HHS Public Access

Author manuscript

Arterioscler Thromb Vasc Biol. Author manuscript; available in PMC 2022 October 16.

Published in final edited form as:

Arterioscler Thromb Vasc Biol. 2020 November ; 40(11): 2577–2585. doi:10.1161/ATVBAHA.120.313831.

SKELETAL MUSCLE PATHOLOGY IN PERIPHERAL ARTERY DISEASE: A BRIEF REVIEW

Mary M. McDermott, MD[1], **Luigi Ferrucci, MD, PhD**[2], **Marta Gonzalez-Freire, PhD**[3], **Kate Kosmac, PhD**[4], **Christiaan Leeuwenburgh, PhD**[5], **Charlotte A. Peterson, PhD**[4], **Sunil Saini, PhD**[5], **Robert Sufit, MD**[6]

[1]Northwestern University Feinberg School of Medicine Department of Medicine and Preventive Medicine

[2]National Institute on Aging Division of Intramural Research

[3]Health Research Institute of the Balearic Islands (IdISBa)

[4]University of Kentucky Center for Muscle Biology

[5]University of Florida, Department of Aging and Geriatric Research

[6]Northwestern University Feinberg School of Medicine, Department of Neurology

Abstract

This brief review summarizes current evidence regarding lower extremity peripheral artery disease (PAD) and lower extremity skeletal muscle pathology. Lower extremity ischemia is associated with reduced calf skeletal muscle area and increased calf muscle fat infiltration and fibrosis on computed tomography (CT) or magnetic resonance imaging. Even within the same individual, the leg with more severe ischemia has more adverse calf muscle characteristics than the leg with less severe ischemia. More adverse CT-measured calf muscle characteristics, such as reduce calf muscle density, are associated with higher rates of mobility loss in people with PAD. Calf muscle in people with PAD may also have reduced mitochondrial activity compared to those without PAD, although evidence is inconsistent. Muscle biopsies document increased oxidative stress in PAD. Reduced calf muscle perfusion, impaired mitochondrial activity, and smaller myofibers are associated with greater walking impairment in PAD. Preliminary evidence suggests that calf muscle pathology in PAD may be reversible. In a small uncontrolled trial, revascularization improved both the ankle brachial index and mitochondrial activity, measured by calf muscle phosphocreatine recovery time. A pilot clinical trial showed that cocoa flavanols increased measures of myofiber health, mitochondrial activity, and capillary density while simultaneously improving six-minute walk distance in PAD. Calf muscle pathologic changes are associated with impaired walking performance in people with PAD, and interventions that both increase calf perfusion and improve calf muscle health are promising therapies to improve and walking performance in PAD.

Potential conflicts: Dr. McDermott reports other research support from ReserveAge, Helixmith, Chromadex, ArtAssist, Mars, and Hershey. Dr. McDermott reports research support from Regeneron.

Lower extremity peripheral artery disease (PAD) affects approximately 8.5 million people in the United States and nearly 200 million worldwide (1,2). PAD is defined by atherosclerotic blockages of arterial supply of the lower extremities and is characterized by reduced oxygen and energy delivery to lower extremity skeletal muscle during walking activity, resulting in leg discomfort and/or weakness during walking that typically resolve within ten minutes of rest. Because of reduced lower extremity perfusion and reduced energy and oxygen delivery to leg muscles, people with PAD walk significantly shorter distances in a six-minute walk test, and have slower walking velocity and lower physical activity than people without PAD, even after adjusting for potential confounders (3). Over a six-month period, people with PAD typically decline by 10 meters in the distance they are able to walk in six minutes and 20–25% report new mobility loss, defined as difficulty walking up and down a flight of stairs or walking ¼ mile without assistance (4–6).

While PAD is primarily defined by atherosclerotic narrowing of lower extremity arteries, people with PAD also have significant pathology in calf skeletal muscle, including reduced calf muscle area, increased calf muscle fatty infiltration and fibrosis, and metabolic and cellular abnormalities, compared to people without PAD (7–13). This review summarizes evidence regarding the pathologic changes in lower extremity muscle of people with PAD and clinical significance of these skeletal muscle pathologic changes.

Ischemia and calf skeletal muscle

While reduced physical activity levels in people with PAD likely contributes to atrophy of lower extremity muscle, substantial evidence shows that lower extremity ischemia-reperfusion injury may also contribute directly to lower extremity skeletal muscle damage in PAD. Several examples follow. First, in a clinical trial of 26 patients with PAD randomized to treadmill exercise training, lower extremity strength training, or a non-exercising control group for 12 weeks, participants randomized to treadmill exercise training had greater denervation in calf muscle (from 7.6% to 15.6% denervated muscle fibers) at 12-week follow-up, compared to the other two groups (7). Second, in an observational study of 704 people age 59 and older, including 439 with PAD, more severe lower extremity ischemia, measured by lower ankle brachial index (ABI) values, was associated with smaller calf muscle area and greater calf muscle fatty infiltration, measured by CT imaging, even after statistical adjustment for physical activity (8). Mean calf muscle area values were: ABI < 0.50: 5,193 mm^2; ABI 0.50–0.90: 5,536 mm^2; and ABI 0.91–1.30: 5,941 mm^2, P<0.001 and mean percent fat values were: ABI<0.50: 12.8%; ABI 0.50–0.90:11.4%; ABI 0.90–1.30: 9.2% (P trend=0.02). In the subset of participants with PAD without history of lower extremity revascularization whose right and left leg ABI values differed by at least 0.20 (N=92), mean intra-individual comparisons of the lower vs. higher leg ABI values demonstrated more adverse changes in the leg with greater ischemia: (lower vs. higher leg ABI calf muscle area: 5,283 mm^2 (SD: 1,403) vs. 5,511 mm^2 (SD:1,230), P=0.001 and lower vs. higher leg ABI calf muscle fatty infiltration: 11.4% vs. 9.5%, P=0.04) (8). Third, an uncontrolled clinical trial of 10 participants with PAD undergoing unilateral revascularization showed significant improvement in calf muscle phosphocreatine recovery time in the revascularized leg, measured by ^{31}P magnetic resonance spectroscopy (from 91±33 seconds to 52±34 seconds, P<0.003) along with significant improvement in ABI

(from 0.62±0.17 to 0.93±0.25, P<0.003 (14). Together, these data suggest that PAD-related ischemia reperfusion results in calf skeletal muscle damage and that revascularization may improve skeletal muscle damage in people with PAD.

