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Investigating a Model for Acute Ischemic Pain in Humans

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Abstract

Background: The pathophysiology of acute ischemic pain is not well established. The aim of the present study was to investigate acute ischemic pain in humans with a view to establish a scientific model to perform future studies. We examined whether peripheral nerve damage and acute inflammation occur during short episodes of acute ischemia.

Methods: Eleven patients with unilateral peripheral arterial disease (PAD) performed treadmill exercise until intolerable ischemic pain urged them to stop. Blood samples were taken before, immediately after treadmill exercise and after a period of recovery. S100B-serum was detected via Elecsys® S100B Immunoassay and IL-6 levels determined via COBAS® IL6 Elecsys 2010.

Results: Treadmill exercise led to intolerable exercise-induced ischemic pain (Numeric Rating Scale) 9 ± 0.3 (0-10, mean ± SEM)) in the PAD affected leg. Reduced ankle/brachial-index (ABI) (0.13 ± 0.05 mean ± SEM) with a mean ankle pressure of 26 ± 9 mmHg, pO2, pH and increased serum lactate indicated that severe ischemia occurred in the PAD affected leg. The inflammation marker IL-6 increased locally and systemically post exercise (n.s.).

Conclusion: We present an effective method to examine acute ischemic pain in humans. By focusing on changes in metabolic parameters in the affected limb, this model could potentially help to evaluate and detect changes during acute ischemic pain and thus contribute to the understanding of the underlying pathophysiology.

Keywords: Acute ischemia; Pain; Exercise; IL-6; S100B; PAD

Introduction

Atherosclerotic diseases, such as symptomatic peripheral arterial disease (PAD), are major causes of morbidity and mortality worldwide [1]. Atherosclerosis leads to narrowing (stenosis) and occlusion of the vessel potentially leading to reduced perfusion of the PAD affected leg. Intermittent claudication is the clinical presentation of exercise induced acute ischemic pain in PAD patients [2]. The aetiology, diagnosis and interventional and therapeutic options in PAD have been examined extensively [3-12], whereas the pathophysiology of acute ischemic pain in PAD has received considerably less attention. The classification, severity and diagnosis of PAD pain are influenced by the subjective reports of the patient [2]. The acute ischemia and its local impact cause the pain during exercise in PAD patients. A correlation between pain intensity and PAD stage has not been determined yet [2]. As such establishing a relationship between the metabolic processes during acute ischemia and the development of pain could facilitate the diagnosis and therapy of PAD and may help in the therapy of ischemic pain. In particular, a model to examine metabolic changes during acute ischemia directly in the affected limb in order to differentiate local from systemic changes might help to understand the pathophysiology of acute ischemic pain.

The pain associated with PAD is thought to result from chronic ischemia and comprises neuropathic and nociceptive pain component [13,14]. Whether peripheral nerve damage actually occurs during exercise induced acute ischemia is unknown. There is currently no diagnostic laboratory test for the detection of peripheral nerve damage. However, the neuroprotein S100B is reported as a biomarker for central nervous system damage, such as traumatic brain damage [15-18]. This study focused on whether S100B is released from peripheral nerves during exercise induced acute ischemic pain [18,19].

An increase in the concentration of markers of systemic inflammation have been described in PAD [20-22]. IL-6 is a marker of acute inflammation and is used widely in clinical practice [23]. Although IL-6 is one of the best studied inflammation markers in PAD, reports of changes in IL-6 during acute ischemia vary considerably [22]. Signorelli et al. and Andreozzi et al. found increased levels of IL-6 after acute ischemia and acute treadmill exercise, whereas Fiotti et al. discovered lowered IL-6 levels after acute ischemia [20,22,24]. Therefore, it is of great interest to look at changes of IL-6 in PAD patients using the investigated model.

Materials and Methods

Patients

Eleven patients over 40 years with unilateral femoral-popliteal PAD, Fontaine grade II (peripheral arterial disease with claudication during exercise) were included in the study and gave their written informed consent one day before the procedure was performed. Patients were scheduled for catheter intervention the following day. Patients with pain at rest or other pain origin, diabetic polyneuropathy or deep vein thrombosis (as well in patient history) were excluded. The study followed the Declaration of Helsinki and was approved by the local ethics committee of the Ludwig-Maximilians-University Munich. Trial registration: Clinicaltrials.gov NCT02192242. Registered July 15th, 2014.

