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Analysis of ischemic muscle in patients with peripheral artery disease using X-ray spectroscopy

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ARTICLE INFO

Article history:
Received 12 January 2017
Received in revised form
23 May 2017
Accepted 28 June 2017
Available online xxx

Keywords:
Atherosclerosis
X-ray microanalysis
Scanning electron microscopy
Muscle damage
Vascular disease

ABSTRACT

Background: Peripheral artery disease (PAD) is a vascular disease caused by atherosclerosis, resulting in decreased blood flow to the lower extremities. The ankle-brachial index (ABI) is a standard PAD diagnostic test but only identifies reduced blood flow based on blood pressure differences. The early signs of PAD manifest themselves not only at a clinical level but also at an elemental and biochemical level. However, the biochemical and elemental alterations to PAD muscle are not well understood. The objective of this study was to compare fundamental changes in intracellular elemental compositions between control, claudicating, and critical limb ischemia muscle tissue.

Materials and methods: Gastrocnemius biopsies from three subjects including one control (ABI $\geq$ 0.9), one claudicating (0.4 $\leq$ ABI $<$ 0.9), and one critical limb ischemia patient (ABI $<$ 0.4) were evaluated using a scanning electron microscope and energy dispersive X-ray spectroscopy to quantify differences in elemental compositions. Spectra were collected for five myofibers per specimen. An analysis of variance was performed to identify significant differences in muscle elemental compositions.

Results: This study revealed that intracellular magnesium and calcium were lower in PAD compared with control myofibers, whereas sulfur was higher. Magnesium and calcium are antagonistic, meaning, if magnesium concentrations go down calcium concentrations should go up. However, our findings do not support this antagonism in PAD. Our analysis found decreases in sodium and potassium, in PAD myofibers.

Conclusions: These findings may provide insight into the pathologic mechanisms that may operate in ischemic muscle and aid in the development of specialized preventive and rehabilitative treatment plans for PAD patients.

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http://dx.doi.org/10.1016/j.jss.2017.06.073

Introduction

Peripheral artery disease (PAD) is a chronic disease caused by atherosclerosis, affecting more than eight million lives in United States.[1] It can result in the narrowing or blockage of major arteries that supply the lower extremities, and if left untreated, the severity of the disease can lead to limb amputation.[2] The most common symptom of PAD is intermittent claudication, which is characterized by lower extremity pain that is exhibited during mild exercise, such as walking, but resolves after rest.[3] However, some patients with PAD fail to mention these symptoms to a physician, which contributes to the underdiagnoses of PAD.[4] If the disease continues to progress, tissue damage becomes more severe, and the patient experiences critical limb ischemia (CLI), a condition characterized by chronic pain at rest and tissue loss.[2] Once the disease has reached this end stage, hypoxic tissue damage is significant, and the viability of the limb is poor, frequently requiring amputation in the absence of revascularization.[5,6]

The pathophysiology of PAD is complex and involves changes occurring on both physical and biochemical levels such as reduced blood flow, altered metabolic processes, skeletal muscle degeneration, and oxidative damage.[7-11] The ankle-brachial index (ABI) is a simple and noninvasive test that physicians use to diagnose PAD and is performed by determining the ratio of systolic blood pressure in the ankle to that in the arm. However, this method has limitations in monitoring progression or regression of PAD; both abnormal blood flow and its effects on skeletal muscle need to be measured to determine the degree of muscle damage or repair. There is a fundamental lack of detail concerning muscle degeneration and recuperation in response to vascular reperfusion surgeries. Hence, there is a need to investigate the effects of PAD on skeletal muscle at a cellular and molecular level.

Previous work from our group studied morphologic parameters of myofibers including area, roundness, perimeter, diameter, major and minor axes, solidity and fiber density of muscles classified as control, claudicating, and CLI samples.[8] We have also performed spectroscopic studies, aimed at developing techniques for identifying new spectral biomarkers and elucidating biochemical mechanisms critical to hypoxic cell damage.[7]

Scanning electron microscopy and elemental microanalysis techniques have been extensively applied in the field of engineering and biological sciences for analyzing and quantifying all the elements, with the exception of H, He, and Li.[12] Energy dispersive X-ray spectroscopy (EDS) is a method used in analytical chemistry to detect and characterize the elemental composition of a given sample. By exciting a material using a high-energy beam, characteristic X-rays are emitted from the probed atom and is measured as a spectral profile. Scanning electron microscope (SEM)-EDS uses the electron beam formed from the instrument to excite the sample and produce X-rays. This form of X-ray microanalysis has been used in past research as a sensitive method to determine elemental concentrations in skeletal muscle.[13,14] Studies have also investigated the role of ionic concentration changes during cell injury, tenotomy in rat muscles, and human skeletal muscle after knee surgeries and have provided strong evidence that the elemental fluctuations are due to disturbed homeostasis of intracellular concentrations in cells.[15-17] Thus, SEM-EDS was selected as a candidate to ascertain the effects of PAD on muscle function, by determining the change of elemental composition in myofibers.

The central hypothesis for this study was that key changes in the elemental composition can be correlated with the progression of PAD in affected patients. The objective of this study was to compare changes in intracellular elemental compositions such as calcium (Ca), sodium (Na), potassium (K), magnesium (Mg), and sulfur (S) in control, claudicating, and critical ischemic muscle. Ca, Na, K, Mg, and S were selected as the elements of interest in this study because of their biological importance in muscle function. Further, these electrolytes were shown to undergo significant changes in diseased cardiac muscle,[18] driving the motivation to determine if similar changes occurred in PAD muscle.

Materials and methods

Tissue collection

The tissue sample collection protocol was approved by the Institutional Review Board of the VA Nebraska-Western Iowa and the University of Nebraska Medical Center, and all subjects gave informed consent. Muscle tissue specimens were collected from one patient with clinically diagnosed CLI (ABI $<$ 0.4), one patient with clinically diagnosed claudication (0.4 $\leq$ ABI$<$0.9), and one control patient (no lower limb impairment or symptoms of PAD and a resting ABI $\geq$ 0.9).[19] Demographic details are listed in Table. The control patient had 25 pack-years smoking, and quit 2 y before the study. The claudicating patient was at 40 pack-years smoking and was down to one pack per month for the last 5 y. The CLI patient was at 34 pack-years smoking and is currently down to half a pack per day. Muscle biopsies were collected using a 6-mm Bergstrom needle from the anteromedial aspect of the gastrocnemius, approximately 10 cm distal to the tibial tuberosity. Tissue samples were sectioned at 4 μm, fixed in methacarn, and embedded in paraffin in accordance with traditional tissue processing methods.[20,21] Before collecting scanning electron microscopy images, the specimens were

Table – Demographics of the control patient, the patient with claudication, and the patient with CLI.

Characteristics	Control	Claudication	CLI
Age (y)	61	63	70
ABI	1.1	0.4	0.1
Sex	Male	Male	Male
Obesity	Yes	No	Yes
Hypertension	Yes	Yes	Yes
Diabetes mellitus	No	No	No
Smoking	Former	Current	Current