Associations of lower extremity skeletal muscle pathology with walking impairment in PAD

In 370 people with PAD, more adverse calf muscle characteristics, measured by CT imaging, were associated with higher rates of mobility loss, defined as becoming unable to walk up and down a flight of stairs or walk ¼ mile without assistance (9). Rates of mobility loss were 5.4%, 13%, and 25% across tertiles of CT measured calf muscle density, with lower muscle density associated with higher rates of mobility loss (P<0.001). Rates of mobility loss were 25%, 9%, and 10% across tertiles of CT measured calf muscle area, with smaller calf muscle area associated with higher rates of mobility loss (P<0.01) (9). These associations remained statistically significant after adjusting for known and potential confounders, including severity of PAD (9).

In 424 people with PAD, plantar flexion isometric strength and knee extension power were significantly poorer compared to 271 people without PAD after adjusting for confounders (13). There were no significant differences in hand grip or knee extension isometric strength between people with vs. without PAD (13). The planter flexion isometric strength measures calf muscle strength, while knee extension isometric strength measures quadriceps strength. In people with PAD, lower values for calf muscle density, plantarflexion strength, knee extension power, and hand grip strength were each associated with higher rates of mortality (15). However, poorer hand grip strength has also been associated with increased mortality in people without PAD (16).

PAD and calf muscle mitochondrial abnormalities

Pre-clinical and preliminary human evidence demonstrated mitochondrial abnormalities in lower extremity skeletal muscle exposed to lower extremity ischemia. In a mouse model of hind limb ischemia, ischemia and reperfusion of limb skeletal muscle was associated with greater mitochondrial dysfunction, increased oxidative damage (measured by greater abundance of total protein carbonyl and 4-hydroxy-2-nonenal), and reduced electron transport chain complex I, III, and IV activity, compared to absence of hind-limb ischemia (17–19). In humans, Pipinos et al. reported reduced mitochondrial electron transport chain activity in complexes I, III and IV and reduced mitochondrial respiration in calf muscle of people undergoing surgery for advanced PAD (n =25, mean age =63, mean ABI =0.34) compared to control patients undergoing leg operations to treat varicose veins (n =16, mean age =60, mean ABI =1.10) (20). White et al reported a higher prevalence of muscle fibers lacking complex I, IV and succinate dehydrogenase activity (complex II), in 26 people with PAD compared to seven without PAD (21).

However, not all studies consistently showed reduced mitochondrial activity in PAD. Hou et al. reported no difference in ATP production or citrate synthase activity in seven people with PAD (mean age 62 years, mean ABI of 0.68) compared to 11 healthy controls without

PAD (mean age=60.8 years, mean ABI=1.31) (22). Hart et al. reported no difference in mitochondrial or citrate synthase activity in ten people with PAD (mean age=65, ABI = 0.67) compared to 12 healthy controls (mean age=62, mean ABI=1.18) (19). Inconsistencies in reports of presence of mitochondrial dysfunction in people with PAD compared to those without PAD may be due to the smaller sample sizes in the studies showing no differences or may be due to variation in severity of PAD between these studies (20–23). A recent study of 19 patients with critical limb ischemia (mean ABI 0.35 SD:0.30), 27 patients with mild to moderate PAD (mean ABI 0.56, SD: 0.21) and 32 people without PAD (no ABI provided) showed down-regulation of mitochondrial gene expression in the patients with critical limb ischemia but no difference in these measures between participants with mild to moderate PAD vs. those without PAD (24). However, this study also showed lower expression of mitochondrial oxidative phosphorylation proteins in PAD participants with mild to moderate PAD, compared to healthy controls, and suggested that mitochondrial activity was lower in the PAD participants with mild to moderate PAD compared to healthy controls, although specific quantitative data and exact p values for the latter comparisons were not presented (24). None of the studies adjusted for potential confounders (20–24). Further study with larger sample sizes that adjust for potential confounders are needed.

Anderson et al used magnetic resonance imaging (MRI) to measure calf muscle perfusion and ^{31}P magnetic resonance spectroscopy phosphocreatine recovery time constant at peak exercise to measure calf muscle mitochondrial activity in 85 patients with mild to moderate PAD (mean ABI 0.69: SD 0.14) (25). Phosphocreatine recovery time, a measure of mitochondrial health (with lower values indicating better mitochondria health), was significantly and negatively correlated with ABI ($r=-0.32$, $P<0.01$) and with maximal treadmill walking time ($r=-0.22$, $P<0.05$), but was not associated significantly with tissue perfusion or six-minute walk distance. In contrast, calf muscle perfusion was associated significantly with six-minute walk distance ($r=0.32$, $P<0.01$), but not with treadmill walking distance (25). Optimal mitochondrial oxidative capacity requires effective delivery of oxygen and substrate as well as healthy mitochondria. As described above, a small uncontrolled study showed that lower extremity revascularization in PAD significantly improved mitochondrial phosphocreatine recovery time (14).

PAD and Mitochondrial DNA copy number

Most cellular DNA exists in chromosomes within the cell nucleus. However, mitochondria also contain DNA, termed “mitochondrial DNA” (mtDNA), consisting of 37 genes in humans. Normal function of all 37 genes is required for optimal mitochondrial function. Calf muscle exposure to oxidative stress via repeated episodes of ischemia-reperfusion may promote accumulation of DNA damage in people with PAD at a higher frequency than in people without PAD, potentially reducing the number and activity of mitochondria in people with PAD. In nine people with unilateral PAD (mean age=62 years), multiple mtDNA deletion mutations were identified in calf muscle from both the ischemic and non-ischemic limb, with ischemic limb having a greater abundance of mtDNA damage (26,27). The most common mtDNA deletion was a 4977 bp deletion, associated with loss of genes encoding subunits of respiratory chain complexes (ATPase6, ATPase8, COXIII, ND3, ND4, ND4L, and ND5).

