Circulation Research

EDITORIAL

Multiomics Analyses of Peripheral Artery Disease Muscle Biopsies

Ishita Jain,* Beu P. Oropeza,* Ngan F. Huang

Peripheral artery disease (PAD) affects 10 million people in the United States and >230 million worldwide.[1,2] It is associated with impaired vascular perfusion to the lower extremities, leading to limb-threatening amputation in severe cases of critical limb ischemia. Patients with PAD have an inferior prognosis and reduced limb function, when compared with patients without PAD.[3] Very few effective therapies have been identified, in part, because the key biologic pathways associated with functional impairment remain unclear. Current treatments include supervised and home-based walking exercise to improve the mobility in patients with PAD.[4,5] A better understanding of the underlying pathological mechanisms of skeletal muscle damage underlying PAD may help identify new therapeutic opportunities.

Article, see p 1428

In this issue of *Circulation Research*, Ferrucci et al[6] characterized the biological pathways that may be responsible for the functional limitations in PAD. The results confirm the expected role of hypoxia and also identify novel and unexpected findings that may lead to new interventional targets for PAD. The authors performed in-depth transcriptomic and proteomic analyses on gastrocnemius muscle biopsies from patients with PAD and non-PAD control participants. The study consisted of age-matched muscle biopsy samples from 31 PAD donors without diabetes or chronic limb-threatening ischemia, along with samples from 29 non-PAD donors. The inclusion criteria for PAD samples consisted of having an ankle-brachial index <0.90 without chronic limb-threatening ischemia. The gastrocnemius muscle biopsy specimens were dissected from visible fat tissue. This study combined transcriptomic and proteomic data analysis to perform coregulation and network-based analyses to identify transcript-protein pairs that were differentially correlated in muscle from patients with PAD, compared with non-PAD participants, which had not been done before.

Gene set enrichment analysis of RNA sequencing data revealed many pathways that were enriched in PAD samples. Such pathways included those that were related to hypoxia, including PTEN (phosphatase and tensin homolog), PI3K (phosphoinositide 3-kinase), and MAPK (mitogen-activated protein kinase) signaling. WNT, Hedgehog, and Notch were among the key signaling pathways that were involved in the repair of damage caused by chronic hypoxia or ischemia/reperfusion. These pathways are involved in muscle regeneration and angiogenesis and may be important for facilitating the coregulation of target genes necessary for tissue repair. Owing to the chronic hypoxia or ischemia/reperfusion that is associated with PAD, the authors showed that additional tissue damage was observed in the form of increased inflammation. The prolonged hypoxic environment in ischemic muscle was associated with induced mitochondrial damage, reduced ATP production, and stimulated inflammatory responses, including the activation of NF-κB (nuclear factor kappa B). Consistent with these findings, protein set enrichment analysis

Key Words: Editorial ■ chronic limb-threatening ischemia ■ gene expression profiling ■ peripheral arterial disease ■ proteomics

Correspondence to: Ngan F. Huang, PhD, Department of Cardiothoracic Surgery, Stanford University, 300 Pasteur Dr, MC 5407, Stanford, CA 94305.
Email ngantina@stanford.edu
*I. Jain and B.P. Oropeza contributed equally.
For Sources of Funding and Disclosures, see page 1446.

Circulation Research is available at www.ahajournals.org/journal/res

demonstrated that PAD samples were characterized by an increase in inflammation and a decrease in glycolysis. Compared with non-PAD participants, PAD participants had lower levels of rate-limiting glycolytic enzymes like hexokinase that was consistent with diminished glucose metabolism. This finding was intriguing, as glycolysis is critically important for ATP production in hypoxic environments, and so this finding should further be validated by metabolic assays. The combined multiomics analysis suggested the activation of hypoxia compensatory mechanisms in PAD muscle, including inflammation, fibrosis, apoptosis, angiogenesis, unfolded protein response, and nerve and muscle repair (Figure).

