UNIVERSITÉ DE STRASBOURG

École doctorale 414 – Sciences de la Vie et de la Santé

UR3072 – Mitochondries, Stress Oxydant et Protection Musculaire

THÈSE

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Soutenue le 21 octobre 2021

En vue d'obtenir le grade de : **Docteur de l'Université de Strasbourg**

Discipline/Spécialité : Sciences du Vivant - Physiologie

Physiopathologie et traitement de la sarcopénie au cours de l'ischémie critique chronique des membres inférieurs

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REMERCIEMENTS

Je souhaiterais remercier les membres du jury, **Lucie Salomon du Mont**, **Bruno Chenuel**, **Frédéric Derbré** et **Alain Pradignac** pour avoir accepté d'évaluer mon travail.

Fabrice Bertile, je vous remercie pour votre aide précieuse lors de l'évaluation de mon suivi de thèse.

Anne Lejay et **Bernard Geny**, en m'accueillant dans votre équipe, vous avez changé le cours de ma vie. Je vous suis infiniment reconnaissante pour la confiance que vous m'avez accordée avec ce projet de thèse, avec des étudiants, avec des patients. Je vous remercie pour votre implication, votre soutien et vos conseils tout au long de ma thèse.

Durant ma thèse, j'ai eu le plaisir de collaborer avec **Jocelyn Laporte** et son équipe, notamment **Marion**, **Juliana**, **Roberto** et **Coralie**, merci pour votre accueil et vos conseils en western blots. Merci à **Julien Graff** et **Cristina Antal** pour leur persévérance avec mes lames histologiques capricieuses. Merci à **Pascal Kessler**, tu as eu la patience de me former en microscopie et de répondre à mes très (trop) longs mails. Tu es le boss ultime de Fiji.

A tous les membres du service d'explorations fonctionnelles, merci pour votre accueil chaleureux, vous avez toujours répondu présent lorsque j'avais besoin d'aide.

A tous les membres du service de chirurgie vasculaire, merci de m'avoir permis d'élire domicile dans votre salle de réunion pendant quelques semaines.

A tous les membres de l'équipe 3072, merci pour cette ambiance de travail si familiale et positive. Merci pour les discussions, scientifiques et moins scientifiques, pour les sorties, les rencontres.

Mireille G., merci pour votre disponibilité, votre prévoyance et votre écoute.

Joffrey, je te remercie d'avoir pris le temps de me former aux différents enseignements, et de m'avoir fait confiance avec les étudiants.

Alain, merci pour ton temps passé à me former au DXA et à répondre à mes nombreuses questions techniques. Merci de ne pas t'être moqué quand je t'ai dit que la fève hippopotame s'appelait Banette…

Marina, tu as amené le soleil du Brésil dans le labo, même quand il ne faisait pas plus de 18 degrés (ressenti 12) à l'intérieur. Merci d'avoir partagé avec moi les secrets du cerveau et de ses mitochondries.

Joris et **Allan**, Masters of Western Blots (mais pas of TGS), merci pour votre aide pour les extractions, les aliquots, les westerns, les partages d'AC… Merci pour votre bonne humeur et pour tous vos conseils sur mon projet.

Margherita, partenaire de surveillance et de TD, merci pour ton enthousiasme et ton sourire. Merci de nous avoir fait découvrir les tarallis, maintenant on est toutes accros ! Je te souhaite le meilleur pour la suite.

Walid, même si tu trouves toujours les moments les plus tendus pour avoir besoin des oroboros, t'es le chouchou de l'équipe alors on te pardonne. C'était un plaisir de travailler avec toi, merci de m'avoir fait confiance avec tes patients.

Fabienne, **Isabelle**, **Anne-Laure**, vous êtes le cœur de cette équipe. Vous partagez tout : qui vous êtes, votre savoir-faire, votre temps. Je suis extrêmement reconnaissante pour ces 3 années passées à vos côtés. **Fabienne**, reine de l'oroboros, merci pour tous tes conseils et astuces, grâce à toi mes mitochondries ne pouvaient que bien respirer. **Isabelle**, l'animalerie est bien plus amusante quand tu es dans les parages. En écrivant ces quelques lignes, je peux entendre ton rire, qui, j'en suis persuadée, est capable de guérir tous les maux. Après la pluie vient l'arc-en-ciel comme tu le dis si bien. **Anne-Laure**, comment fais-tu pour toujours trouver les bons mots, les bons conseils ? Merci d'avoir supporté mes milliards de « Anne-Lauuuuure, j'ai une question », et surtout, merci d'y avoir toujours répondu avec le sourire.

Léa et **Anouk**, aussi connues sous le nom de Sam et Clover, ou Atchi et Charlotte, vous êtes des collègues et amies en or. Si je devais résumer ces 3 ans avec vous en quelques chiffres, je dirais : 385 « pardon, je range mes pieds maintenant », 37 étagères transportées grâce à nos (énormes) biceps, 1 betterave devenue blanche qui n'aurait pas dû être jetée, 7892 « à tes

souhaits », 61 tentatives de réanimation suite à l'ingestion de sésame, 1000 tasses de thé/café ± 1000 Mopral, 1 transport ultra périlleux d'oroboros en plein confinement, 290 chansons plus que douteuses qui nous restent en tête… Bref, beaucoup beaucoup beaucoup de bons moments. Que la force soit avec vous pour la suite (oubliez pas de me faire un petit coucou de l'ISS). Vous allez me manquer.

Amandine et **Marion**, merci pour toutes ces soirées jeux, et toutes ces soirées jeux où on ne jouait pas. Quand je pense à vous j'ai la musique d'exploding kittens en tête, et ça, ça met forcément de bonne humeur.

Mireille, tu as été le premier visage familier que j'ai retrouvé quand je suis arrivée à Strasbourg. Merci d'avoir été là pour papoter avec moi, c'est toujours un plaisir.

Delphine, **Francis**, **Maxime** et **Charlotte**, vous m'avez accueilli les bras ouverts, je vous remercie pour les nombreux moments de partage, de Oizon à Lisbonne en passant par Villeneuve-lès-Maguelone.

A toute ma famille, parce que c'est ce qu'il y a de plus précieux dans une vie, merci d'avoir toujours été là. À mes parents, **Sophie** et **Diego**, à mon frère **David**, à mes grands-parents **Mado**, **Nona**, **Raymond** et **Nono**, à mes tantes et oncles, **Karine**, **Pascale**, **Cathy, Élise**, **Papus**, **Nicolas** et **Xavier**, à mes cousines et cousin, **Eva**, **Alizé**, **Léa**, **Gabriella** et **Yann**, vous êtes avec moi à chaque instant, dans ma tête et dans mon cœur. Mes réussites sont aussi les vôtres, même si, avouons-le, personne ne comprend ce que je fais dans la vie, ni sur quoi je travaille. Je vous aime fort.

Coralie, à nos aventures passées, présentes et futures. J'ai hâte de voir ce que la vie nous réserve, même si je sais déjà qu'avec toi, elle sera remplie d'amour et de rire. Infini amour infini.

RÉSUMÉ

La sarcopénie est caractérisée par une diminution de la force et de la masse musculaire, parfois associée à une faible performance physique. Elle peut toucher les patients atteints d'ischémie critique chronique (ICC) mais est souvent peu diagnostiquée, voire négligée, puisque les lésions liées à l'ICC sont au premier plan. Néanmoins, la sarcopénie associée à l'ICC est un facteur de mauvais pronostic, que ce soit en termes de survie ou de sauvetage de membre. Chez l'Homme, nous avons identifié le ratio inflammatoire plaquettes/lymphocytes comme marqueur simple et rapide de sarcopénie chez les patients atteints d'ICC, ces patients devant alors être considérés à haut-risque chirurgical, du fait de leur mauvais pronostic post-opératoire. Cependant, sa faible sensibilité limite son utilisation en routine.

L'étude de la sarcopénie dans notre modèle murin d'ICC nous a permis de mieux comprendre les mécanismes physiopathologiques sous-jacents, à savoir une fibrose musculaire, une altération des voies de dégradation des protéines musculaires, ainsi qu'une augmentation des paramètres inflammatoires, et de mettre en place un protocole d'exercice visant à protéger le muscle squelettique sarcopénique chez la souris. L'exercice de faible intensité est ainsi une approche thérapeutique protectrice permettant de réduire l'atrophie et la fibrose musculaire, et de restaurer la force et la capacité de marche dans notre modèle murin. Il serait intéressant d'étudier si un protocole d'exercice de faible intensité chez l'Homme pourrait améliorer voire reverser les effets délétères de la sarcopénie.

ABSTRACT

Sarcopenia is characterized by a decrease in muscle strength and mass, sometimes associated with low physical performance. It can affect patients with chronic limb-threatening ischemia (CLTI) but it is often undiagnosed or even overlooked, since CLTI-related lesions are prominent. Nevertheless, sarcopenia associated with CLTI is a factor of poor prognosis, either in terms of survival or limb salvage. In humans, we have identified the inflammatory platelet/lymphocyte ratio as a simple and rapid marker of sarcopenia in patients with CLTI, these patients should then be considered at high surgical risk, due to their poor postoperative prognosis. However, its low sensitivity limits its routine use.

The study of sarcopenia in our mouse model of CLTI allowed us to better understand the underlying pathophysiological mechanisms, namely muscle fibrosis, alteration of muscle protein degradation pathways, as well as increase in inflammatory parameters, and to set up an exercise protocol aimed at protecting sarcopenic skeletal muscles in mice. Low-intensity exercise is thus a protective therapeutic approach to reduce muscle atrophy and fibrosis, and to restore strength and walking ability in our model. It would be interesting to study whether a low-intensity exercise regimen in humans could improve or even reverse the deleterious effects of sarcopenia.

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Abréviations

4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1
Akt	Protein kinase B
ATP	Adénosine triphosphate
Bak	Bcl-2 homologous antagonist/killer
Bax	Bcl-2–associated X
Ca^{2+}	Calcium
Cdnkn2a	Cyclin Dependent Kinase Inhibitor 2A
DEXA	Absorption biphotonique à rayons X
DPH	Dihydropyridine
E1s	Ubiquitin-activating enzymes 1
eIF-4E	Eukaryotic translation initiation factor 4E
FAP	Progéniteur fibro-adipogénique
ICC	Ischémie critique chronique
IL-12	Interleukine 12
IL-1β	Interleukine 1 bêta
MAFbx	Muscle Atrophy F-box
MSA	Masse squelettique appendiculaire
mTOR	Mammalian target of rapamycin
MurF1	Muscle ring finger-1
P21	Cyclin-dependent kinase inhibitor 1
p70S6K	Ribosomal protein S6 kinase bêta
PI3K	Phosphoinositide 3-kinase
RLO	Radicaux libres de l'oxygène
RPL	Ratio plaquettes/lymphocytes
rpS6	Ribosomal protein S6
SPPB	Short Physical Performance Battery
TGFβ	Transforming growth factor bêta
TNFα	Tumor necrosis factor alpha
Trp53	Transformation related protein 53
TUG	Timed Up and Go
VEGF	Vascular Endothelial Growth Factor

Publications faisant l'objet de la thèse

Articles originaux publiés

Pizzimenti, M., Charles, A. L., Riou, M., Thaveau, F., Chakfé, N., Geny, B., & Lejay, A. (2021). Usefulness of Platelet-to-Lymphocyte Ratio as a Marker of Sarcopenia for Critical Limb Threatening Ischemia. *Annals of Vascular Surgery*, *72*, 72-78.

Pizzimenti, M., Meyer, A., Charles, A. L., Giannini, M., Chakfé, N., Lejay, A., & Geny, B. (2020). Sarcopenia and peripheral arterial disease: a systematic review. *Journal of Cachexia, Sarcopenia and Muscle*, *11*(4), 866-886.

Articles originaux soumis

Pizzimenti, M., Debrut, L., Charles, A. L., Mallard, J., Pagano, A., Georg, I., Goupilleau, F., Graff, J., Kessler, P., Borne, C., Ciancia, M., Laporte, J., Favret, F., Geny, B., & Lejay, A. Effect of short-term low-intensity exercise training on sarcopenia in a mouse model of critical limb threatening ischemia.