MtDNA damage stimulates mitochondrial biogenesis (28). McDermott et al. reported an inverse association of ABI with mtDNA copy number in 34 people with PAD (mean age 73.5, mean ABI 0.67) and 10 without PAD (mean age =73.1, ABI =1.14)) (29). Higher mtDNA copy number was associated with better six-minute walk distance in those without PAD and in those with mild to moderate PAD, but not in participants with severe PAD. Although this study was cross-sectional and no causal inferences can be made, one possible explanation for these findings is that mechanisms compensating for mtDNA damage may become exhausted in people with severe or long-standing PAD and fail to overcome the adverse effects of ischemia on lower extremity mitochondria. Longitudinal study is needed to explore this hypothesis.

Mitochondrial DNA heteroplasmy

The D-loop of mtDNA is a non-coding region essential for normal mtDNA replication that is particularly susceptible to damage from ischemia. When mtDNA is damaged and not fully repaired, the result is mitochondria containing both wild type and mutated mtDNA, a condition termed “heteroplasmy”. Twenty-five percent of people without chronic disease have detectable heteroplasmy in blood and saliva, particularly in the non-coding mtDNA D-loop (30). Patients with coronary artery disease have an increased abundance of heteroplasmy in myocardial mitochondria compared to controls (31) and 22 human aorta specimens with atherosclerosis contained a greater abundance of mitochondrial mutations compared to human aorta sections without atherosclerosis (32). In calf muscle biopsies from 33 people with PAD (mean age 73.7 years, mean ABI 0.67) and nine without PAD (mean age 73.3 years, mean ABI 1.14), participants with PAD had a significantly higher abundance of heteroplasmy in muscle compared to participants without PAD (33). MtDNA heteroplasmy was more abundant in the D-loop and cytochrome b coding regions in participants with PAD compared to those without PAD (P=0.037) (33). PAD participants with both lower abundance of mtDNA heteroplasmy and lower mtDNA copy number had faster walking speed than PAD participants without both of these mtDNA characteristics (33).

Calf muscle histopathology in PAD

Although PAD is defined by atherosclerotic narrowing of lower extremity arteries, some studies reported higher capillary density while others reported lower capillary density in people with PAD compared to those without PAD (9,21,34-38). However, in two of the studies reporting lower capillary density in participants with PAD, these differences were no longer statistically significant after adjusting for fiber area or number (36,37). A third study reporting lower capillary density in people with PAD compared to those without PAD, was limited by a 12 year age difference between those with vs. without PAD (38).

White et al. reported a higher number of capillaries per fiber in muscle from people with PAD compared to those without PAD (21). However, greater capillary density was not associated with better functional performance (21). It is possible that increased capillary density in people with PAD represents a compensatory phenomenon, similar to increased numbers and size of collateral vessels observed in people with more severe PAD (39),

but these compensatory phenomena are not sufficient to overcome the adverse effects of muscle ischemia in PAD. In a clinical trial in which 35 participants with PAD were randomized to supervised vs. home-based exercise training for 12 weeks, capillary density significantly increased among participants randomized to supervised exercise training at 3-week follow-up (216 ±66 vs. 284 ± 77, P<0.01), but there were no significant differences in capillary density between the supervised and home-based exercise groups and there were no within group differences in change in capillary density per myofiber at either 3 or 12 week follow-up in participants randomized to supervised exercise (1.72±0.5 vs. 1.87 ± 0.70, P=0.08) (40).

Fiber atrophy has been observed in muscle biopsies from people with PAD (21,41,42). However, some studies reported that PAD was associated with atrophy in type II fibers, the muscle fibers that rely on anaerobic respiration and are responsible for short quick bursts of speed (41,42), while other studies reported that people with PAD have atrophy in type I fibers, the muscle fibers that rely on aerobic metabolism and are recruited during endurance activity (21). Muscle fiber shape also varies within PAD muscles compared to control (42–45). Koutakis et al. collected muscle biopsies from 154 patients with PAD undergoing vascular surgery (mean age 62.9 (SD:7.5), mean ABI 0.51 (SD:0.25)) and 85 controls undergoing non-vascular surgery (mean age 62.5 (SD:9.8), mean ABI 1.08 (SD:0.08)) (43). Muscle from patients with PAD had significantly smaller myofiber area and perimeter than those without PAD (43).

During normal aging, type II fibers can become denervated and may be reinnervated with a slow motor neuron, thereby converting the fiber to type I, potentially rescuing the muscle fiber. Grouping of type I fibers indicates type II re-innervation and conversion to type I. Myofiber atrophy due to denervation is characterized by small, tightly packed, angular appearing myofibers with a crowded angular appearance. Reinnervation is characterized by appearance of the myofibers in groups (i.e. ‘grouping’). Angular and grouped muscle fibers have been described in PAD, and angular fibers consistent with denervation are more common in people with lower ABI values (7,42,45). White et al reported type I fiber grouping in calf muscles from some individuals with PAD, and larger type I myofibers were associated with faster walking velocity (21). However, extreme variability in calf muscle fiber type composition was observed, with type I fibers comprising 9% to 81% of total muscle fibers (21). This variability may partially explain prior conflicting reports regarding the predominant myofiber type in PAD, with some showing higher muscle area occupied by type I fibers in PAD compared to age-matched controls while others report fewer type I muscle fibers in people with PAD along with an increased abundance of IIX fibers, normally associated with inactivity (17,21,41,42,45–48).

