Peripheral artery disease (PAD) characterized by atherosclerotic blockages of the arteries supplying blood to lower extremities and affects approximately 10 million lives in the United States. Patients diagnosed with PAD have increased risks of limb loss and mortality. The classic symptom of PAD is intermittent claudication (IC), defined as walking-induced calf pain and gait dysfunction relieved by rest. In more advanced disease (critical limb ischemia), the patient experiences pain at rest and/or tissue loss. Functional testing, such as the ankle brachial index (ABI), measured as the ratio of systolic blood pressure in the ankle to that in the arm, is the most common test for the diagnosis of PAD. The ABI can identify reduced blood flow (due to blockages in the arteries) based on blood pressure differences. However, there is a need to measure more than just abnormal blood flow, there is a need to measure the secondary effects on the end organ (skeletal muscle). In this study, we evaluated the hypothesis that differences in muscle elemental composition correlate with clinical diagnosis and may be used to characterize severity of muscle damage. The objective of this study was to compare elemental composition including sodium, potassium, calcium, magnesium and sulfur in myofibers of gastrocnemius biopsies from control subjects and PAD patients at different stages of disease. We evaluated gastrocnemius biopsies from three subjects including one control (person without PAD), one claudicating patient (ABI<0.9) and one critical limb ischemia patient (ABI<0.4). Using a scanning electron microscope and energy dispersive X-ray spectroscopy (EDS), differences in elemental concentrations between control and PAD muscle samples were quantified. In total, 15 myofibers were analyzed, 5 from each tissue specimen. An analysis of variance was performed to identify significant differences in muscle elemental concentration. A statistical analysis of variance revealed significant differences in elemental concentrations for sodium (p=0.0001), potassium (p=0.0094), calcium (p=0.003), magnesium (p=0.0001) and sulfur (p=0.004) among control, claudicating and critical limb ischemic muscle samples. Scanning electron microscopy and EDS were able to characterize changes to the elemental concentration in PAD muscle, which correlated with clinical diagnosis of PAD. These findings may aid in providing a foundation for the development of specialized preventive and rehabilitative treatment plans by providing new targets for treatment based on the underlying altered elemental concentrations.