Communications orales

Pizzimenti, M., Lejay, A., Charles, A. L., Thaveau, F., Georg, Y., Steinmetz, L., Chakfé, N., & Geny, B. (2020). Impact of sarcopenia on morbi-mortality after infra-inguinal revascularization for critical ischemia. Congrès annuel de la société de Chirurgie Vasculaire et Endovasculaire 2021.

Pizzimenti, M., Lejay, A., Charles, A. L., Thaveau, F., Georg, Y., Steinmetz, L., Chakfé, N., & Geny, B. (2020). The platelets/lymphocytes ratio as marker of sarcopenia in critical chronic ischemia. Congrès annuel de la société de Chirurgie Vasculaire et Endovasculaire 2021.

Autres publications

Riou, M., **Pizzimenti, M**., Enache, I., Charloux, A., Canuet, M., Andres, E., Talha, S., Meyer, A., & Geny, B. (2020). Skeletal and respiratory muscle dysfunctions in pulmonary arterial hypertension. *Journal of clinical medicine*, *9*(2), 410.

Pizzimenti, M., Riou, M., Charles, A. L., Talha, S., Meyer, A., Andres, E., Chakfé, N., Lejay, A., & Geny, B. (2019). The rise of mitochondria in peripheral arterial disease physiopathology: experimental and clinical data. *Journal of clinical medicine*, *8*(12), 2125.

Leardini-Tristao, M., Charles, A. L., Lejay, A., **Pizzimenti, M**., Meyer, A., Estato, V., Tibiriçá, E., Andres, E., & Geny, B. (2019). Beneficial effect of exercise on cognitive function during peripheral arterial disease: potential involvement of myokines and microglial anti-inflammatory phenotype enhancement. *Journal of clinical medicine*, *8*(5), 653.

INTRODUCTION ET OBJECTIFS

Introduction et objectifs

Les maladies vasculaires sont une cause majeure de décès dans le monde, avec une prévalence augmentant progressivement à mesure que l'espérance de vie augmente. Parmi celles-ci, l'artériopathie des membres inférieurs est définie par une réduction ou une obstruction du flux sanguin dans les artères, avec des symptômes allant de la claudication intermittente à une ischémie critique chronique (ICC), caractérisée par des douleurs de décubitus et/ou des troubles trophiques des membres inférieurs.

L'artériopathie, essentiellement l'ICC, peut être accompagnée d'anomalies musculo-squelettiques, notamment une perte généralisée de la force et de la masse musculaire, associée ou non à une faible performance physique – aussi appelée sarcopénie. L'ICC et la sarcopénie peuvent évoluer en parallèle, de nombreux patients souffrant d'ICC présentant une sarcopénie. Néanmoins la sarcopénie est peu diagnostiquée chez ces patients, voire négligée, puisque l'atteinte liée aux troubles trophiques est souvent au premier plan. Pourtant, la sarcopénie associée à l'ICC est de mauvais pronostic, puisqu'elle aggrave d'une part la qualité de vie déjà altérée de ces patients, et qu'elle met en jeu le pronostic vital et fonctionnel de ces patients. Chez ces patients, même si la revascularisation reste le traitement de référence, la sarcopénie ne doit pas être négligée, et devrait également être traitée. Il est donc essentiel de diagnostiquer précocement la sarcopénie chez les patients en ICC, mais également de mieux comprendre comment l'ICC et la sarcopénie peuvent altérer le muscle squelettique, afin de développer des protocoles permettant de protéger et réadapter le muscle squelettique sarcopénique.

Pour cela, mon travail de thèse a plusieurs **objectifs** :

- Identifier un marqueur simple de sarcopénie chez les patients présentant une ICC, afin de déterminer un sous-groupe de patients à haut-risque
- Déterminer les voies de signalisation impliquées dans la genèse de la sarcopénie dans un modèle murin d'ICC (étude des voies d'anabolisme et de catabolisme musculaire, fibrose, inflammation)
- Déterminer si l'exercice de courte durée et faible intensité peut améliorer les paramètres musculaires et par quelles voies

POINT SUR LA LITTÉRATURE

Point sur la littérature

1 Le muscle squelettique

1.1 Généralités et vue d'ensemble de son organisation

Avec plus de 600 muscles, le tissu musculaire squelettique est l'un des plus abondant de l'organisme humain et représente environ 40% de la masse corporelle totale d'un adulte. Les muscles squelettiques jouent un rôle crucial dans la locomotion, le maintien de la posture, la thermogenèse ou la régulation du métabolisme lipidique et glucidique (Frontera and Ochala 2015; Pedersen and Febbraio 2012).

Les muscles squelettiques sont attachés aux os par des tendons et sont composés d'un ensemble de faisceaux de fibres musculaires. Le maintien du muscle est assuré par trois couches successives :

- L'épimysium qui isole le muscle entier de son environnement et englobe plusieurs faisceaux musculaires,
- Le périmysium qui entoure chaque faisceau musculaire et qui contient des nerfs et des capillaires,
- L'endomysium, une fine matrice de collagène qui s'intercale entre chaque fibre musculaire (McCuller, Jessu, and Callahan 2021) (Figure 1).

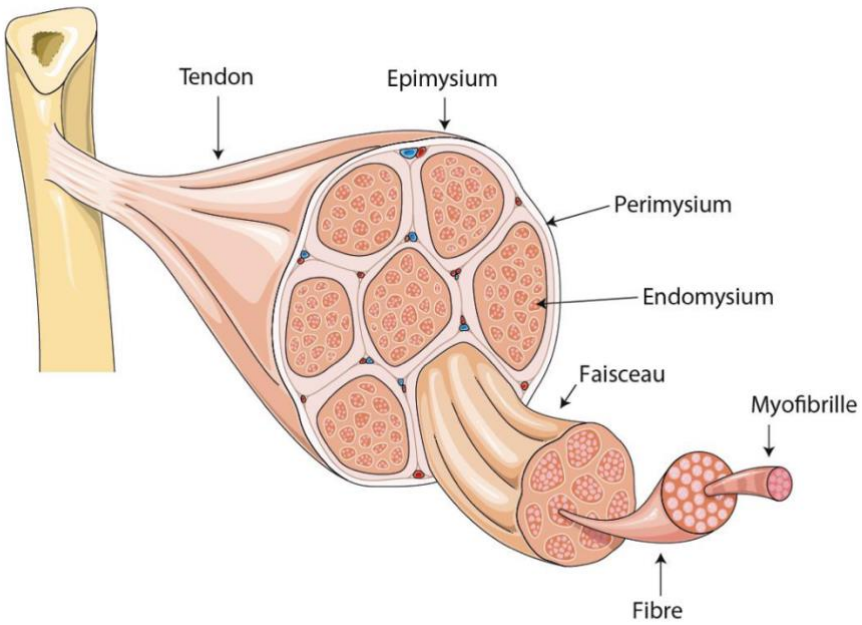

Figure 1 : organisation du muscle strié squelettique. Adapté de Basic and Applied Biology – 2nd Edition

1.2 Structure et développement de fibres musculaires

La fusion des myoblastes (des cellules souches précurseurs mononucléées) aboutie à la création de fibres musculaires matures. Ces fibres sont des longues cellules plurinucléées cylindriques allant de 10 à 100 µm de diamètre et de 1 mm à 40 cm de longueur. Elles sont constituées de myofibrilles qui participent à la contraction du muscle grâce aux structures contractiles répétées qui les compose : les sarcomères. Les sarcomères sont composés de protéines contractiles : l'actine et la myosine (Ceafalan et al. 2018).

1.3 Types de fibres musculaires

Le muscle squelettique est composé de différents types de fibres musculaires qui diffèrent selon leur propriété métabolique, leur vitesse de contraction et l'expression de la chaîne lourde de myosine. Les muscles des mammifères peuvent être composés de fibres lentes de type I (ce sont des fibres exprimant la chaîne lourde de la myosine I), ou de fibres rapides de type IIX, IIA ou IIB (exprimant la chaîne lourde de la myosine IIX, IIA ou IIB, respectivement). Les fibres de type IIB n'existent pas chez l'homme. Des fibres « hybrides » contenant deux types différents comme I/IIA, IIAX, IIBX peuvent également être présentes dans le muscle (Bloemberg and Quadrilatero 2012).

Les fibres de **type I** sont des fibres à contraction lentes. Elles sont riches en mitochondries, et reposent principalement sur la phosphorylation oxydative pour générer de l'ATP, elles peuvent donc produire de l'énergie en conditions aérobie. Cela permet une contraction continue et une forte résistance à la fatigue : elles sont donc utilisées lors d'exercices peu puissants et prolongés. Elles sont très vascularisées, et ont donc un aspect rouge.

Les fibres de **type II** sont des fibres à contraction rapides. Elles possèdent moins de mitochondries que les fibres de type I, et utilisent principalement du glycogène pour produire de l'ATP. Ce sont des fibres qui fatiguent rapidement lors de l'épuisement des réserves en glycogène : elles sont sollicitées lors des exercices brefs mais intenses. Elles sont peu vascularisées, et ont donc un aspect blanc. Les fibres de **type IIX** sont très rapides, glycolytiques et peu vascularisées. Les fibres de **type IIA** et **IIB** sont rapides, oxydatives et glycolytiques et modérément vascularisées, ce sont des fibres intermédiaires entre les fibres de type I et les fibres de type IIX (Schiaffino and Reggiani 2011)(Figure 2).

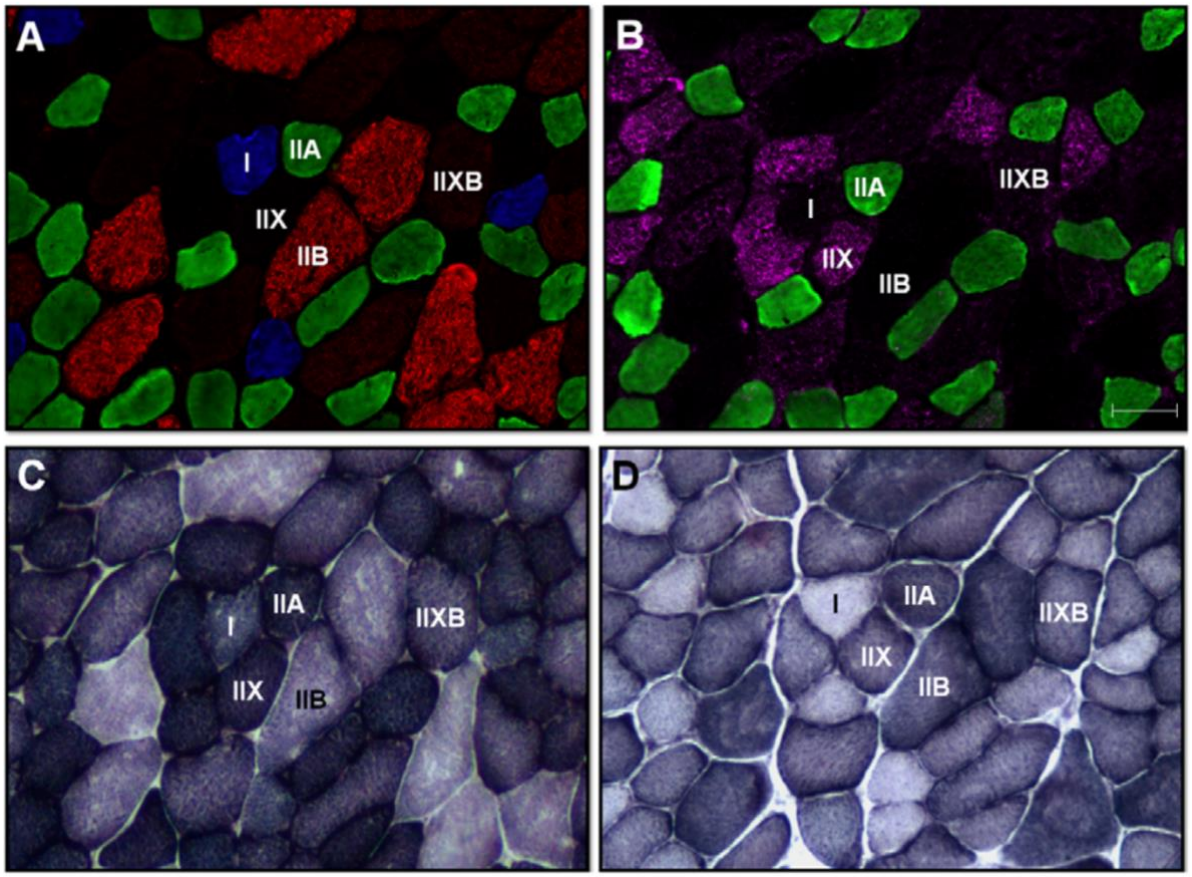

Figure 2 : visualisation des différents types de fibres musculaires sur muscle gastrocnémien de souris Immunofluorescence dirigé contre les isoformes des chaînes lourdes de la myosine I, IIX, IIA, IIB (A-B) et coloration succinate déshydrogénase (C-D). Bloemberg et al. 2012

1.4 Machinerie d'excitation – contraction musculaire

Après un stimulus neuronal, le neurotransmetteur acétylcholine est libéré par l'axone terminal au niveau de la jonction neuromusculaire. L'activation des récepteurs de l'acétylcholine entraîne une dépolarisation locale, et la propagation d'un potentiel d'action le long de la fibre musculaire et dans les invaginations membranaires profondes appelées tubule T. Le potentiel d'action active les récepteurs dihydropyridines (DPH) situé sur le tubule T, et entraîne un changement de leur conformation, leur permettant ainsi d'interagir avec les récepteurs ryanodines situés sur le réticulum sarcoplasmique. Le réticulum sarcoplasmique libère ainsi du Ca^{2+}, ce qui déclenche le raccourcissement du sarcomère et donc la contraction musculaire (Calderón, Bolaños, and Caputo 2014) (Figure 3).

