle, which leads to an increase in blood flow in the feeding arteries.[17,33] Accordingly, a recent study demonstrated that the blood flow increases during isotonic muscle exercise under varying grades of cuff stenoses compared with baseline at rest but that it decreases with compression of the superficial femoral artery compared with exercise without stenosis.[17] Similar results were observed in an experimental study in dogs that demonstrated that the increase in muscle perfusion due to contractile exercise decreases with stenosis severity.[35] Therefore, the increase in oxygen supply induced by muscle exercise is limited by a peripheral stenosis and does not adequately meet the metabolic demand, which obviously depends on the severity of stenosis or exercise intensity. Consequently, the PCr concentration further decreases progressively until exercise stops or passes into a steady state with a prolongation of the PCr time constant.[17,18]

In our study, the symptomatic legs of the PAD patients showed increased PCr time constants and extent of PCr changes during the first three increments compared with normal controls and asymptomatic legs. During the last exercise increment, PCr time constants of only two symptomatic legs could be calculated, so a statistical comparison of these two legs was not useful. The remaining 15 symptomatic legs were exhausted and showed a progressive PCr decrease before exhaustion, as detected during visual inspection of the PCr time curves. Therefore, we were not amazed to observe significantly lower PCr levels at the end of exercise compared with the asymptomatic legs and the healthy controls. This finding was already reported by the previous ^{31}P MRS studies that investigated PAD patients.[20-25] Similar results were also observed by several previous ^{31}P MRS studies that investigated the effect of hypoxia on muscle metabolism during isotonic exercise. Under hypoxia, these studies detected lower PCr levels at comparable time points of exercise and longer PCr recovery times compared with normoxia.[36,37]

It is interesting to note that the asymptomatic legs exhibited increased PCr time constants during the last exercise increment compared with normal controls, thus indicating that the asymptomatic legs, showing a median ABPI of 1.05 with a range between 0.77 and 1.21 and a median ROR of 6.0 with a range between 4.0 and 12.0,

Table. The Spearman correlation coefficients *r* indicate the correlations between the runoff resistance (ROR) and the ankle-brachial pressure indices (ABPI) and the parameters of the high-energy phosphate metabolism.

R	*ROR*	*ABPI*	τPCr_{rec} [$]	ΔPCr_{ssrec} [2]
τPCr_{2W} [§]	.28*	−0.22*		
τPCr_{3W} [§]	.59†	−0.45†		
τPCr_{4W} [§]	.39†	−0.32*		
τPCr_{5W} [§]	.61†	−0.62†		
τPCr_{rec} [$]	.56†	−0.58†		
PCr_{end} [+]	.58†	−0.36†		
ΔPCr_{ss2w} [1]	.51†	−0.39*		
ΔPCr_{ss3w} [1]	.64†	−0.38†		
ΔPCr_{ss4w} [1]	.57†	−0.51†		
ΔPCr_{ss5w} [1]	.72†	−0.64†		
ΔPCr_{ssrec} [2]	.56†	−0.34*		
pH_{end} [3]	−.45†	0.47†	−0.64†	0.55†
pH_{30} [4]	−.53†	0.42†	−0.67†	0.63†
pH_{60} [4]	−.53†	0.54†	−0.67†	0.64†
pH_{90} [4]	−.57†	0.56†	−0.68†	0.63†
pH_{120} [4]	−.48†	0.53†	−0.49†	0.49†
pH_{150} [4]	−.44†	0.43†	−0.46†	0.44†

†Significant ($p < .05$).
*non significant.
§PCr time constants of the exercise increments with a work intensity ranging from 2 to 5W.
$PCr time constant of recovery.
+PCr level at the end of exercise.
[1]Differences between baseline PCr level at the beginning of each increment and the steady-state PCr level during the work intensities ranging from 2 to 5W.
[2]Difference between baseline PCr level at the beginning of recovery and the steady-state PCr level.
[3]pH value at the end of exercise.
[4]pH values at 30, 60, 90, 120 and 150 s of recovery.

were also diseased. However, the asymptomatic legs presented abnormalities in PCr time constants at higher workloads compared with the symptomatic legs; this probably means that the asymptomatic legs were less affected by PAD than the symptomatic legs. Because the median ABPIs of most asymptomatic legs were in the normal range and the performance on the treadmill test depended on the symptomatic leg, which determines the overall walking distance, the degree of PAD in the asymptomatic legs was not recognized on routine clinical examination.

During recovery, we detected significant differences in PCr time constants between the symptomatic legs and the asymptomatic legs, as well as normal controls, but no differences between the asymptomatic legs and the normal controls and no significant differences in the extent of PCr changes. Prolonged PCr recovery times after exhaustive exercise in PAD patients are controversially discussed in the literature. One aspect is the peripheral stenosis that limits the blood flow and oxygen supply during exercise, when the metabolic demand is increased and cannot be satisfied by an appropriate increase in oxygen supply.[20,35] A mitochondrial impairment due to chronic ischemia is also discussed in the literature as a possible explanation of the increased PCr recovery rate[21] and is further supported by histologic evaluations of mitochondria in PAD patients with altered mitochondrial function.[38] However, the PCr time constants and the extent of PCr changes during recovery showed a significant correlation with the RORs and the ABPIs, as already revealed for the exercise increments. Our findings indicate a close correlation between the hemodynamic parameters and PCr kinetics and appear to be similar to the results obtained during muscle exercise under hypoxia, especially the behaviour of PCr.[36,37] In light of these considerations, our results can be completely explained by a lack of oxygen supply with increasing exercise intensity in PAD patients.

The pH levels showed significant differences between the symptomatic legs and the asymptomatic legs, as well as the normal controls, at the end of exercise and during the first 150 seconds of the recovery phase, with a significant correlation to the PCr recovery rate. Corresponding to our results, a recent article[39] indicated that the PCr level at the end of exercise and the pH kinetics during recovery affect the PCr recovery rate. When additionally considering that the [H+] are principally generated by the adenosine triphosphate hydrolysis during muscle exercise, the extent of [H+] generation depends on the work intensity, which is also linearly related to the extent of PCr hydrolysis.[13,10] Therefore, the significant correlations between PCr recovery rate and pH at the end of exercise and during the first 150 seconds of recovery are not amazing. Generally, the intramuscular pH reflects the balance among the amount of adenosine triphosphate that has been hydrolyzed due to contraction, the capacity of oxidative rephosphorylation that binds [H+], and the amount of [H+] that has been discharged from the cytosol to the extracellular space.[13]

In conclusion, our study demonstrated prolonged PCr time constants and an increased amount of PCr hydrolysis in PAD patients during exercise and recovery; these correlated with the RORs and, somewhat more weakly, with the ABPIs. Furthermore, we observed impaired PCr time constants in clinically asymptomatic legs of PAD patients at a work intensity of 5 W compared with normal controls, thus indicating that the asymptomatic legs were also diseased. Several previous studies investigating the effect of hypoxic muscle metabolism observed similar results, and this strongly suggests that our findings were mainly caused by an impaired oxygen supply. However, we cannot clearly define the possible role of a mitochondrial dysfunction due to chronic ischemia in our patients. This should be a focus of future research by investigating the effect of revascularization or angioplasty on high-energy phosphate metabolism. In our opinion, it is important to prove the benefit of different established and new therapies on muscle metabolism and function, which might also contribute to a further improvement of the limb amputation rate in PAD patients.[40]

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