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Study design

Intravenous catheters were placed into the cubital vein and the right and left femoral vein after local anaesthesia for the entire study. Blood samples were taken at baseline, immediately after exercise and after recovery from the PAD affected leg, the non-affected leg and the arm which served as a systemic control. Systemic blood pressure and the ankle-brachial index (ABI) were measured simultaneously during the blood sampling. Systolic blood pressure was determined using the Doppler method (logidop, Fa. Elcat, Wolfratshausen, Germany).

Following venous access, baseline blood sample and clinical examination, patients performed treadmill exercise (Fa. Woodway, Serie **PPS 43MED,** Weil am Rhein, Germany) at a speed of 3.2 km/h up an incline slope of 12%. The time and distance until the first appearance of pain and time and distance until stopping the exercise because of maximum pain in the PAD affected leg were measured. Immediately after termination of the exercise and after recovery (20 minutes later) the above mentioned parameters were obtained again. The patients rated their pain at each point (baseline, immediately after exercise and after recovery) on a numerical rating scale with 0 indicating the absence of pain, while 10 represented the worst pain imaginable. Following the study completion, patients were monitored on the ward.

Blood gas analysis (BGA)

BGA was analysed via an automatic blood gas analysis tool ABL 700 (Brønshøj, Dänemark). The pO_2, pCO_2, pH, venous oxygen saturation, base excess, HCO_3 and lactate were analysed in venous blood.

Ankle-brachial index (ABI)

ABI was used to document the presence of PAD, defined by an ABI ≤0.90. The ABI was measured via conventional Doppler method and calculated as the highest detectable systolic Doppler measurement of the anterior and posterior tibial artery and the highest systolic pressure in the arms.

Analysis of S100B

S-100B-serum levels were detected via Elecsys® S-100B Immunoassay (Roche Diagnostics, Penzberg Deutschland). The total duration of the assay was 18 minutes at 37°C. The test works via a sandwich principle. In the first incubation step the antigen in the sample formed a sandwich complex with a biotinylated monoclonal S100-specific antibody and a monoclonal S100-specific antibody labeled with a ruthenium complex. In the second step streptavidin-coated microparticles were added and the complex bound to the solid phase via interaction of biotin and streptavidin. The electrochemiluminescence measuring cell was constructed as a flow chamber and had three main tasks:

1. The separation of unbound and bound substances. The streptavidin-coated microparticles loaded with immune complexes were drawn to the surface of the electrode with the help of a magnet and held there temporarily. Unbound reagent components and excess sample material were then removed from the measuring cell with ProCell system buffer.

2. The Generation of electrochemiluminescence. Application of a defined voltage induces the electrochemiluminescent reaction and the resulting light emission was measured directly by the photo-multiplier.

3. At the end of the electrochemiluminescent reaction the microparticles were removed with the measuring cell cleaning solution. The measuring cell was ready for the next measurement.

4. The electrochemiluminescent label was a ruthenium complex. A photomultiplier measured the signal intensity in **R**elative **L**ight **U**nits (RLU) [25].

Analysis of IL-6

IL-6 levels were detected via COBAS® IL6 Elecsys 2010 (GNR 511, 512) (Roche Diagnostics Mannheim, Deutschland). The method employs the sandwich principle described for S100B [26].

Statistical analysis

A mixed effect model was performed to determine the significance of changes in IL-6, S100B, BGA and lactate across the time points baseline, directly after exercise and recovery on the affected and non-affected leg and arm. The significance level was set at $p<0.05$. Data are given as means ± SEM.

Results

Basic patient characteristics and risk factors are summarized in Table 1A.

Treadmill exercise and pain

To induce acute ischemic pain, patients performed treadmill exercise until intolerable pain developed. Treadmill exercise results are summarized in Table 1B. At baseline the mean reported pain was 0. Directly after treadmill exercise, pain was rated at 9.0 ± 0.3. After recovery reported pain was 0.1 ± 0.1.