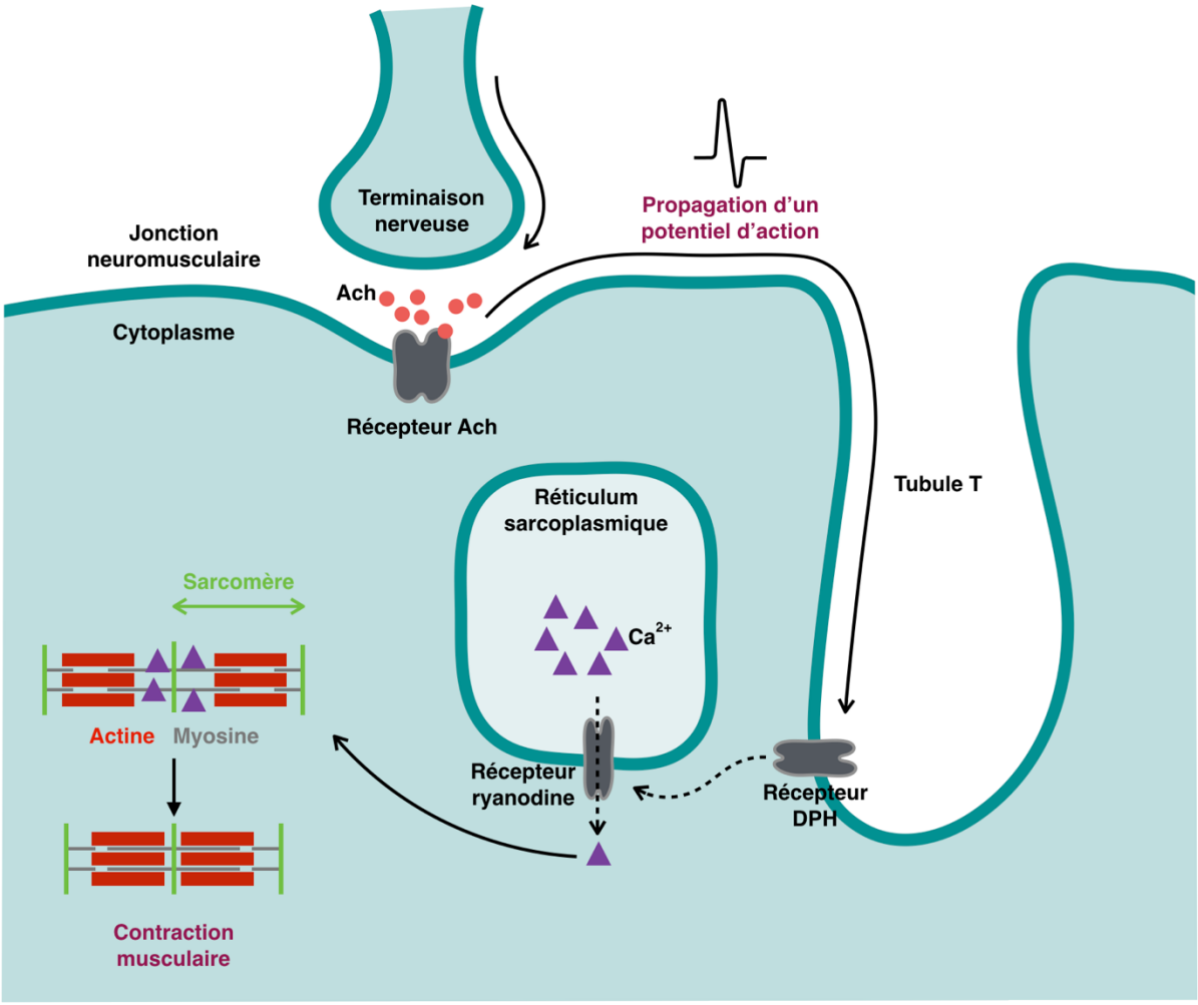

Figure 3 : schéma représentant les étapes d'excitation – contraction musculaire.
Ach : acétylcholine, DPH : dihydropyridine. Adapté de Kuo et al. 2015

2 La sarcopénie

2.1 Présentation clinique

Longtemps considérée comme un syndrome gériatrique, la sarcopénie est depuis 2016 reconnue par l'organisation mondiale de la santé comme une maladie à part entière, ayant de nombreuses causes contribuant à son développement au-delà du vieillissement. La sarcopénie est caractérisée par une faible force et masse musculaire. Elle augmente les risques d'amputation, d'hospitalisation et de mortalité. Il est donc essentiel pour les professionnels de santé de mieux reconnaître cette condition. Dans cette optique et au cours de la dernière décennie, des efforts de collaboration ont été déployés dans le monde entier, par exemple en Europe (Cruz-Jentoft et al. 2010, 2019), en Amérique (Morley et al. 2011) et en Asie (L.-K. Chen et al. 2014, 2016) afin de parvenir à une définition consensuelle et des critères de diagnostic de la sarcopénie. A ce jour, il semble admis par les différents groupes de travail que la sarcopénie doit être définie à travers trois critères principaux : 1) une faible force musculaire, 2) une faible quantité ou qualité musculaire, et 3) une faible performance physique. Seul, le critère 1 est considéré comme le plus fiable et doit orienter vers le diagnostic de sarcopénie. Lorsqu'ils sont combinés, les critères 1 et 2 rendent compte de la certitude du diagnostic. Enfin, si les trois critères sont remplis, la sarcopénie est considérée comme sévère grâce au lien entre une faible performance physique et un mauvais pronostic.

2.2 La sarcopénie en quelques chiffres

La prévalence de la sarcopénie dans la population générale mondiale est estimée aux alentours de 10% (Shafiee et al. 2017). Elle varie de 5 à 13% chez les personnes entre 60 et 70 ans, et de 11 à 50% chez les plus de 80 ans (von Haehling et al. 2017). C'est une maladie qui touche plus de 50 millions d'individus dans le monde à ce jour, et ce chiffre pourrait atteindre 200 millions d'ici à 2050 avec l'augmentation continue de l'espérance de vie (Cruz-Jentoft et al. 2010).

La sarcopénie augmente significativement le risque de chutes et de fractures (Yeung et al. 2019; X. Zhang et al. 2020), de maladies cardiovasculaires (Curcio et al. 2020; Y. Zhang et al. 2021), de maladies respiratoires (Benz et al. 2019; Sepúlveda-Loyola et al. 2020) d'altérations cognitives (Peng et al. 2020; Scisciola et al. 2021), de perte qualité de vie (dos Santos et al. 2017), et de mortalité (Arango-Lopera et al. 2013; Bachettini et al. 2020).

La sarcopénie s'accompagne donc de coûts élevés en termes de soins de santé (Bruyère et al. 2019; Goates et al. 2019). En effet, les coûts d'hospitalisation et de soins d'un patient

sarcopénique sont 2 à 5 fois plus élevés que ceux d'un patient non-sarcopénique (Antunes et al. 2017; Steffl et al. 2017).

2.3 Diagnostic de la sarcopénie

2.3.1 Identifier des individus à risque

Le questionnaire SARC-F peut être utilisé pour identifier des individus à risque de sarcopénie. Il s'agit d'une auto-évaluation, basée sur la perception par un individu de ses limitations de force, de marche, de lever de chaise, de monter d'escaliers, et d'expériences de chutes. Chaque composante est notée de 0 à 2. Un score supérieur ou égal à 4 points (sur 10) serait prédictif de sarcopénie et doit orienter vers des examens complémentaires (Malmstrom et al. 2016) (Tableau 1).

Tableau 1 : Questionnaire SARC-F pour identifier des individus à risque de sarcopénie

Composante	Question	Score
Force	Quel est votre niveau de difficulté lorsque vous portez et soulevez 4,5 kg ?	Aucun = 0 Modéré = 1 Élevé ou incapable = 2
Aide à la marche	Quel est votre niveau de difficulté lorsque vous marchez dans une pièce ?	Aucun = 0 Modéré = 1 Élevé, besoin d'assistance ou incapable = 2
Lever de chaise	Quel est votre niveau de difficulté lorsque vous vous levez d'une chaise ou d'un lit ?	Aucun = 0 Modéré = 1 Élevé ou incapable sans assistance = 2
Montée des marches d'escalier	Quel est votre niveau de difficulté lorsque vous devez monter 10 marches d'escalier ?	Aucun = 0 Modéré = 1 Élevé ou incapable = 2
Chutes	Combien de fois êtes-vous tombé au cours des 12 derniers mois ?	Aucun = 0 1 à 3 chutes = 1 4 chutes ou plus = 2

2.3.2 Évaluer la force

La méthode standard pour évaluer une faiblesse musculaire est le **test de force de préhension**. Le test consiste à mesurer la force (en kilogrammes) exercée par la main d'un sujet lors de la

préhension d'un dynamomètre. Une faible force musculaire est définie par des seuils inférieurs à 27 kg pour les hommes, et 16 kg pour les femmes. Le **test du lever de chaise** peut être utilisé pour mesurer la diminution de la force du bas du corps. Une faible force musculaire intervient si le temps nécessaire pour effectuer cinq élévations consécutives dépasse 15 secondes. Enfin, pour des cas particuliers ou des études cliniques le **test de flexion/extension du genou** peut être utilisé pour évaluer la perte de puissance musculaire (Cruz-Jentoft et al. 2019).

2.3.3 Évaluer la masse

Une faible quantité/qualité de muscle peut être évaluée par des mesures de composition corporelle par **impédancemétrie** ou par **absorptiométrie biphotonique (DEXA)**. Ces techniques permettent la mesure de masse squelettique appendiculaire (MSA), ou d'indice de MSA (MSA/taille^2). Une faible quantité/qualité musculaire est définie par des seuils inférieurs à 20 kg ou 7 kg/m^2 chez l'homme, et 15 kg ou 5,5 kg/m^2 chez la femme. L'imagerie par résonance magnétique ou la tomodensitométrie peuvent également être utilisées pour apprécier la quantité/qualité du muscle, cependant, les valeurs seuils appropriées ne sont pas bien définies (Cruz-Jentoft et al. 2019).

2.3.4 Évaluer la performance physique

Une faible performance physique peut être évaluée par le **test de vitesse de marche**. Une vitesse inférieure à 0,8 m/s est un indicateur d'une altération de la fonction musculaire. Le **test SPPB (Short Physical Performance Battery)** regroupe une batterie de tests de vitesse de marche, d'équilibre, et de lever de chaise. Un score inférieur ou égal à 8 points (sur 12) est un indicateur d'une altération de la fonction musculaire (Guralnik et al. 1994) (Figure 4). Enfin, le **test TUG (Get up and Go)** permet l'évaluation de la motricité et de l'équilibre d'un sujet. Le sujet est invité à se lever d'une chaise, marcher sur une distance de 3 mètres, faire demi-tour et se rassoir sur la chaise. Un temps supérieur à 30 secondes est un indicateur d'une altération de la fonction musculaire (Cruz-Jentoft et al. 2019).