Muscle fiber contractile properties and oxidative capacity are closely associated with mitochondria activity within skeletal muscles, with slow-twitch, type I fibers having greater mitochondrial content and activity compared to fast-twitch, type II fibers (49). As described above, mitochondrial dysfunction has been documented in PAD and may be a major contributor to PAD myopathy (19–25). In 26 calf muscle biopsies from patients with PAD, White et al. reported absence of intermyofibrillar mitochondrial activity in up to 17% of oxidative, type I fibers (21). Mitochondrial transmembrane proteins were absent in these

muscle fibers, indicating a complete loss of mitochondria within these fibers (21). The autophagosome marker LC3 was observed in fiber areas lacking mitochondria, suggesting mitochondrial degradation or recycling (21). Larger fiber size was positively associated with the proportion of fibers lacking mitochondria in PAD, suggesting mitochondrial destruction in the center of larger fibers where oxygen deficiencies are most severe (Figure 1).

Abnormal actin and myosin in PAD

Abnormalities in actin and myosin, proteins responsible for muscle contraction, have also been reported in PAD (46,50). In healthy muscle, actin and myosin are highly organized within the muscle sarcomere, facilitating coordinated contraction of a muscle fiber. Sjostrom et al. reported sarcomere disruptions within muscle fibers from PAD patients, consisting of irregularly packed myofibrils and Z-disk abnormalities, evidence of disorganization in normal muscle fiber structure (46). The protein desmin, integral for myofibrillar alignment at the Z-discs, was also disorganized in calf muscle from people with PAD (50). A recent clinical trial showed that PAD muscle fibers contain a high proportion of central nuclei, which may also disrupt normal contractile function (51). Together, these findings document alterations of basic muscle fiber properties with PAD. Delineating how these changes effect muscle performance and contribute to functional impairment with PAD requires further study.

Implications of ischemia-related muscle pathology for treatment of PAD

Randomized trials have tested whether lower extremity muscle strengthening improves walking performance in people with PAD (52–54). McGuigan et al. randomized 20 participants with PAD to resistance training or a control group for 24 weeks. Resistance training increased type I and type II muscle fiber area, capillary density, maximum leg press, calf press strength, and treadmill walking distance compared to the control group (52). However, in a separate trial of 156 participants with PAD randomized to supervised treadmill exercise, supervised lower extremity resistance training, or attention control, those randomized to supervised treadmill exercise improved six-minute walk distance by 35.9 meters (95% CI: +15.3, +56.5, $p<0.001$) while those randomized to resistance training improved their six-minute walk distance by 12.4 meters (95% CI: −8.42, +33.3, P=0.24) compared to control, a difference that was not statistically significant but was consistent with a clinically meaningful effect (53,54). A meta-analysis of 15 randomized trials including 826 participants with PAD randomized to resistance training or a comparator concluded that resistance training significantly improved treadmill walking compared to a control group, but did not improve six-minute walk distance (55). Improvement in six-minute walk may be more meaningful to patients and relevant to walking in daily life than improvement in maximal treadmill walking (56,57). Older patients with PAD can find treadmill walking particularly difficult and the treadmill test is associated with a substantial learning effect (55,56). For these reasons, and because the effects of strength training on six-minute walk are not as great as the effects of walking exercise on six minute walk, current evidence supports walking exercise as a more effective therapy for PAD than strength training.

Therapeutic interventions that improve perfusion simultaneously with calf skeletal muscle abnormalities may have the greatest effects on walking impairment in people with PAD (58). Consistent with this hypothesis, randomized trials have consistently demonstrated that combining lower extremity revascularization with supervised treadmill exercise improves walking performance more than lower extremity revascularization or supervised treadmill exercise alone (58). A recent pilot study of 44 participants with PAD randomized to a cocoa-flavanol beverage vs. placebo demonstrated that the cocoa flavanol beverage significantly improved six-minute walk distance at 6-month follow-up while simultaneously improving calf muscle perfusion and calf muscle biopsy measured capillary density, COX enzyme activity, and reducing the proportion of myofibers with central nuclei (51).

Additional Considerations

Many aspects of the pathophysiologic effects of lower extremity ischemia on skeletal muscle remain unclear. First, walking exercise substantially improves walking ability in people with PAD but simultaneously exposes calf muscle to ischemia reperfusion and can promote calf muscle denervation (7). Reasons for this inconsistency require further investigation. It is likely that benefits of walking exercise on perfusion and skeletal muscle overcome the possible adverse effects of increased denervation. Second, while it is possible that lower extremity skeletal muscle damage in PAD presents a “final common pathway” common to other disease states associated with lower extremity muscle damage such as chronic obstructive pulmonary disease and heart failure, current evidence supports a unique and specific effect of PAD on skeletal muscle pathology. For example, while PAD was associated with increased calf muscle mitochondrial DNA, increased capillary density, and heterogeneous proportions of Type 1 and Type 2 myofibers compared to people without PAD, COPD was associated with reduced lower extremity skeletal muscle mitochondria abundance, lower capillary density, and reduced abundance of Type 1 myofibers compared to people without COPD (59–62). Third, current evidence suggests that novel therapies for PAD should aim to improve calf muscle perfusion, mitochondria activity, and myofiber size and quality of function. However, the most important skeletal muscle targets for therapeutic interventions to improve walking performance remain unclear. Fourth, while smaller calf muscle area, lower muscle density, and increased calf muscle fat infiltration were associated with a greater abundance of circulating inflammatory biomarkers and homocysteine in PAD (63), to our knowledge circulating biomarkers associated with the histopathologic and mitochondrial deficiencies have not been identified.

In summary, people with PAD have pathologic changes in lower extremity skeletal muscle compared to people without PAD. Identifying skeletal muscle targets for novel therapies has the potential to improve walking performance and prevent mobility loss in the large and growing number of people disabled by PAD.

ACKNOWLEDGEMENTS

Funding:

The American Heart Association Strategically Focused Research Network in Vascular Diseaes: SFRN 33900097, SFRN33900136, SFRN33970010.