Ischemia

BGA, ABI values and the post exercise decrease of ankle pressures were evaluated to examine and validate acute ischemia. ABI values in the PAD affected leg were significantly lower than those in the non-affected leg. A mean perfusion pressure of 26 ± 9 mmHg was measured post exercise in the ankle arteries of the PAD affected leg. Venous BGA values in the PAD affected leg showed typical ischemic changes with an increase in venous pCO_2 and base excess and a decrease in pH, HCO_3, venous oxygen saturation and pO_2. These changes were not observed in the non-affected leg nor in blood samples from the arm that served as the systemic control. The interaction of points of time and measure points showed statistically significant changes in pO_2, pCO_2, pH, lactate and venous oxygen saturation. Ischemic changes are summarized in Tables 2 and 4 as well as Figures 1-3.

Data of BGA and lactate in the PAD affected leg demonstrated acute ischemia after acute treadmill exercise (Table 2 and Figures 1-3). Comparing baseline and acute treadmill exercise, values derived from the BGA showed the greatest changes in the PAD affected leg (Table 2). Lactate, pCO_2, pH, HCO_3, venous oxygen saturation and pO_2 showed statistically significant differences in comparing locations and points of time (Tables 2 and 4).

S100B

S100B levels in the PAD affected leg were highest at baseline and decreased at the end of acute treadmill exercise and further after a period of recovery. S100B values determined from the control leg and the systemic control increased minimally after treadmill exercise and decreased during recovery. Data are illustrated in Table 3 and Figure 4.

IL-6

The baseline level of IL-6 was higher in the affected leg compared to the control leg. After treadmill exercise although IL-6 levels increased in the affected and control leg as well as for the systemic control, the

A: Patient Characteristics (n=11)		
Age	years (mean ± SD)	65 ± 2
Gender	male/female	8/3
Diabetes mellitus	yes/no	2/9
Arterial Hypertension	yes/no	8/3
Nicotine	yes/no	6/5
Hyperlipidaemia	yes/no	6/5
B: Treadmill Exercise (n=11)		
Distance until pain appearance meters	74 ± 11	
Total distance (meters)	263 ± 43	
Time until pain appearance (seconds)	92 ± 13	
Total time (on treadmill) (seconds)	301 ± 50	
All data are given as mean ± SEM		

Table 1: Patient characteristics (A) and results of the treadmill exercise (B).

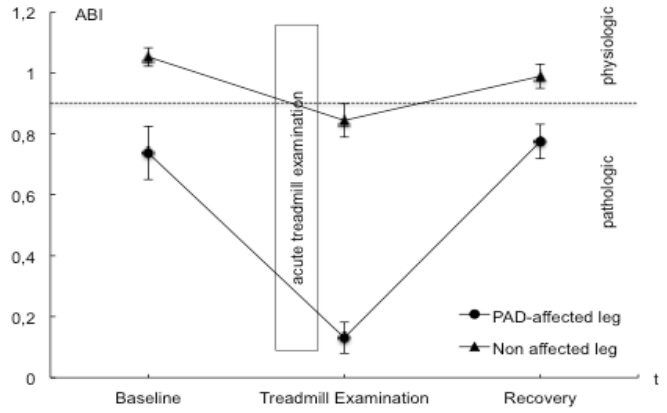

Figure 1: The time course of the effect of acute treadmill exercise on ABI is illustrated. At baseline, ABI was lower in the affected than in the non-affected leg, showing pathological ABI values in the PAD affected leg. At acute treadmill exercise, ABI was lowered to pathological values in both legs, with a greater decrease in the affected leg. Baseline BI levels were restored at recovery.

increase in the affected leg were higher than that in the control leg (Table 3 and Figure 4).

Discussion

The aim of this study was to establish a model to examine acute ischemic pain in humans. During treadmill exercise, acute ischemia and the associated pain were evident in the PAD affected leg but not in non-affected leg nor in the parameters monitoring the systemic response. As an example of use, we evaluated whether S100B levels and Il-6 levels changed during acute ischemic pain.

Model to examine acute ischemia

In order to detect local metabolic changes in metabolites during acute ischemia it was of great importance that ischemia developed in the PAD affected leg and not in the non-affected leg or the systemic control. Additionally, these metabolic changes had to be immediately measured. Blood samples were taken simultaneously at all three locations which was achieved by placement of peripheral venous catheters in the cubital vein and the right and left femoral vein. Catheters were installed at study baseline and remained in situ for the entire study. This way allowed immediate blood sampling following acute treadmill exercise. Blood samples from the cubital vein served as systemic controls, while blood samples from the femoral vein in the PAD affected leg reflected acute ischemia. Whereas blood samples from the femoral vein of the non-affected leg served as exercise controls. The outcome measures ABI, BGA and lactate were evaluated at baseline, immediately after treadmill exercise to detect acute ischemia and after recovery.