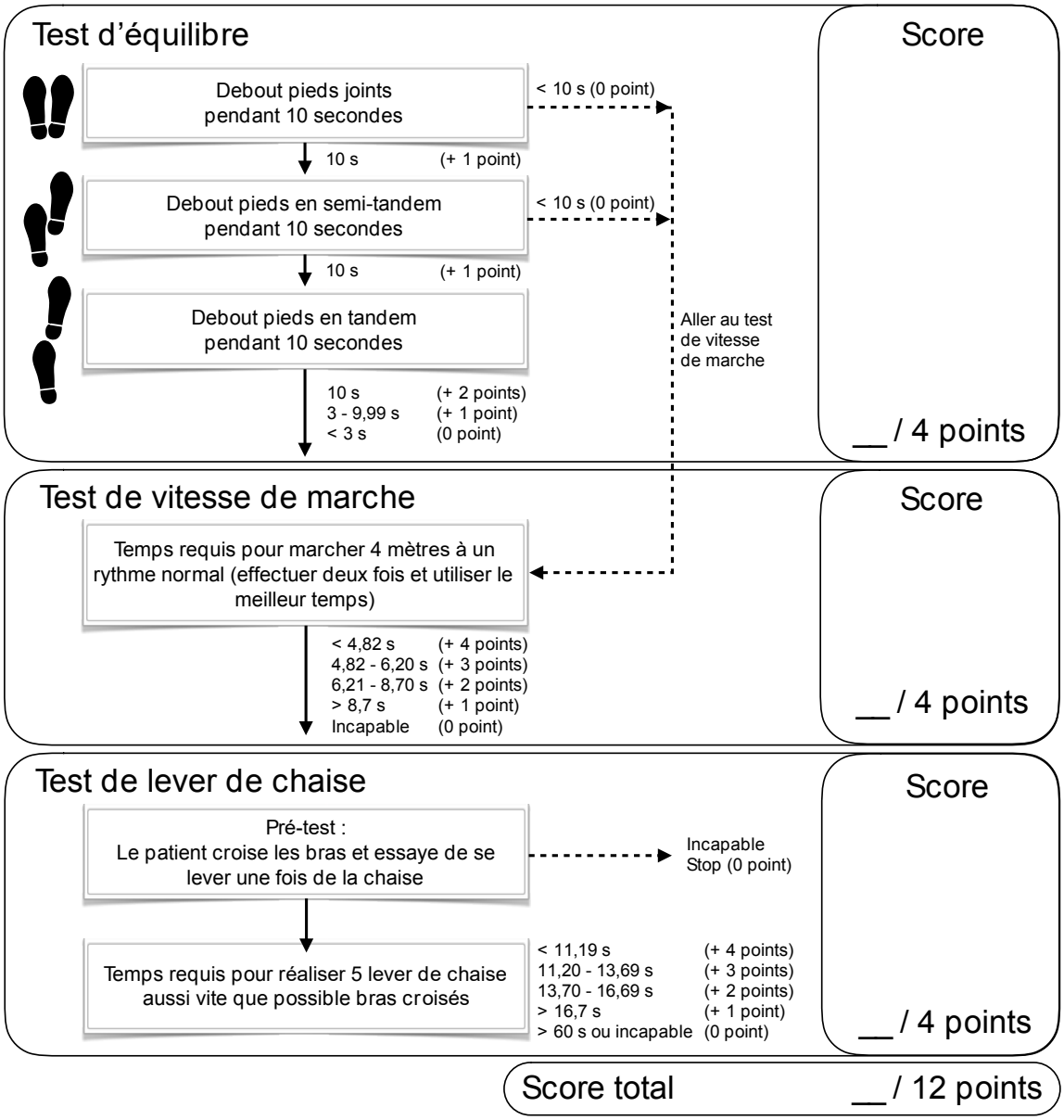

Figure 4 : test Short Physical Performance Battery (SPPB) pour évaluer la performance physique. Adapté de Riskowski et al. 2012

2.3.5 Difficultés du diagnostic de sarcopénie

Les différents groupes de travail sur la sarcopénie ont permis une plus grande reconnaissance de la maladie, et une harmonisation des critères diagnostiques. Cependant, les critères sont

nombreux et demandent l'utilisation d'outils spécifiques, rendant l'évaluation de la sarcopénie rare en pratique clinique courante (Figure 5).

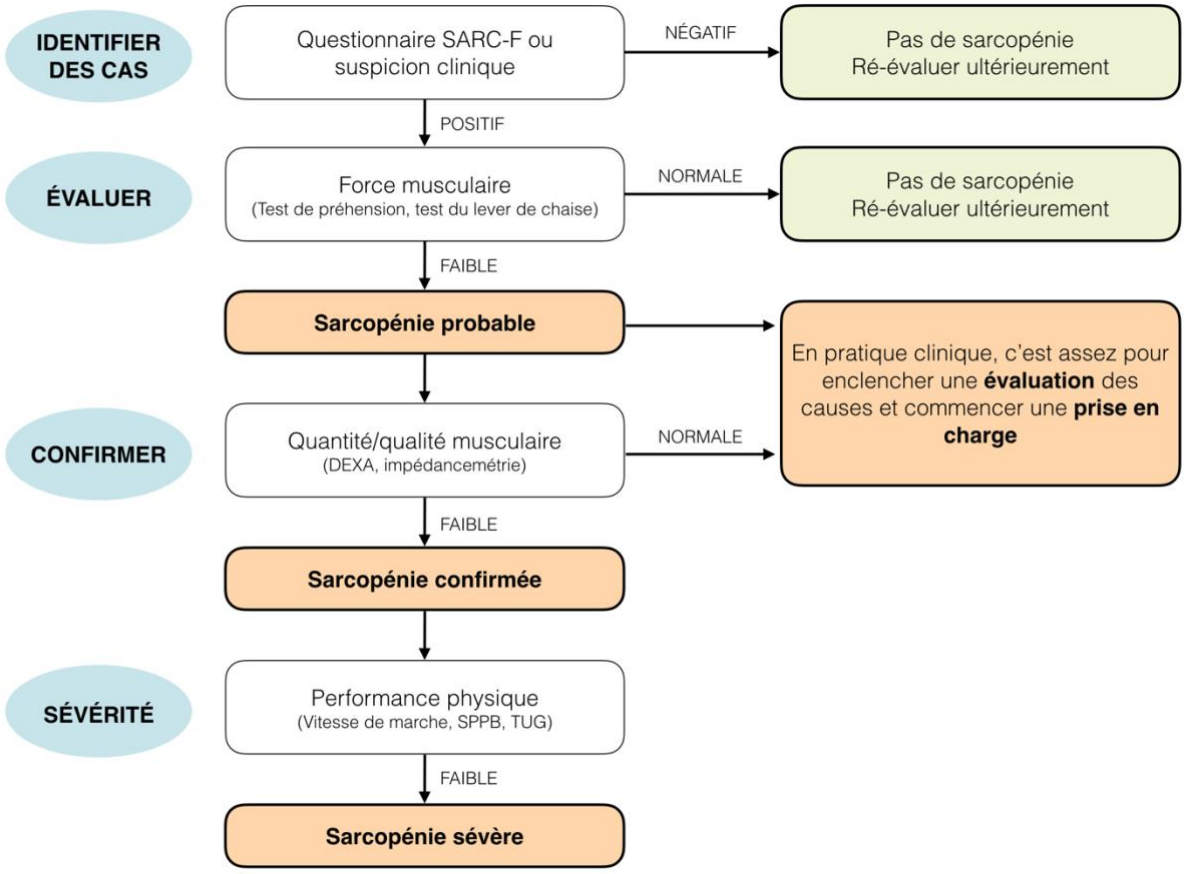

Figure 5 : Diagramme pour la recherche de cas, l'établissement d'un diagnostic et la quantification de la sévérité de la sarcopénie en pratique clinique. Adapté de Cruz-Jentoft et al. 2019

2.4 Facteurs de risques

Le vieillissement est un facteur de risque majeur de sarcopénie qu'on appelle alors « sarcopénie primaire ». D'autres facteurs de risques comme une maladie chronique ou l'inactivité peuvent également conduire à une sarcopénie appelée « sarcopénie secondaire ».

2.4.1 Sarcopénie primaire

La sarcopénie primaire se manifeste en l'absence de cause évidente autre que l'âge. En effet, le vieillissement entraîne une réduction de la force musculaire, une atrophie musculaire prononcée, et une altération de la performance physique (Goodpaster et al. 2006; Mitchell et al. 2007; Russ et al. 2012).

2.4.2 Sarcopénie secondaire

La sarcopénie est considérée comme secondaire lorsque d'autres facteurs que le vieillissement sont évidents. La sarcopénie peut survenir secondairement, soit :

- A la suite d'une maladie systémique, en particulier une maladie inflammatoire ou endocrinienne, un cancer ou une défaillance d'organe (cœur, poumon, foie, rein, cerveau) (Borba et al. 2019).
- A cause d'une activité réduite, qui peut être liée à un alitement, un mode de vie sédentaire, un handicap lié à une maladie ou un environnement sans gravité. Cette perte d'activité accélère l'apparition du vieillissement et donc de la sarcopénie (Evans 2010).
- A cause d'un problème nutritionnel, comme une anorexie, une malabsorption, des troubles gastro-intestinaux ou une obésité. Une sous-nutrition entraîne une carence ou un déséquilibre en énergie, protéines et nutriments, et conduit à une perte de masse et de force musculaire. Une surnutrition favorise l'inflammation, la résistance à l'insuline, et le gain de masse grasse au détriment de masse musculaire (obésité sarcopénique) (Batsis et al. 2013; Beaudart et al. 2019).

2.5 Mécanismes impliqués dans le développement de la sarcopénie

Les mécanismes impliqués dans le développement de la sarcopénie sont surtout étudiés dans le contexte de sarcopénie primaire.

Au niveau myocellulaire, les études montrent que les fibres de type II (à contraction rapide et responsables des activités de force) sont particulièrement sensibles au vieillissement, avec une réduction significative de 10 à 40% de leur taille (mais pas de leur nombre) chez des sujets âgés comparé à des sujets jeunes. La taille des fibres de type I (à contraction lente et responsables des activités d'endurance) ne change pas avec l'âge (Larsson 1978; Martel et al. 2006; Nilwik et al. 2013; Snijders, Verdijk, and van Loon 2009). La réduction de la taille des fibres de type II, indépendamment de leur nombre, entraîne donc une baisse de force musculaire et de performance physique chez les personnes âgées.

Au niveau de la machinerie d'excitation-contraction musculaire, l'âge altère la libération d'ions Ca^{2+} dans le muscle squelettique, ce qui entraîne un défaut d'activation de l'appareil contractile, et une baisse de force musculaire. L'âge conduit également à un défaut de recapture de ces ions Ca^{2+} après la contraction, ce qui entraîne un problème de relaxation musculaire et donc des défauts de coordination motrice et de fatigue (Payne et al. 2009).

Au niveau neuromusculaire, les études montrent que l'âge entraîne une réduction significative du nombre de motoneurones alpha (innervant les fibres de type II), entraînant donc une perte de fonction et de force musculaire (Campbell, McComas, and Petito 1973; Hunter, Pereira, and Keenan 2016).

Au niveau du renouvellement cellulaire, l'âge entraîne une perturbation de la régulation du renouvellement des protéines musculaires : avec une diminution de la synthèse et une augmentation de la dégradation des protéines musculaires (Altun et al. 2010; Koopman and van Loon 2009; Mallinson and Murton 2013).

Au niveau de l'architecture musculaire, les études montrent que l'âge entraîne une importante infiltration de tissu fibreux et adipeux au niveau du muscle squelettique (Brack et al. 2007; Zoico et al. 2010). Cela contribue à une perte de mouvements musculaires et donc de performance physique (Beavers et al. 2013; Marcus et al. 2012).

Au niveau mitochondrial, l'âge entraîne des défauts de fonctionnement de la mitochondrie. Avec l'âge, le stress oxydant induit par les radicaux libres de l'oxygène (RLOs) s'accumule et altère la respiration mitochondriale et donc la production d'ATP. La biogenèse mitochondriale est diminuée, et la mitophagie est inhibée, entraînant une accumulation de mitochondries dysfonctionnelles (Son and Lee 2021).