Nonstandard Abbreviations and Acronyms

ABI	Ankle brachial index
CT	Computed tomography
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA
PAD	Peripheral artery disease

REFERENCES

1. Fowkes FG, Rudan D, Rudan I, Aboyans V, Denenberg, JO, McDermott MM, Norman PE, Sampson UK, Williams, LJ, Mensah GA, Criqui MH. Comparison of global estimates of prevalence and risk factors for peripheral artery disease in 2000 and 2010: a systematic review and analysis. Lancet 2013;382:1329–1340. [PubMed: 23915883]
2. Brass EP, Koster D, Hiatt WR, Amato A. A systematic review and meta-analysis of propionyl-L-carnitine effects on exercise performance in patients with claudication. Vasc Med 2013;18:3–12. [PubMed: 23321261]
3. McDermott MM, Greenland P, Liu K, Guralnik JM, Celic L, Criqui MH, Chan C, Martin GJ, Schneider J, Pearce WH, Taylor LM, Clark E. The ankle brachial index is associated with leg function and physical activity: the Walking and Leg Circulation Study. Ann Intern Med 2002;136:873–883. [PubMed: 12069561]
4. McDermott MM, Liu K, Greenland P, Guralnik JM, Criqui MH, Chan C, Pearce WH, Schneider JR, Ferrucci L, Celic L, Taylor LM, Vonesh E, Martin GJ, Clark E. Functional decline in peripheral arterial disease: associations with the ankle brachial index and leg symptoms. JAMA 2004;292:453–461. [PubMed: 15280343]
5. McDermott MM, Guralnik J, Tian L, Zhao L, Polonsky TS, Kibbe MR, Criqui MH, Zhang D, Conte MS, Domanchuk K, Li L, Sufit R, Leeuwenburgh C, Ferrucci L. Comparing 6-minute walk versus treadmill walking distance as outcomes in randomized trials of peripheral artery disease. J Vasc Surg 2019;3:988–1001.
6. McDermott MM, Guralnik JM, Criqui MH, Ferrucci L, Liu K, Spring B, Tian L, Domanchuk K, Kibbe M, Zhao L, Lloyd Jones D, Liao Y, Gao Y, Rejeski WJ. Unsupervised exercise and mobility loss in peripheral artery disease: a randomized controlled trial. J Am Heart Assoc 2015;4;1–13.
7. Hiatt WR, Regensteiner JG, Wolfel EE, Carry MR, Brass EP. Effect of exercise training on skeletal muscle histology and metabolism in peripheral arterial disease. J Appl Physiol 1996;81:780–788. [PubMed: 8872646]
8. McDermott MM, Hoff F, Ferrucci L, Pearce WH, Guralnik JM, Tian L, Liu K, Schneider JR, Sharma L, Tan J, Criqui MH. Lower extremity ischemia, calf skeletal muscle characteristics, and functional impairment in peripheral arterial disease. J Am Geriatr Soc 2007;55:400–406. [PubMed: 17341243]
9. Kosmac K, Gonzalez-Freire M, McDermott MM, et al. Correlations of calf muscle macrophage content with muscle properties and walking performance in peripheral artery disease. J Am Heart Assoc 2020;9:e015929. [PubMed: 32390569]
10. Lin YC, Chuang WY, Wei FC, et al. Peripheral arterial disease: The role of extracellular volume measurements in lower limb muscles with MRI. Eur Radiol 2020;30(7):3943–3950. [PubMed: 32166488]
11. Pipinos I, Shepard AD, Aangnostopoulos PV, Katsamouris A, Boska BD. Phosphorus 31 nuclear magnetic resonance spectroscopy suggests a mitochondrial defect in claudicating skeletal muscle. J Vasc Surg 2000;31:944–952. [PubMed: 10805885]
12. McDermott MM, Ferrucci L, Guralnik J, Tian L, Liu K, Hoff F, Liao Y, Criqui MH. Pathophysiological changes in calf muscle predict mobility loss at 2-year follow-up in men and women with peripheral arterial disease. Circulation 2009;120:1048–1055. [PubMed: 19738138]