At baseline, ABI values were elevated in the PAD affected leg indicating an arterial stenosis. The control leg presented physiological values. In PAD affected limbs, acute treadmill exercise reduced ABI values as well as absolute perfusion pressures, representing an inadequate blood supply to the poststenotic area and an inadequate perfusion [3]. Even though ABI values in the non-affected leg also decreased to pathological values (0.84 ± 0.05, mean ± SEM) (normal values 0.9-1.3), these changes were minor in comparison to the PAD affected leg (0.13 ± 0.05, mean ± SEM) [3]. The absolute values of ankle pressure were 133 ± 10 mmHg indicating absence of ischemia. Therefore, ischemia with an inadequate poststenotic perfusion, defined as an ankle occlusive pressure below 50 mmHg was documented for the affected leg during acute treadmill exercise, can be assumed.

The changes in BGA and lactate are in accordance to earlier described changes during acute ischemia [2,27]. These results support the idea that in the PAD affected leg acute ischemia results in an inadequate perfusion in the poststenotic tissue which represents a deficit in oxygen and substrate supply and a deficient elimination of metabolic products [2]. These findings were less expressed in the non-affected leg and could not observe in the systemic control.

A previous study from Neumann et al. used a similar approach [28]. They investigated 17 patients with PAD, performed venous and arterial cannulation and looked at activation and deformability of neutrophils. Claudication was achieved by consecutive toe stands instead of the treadmill testing in the present study. Ischemia was determined by ABI and lactate concentration, further parameters were not documented. Changes in the arterial system were either less pronounced than those in the venous system (neutrophils) or not affected (lactate). These findings support the determination of metabolic changes in the venous system as a method to document ischemia. In the current study, the ischemic state was more rigorously documented by performing a blood gas analysis to assess changes in pO_2 and pH.

Ischemic pain

Pain was rated subjectively by the patient. In all patients, treadmill exercise was terminated only because of maximum pain development in the PAD affected leg and not because of other reasons i.e. distress or chest pain. In all patients the maximum tolerable pain in the PAD affected leg could be observed during acute treadmill exercise and an ankle occlusion pressure below 50 mmHg was reached.

S100B

Using S100B levels as an index for peripheral nerve damage, short-term acute ischemia did not induce nerve damage. Whether S100B can serve as a surrogate marker for peripheral nerve damage remains to be determined. In general, S100B levels<0.100 µg/l are considered physiological, indicating minor probability of cerebrovascular change or central nerve system damage [29]. Previous studies indicated that physiological S100B levels are lower than the commonly used reference value of <0.100 µg/l [29,30]. At baseline only 2 out of 11 patients and after acute treadmill examination only 3 out of 11 patients in our study showed S100B levels above 0.1 µg/l. Hence, the reference value for S100B levels is not sensitive for the detection of changes in the peripheral nervous system with a lower absolute nerve fibre density