2.6 Prise en charge

Prévenir la sarcopénie est un enjeu médical et économique. Pour tous les patients, le traitement de référence de la sarcopénie est l'entraînement contre résistance. L'entraînement contre résistance permet d'augmenter la force, la masse musculaire, l'équilibre et l'endurance chez les personnes âgées (Law, Clark, and Clark 2016). Pour être efficaces, les programmes d'exercice doivent être adaptés à chaque individu, être progressifs, et cibler en priorité les membres inférieurs qui sont plus importants pour l'équilibre et la mobilité (Giallauria et al. 2016). Bien que moins bien documentée, une supplémentation en macronutriments, en vitamines (vitamine C, vitamine D), en minéraux (calcium, sélénium), en antioxydants (oméga-3) et en différents groupes d'aliments (produits laitier, fruits, légumes) semblerait bénéfique – particulièrement pour les personnes atteintes de sarcopénie secondaire liée à la nutrition (Ganapathy and Nieves

2020). D’autres thérapies pouvant cibler la dégradation protéique ont été testées, avec pour but de réduire l’atrophie musculaire médiée par la myostatine (Becker et al. 2015; Wei et al. 2016).

3 Ischémie critique chronique

3.1 Présentation clinique

L'ischémie critique chronique (ICC) des membres inférieurs correspond à la forme la plus sévère de l'artériopathie oblitérante des membres inférieurs, caractérisée par des douleurs de décubitus et/ou des troubles trophiques. L'ICC est une maladie hautement morbide qui augmente le risque d'amputation majeure et engage le pronostic vital du patient.

3.2 L'ischémie critique chronique en quelques chiffres

Bien que l'ICC soit un problème de santé publique croissant, les données épidémiologiques restent extrêmement limitées. On estime à 1% la population mondiale d'adultes souffrant d'ICC. Jusqu'à 10% des patients atteints d'une artériopathie évolueront vers une ICC (Farber and Eberhardt 2016; Norgren et al. 2007). Ces chiffres pourraient augmenter dans le futur avec le vieillissement des populations et l'accumulation des facteurs de risques.

Les patients atteints d'ICC sont à très haut risque d'amputations majeures, d'événements cardiovasculaires et de décès. A un an de la prise en charge initiale, 25% des patients en ICC sont décédés, 30% ont subi une amputation majeure, et seulement 45% sont en vie sans amputation (Norgren et al. 2007). A 2 ans de la prise en charge initiale, 40% des patients sont décédés (Soga et al. 2014). A 4 ans de la prise en charge initiale, 35 à 67% des patients en ICC ont subi une amputation majeure (Reinecke et al. 2015).

L'ICC s'accompagne de coûts élevés en termes de soins de santé, du fait de taux élevés d'amputation, de réinterventions chirurgicales, de comorbidités et d'invalidité (Luengo-Fernandez et al. 2018). En effet, le traitement initial des patients est : une amputation majeure dans 25% des cas, une revascularisation chirurgicale dans 50% des cas, ou un traitement médical si un geste de revascularisation est impossible dans 25% des cas (Duff et al. 2019; Mustapha et al. 2018).

3.3 Diagnostic

Le diagnostic d'ICC nécessite la présence de douleurs de décubitus ou de troubles trophiques associée à des paramètres hémodynamiques anormaux. La durée des symptômes doit être supérieure à deux semaines (Aboyans et al. 2018).

3.3.1 Douleurs de décubitus ou troubles trophiques

Les douleurs de décubitus et troubles trophiques sont évalués lors d'un **examen clinique**.

Les douleurs de décubitus – aussi connue sous le nom de « douleur au repos » – affectent principalement l'avant-pied (orteils ou tête de métatarses). Les douleurs de décubitus apparaissent ou s'aggravent si le pied est incliné ou élevé, et sont soulagées en position assise ou debout. Au départ, les douleurs sont décrites comme des engourdissements ou des picotements qui s'estompent en mettant le talon hors du lit. Puis, les douleurs ne peuvent être soulagées qu'en faisant pendre la jambe hors du lit pour dormir. Enfin, les douleurs obligent à rester en position assise pour trouver le sommeil. Dans ce cas, un œdème du pied et de la cheville se développe.

Les troubles trophiques correspondent à des plaies non cicatrisantes ou des gangrènes liées directement à l'insuffisance artérielle. Ils sont distaux, touchant principalement les zones de pression ou de frottement comme les orteils, les talons ou les malléoles. Ils sont généralement douloureux, et responsables de complications comme une infection locale et une inflammation.

3.3.2 Quantification hémodynamique

Pour confirmer un diagnostic, il est essentiel de réaliser des mesures de pressions distales à la cheville et au gros orteil, notamment par **laser Doppler**. Pour les patients présentant des douleurs de décubitus, un diagnostic d'ICC survient si la pression est <50 mm Hg à la cheville, ou <30 mm Hg à l'orteil. Pour les patients présentant des troubles trophiques, un diagnostic d'ICC survient si la pression est <70 mm Hg à la cheville, ou <50 mm Hg à l'orteil (Figure 6).

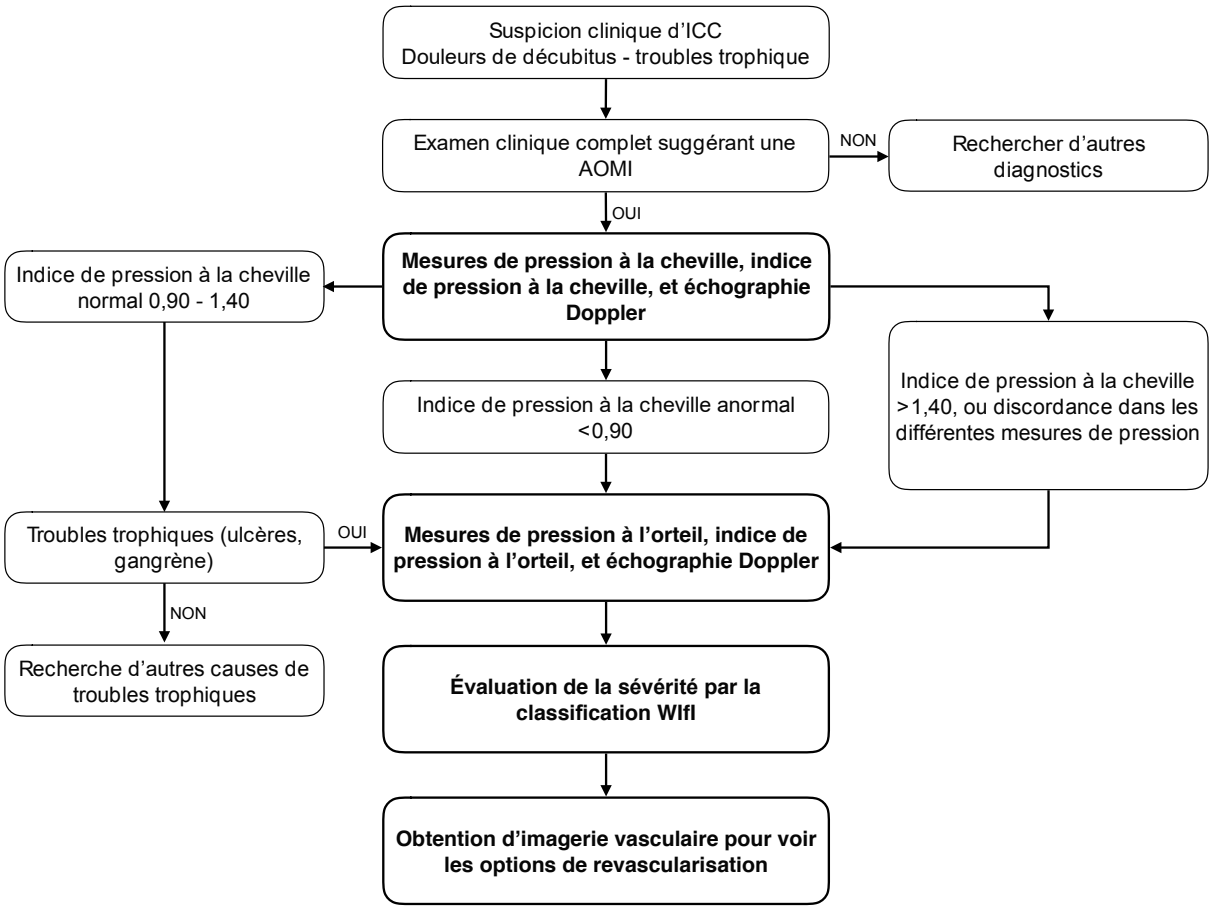

Figure 6 : Diagramme pour l'examen des patients présentant une suspicion d'ICC. Adapté de Conte et al. 2019

3.3.3 Durée des symptômes

L'ICC est diagnostiquée lorsque les signes cliniques validés hémodynamiquement existent depuis au moins deux semaines.

3.4 Facteurs de risque

Les facteurs de risque pour les patients en ICC sont l'âge, le diabète (Lilja et al. 2021; Malmstedt et al. 2008; Virkkunen et al. 2004), l'hypertension (Emdin et al. 2015; Howard et al. 2015), le tabagisme (Joosten et al. 2012) et la dyslipidémie (Martinez-Aguilar et al. 2017). Ces patients sont à très haut risque de progression de l'ICC, d'amputation et de mortalité.

3.5 Mécanismes impliqués dans le développement de l'ischémie critique chronique

Au départ, les plaques d'athérosclérose qui s'accumulent rétrécissent le diamètre du réseau artériel et réduisent donc le débit artériel. Ainsi, une perfusion insuffisante des tissus provoque un dysfonctionnement endothélial, une inflammation chronique et du stress oxydant. Toutes ses

altérations participent aux lésions des fibres musculaires, à l'accumulation de fibrose musculaire et au développement de gangrènes (Simon et al. 2018).

3.6 Prise en charge

Le traitement de l'ICC vise à améliorer la perfusion des membres. La revascularisation, lorsqu'elle est possible, est le traitement de référence. Le traitement des facteurs de risque associés doit également être instauré.

4 La sarcopénie dans l'ischémie critique chronique

4.1 Implications et chiffres

L'ICC et la sarcopénie peuvent évoluer en parallèle, de nombreux patients souffrant d'ICC étant également sarcopéniques. On estime qu'un quart des patients en ICC sont sarcopéniques, mais ce chiffre est probablement sous-estimé car la sarcopénie est peu diagnostiquée voire totalement négligée dans le contexte de l'ICC.

Pourtant, la sarcopénie est un facteur de mauvais pronostic pour les patients atteints d'artériopathie, et particulièrement au stade d'ICC. En effet, plusieurs études ont montré que la sarcopénie – diagnostiquée uniquement sur la base d'une masse musculaire faible – est associée à un taux élevé d'événements cardiovasculaires (Matsubara et al. 2017), et à une survie plus faible chez les patients en ICC (Matsubara et al. 2015; Morisaki et al. 2017; Shimazoe et al. 2019; Taniguchi et al. 2019).

4.2 Mécanismes moléculaires potentiellement impliqués

Plusieurs mécanismes communs pourraient expliquer l'atteinte musculaire dans l'ICC, comme un déséquilibre entre la synthèse et la dégradation des protéines musculaires, une fibrose musculaire ou des altérations mitochondriales.

4.2.1 Régulateurs de croissance musculaire

L'homéostasie musculaire est étroitement liée au renouvellement des protéines musculaires. Si ce processus est ralenti – comme décrit lors du vieillissement – il entraîne un déséquilibre entre la synthèse et la dégradation des protéines musculaires. Ce déséquilibre altère négativement la fonction musculaire en induisant une perte de protéines et une accumulation de protéines endommagées (Metter et al. 1999).

4.2.1.1 Altération de la synthèse protéique

La voie de signalisation IGF-1/PI3K/Akt/mTOR est un acteur clé de la croissance musculaire. La liaison de l'IGF-1 à son récepteur provoque son autophosphorylation et l'activation subséquente de PI3K. Cet événement induit un recrutement d'AKT à la surface de la membrane plasmique. Une fois activée, AKT favorise la croissance musculaire en phosphorylant mTOR et ses effecteurs en aval 4EBP1, p70S6K, rpS6 et eIF-4E (Chakravarthy, Davis, and Booth 2000; Sacheck et al. 2004; Svanberg et al. 1996)(Svanberg et al., 1996).