However, there was also notable inconsistency in findings between the proteomics and transcriptomics data, especially in mitochondrial mRNAs-proteins. Among the transcripts-proteins that were significantly differentially expressed at both the mRNA and protein levels, 17 of them had downregulated transcripts but were at higher protein levels in PAD samples, compared with non-PAD samples. Interestingly, 9 out of the 17 were mitochondrial proteins such as the TRAM (mitochondrial transcription factor A). The authors reasoned that the higher abundance of protein levels may be due to impaired mitophagy and fragmented protein complexes, ultimately leading to transcriptional inhibition of related genes. They performed a coregulation network analysis of the mitochondrial proteins, in which protein complexes were found to be more loosely connected in PAD samples, compared with non-PAD samples. In PAD samples, the mitochondrial respiratory proteins had abnormal stoichiometric proportions, compared with non-PAD samples. This stoichiometric imbalance suggested that the respiratory proteins were not part of complete functional units, but were not removed because of impaired mitophagy, and that the accumulation of these proteins could impede the transcription of other related genes.

The authors also uncovered additional biological mechanisms that were altered in PAD samples. They found 128 transcript-protein pairs that showed a higher correlation of mRNA and the corresponding protein in PAD samples, relative to non-PAD. Among the proteins was RBX1 (ring-box 1), which is a constituent of the multiprotein complex that is involved in *HIF-1α* regulation. Furthermore, gene set enrichment analysis of these 128 genes revealed that the most highly enriched gene set was microtubulins that mediate *HIF-1α* nuclear translocation and protein localization. Another highly enriched gene set was extracellular organelle that includes extracellular vesicles. Overall,