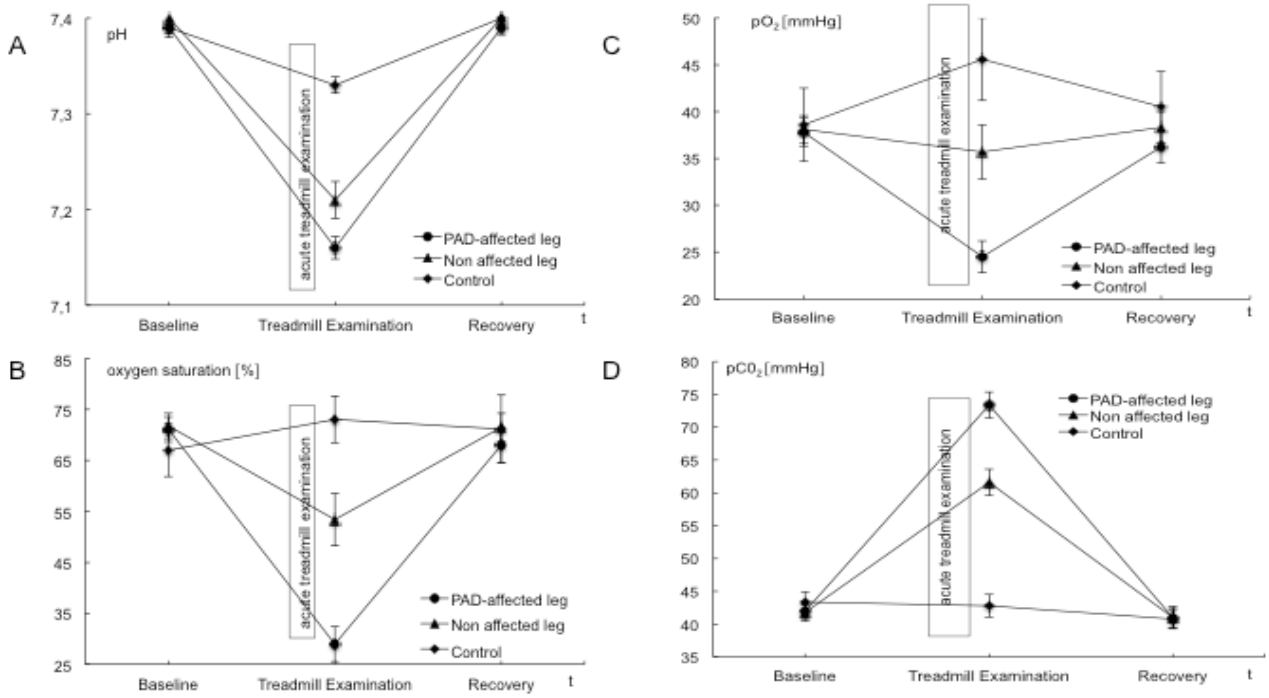

Figure 2: A: Time course of the effect of acute treadmill exercise on pH-levels in venous blood samples is illustrated. The most pronounced lowering in pH during acute treadmill exercise was found in the affected leg, followed by the non-affected leg. The control showed slight lowering of pH level. At recovery, baseline pH levels were restored. B: Time course of the effect of acute treadmill exercise on oxygen saturation [%] in venous blood take is illustrated. Oxygen saturation in the affected leg decreased more than in the non-affected leg. At recovery, baseline oxygen saturation levels were restored. C: Time course of the effect of acute treadmill exercise on pCO2 [mmHg] levels in venous blood samples is illustrated. Acute treadmill exercise led to an increase in pCO2 in the affected leg as well as in the non-affected leg. pCO2 levels in the control decreased minimally. At recovery, baseline pCO2 levels were restored. D: Time course of the effect of acute treadmill exercise on pO2 [mmHg] levels in venous blood take is illustrated. Acute treadmill exercise led to a relevant decrease in pO2 in the affected leg. Decrease in pO2 in the non-affected leg was minimal. The control showed an increase in pO2 levels. At recovery, baseline pO2 levels were nearly restored.

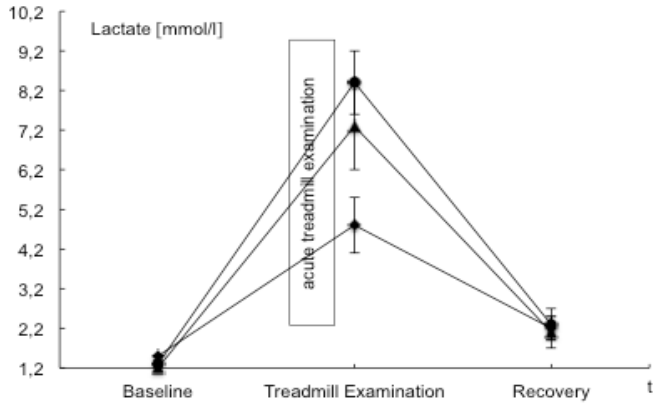

Figure 3: Time course of the effect of acute treadmill exercise on lactate [mmol/l] levels in venous blood samples is illustrated. Acute treadmill exercise led to an increase in lactate in the affected leg, followed by the non-affected leg and systemic control. At recovery, lactate levels remained slightly elevated.