4.2.1.2 Altération de la dégradation protéique

La perte de masse squelettique est largement contrôlée par l'activation de la dégradation des protéines par le système ubiquitine/protéasome, à travers deux étapes principales : premièrement, une chaîne de molécules d'ubiquitine est fixée de manière covalente sur les protéines pour les marquer pour la dégradation, grâce à trois composants enzymatiques E1s (enzymes activant l'ubiquitine), E2s (enzymes de conjugaison d'ubiquitine) et E3s (enzymes ubiquitine ligase). Deuxièmement, les protéines poly-ubiquitinées sont reconnues et dégradées en petits peptides par le protéasome 26S. Les enzymes ubiquitine ligase, notamment MuRF1 et MAFbx sont donc considérées comme des marqueurs de la dégradation des protéines musculaires par le système ubiquitine/protéasome (Cai et al. 2004; de Palma et al. 2008; Tanner et al. 2015).

4.2.2 Fibrose musculaire

Lors de blessures ou d'inflammation, la régénération musculaire est initiée par une infiltration inflammatoire (macrophages pro-inflammatoire et cytokines TNFα, IL-1β, IL-12) qui permet une dégénérescence musculaire et une phagocytose des fibres musculaires lésées (Novak and Koh 2013). Par la suite, un tissu de cicatrisation riche en facteurs de croissance, en macrophages anti-inflammatoire et en collagène I et III se forme (Mann et al. 2011; Pillon et al. 2013; Shono et al. 2013). Lorsque la régénération est normale, ce tissu de cicatrisation est progressivement éliminé. Cependant, lorsque les atteintes sont répétées comme dans certaines maladies chroniques, il arrive que ce tissu persiste et finisse par remplacer le tissu sain : on appelle cela la fibrose.

La fibrose musculaire entraîne une rigidité musculaire, une perte de capacité à l'exercice, et affecte négativement la régénération musculaire : c'est une cause majeure de faiblesse musculaire (Lieber and Ward 2013).

4.2.3 Altérations mitochondriales

Avec la maladie et/ou l'âge, les dysfonctionnements mitochondriaux s'accumulent, perturbant ainsi les activités vitales dépendantes des mitochondries. Un déséquilibre entre les activités pro et antioxydantes, également appelé stress oxydatif, est responsable de l'accumulation d'espèces réactives de l'oxygène (RLOs), des dysfonctionnements de la chaîne respiratoire mitochondriale et des dommages oxydatifs de l'ADN (Lejay et al. 2019; Paradis et al. 2019; Pottecher et al. 2018).

4.3 Options de traitements

4.3.1 Revascularisation

La chirurgie de revascularisation est le traitement de choix pour les patients souffrant d'ICC, permettant la restauration du flux sanguin et le sauvetage du membre, tout en inversant certaines caractéristiques sarcopéniques comme la force et la performance physique (Landry et al. 2014). Cependant, la sarcopénie étant très peu diagnostiquée dans la pratique clinique, il est difficile de savoir si les altérations musculaires liées à l'ICC sont normalisées ou si des séquelles subsistent.

4.3.2 Exercice

L'exercice fait partie de l'arsenal thérapeutique dans l'artériopathie, même si la revascularisation est le traitement de référence dans l'ICC. L'exercice peut être complémentaire, pour diminuer les effets de la sarcopénie ou améliorer l'état du muscle squelettique (Gardner et al. 2014; Mary M. McDermott et al. 2009; Schieber et al. 2019; Vun et al. 2016) sous réserve qu'il soit de faible intensité et de courte durée (A. Lejay et al. 2017). En effet, l'ICC étant un stade avancé d'artériopathie, avec souvent des troubles trophiques évolués, les protocoles d'exercices doivent être adaptés à la sévérité de la maladie.

4.3.3 Autres options

À ce jour, outre la revascularisation, la meilleure stratégie thérapeutique inclut l'exercice, mais les approches ciblant les mécanismes sous-jacents (cf partie 4.2 Mécanismes moléculaires potentiellement impliqués) méritent encore des études plus approfondies.

4.4 Notre modèle expérimental d'étude

Plusieurs modèles d'ICC existent et sont basés sur une seule ligature artérielle. Le modèle le plus utilisé consiste en une ligature isolée de l'artère fémorale (Couffinhal et al. 1998; Hellingman et al. 2010; Limbourg et al. 2009; Lotfi et al. 2013; Tang et al. 2005; Waters et al. 2004). Cependant, cette méthode laisse intacte la majeure partie de la circulation collatérale vers le membre inférieur et, par conséquent, le flux sanguin est complètement restauré en 7 jours (Hellingman et al. 2010). Un autre modèle consiste en l'excision totale de l'artère fémorale (Masaki et al. 2002). Cependant, le flux sanguin est progressivement restauré, notamment grâce aux vaisseaux collatéraux provenant de l'artère iliaque interne.

Pour pallier à cette collatéralité artérielle, nous utilisons un modèle murin d'ICC induite par une procédure de ligatures séquentielles (A. Lejay et al. 2015). Dans un premier temps, nous

réalisons une ligature de l'artère fémorale droite (réalisée à mi-chemin entre l'artère épigastrique superficielle et la bifurcation des artères poplitée et saphène) et de ses collatérales. Dans un deuxième temps, nous réalisons 4 jours plus tard une ligature de l'artère iliaque commune droite à 0,5 cm en aval de son origine. Cette seconde ligature réduit la perfusion collatérale fournie par l'artère iliaque interne, et permet donc une hypoperfusion artérielle qui dure plus de deux semaines (Figure 7).

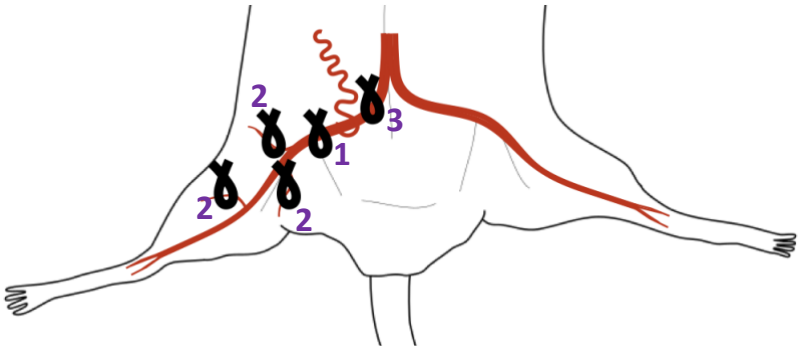

Figure 7 : schéma expérimental de la procédure chirurgicale d'ischémie critique chronique 1 = ligature de l'artère fémorale droite, 2 = ligatures des collatérales de l'artère fémorale droite, 3 = ligature de l'artère iliaque commune droite

RÉSULTATS

Résultat I Le ratio plaquettes/lymphocytes comme marqueur de sarcopénie dans l'ischémie critique chronique

Objectifs : La sarcopénie est un facteur de mauvais pronostic pour les patients atteints d'ischémie critique chronique (ICC). Cependant, son diagnostic nécessite des mesures sur imagerie qui sont complexes, longues et difficiles à standardiser en routine. L'inflammation étant un pathomécanisme commun à la sarcopénie et à l'ICC, nous avons cherché à évaluer si le ratio plaquettes/lymphocytes (RPL) pouvait être un marqueur rapide de sarcopénie chez les patients présentant une ICC.

Méthodes : Les patients traités pour ICC entre janvier 2019 et juillet 2019 ont été inclus dans cette étude rétrospective monocentrique. La sarcopénie a été définie sur la base d'un indice d'aire de psoas <5.5 cm^2/m^2 chez les hommes et <4.0 cm^2/m^2 chez les femmes. Le RPL a été calculé pour tous les patients sur la base de leur analyse sanguine préopératoire. La puissance diagnostique du RPL a été calculée au moyen d'une courbe ROC. Les données concernant la morbidité et la mortalité à 30 jours ont été récupérées.

Résultats : 64 patients sont inclus dans l'étude : 48 non-sarcopéniques (indice d'aire de psoas moyen de 7.34 cm^2/m^2) et 16 sarcopéniques (indice d'aire de psoas moyen de 4.30 cm^2/m^2). Il n'y a aucune différence entre les groupes non-sarcopéniques et sarcopéniques en termes de démographies, caractéristiques cliniques, risques cardiovasculaires, comorbidités, ou modalités de revascularisation. Le RPL est significativement plus élevé dans le groupe sarcopénique (332.1 en moyenne) comparé au groupe non-sarcopénique (204.6 en moyenne) ($p=0.02$). Une valeur seuil ≥292.5 s'est avérée être un marqueur diagnostic de sarcopénie sur la base d'une courbe ROC (sensibilité de 31.3%, spécificité de 91.7%). La mortalité à 30 jours était de 12.5% dans le groupe sarcopénique et de 2.1% dans le groupe non-sarcopénique ($p=0.15$). La morbidité à 30 jours était de 56.3% dans le groupe sarcopénique et de 10.4% dans le groupe non-sarcopénique ($p<0.001$).

Conclusions : Nous avons pu montrer que la sarcopénie était associée à un RPL élevé et à un mauvais pronostic en termes de morbidité postopératoire. Cependant, le RPL ne peut être à lui seul utilisé comme marqueur de sarcopénie chez les patients atteints d'ICC du fait de sa faible sensibilité (31.3%), limitant son utilisation en pratique clinique courante.

ELSEVIER

Clinical Research

Usefulness of Platelet-to-Lymphocyte Ratio as a Marker of Sarcopenia for Critical Limb Threatening Ischemia

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Background: Sarcopenia is a factor of poor prognosis for patients with critical limb threatening ischemia (CLTI), but its diagnosis requires imaging measurements and is time consuming. We investigated whether preoperative platelet-to-lymphocyte ratio (PLR) could be an easy and rapid marker of sarcopenia.

Methods: Patients treated for CLTI between January 2019 and July 2019 were included in this single-center retrospective study. Sarcopenia was defined by a psoas muscle index (PMI) <5.5 cm^2/m^2 in men, and <4.0 cm^2/m^2 in women. PLR was calculated for all patients based on their systematic preoperative blood analysis. The diagnostic power of PLR was analyzed through a receiver operating characteristic (ROC) curve. Early outcomes of sarcopenic patients in terms of 30-day mortality and 30-day morbidity were retrieved.

Results: Sixty-four patients were included in the study: 48 nonsarcopenic patients (mean PMI 7.34 cm^2/m^2; interquartile range [IQR] 6.58–8.01) and 16 sarcopenic patients (mean PMI 4.30 cm^2/m^2; IQR 3.45–5.17). No difference was found between both groups regarding patient demographics, clinical characteristics, cardiovascular risk factors, comorbidities, or revascularization modalities. PLR was significantly higher in the sarcopenic group (mean 332.1; IQR 158.2–320.7) compared with the nonsarcopenic group (mean 204.6; IQR 133.8–265.6) ($P = 0.02$). A PLR value ≥292.5 was shown to be a diagnostic marker for sarcopenia based on the ROC curve (sensitivity 31.3%, specificity 91.7%). Thirty-day mortality was 12.5% in the sarcopenic group and 2.1% in the nonsarcopenic group ($P = 0.15$); 30-day morbidity was 56.3% in the sarcopenic group and 10.4% in the nonsarcopenic group ($P < 0.001$).

Conclusions: PLR might help identifying a subgroup of CTLI patients associated with poor prognosis but does not seem appropriate to be used as a marker of sarcopenia given its low sensitivity.

INTRODUCTION

Critical limb threatening ischemia (CLTI) represents the end stage of peripheral arterial disease. CLTI is highly morbid, incurring significant mortality, limb loss, pain, and decreased quality of life among those afflicted patients.[1] Because vascular specialists are today given the opportunity to choose between a variety of modern and efficient technologies to treat CLTI, guidelines provide the current state of evidence for guiding treatment decisions in CLTI patients.[2] However, this global approach should be tailored to the physiological and anatomical

Conflict of Interest Statement: None declared.

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Ann Vasc Surg 2020; ■: 1–7
https://doi.org/10.1016/j.avsg.2020.05.027

Manuscript received: April 15, 2020; manuscript accepted: May 21, 2020; published online: ■ ■ ■

specificities of the patients and it appears mandatory to identify subgroups of patients who would require specific attention due to poor outcomes.