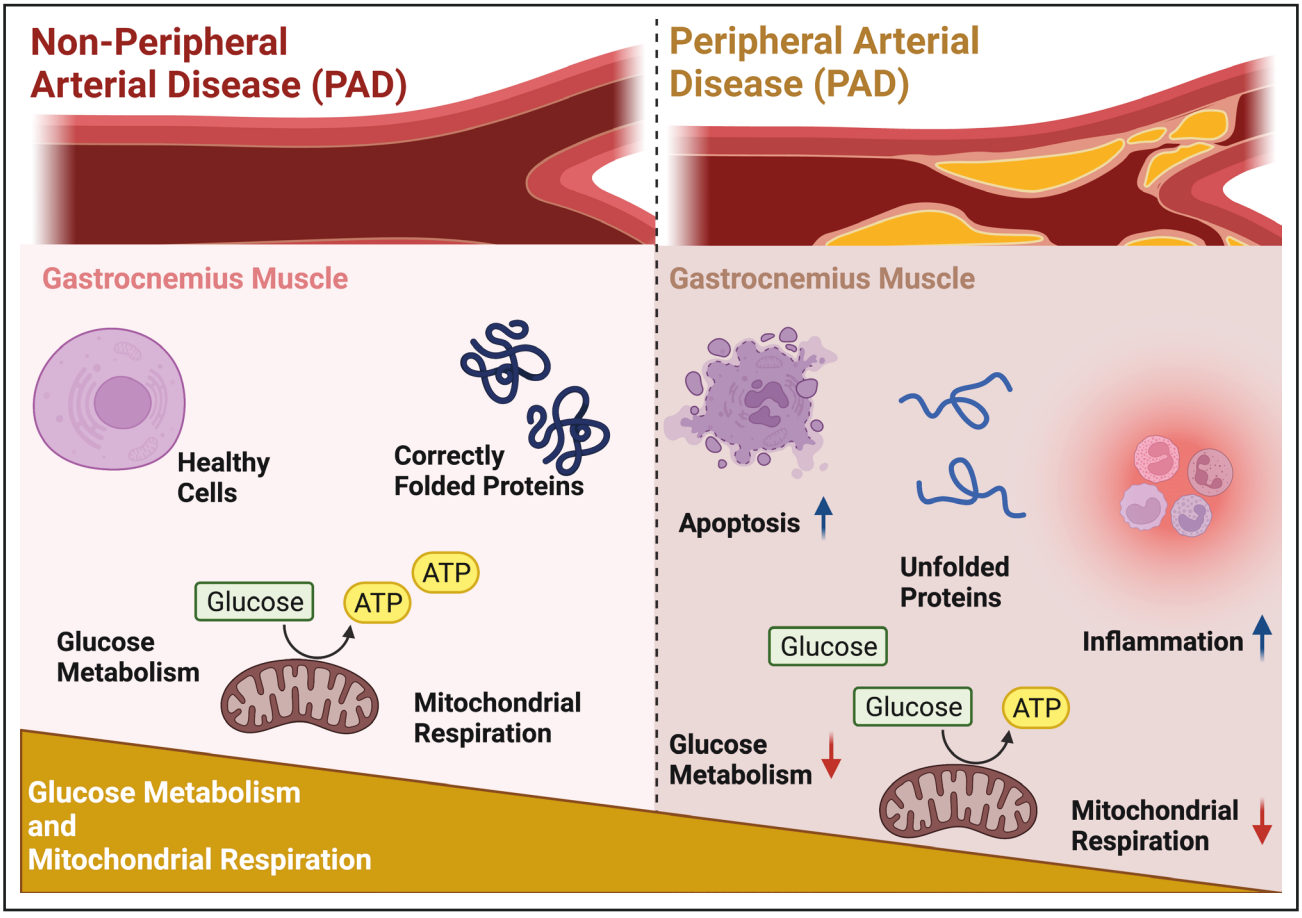

Figure. Peripheral artery disease (PAD) muscle biopsy samples experience increased inflammation, aberrant protein folding, reduced mitochondrial respiration, and impaired glucose metabolism, compared with samples from non-PAD participants.

these findings suggest that, as part of the response to hypoxia and stress that takes place in the setting of PAD, there is notable cytoskeletal remodeling and extracellular vesicle production, as well as a looser association between transcription and translation. The finding of cytoskeletal reorganization concurs with prior publications, in which other cytoskeletal factors like desmin were found to be aberrant in PAD muscle.[7] However, the pathological importance of enhanced extracellular vesicle production in PAD is largely unknown and could be a potential target for therapeutic intervention.

Although there is a current lack of studies with comprehensive proteomic and transcriptomic analysis in patients with PAD, the authors compared their findings to other existing reports of gene expression differences in patients with PAD, compared with non-PAD patients.[8–11] The authors listed multiple discrepancies between their results and those from existing publications. The authors point out the differences in the patient characteristics, sample sizes, and sequencing depth in the existing studies, in addition to the apparent heterogeneity generally seen in PAD patients as contributing factors to the observed discrepancies.

The authors also noted limitations and future directions of this study. They acknowledge that this exploratory study had relatively small samples sizes that were appropriate for hypothesis generation, but not for suggesting causation, in the absence of subsequent detailed investigation. Additionally, multiple avenues for future work were discussed, including the need for direct measurements of the biological pathways found to affect PAD pathology in the current study. Specifically, studying the hypoxia response by quantification of genes/proteins such as *HIF-1α*, AMPK (5'adenosine monophosphate-activated protein kinase), and PGC-1α would validate some of the findings from this study. Data from this study also suggest that the modulation of autophagy or glycolysis may be potential therapeutic strategies to treat PAD.

In summary, this study identified numerous biological pathways that were differentially expressed in PAD muscle biopsies. Salient findings included hypoxia-induced mechanisms, reduced glucose metabolism due to rate-limiting enzymes, cytoskeletal reorganization, aberrant mitochondria respiratory proteins, and a looser association between transcription and translation. These mechanisms each have immense potential for further investigation to develop effective interventions for the treatment of PAD.

ARTICLE INFORMATION

Affiliations

Department of Cardiothoracic Surgery (I.J., B.P.O., N.F.H.), Stanford Cardiovascular Institute (I.J., B.P.O., N.F.H.), and Department of Chemical Engineering (N.F.H.), Stanford University, CA. Center for Tissue Regeneration, Repair and Restoration, Veterans Affairs Palo Alto Health Care System (I.J., B.P.O., N.F.H.).

Sources of Funding

This work was supported, in part, by grants to N.F. Huang from the US National Institutes of Health (R01 HL127113 and R01 HL142718), the US Department of Veterans Affairs (1I01BX004259 and RX001222), the National Science Foundation (1829534 and 2227614), and the American Heart Association (20IPA35360085 and 20IPA35310731). N.F. Huang is the recipient of a Research Career Scientist Award (IK6BX006309) from the Department of Veterans Affairs. The figure was prepared using Biorender.