13. McDermott MM, Tian L, Ferrucci L, Liu K, Guralnik JM, Liao Y, Pearce WH, Criqui MH. Associations between lower extremity ischemia, upper and lower extremity strength, and functional impairment in peripheral arterial disease. J Am Geriatr Soc 2008;56:724–729. [PubMed: 18284536]
14. West AM, Anderson JD, Epstein FH, Meyer CH, Hagspiel KD et al. Percutaneous intervention in peripheral artery disease improves calf muscle phosphocreatine recovery kinetics: A pilot study. Vasc Med 2012;17:3–9. [PubMed: 22363013]
15. McDermott MM, Liu K, Tian L, Guralnik JM, Criqui MH, Liao Y, Ferrucci L. Calf muscle characteristic, strength measures, and mortality in peripheral arterial disease: a longitudinal study. J Am Coll Cardiol 2012;59:1159–1167. [PubMed: 22440216]
16. Cooper R, Kuh D, Hardy R, Mortality Review Group: FALCon and HALCyon Study Teams. Objectively measured physical capabilities and mortality. Systematic review and meta-analysis. BMJ 2010;341:C4467. [PubMed: 20829298]
17. Pipinos II, Swanson SA, Zhu Z, Nella AA, Weiss DJ, Gutti TL, McComb RD, Baxter BT, Lynch TG, Casale GP. Chronically ischemic mouse skeletal muscle exhibits myopathy in association with mitochondrial dysfunction and oxidative damage. Am J Physiol Regul Integr Comp Physiol 2008;295:290–296.
18. Weiss DJ, Casale GP, Koutakis P, Nella AA, Swanson SA, Zhu Z, Miserlis D, Johanning JM, Pipinos II. Oxidative damage and myofiber degeneration in the gastrocnemius of patients with peripheral arterial disease. J Transl Med 2013;11:1–9. [PubMed: 23281771]
19. Thaveau F, Zoll J, Bouitbir J, Ribera F, Marco PD, Chakfe N, Kretz JG, Piquard F, Geny B. Contralateral leg as a control during skeletal muscle ischemia-reperfusion. J Surg Res 2009;155:65–69. [PubMed: 19159910]
20. Pipinos II, Judge AR, Zhu Z, Selsby JT, Swanson SA, Johanning JM, Baxter BT, Lynch TG, Dodd SL. Mitochondrial defects and oxidative damage in patients with peripheral arterial disease. Free Radic Biol Med 2006;41:262–269. [PubMed: 16814106]
21. White SH, McDermott MM, Sufit RL, et al. Walking performance is positively correlated to calf muscle fiber size in peripheral artery disease subjects, but fibers show aberrant mitophagy: An observational study. J Transl Med 2016;14:284. [PubMed: 27687713]
22. Hou XY, Green S, Askew CD, Barker G, Green A, Walker PJ. Skeletal muscle mitochondrial ATP production rate and walking performance in peripheral arterial disease. Clin Physiol Funct Imaging 2002;22:226–232. [PubMed: 12076351]
23. Hart CR, Layec G, Trinity JD, Kwon OS, Zhao J, Reese VR, Gifford JR, Richardson RS. Increased skeletal muscle mitochondrial free radical production in peripheral arterial disease despite preserved mitochondrial respiratory capacity. Exp Physiol 2018;103:838–850. [PubMed: 29604234]
24. Ryan TE, Yamaguchi DJ, Schmidt CA, et al. Extensive skeletal muscle cell mitochondriopathy distinguishes critical limb ischemia patients from claudicants. JCI Insight 2018;3:1–18.
25. Anderson JD, Epstein FH, Meyer CH, Hagspiel KD, Wang H, Berr SS, Harthun NL, Weltman A, Dimaria JM, West AM, Kramer CM. Multifactorial determinants of functional capacity in peripheral arterial disease: Uncoupling of calf muscle perfusion and metabolism. J AM Coll Cardiol 2009;54:628–635. [PubMed: 19660694]
26. Brass EP, Wang H, Hiatt WR. Multiple skeletal muscle mitochondrial DNA deletions in patients with unilateral peripheral arterial disease. Vasc Med 2000;5:225–230. [PubMed: 11213234]
27. Bhat HK, Hiatt WR, Hoppel CL, Brass EP. Skeletal muscle mitochondrial DNA injury in patients with unilateral peripheral arterial disease. Circulation 1999;99:807–812. [PubMed: 9989967]
28. Pickles S, Vigie P, Youle RJ. Mitophagy and quality control mechanisms in mitochondrial maintenance. Curr Biol 2018;28:R170–R185. [PubMed: 29462587]
29. McDermott MM, Peterson CA, Sufit R, Ferrucci L, Guralnik JM, Kibbe MR, Polonsky TS, Tian L, Criqui MH, Zhao L, Stein JH, Li L, Leeuwenburgh. Peripheral artery disease, calf skeletal muscle mitochondrial DNA copy number, and functional performance. Vasc Med 2018;23:340–348. [PubMed: 29734865]

30. Li M, Schönberg A, Schaefer M, Schroeder R, Nasidze I, Stoneking M. Detecting heteroplasmy from high-throughput sequencing of complete human mitochondrial DNA genomes. Am J Hum Genet 2010;87:237–249. [PubMed: 20696290]

31. Hefti E, Blanco JG. Mitochondrial DNA heteroplasmy in cardiac tissue from individuals with and without coronary artery disease. Mitochondrial DNA A DNA Mapp Seg Anal 2018;29:587–593.

32. Sobenin IA, Sazonova MA, Postnov AY, Bobryshev YV, Orekhov AN. Mitochondrial mutations are associated with atherosclerotic lesions in the human aorta. Clin Dev Immunol 2012;2012:1–5.

33. Gonzalez-Freire M, Zenobia Moore A, Peterson CA, Kosmac K, McDermott MM, Sufit RL, Guralnik JM, Polonsky T, Tian L, Kibbe MR, Criqui MH, Li L, Leeuwenburgh C, Ferrucci L. Associations of peripheral artery diseaes with calf skeletal muscle mitochondrial DNA heteroplasmy. J Am Heart Assoc (In press).

34. Ho TK, Rajkumar V, Black CM, Abraham DJ, Baker DM. Increased angiogenic response but deficient arteriolization and abnormal microvessel ultrastructure in critical leg ischaemia. Br J Surg 2006;93:1368–1376. [PubMed: 16952207]

35. McGuigan MR, Bronks R, Newton RU, Sharman MJ, Graham JC, Cody DV, Kraemer WJ. Muscle fiber characteristics in patients with peripheral arterial disease. Med Sci Sports Exerc 2001;33:2016–2021. [PubMed: 11740293]

36. Askew CD, Green S, Walker PJ, Kerr GK, Green AA, Williams AD, Febbraio MA. Skeletal muscle phenotype is associated with exercise tolerance in patients with peripheral arterial disease. J Vasc Surg 2005;41:802–807. [PubMed: 15886664]

37. Baum O, Torchetti E, Malik C, Hoier B, Walker M. Walker PJ, Odriozola A, Graber F, Tschanz SA, Bangsbo J, Hoppeler J, Askew CD, Hellsten Y. Capillary ultrastructure and mitochondrial volume density in skeletal muscle in relation to reduced exercise capacity of patients with intermittent claudication. Am J Physiol Regul Integr Comp Physiol 2016;310:943–951.

38. Jones WS, Duscha BD, Robbins JL, Duggan NN, Regensteiner JG, Kraus WE, Hiatt WR, Dokun AO, Annex BH. Alteration in angiogenic and anti-angiogenic forms of vascular endothelial growth factor-A in skeletal muscle of patients with intermittent claudication following exercise training. Vasc Med 2012;17:94–100. [PubMed: 22402934]

39. Keeling AN, Carroll TJ, McDermott MM, Liu K, Liao Y, Farrelly CT, Pearce WH, Carr J. Clinical correlates of size and number of collateral vessels in peripheral artery disease. Vasc Med 2012;17:223–230. [PubMed: 22738758]

40. Duscha B, Robbins JL, Jones WS, Kraus WE, Lye RJ et al. Angiogenesis in skeletal muscle precede improvements in peak oxygen uptake in peripheral artery disease patients. Arterioscler Thromb Vasc Biol 2011;31:2742–2748. [PubMed: 21868709]