When associated with sarcopenia, both quality of life and prognosis of patients with CLTI are significantly worsened.[3–6] However, although critical for patients' care, the diagnosis of sarcopenia is often overlooked in these patients.

To date, a large majority of patients experiencing CLTI are investigated with preoperative conventional tomography angiography (CTA), which could be a way to conduct systematic and automated assessment of sarcopenia. Nevertheless, CTA analysis might be difficult to standardize and can be considered time consuming. That is why it is important to find other sarcopenic markers suitable for daily routine practice.

Given the role of inflammation and blood flow alterations in the pathophysiology of CLTI and sarcopenia,[7] we hypothesized that platelet-to-lymphocyte ratio (PLR) could be a simple and easy predictive indicator of sarcopenia, as it has been shown in oncology or digestive surgery[8,9] or in the community-dwelling older adults.[10] To our knowledge, no study has assessed the relationship between PLR and sarcopenia in the setting of CLTI. Therefore, the aim of the study is to investigate if PLR can be considered as a marker of sarcopenia in CLTI patients and to evaluate early outcomes of sarcopenic CLTI patients.

METHODS

Population

Medical records of all patients treated for CLTI in our institution between January 2019 and July 2019 were retrieved. The inclusion criteria were patients (1) who underwent infrainguinal surgical revascularization and (2) patients in whom preoperative CTA and blood analysis including platelet and lymphocyte counts were performed.

Preoperative Parameters

The following preoperative parameters were recorded: demographic data (age, gender), clinical data (weight, height, body mass index [BMI], rest pain or ulcers, hemodynamic evaluation), cardiovascular risk factors (tobacco use or smoking history defined as smoking cessation for more than 1 year, diabetes, hypertension, dyslipidemia, obesity), comorbidities (cardiac, renal, or pulmonary comorbidities), history of cancer, chronic inflammatory disease or hematologic disorders, skeletal muscle mass, and blood parameters (platelet, neutrophil, lymphocyte counts).

Skeletal muscle mass was obtained from preoperative transverse cross-sectional CTA images. The left and right psoas muscles were outlined at the inferior border of the fourth lumbar vertebrae. The areas of the left and right psoas muscles were summed to compute total psoas muscle area (TPMA). TPMA was later standardized by height to obtain psoas muscle index (PMI) (reported as cm^2/m^2).[11] Low PMI was considered as an indicator of sarcopenia. Low PMI was defined as <5.5 cm^2/m^2 in men and <4.0 cm^2/m^2 in women, based on previous reports defining sarcopenia in vascular patients.[12–14]

Study Design

Patients were divided into 2 groups according to skeletal muscle mass: sarcopenic group (PMI <5.5 cm^2/m^2 in men or <4.0 cm^2/m^2 in women) and nonsarcopenic group (PMI ≥ 5.5 cm^2/m^2 in men or ≥ 4.0 cm^2/m^2 in women).

Peroperative Parameters

Procedural data included type of surgery, length of the procedure, and need for blood transfusion.

Postoperative Parameters

The following postoperative parameters were recorded: 30-day mortality and 30-day morbidity. Morbidity was defined as surgery-related morbidity (major amputation or wound complications such as lymphocele and hematoma) or systemic morbidity (cardiac, renal, or pulmonary). Cardiac morbidity was defined as myocardial infarction, renal morbidity as acute kidney failure requiring dialysis, and pulmonary morbidity as lung decompensation requiring ventilator support. Clinical and hemodynamic examination was systematically performed at 4 weeks.

Early Outcomes

Primary outcome was the effectiveness of PLR to be a marker of sarcopenia in CLTI patients. Secondary outcomes were early outcomes of sarcopenic CLTI patients: 30-day mortality and morbidity.

Statistical Analysis

The D'Agostino-Pearson test was carried out to confirm the normality of continuous parameters. Depending on their distribution, continuous variables were either analyzed using the Mann–Whitney *U*-test, or the Student's *t*-test, and were

Table I. Patient demographics and clinical characteristics

Factors	Overall ($n = 64$)	Nonsarcopenic group ($n = 48$)	Sarcopenic group ($n = 16$)	P-value
Demographic data				
Age (years)	73.3 (71.5; 65.3–84.5)	72.3 (71.0; 66.0–81.0)	76.3 (77.5; 64.3–88.3)	0.2588
Sex ratio	1.9	1.7	3.0	0.5445[a]
Clinical data				
Weight (kg)	73.78 (70.0; 64.0–80.0)	73.91 (70.0; 64.0–82.0)	73.40 (69.50; 64.0–78.25)	0.5908
Height (m)	1.69 (1.70; 1.61–1.75)	1.68 (1.69; 1.60–1.74)	1.72 (1.73; 1.66–1.82)	0.0685
BMI (kg/m^2)	25.92 (24.91; 22.79–27.34)	26.37 (25.35; 23.03–27.82)	24.61 (24.14; 22.07–25.96)	0.1858
Rest pain	23 (35.9%)	19 (39.6%)	4 (25%)	0.3749[a]
Ulcers	48 (75%)	34 (70.8%)	14 (87.5%)	0.3173[a]
$TCPO_2$	5.4 (5.0; 3.0–7.0)	5.4 (5.0; 3.0–7.2)	5.6 (5.5; 3.0–7.0)	0.2453
Cardiovascular risk factors				
Smoking	29 (45.3%)	20 (41.7%)	9 (56.3%)	0.3102[b]
Diabetes	32 (50%)	23 (47.9%)	9 (56.3%)	0.5637[b]
Hypertension	46 (71.9%)	36 (75%)	10 (62.5%)	0.3355[b]
Dyslipidemia	27 (42.2%)	21 (43.8%)	6 (37.5%)	0.6611[b]
Obesity	9 (14.1%)	7 (14.6%)	2 (12.5%)	1.0000[a]
Comorbidities				
Cardiac	28 (43.8%)	20 (41.7%)	8 (50%)	0.5606[b]
Renal	3 (4.7%)	2 (4.2%)	1 (6.3%)	1.0000[a]
Pulmonary	4 (6.3%)	2 (4.2%)	2 (12.5%)	0.2582[a]
Past medical history				
Cancer	4 (6.3%)	3 (6.3%)	1 (6.3%)	1.0000[a]
Chronic inflammatory disease	2 (3.1%)	1 (2.1%)	1 (6.3%)	0.4405[a]
Hematologic disorders	1 (1.6%)	1 (2.1%)	0 (0.0%)	1.0000[a]
Skeletal muscle mass				
TPMA (cm^2)	18.83 (19.62; 14.96–22.82)	20.76 (20.68; 18.08–23.22)	13.05 (13.83; 8.95–16.51)	<0.0001[c]
PMI (cm^2/m^2)	6.58 (6.75; 5.30–7.77)	7.34 (7.11; 6.58–8.01)	4.30 (4.43; 3.45–5.17)	<0.0001[c]
Blood parameters				
Platelet count	320.7 (300.0; 236.8–372.0)	302.6 (275.5; 236.8–341.3)	374.8 (334.0; 243.3–417.3)	0.1824
Neutrophil count	6.60 (5.65; 4.57–7.62)	6.25 (5.5; 4.41–7.62)	7.65 (5.93; 4.68–11.42)	0.4128
Lymphocyte count	1.74 (1.53; 1.18–2.11)	1.77 (1.53; 1.17–2.17)	1.65 (1.62; 1.22–2.11)	0.9382
NLR	5.32 (4.25; 2.26–5.79)	4.48 (4.25; 2.12–5.76)	7.85 (4.21; 2.79–6.0)	0.5137
PLR	236.5 (199.6; 141.8–269.9)	204.6 (193.4; 133.8–265.6)	332.1 (220.4; 158.2–320.7)	0.0228[c]

Values are presented as mean (median; interquartile range), or number (%).
$TCPO_2$, transcutaneous oxygen pressure; NLR, neutrophil-to-lymphocyte ratio.
[a]Fisher's exact test.
[b]Chi-squared test.
[c]Statistically significant ($P < 0.05$).

presented as mean, median, and interquartile range (IQR). Categorical variables were analyzed using the chi-squared test or the Fisher's exact test, and reported as absolute numbers and percentages. The diagnostic power of PLR was assessed using the area under the receiver operating characteristic (ROC) curve, and expressed as plots of the test sensitivity versus 1−specificity. A P value <0.05 was considered statistically significant. All statistical analyses were performed using GraphPad software.

RESULTS

Population

In total, 64 patients met the inclusion criteria: 42 men (65.6%) and 22 women (34.4%). The nonsarcopenic group was constituted of 48 patients (75.0%) and the sarcopenic group of 16 patients (25.0%). Mean PMI was 7.34 cm^2/m^2 (median 7.11; IQR 6.58−8.01) in the nonsarcopenic group and 4.30 cm^2/m^2 (median 4.43; IQR 3.45−5.17) in the sarcopenic group.

Preoperative Parameters

Patient demographics and clinical characteristics, cardiovascular risk factors, comorbidities and history of cancer, chronic inflammatory disease, or hematologic disorders were similar between both groups. Among blood markers, PLR (P = 0.0228) was significantly higher in sarcopenic patients compared to those without sarcopenia. Platelet, neutrophil, or lymphocyte counts alone were not relevant with sarcopenia (Table I).

The area under the ROC curve was used as a measure for the effectiveness of PLR as diagnostic marker. Figure 1 shows the area under the curve of preoperative PLR (0.6068, 95% confidence interval 0.437−0.777).

Based on the ROC curve, the cut-off value of PLR for sarcopenia was set as 292.5 (sensitivity 31.3%, specificity 91.7%). Groups were dichotomized as "low PLR (≤292.5)" and "high PLR (>292.5)." High PLR was found in 9 patients (14.1%). Higher PLR was significantly associated with elevated cases of sarcopenia (P = 0.0364), lower BMI (P = 0.0285), and a higher proportion of ulcers (P < 0.0001) (Table II).

Peroperative Parameters

No difference was found between sarcopenic and nonsarcopenic groups regarding revascularization modalities (Table III).

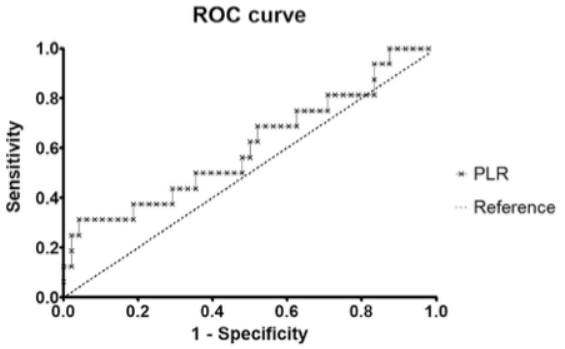

Fig. 1. ROC curve for PLR inflammatory marker according to sarcopenia.

Thirty-day Morbidity and Mortality

No patient was lost of follow-up. Thirty-day mortality was 2.1% vs. 12.5% in nonsarcopenic and sarcopenic groups, respectively (P = 0.1517). A statistical difference was noted concerning overall morbidity: 10.4% and 56.3% in nonsarcopenic and sarcopenic groups, respectively (P = 0.0004) (Table IV).

DISCUSSION

The main result of the present study is that sarcopenia is associated with elevated PLR and bad prognosis in terms of postoperative morbidity. However, a PLR cut-off value of 292.5 does not favor the diagnosis of sarcopenia given its low sensitivity, limiting its use as a simple marker of sarcopenia in CLTI patients.