than the CNS [29,30]. Reference values for S100B after peripheral nerve damage do not exist. In our PAD patients peripheral nerve damage was not detectable using S100B (Table 3 and Figure 4). Additional difficulties with the detection of S100B following acute ischemia may be related to the time of analysis following ischemia. Biberthaler et al. reported increased S100B levels after severe brain injury. In contrary to our study, blood withdrawal was 116 ± 18.8 minutes after trauma [29] and not immediately after the assumed trauma (like in the presented study). Anderson et al. measured elevated S100B levels intraoperatively and immediately postoperatively during coronary artery bypass [29,30] and observed a rapid increase in S100B from damaged tissues. Nevertheless it remains difficult to compare the severity of a coronary artery bypass or severe brain injury to exercise induced acute ischemia possibly affecting peripheral nerves. In the periphery, S100B is not only released by Schwann cells but also from muscle and adipose cells [30,31]. An increase in S100B proteins could result from peripheral nerve damage, but also from damages to other tissues. Further studies to establish S100B reference values for peripheral nerve damage and guidelines for the time of sampling would be beneficial.

IL-6

IL-6 levels served as a marker for inflammation. Previous reports of changes in IL-6 during acute ischemia are somewhat varied with some groups reporting increases and others decreased levels of IL-6 after acute ischemia and acute treadmill exercise [20,22,24]. Even though the IL-6 level changes were not significant in this study, an increase after acute treadmill exercise as described by Andreozzi et al. und Signorelli et al. was noted [22,24]. Although the experimental setup in this model was similar, significant changes could not be found in our analysis. Earlier studies looked at patients with more severe PAD, thus it might be possible that IL-6 levels progressively increase with advancing stages of the disease.

In summary, exercise in PAD patients led to intolerable exercise-induced ischemic pain and this was associated with acute local ischemia (changes in ABI, ankle occlusive pressure, pO_2, pH and serum lactate) and an increase in S100B was also observed although the absolute

Time	Baseline	After Treadmill	Recovery
pO_2 [mmHg]			
PAD-affected leg	37.8 ± 1.5	24.5 ± 1.7	36.2 ± 1.7
non-affected leg	38.1 ± 1.5	35.7 ± 2.9	38.3 ± 1.7
control	38.6 ± 3.9	45.6 ± 4.4	40.5 ± 3.8
pCO_2 [mmHg]			
PAD-affected leg	42.0 ± 1.1	73.4 ± 2.0	41.0 ± 1.5
non-affected leg	41.8 ± 1.4	61.6 ± 2.1	41.0 ± 1.8
control	43.3 ± 1.6	42.8 ± 1.7	40.8 ± 1.3
pH			
PAD-affected leg	7.39 ± 0.01	7.16 ± 0.01	7.39 ± 0.01
non-affected leg	7.40 ± 0.01	7.21 ± 0.02	7.40 ± 0.01
control	7.39 ± 0.01	7.33 ± 0.01	7.40 ± 0.01
Oxygen Saturation [%]			
PAD-affected leg	71 ± 2.5	29 ± 3.4	68 ± 3.4
non-affected leg	72 ± 2.6	53 ± 5.4	72 ± 2.9
control	67 ± 5.6	73 ± 5.2	71 ± 4.6
Base Excess [mmol/l]			
PAD-affected leg	1.1 ± 0.6	-2.7 ± 0.9	0.2 ± 0.8
non-affected leg	1.3 ± 0.6	-3.2 ± 1.1	0.4 ± 0.7
control	1.4 ± 0.6	-3.1 ± 1.0	0.5 ± 0.6
HCO_3 [mmol/l]			
PAD-affected leg	24.6 ± 0.5	19.1 ± 0.6	23.8 ± 0.6
non-affected leg	24.8 ± 0.5	19.9 ± 0.8	24.1 ± 0.5
control	24.9 ± 0.4	21.3 ± 0.6	24.3 ± 0.5
Lactate [mmol/l]			
PAD-affected leg	1.3 ± 0.2	8.4 ± 0.8	2.3 ± 0.4
non-affected leg	1.2 ± 0.2	7.3 ± 1.1	2.1 ± 0.4
control	1.5 ± 0.2	4.8 ± 0.7	2.2 ± 0.3
All data are given as mean ± SEM			

Table 2: Presented are the results of the Blood Gas Analysis (pO_2, pCO_2, pH, oxygen saturation, base excess, HCO_3, and lactate at baseline, after treadmill exercise and after recovery. Blood gas analysis (n=11).