41. Koutakis P, Weiss DJ, Miserlis D, Shostrom VK, Papoutsi E, Ha DM, Carpenter LA, McComb RD, Casale GP, Pipinos, II. Oxidative damage in the gastrocnemius of patients with peripheral artery disease is myofiber type selective. Redox Biol 2014;2:921–928. [PubMed: 25180168]

42. Regensteiner JG, Wolfel EE, Brass EP, Carry MR, Ringel SP, Hargarten ME, Stamm ER, Hiatt WR. Chronic changes in skeletal muscle histology and function in peripheral arterial disease. Circulation 1993;87:413–421. [PubMed: 8425290]

43. Koutakis P, Myers SA, Cluff K. Abnormal myofiber morphology and limb dysfunction in claudication. J Surg Res 2015;196:172–179. [PubMed: 25791828]

44. Cluff K, Miserlis D, Naganathan GK, Pipinos, II, Koutakis P, Samal A, McComb RD, Subbiah J, Casale GP. Morphometric analysis of gastrocnemius muscle biopsies from patients with peripheral arterial disease: Objective grading of muscle degeneration. Am J Physiol Regul Integr Comp Physiol 2013;305:291–299.

45. England JD, Regensteiner JG, Ringel SP, Carry MR, Hiatt WR. Muscle denervation in peripheral arterial disease. Neurology 1992;42:994–999. [PubMed: 1579257]

46. Sjostrom M, Angquist KA, Rais O. Intermittent claudication and muscle fiber fine structure: Correlation between clinical and morphological data. Ultrastruct Pathol 1980;1:309–326. [PubMed: 7233586]

47. Hedberg B, Angquist KA, Sjostrom M. Peripheral arterial insufficiency and the fine structure of the gastrocnemius muscle. Int Angiol 1988;7:50–59. [PubMed: 3385269]

48. Fry CS, Noehren B, Mula J, Ubele MF, Westgate PM, Kern PA, Peterson CA. Fiber type-specific satellite cell response to aerobic training in sedentary adults. J Physiol 2014;592:2625–2636. [PubMed: 24687582]
49. Pette D, Staron RS. Molecular basis of the phenotypic characteristics of mammalian muscle fibres. Ciba Found Symp 1988;138:22–34. [PubMed: 3058430]
50. Koutakis P, Miserlis D, Myers SA. Abnormal accumulation of desmin in gastrocnemius myofibers of patients with peripheral artery disease: Associations with altered myofiber morphology and density, mitochondrial dysfunction and impaired limb function. J Histochem Cytochem 2015;63:256–269. [PubMed: 25575565]
51. McDermott MM, Criqui MH, Domanchuk K, Ferrucci L, Guralnik JM, Kibbe MR, Kosmac K, Kramer CM, Leeuwenburgh C, Li L, et al. Cocoa to improve walking performance in older people with peripheral artery disease: The COCOA-PAD pilot randomized clinical trial. Circulation Res 2020;126:589–599. [PubMed: 32078436]
52. McGuigan MR, Bronks R, Newton RU, Sharman MJ, Graham JC, Cody DV, Kraemer WJ. Resistance training in patients with peripheral arterial disease: Effects on myosin isoforms, fiber type distribution, and capillary supply to skeletal muscle. J Gerontol A Biol Sci Med Sci 2001;56:302–310.
53. McDermott MM, Ades P, Guralnik JM et al. Treadmill exercise and resistance training in patients with peripheral arterial disease with and without intermittent claudication: A randomized controlled trial. JAMA 2009;301:165–174. [PubMed: 19141764]
54. McDermott MM, Tian L, Criqui MH et al. Meaningful change in 6-minute walk in people with peripheral artery disease. J Vasc Surg 2020;S0741 −5214:31053–3.
55. Parmenter BJ, Mavros Y, Ritti Dias R et al. Resistance training as a treatment for older persons with peripheral artery disease: A systematic review and meta-analysis. Br J Sports Med 2019;0:1–11.
56. McDermott MM, Guralnik JM, Criqui MH, Liu K, Kibbe MR, Ferrucci L. Six-minute walk is a better outcome measure than treadmill walking tests in therapeutic trials of patients with peripheral artery disease. Circulation 2014;130:61–68. [PubMed: 24982117]
57. McDermott MM, Guralnik JM, Tian L, Zhao L, Polonsky TS, Kibbe MR, Criqui MH, Zhang D, Conte MS, Domanchuk K, Li L, Sufit R, Leeuwenburgh C, Ferrucci L. Comparing 6-minute walk versus treadmill walking distance as outcomes in randomized trials of peripheral artery disease. J vasc Surg 2020;71:988–1001. [PubMed: 31870756]
58. McDermott MM, Kibbe MR. Improving lower extremity functioning in peripheral artery disease: Exercise, endovascular revascularization, or both? JAMA 2017;317:689–690. [PubMed: 28241363]
59. Gosker HR, Hesselink MKC, Duimel H, Ward KA, Schols AMWJ. Eur Respir J 2007;30:73–79. [PubMed: 17428811]
60. Jobin J, Maltais F, Doyon JF, LeBlanc P, Simard PM, Simard AA, Simard C. Chronic obstructive pulmonary disease: capillarity and fiber-type characteristics of skeletal muscle. J Cardiopulm Rehabil 1998;18:432–437. [PubMed: 9857275]
61. Maltais F, Sullivan MJ, Leblanc P, et al. Altered expression of myosin heavy chain in the vastus lateralis muscle in patients with COPD. Eur Respir J 1999;13:850–854. [PubMed: 10362052]
62. Whittom F, Jobin J, Simard PM, Leblanc P, Simard C, Bernard S, Belleau R, Maltais F. Histochemical and morphological characteristics of the vastus lateralis muscle in patients with chronic obstructive pulmonary disease. Med Sci Sport Exerc 1998;30;1467–1474
63. McDermott MM, Ferrucci L, Guralnik JM, Tian L, Green D, Liu K, Tan J, Liao Y, Pearce WH, Schneider JR, Ridker P, Rifai N, Hoff F, Criqui MH. Elevated levels of inflammation, d-dimer, and homocysteine are associated with adverse calf muscle characteristics and reduced calf strength in peripheral arterial disease. J Am Coll Cardiol 2007;50:897–905. [PubMed: 17719478]