Disclosures

None.

REFERENCES

1. Aday AW, Matsushita K. Epidemiology of peripheral artery disease and polyvascular disease. *Circ Res*. 2021;128:1818–1832. doi: 10.1161/CIRCRESAHA.121.318535
2. Fowkes FGR, Aboyans V, Fowkes FJI, McDermott MM, Sampson UKA, Criqui MH. Peripheral artery disease: epidemiology and global perspectives. *Nat Rev Cardiol*. 2016;14:156–170. doi: 10.1038/nrcardio.2016.179
3. Criqui MH, Matsushita K, Aboyans V, Hess CN, Hicks CW, Kwan TW, McDermott MM, Misra S, Ujueta F; American Heart Association Council on Epidemiology and Prevention; Council on Arteriosclerosis, Thrombosis and Vascular Biology; Council on Cardiovascular Radiology and Intervention; Council on Lifestyle and Cardiometabolic Health; Council on Peripheral Vascular Disease; and Stroke Council. Lower extremity peripheral artery disease: contemporary epidemiology, management gaps, and future directions: a scientific statement from the American Heart Association. *Circulation*. 2021;144:E171–E191. doi: 10.1161/CIR.0000000000001005
4. Harwood AE, Smith GE, Cayton T, Broadbent E, Chetter IC. A systematic review of the uptake and adherence rates to supervised exercise programs in patients with intermittent claudication. *Ann Vasc Surg*. 2016;34:280–289. doi: 10.1016/j.avsg.2016.02.009
5. Dua A, Gologorsky R, Savage D, Rens N, Gandhi N, Brooke B, Corriere M, Jackson E, Aalami O. National assessment of availability, awareness, and utilization of supervised exercise therapy for peripheral artery disease patients with intermittent claudication. *J Vasc Surg*. 2020;71:1702–1707. doi: 10.1016/j.jvs.2019.08.238
6. Ferrucci L, Candia J, Ubaida-Mohien C, Lyaskov A, Banskota N, Leeuwenburgh C, Wohlgemuth S, Guralnik JM, Kaileh M, Zhang D, et al. Transcriptomics and proteomics of gastrocnemius muscle biopsies in peripheral artery disease. *Circ Res*. 2023;132:1428–1443. doi: 10.1161/CIRCRESAHA.122.322325
7. Koutakis P, Miserlis D, Myers SA, Kim JKS, Zhu Z, Papoutsi E, Swanson SA, Haynatzki G, Ha DM, Carpenter LA, et al. 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. doi: 10.1369/0022155415569348
8. Saini SK, Pérez-Cremades D, Cheng HS, Kosmac K, Peterson CA, Li L, Tian L, Dong G, Wu KK, Bouverat B, et al. Dysregulated genes, MicroRNAs, biological pathways, and gastrocnemius muscle fiber types associated with progression of peripheral artery disease: a preliminary analysis. *J Am Heart Assoc*. 2022;11:e023085. doi: 10.1161/JAHA.121.023085
9. Newman JD, Cornwell MG, Zhou H, Rockman C, Heguy A, Suarez Y, Cheng HS, Feinberg MW, Hochman JS, Ruggles KV, et al. Gene expression signature in patients with symptomatic peripheral artery disease. *Arterioscler Thromb Vasc Biol*. 2021;41:1521–1533. doi: 10.1161/ATVBAHA.120.315857
10. Cong G, Cui X, Ferrari R, Pipinos II, Casale GP, Chattopadhyay A, Sachdev U. Fibrosis distinguishes critical limb ischemia patients from claudicants in a transcriptomic and histologic analysis. *J Clin Med*. 2020;9:3974. doi: 10.3390/jcm9123974
11. Ryan TE, Yamaguchi DJ, Schmidt CA, Zeczycki TN, Shaikh SR, Brophy P, Green TD, Tarpey MD, Karnekar R, Goldberg EJ, et al. Extensive skeletal muscle cell mitochondriopathy distinguishes critical limb ischemia patients from claudicants. *JCI Insight*. 2018;3:e123235. doi: 10.1172/JCI.INSIGHT.123235