Location	Before Treadmill	After Treadmill	Recovery
S100B [µg/l]			
Affected leg	0.090 ± 0.04	0.088 ± 0.02	0.072 ± 0.02
Non-affected leg	0.068 ± 0.02	0.080 ± 0.02	0.054 ± 0.01
Control	0.069 ± 0.1	0.074 ± 0.1	0.054 ± 0.0
Interleukin 6 [pg/ml]			
Affected leg	4.1 ± 0.8	4.6 ± 1.1	4.4 ± 0.7
Non-affected leg	3.3 ± 0.4	3.6 ± 0.5	4.1 ± 0.7
Control	3.8 ± 0.7	4.0 ± 0.9	4.5 ± 0.9
All data are given as mean ± SEM			

Table 3: The data of S100B [µg/l] and Interleukin 6 [pg/ml] are given at baseline, after treadmill exercise and after recovery (S100B and IL-6).

	Time	Location	Interaction Time/Location
S100B	0.0007	0.1790	0.8689
Il-6 pg/ml]	0.2926	0.6391	0.8874
pO_2	0.2665	0.0003	0.0021
$pC0_2$	<0.0001	<0.0001	<0.0001
pH	<0.0001	<0.0001	<0.0001
Venous oxygen saturation	<0.0001	<0.0001	<0.0001
Base Excess	<0.0001	0.94	0.85
HCO_3	<0.0001	0.0029	0.0751
The mixed effect model was used for statistical analysis. The significance level was set at P<0.05			

Table 4: The Results of the statistical analysis. The mixed effect model was used for statistical analysis. The significance level was set at p<0.05. HCO_3, pO_2, pCO_2, pH, and venous oxygen saturation showed statistically significant differences in comparing locations and points of time.

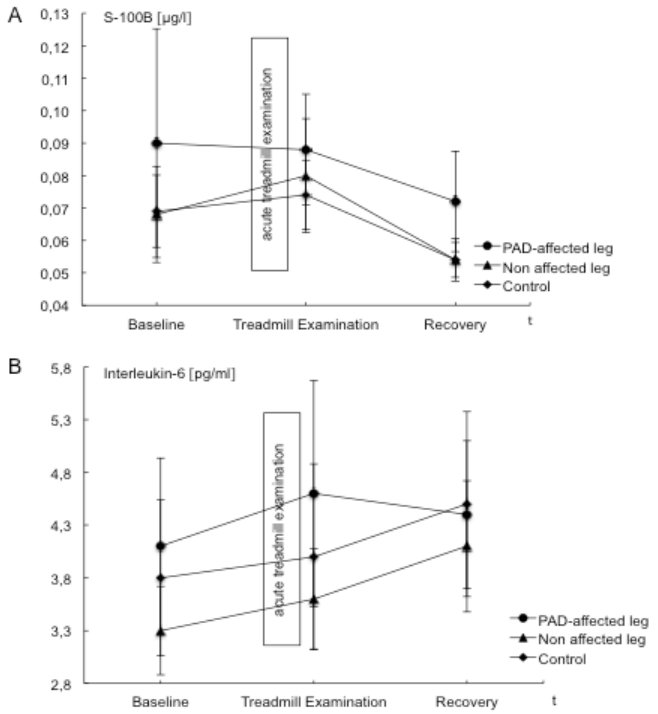

Figure 4: A: Time course of the effect of acute treadmill exercise on S100B [µg/l] levels in venous blood samples is illustrated. S100B levels in the affected leg were highest at baseline, decreasing after acute treadmill exercise and after recovery. Acute treadmill exercise led to an increase in S100B in the non-affected leg and systemic control, decreasing at recovery. B: Time course of the effect of acute treadmill exercise on Interleukin-6 [pg/ml] levels in venous blood samples is illustrated. Acute treadmill exercise led to an increase in Interleukin-6 levels in the systemic control and control leg which further increased at recovery. In the PAD affected leg, Interleukin-6 levels were increased at treadmill exercise whereas a decrease was seen at recovery.

S100B level was below that normally used to evaluate nerve damage in the central nervous system.

We present an effective model to examine acute ischemia and acute ischemic pain in humans. It offers the opportunity to look at metabolites during acute treadmill exercise and compare the local effect of exercise and acute ischemia against a systemic control. This research might be useful in evaluating different pathophysiological states, such as chronic compartment syndrome or further more.

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