HIGHLIGHTS

- Lower extremity peripheral artery disease is associated with reduced calf muscle area and increased calf muscle fatty infiltration and fibrosis.
- Calf muscle biopsy abnormalities in PAD include mitochondrial dysfunction, signs of denervation, and fibers lacking mitochondria centrally
- Calf muscle abnormalities in PAD are associated with functional impairment and mobility loss.
- Preliminary evidence suggests that interventions that reverse calf muscle abnormalities can improve walking performance in PAD

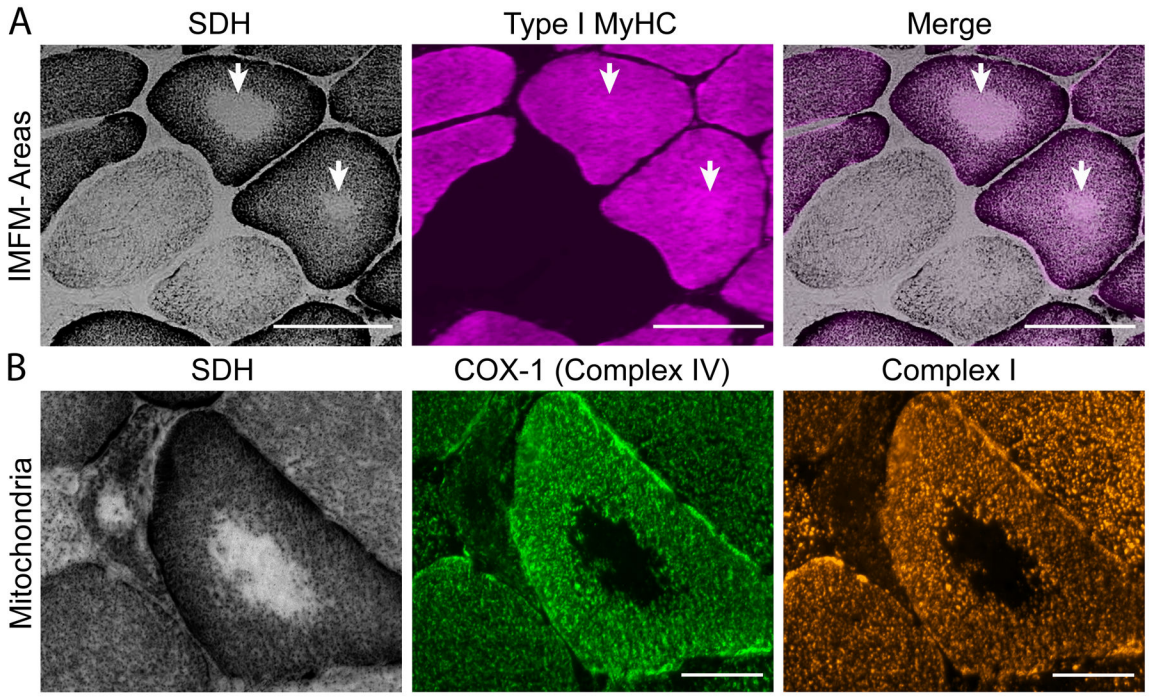

Figure 1. Fiber areas lacking intermyofibrillar mitochondria in gastrocnemius muscle from PAD patients.

A. Histochemistry for succinate dehydrogenase (SDH) and immunohistochemistry (IHC) identifying type I myosin heavy chain (MyHC; pink) showing mitochondrial cavities in type I fibers within the gastrocnemius muscle of a PAD patient. Representative region of interest from an image acquired at 100x magnification. Scale bars = 100 μm. B. SDH and IHC for mitochondrial membrane COX-1 (complex IV; green) and complex I (orange) showing an absence of mitochondria and mitochondrial activity in the center of a gastrocnemius muscle fiber from a PAD patient. Image acquired at 400x magnification. Scale bars = 50 μm.

Table.

Calf skeletal muscle pathology in peripheral artery disease

	Calf muscle pathologic changes in PAD	Associations with functional impairment and decline	Additional information or controversies
Computed tomography imaging	Greater leg ischemia is associated with smaller muscle area and greater fat infiltration.	Smaller muscle area and greater fat infiltration of calf muscle were associated with greater functional impairment and increased rates of mobility loss	
Mitochondrial activity	Lower extremity ischemia is associated with reduced mitochondrial activity.	Reduced mitochondrial activity has been associated with poorer treadmill walking time but not with six-minute walk distance.	Data on the associations of mitochondrial activity with functional performance are mixed, with some studies showing a direct association and others showing no association.
Mitochondrial DNA damage	People with PAD have increased mitochondrial DNA mutations compared to those without PAD	Preliminary evidence shows that among people with PAD, those with lower mitochondrial copy number combined with lower mitochondrial DNA heteroplasmy have faster walking velocity.	Mitochondrial DNA damage was observed even in the unaffected leg of patients with unilateral PAD. However, the abundance of mitochondrial DNA damage was greater in the leg with ischemia.
Capillary density	Studies both show higher and lower capillary density in people with PAD. Greater capillary density may be a compensatory mechanism in PAD.	No association of capillary density and walking impairment has been demonstrated in people with PAD.	At least one study showing a lower capillary density in people with PAD compared to those without PAD was limited by a 12 year difference in age between those with vs. without PAD. Capillary density decreases with age in older people.
Histopathologic changes	Changes in myofiber typing are heterogeneous between people with PAD. Increases in type I and type II myofibers have been demonstrated in PAD. Leg ischemia is associated with changes of denervation. Larger myofibers have ‘holes’ that are devoid of mitochondrial activity, with evidence of autophagic marker accumulation in the holes. Central nuclei are also increased in muscle of people with PAD.	Larger myofibers are associated with faster walking velocity.	There is substantial heterogeneity in the relative abundance of type 1 vs. type 2 fibers in people with PAD.