Peripheral artery disease (PAD) is characterized by atherosclerotic blockages of the arteries supplying blood to lower extremities and affects approximately 10 million lives in the United States. Functional testing, such as ankle brachial index (ABI), can identify reduced blood flow (due to blockages in the arteries) based on blood pressure differences between the ankle and arm. 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 severity of muscle damage can be characterized by analyzing the differences in muscle elemental concentration. The objective of this study was to compare elemental concentration including sodium, potassium, calcium, magnesium and sulfur in myofibers of gastrocnemius biopsies from control subjects and PAD patients. We obtained gastrocnemius biopsies from three subjects including control, claudicating and critical limb ischemia (CLI) patients. In total, 15 myofibers were analyzed, 5 from each tissue specimen. Using a scanning electron microscope (SEM) and energy dispersive X-ray spectroscopy (EDS), differences in elemental concentrations between control and PAD muscle samples were quantified. An analysis of variance was performed and significant differences (p<0.05) in muscle elemental concentration were found. SEM 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 the identification of neglected therapeutic targets and the development of specialized preventive or rehabilitative treatment plans.