The relationship between PLR and sarcopenia is unclear but might involve the inflammatory status of sarcopenic patients with CLTI. Altered inflammatory process is an important pathophysiological mechanism in CLTI, and likely contributes to the genesis of sarcopenia. Indeed, several vascular inflammatory markers such as tumor necrosis factor-α, interleukin 6, interleukin 1 receptor antagonist, fibrinogen, vascular cell adhesion protein 1, intercellular adhesion molecule 1, selectins, and C-reactive protein were found elevated in CLTI patients, compared with controls subjects.[15,16] Furthermore, elevated levels of these markers were associated with reduced muscle strength, muscle quality, and poorer physical performance in CLTI.[17−19] This systemic inflammation could be attributable to (1) the activation of platelet (as seen in thrombosis injuries) because they are responsible for the release of cytokines, proinflammatory chemokines, and growth factors and (2) the reduction in lymphocyte counts due to the physiological stress and/or poorer health

Table II. Clinical characteristics according to PLR

Factors	Low PLR ($n = 55$)	High PLR ($n = 9$)	*P*-value
Demographic data			
Age (years)	72.2 (71.0; 65.0–82.0)	79.7 (86.0; 65.0–90.5)	0.0885
Sex ratio	1.9	2	1.0000[a]
Clinical data			
Weight (kg)	74.80 (70.0; 64.75–82.25)	67.67 (69.0; 60.0–76.5)	0.2010
Height (m)	1.69 (1.70; 1.60–1.75)	1.71 (1.70; 1.66–1.77)	0.4489
BMI (kg/m^2)	26.38 (25.46; 23.12–27.51)	23.14 (22.21; 21.18–25.20)	0.0285[b]
Rest pain	21 (38.2%)	2 (22.2%)	0.4699[a]
Ulcers	16 (29.1%)	9 (100%)	<0.0001[a,b]
$TCPO_2$	5.5 (5.0; 3.0–7.0)	5.0 (5.0; 3.0–7.0)	0.5669
Cardiovascular risk factors			
Smoking	27 (49.1%)	2 (22.2%)	0.1660[a]
Diabetes	29 (52.7%)	3 (33.3%)	0.4741[a]
Hypertension	37 (67.3%)	9 (100%)	0.0515[a]
Dyslipidemia	24 (43.6%)	3 (33.3%)	0.7222[a]
Obesity	9 (16.4%)	0 (0%)	0.3369[a]
Comorbidities			
Cardiac	24 (43.6%)	4 (44.4%)	1.0000[a]
Renal	2 (3.6%)	1 (11.1%)	0.3703[a]
Pulmonary	3 (5.5%)	1 (11.1%)	0.4632[a]

Values are presented as mean (median; interquartile range), or number (%).
$TCPO_2$, transcutaneous oxygen pressure.
[a]Fisher's exact test.
[b]Statistically significant ($P < 0.05$).

conditions of sarcopenic patients.[20,21] The main advantage of PLR results from an easy and simple blood test and reflects variations in platelet and lymphocyte levels. It is easy to assess, and quicker than imaging measurements. As it is a ratio, PLR is relatively more stable than individual blood parameters. Consequently, our study shows an association between sarcopenia and PLR in CLTI patients, but not between sarcopenia and platelet or lymphocyte counts alone. This might also explain the high proportion of ulcers in high PLR patients. Indeed, infection and acute inflammation related to ulcers might play an important role.

This study explored the link between PLR, sarcopenia, and the prognosis of patients with CTLI. Previous studies demonstrated the prognosis value of PLR for vascular patients. Indeed, PLR was shown to predict limb salvage in CLTI patients following a revascularization procedure, and in patients for whom surgery was not possible. Indeed, Huang et al.[22] reported that CLTI patients with high PLR (≥130.337) displayed shorter amputation-free survival time (18.0 vs. 109.9 months for controls, $P < 0.001$) and higher risks of low extremity amputation (rates of 52.5% vs. 15.0% for controls, $P < 0.0001$) compared with CLTI patients with lower PLR following endovascular revascularization. Furthermore, 2 independent studies found that high PLR (≥160 or ≥237.14) was associated with poorer overall limb salvage in CLTI patients not eligible for surgical revascularization.[23,24] PLR was also associated with increased cardiovascular events in patients with CLTI, as patients with high PLR (≥142 or ≥150) presented with higher long-term cardiovascular mortality (31.6% vs. 17.2% for the low PLR group, $P < 0.001$) and myocardial infarction rates (5.7% vs. 3.5%, $P = 0.02$).[25,26] These studies highlight the significance of PLR in vascular diseases. It should be noted that these large disparities in PLR cut-off values are likely due to the heterogeneity in the population and the outcome of interest studied. In our study, PLR might help identifying a subgroup of patients associated with poor prognosis, even PLR does not seem appropriate to be used as a marker of sarcopenia.

The diagnosis of sarcopenia represents a challenge due to the complex assessment of muscle condition in CLTI patients. Indeed, appropriate cut-off values are not well defined for measurements of low muscle mass. Some studies used previously published thresholds, while others define sarcopenia based on the lowest quartile or ROC analysis. Here, we decided to use the previously published

Table III. Revascularization modalities according to sarcopenia

Factors	Nonsarcopenic group ($n = 48$)	Sarcopenic group ($n = 16$)	*P*-value
Type of surgery			
Open surgery	13 (27.1%)	5 (31.2%)	0.7563[a]
Endovascular surgery	29 (60.4%)	8 (50.0%)	0.4650[b]
Hybrid procedure	6 (12.5%)	3 (18.8%)	0.6792[a]
Length of procedure (min)	116 (95; 76.5–113.5)	136 (135; 105–165)	0.3986
Need for blood transfusion	2 (4.2%)	1 (6.2%)	1.0000[a]

Values are presented as mean (median; interquartile range), or number (%).
[a]Fisher's exact test.
[b]Chi-squared test.

Table IV. Thirty-day morbidity and mortality according to sarcopenia

Factors	Overall ($n = 64$)	Nonsarcopenic group ($n = 48$)	Sarcopenic group ($n = 16$)	*P*-value[a]
Thirty-day mortality	3 (4.7%)	1 (2.1%)	2 (12.5%)	0.1517
Major amputation	2 (3.1%)	1 (2.1%)	1 (6.3%)	0.4405
Local complications (lymphocele, hematoma)	7 (10.9%)	3 (6.3%)	4 (25%)	0.0592
General complications				
Cardiac decompensation	2 (3.1%)	0 (0%)	2 (12.5%)	0.0595
Renal decompensation with dialysis	3 (4.7%)	1 (2.1%)	2 (12.5%)	0.1517
Pulmonary decompensation	0 (0%)	0 (0%)	0 (0%)	NA
Overall 30-day morbidity	14 (21.9%)	5 (10.4%)	9 (56.3%)	0.0004[b]

Values are presented as number (%).
NA, not applicable.
[a]Fisher's exact test.
[b]Statistically significant ($P < 0.05$).

gender-specific cutoff of <5.5 cm^2/m^2 in men, and <4.0 cm^2/m^2 in women for low PMI, rather than creating our own cutoff.[11–14] This threshold was based on a western population of vascular patients, highly similar to our population of patients. It could represent a universal cut-off value for defining sarcopenia in vascular patients, allowing a generalizability of the resulting data. Our study shows that PLR is a simple screening test that could help with the identification of subgroups of patients who would require personalized treatments or specific therapeutic strategies, such as sarcopenic patients. Eventually, we hypothesize that patients' quality of life and overall prognosis could be improved by reversing the sarcopenic features seen in CLTI patients, notably through exercise or modulation of muscle mitochondrial dysfunction.[7,27]

This study suffers from several limitations. First, the study design is retrospective. Moreover, the diagnosis of sarcopenia was solely based on muscle mass measurements and did not include data on muscle strength or physical performance, even if, it has been shown to be a reliable parameter of sarcopenia in the setting of CLTI.[11] Furthermore, the cut-off value for low or high PLR was set at 292.5. As it is a high threshold, the risk of mistakenly diagnosed sarcopenia in patients who do not have it is extremely low (as shown by the high specificity of the screening test at 91.7%), but the risk of missing some of the patients who are indeed sarcopenic is high (as reflected by the low sensibility of the screening test at 31.3%). Finally, the small sample size of this study might have impacted the statistical

power of the analysis, limiting the robustness of the results.

CONCLUSION

CLTI is a heterogeneous entity urging for tailored treatment decisions based on patients' physiological and anatomical specificities. Particularly, sarcopenic patients should be considered as a subgroup of high-risk patients and the PLR might help to depict this subgroup of patients, requiring personalized treatments and probably specific therapeutic strategies.

REFERENCES

1. Aboyans V, Ricco J-B, Bartelink M-LEL, et al. Editor's choice - 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS). Eur J Vasc Endovasc Surg 2018;55:305–68.
2. Conte MS, Bradbury AW, Kolh P, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia. J Vasc Surg 2019;69:3S–125S.e40.
3. Morisaki K, Matsumoto T, Matsubara Y, et al. Prognostic factor of the two-year mortality after revascularization in patients with critical limb ischemia. Vascular 2017;25:123–9.
4. Taniguchi R, Deguchi J, Hashimoto T, et al. Sarcopenia as a possible negative predictor of limb salvage in patients with chronic limb-threatening ischemia. Ann Vasc Dis 2019;12:194–9.
5. Matsubara Y, Matsumoto T, Inoue K, et al. Sarcopenia is a risk factor for cardiovascular events experienced by patients with critical limb ischemia. J Vasc Surg 2017;65:1390–7.
6. Matsubara Y, Matsumoto T, Aoyagi Y, et al. Sarcopenia is a prognostic factor for overall survival in patients with critical limb ischemia. J Vasc Surg 2015;61:945–50.
7. Pizzimenti M, Meyer A, Charles A-L, et al. Sarcopenia and peripheral arterial disease: a systematic review. J Cachexia Sarcopenia Muscle 2020 (in press).
8. Lin J, Zhang W, Huang Y, et al. Sarcopenia is associated with the neutrophil/lymphocyte and platelet/lymphocyte ratios in operable gastric cancer patients: a prospective study. Cancer Manag Res 2018;10:4935–44.
9. Kitano Y, Yamashita Y-I, Saito Y, et al. Sarcopenia affects systemic and local immune system and impacts postoperative outcome in patients with extrahepatic cholangiocarcinoma. World J Surg 2019;43:2271–80.
10. Liaw F-Y, Huang C-F, Chen W-L, et al. Higher platelet-to-lymphocyte ratio increased the risk of sarcopenia in the community-dwelling older adults. Sci Rep 2017;7:16609.
11. Cruz-Jentoft AJ, Sayer AA. Sarcopenia. Lancet 2019;393:2636–46.
12. Waduud MA, Wood B, Keleabetswe P, et al. Influence of psoas muscle area on mortality following elective abdominal aortic aneurysm repair. Br J Surg 2019;106:367–74.
13. Heard R, Black D, Ramsay G, et al. The prevalence of sarcopaenia in a vascular surgical patient cohort and its impact on outcome. Surgeon 2018;16:325–32.
14. Jones K, Gordon-Weeks A, Coleman C, et al. Radiologically determined sarcopenia predicts morbidity and mortality following abdominal surgery: a systematic review and meta-analysis. World J Surg 2017;41:2266–79.
15. Signorelli SS, Mazzarino MC, Di Pino L, et al. High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test. Vasc Med 2003;8:15–9.
16. McDermott MM, Guralnik JM, Corsi A, et al. Patterns of inflammation associated with peripheral arterial disease: the InCHIANTI study. Am Heart J 2005;150:276–81.
17. McDermott MM, Greenland P, Green D, et al. D-dimer, inflammatory markers, and lower extremity functioning in patients with and without peripheral arterial disease. Circulation 2003;107:3191–8.
18. McDermott MM, Ferrucci L, Guralnik JM, et al. 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.
19. Nylaende M, Kroese A, Stranden E, et al. Markers of vascular inflammation are associated with the extent of atherosclerosis assessed as angiographic score and treadmill walking distances in patients with peripheral arterial occlusive disease. Vasc Med 2006;11:21–8.
20. Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. J Clin Invest 2005;115:3378–84.
21. Michaud M, Balardy L, Moulis G, et al. Proinflammatory cytokines, aging, and age-related diseases. J Am Med Dir Assoc 2013;14:877–82.
22. Huang H-L, Juang J-MJ, Hsieh C-A, et al. Risk stratification for low extremity amputation in critical limb ischemia patients who have undergone endovascular revascularization: a survival tree analysis. Medicine (Baltimore) 2019;98:e16809.
23. Taşoğlu İ, Sert D, Colak N, et al. Neutrophil-lymphocyte ratio and the platelet-lymphocyte ratio predict the limb survival in critical limb ischemia. Clin Appl Thromb Hemost 2014;20:645–50.
24. Wang Q, Liu H, Sun S, et al. Neutrophil-to-lymphocyte ratio is effective prognostic indicator for post-amputation patients with critical limb ischemia. Saudi Med J 2017;38:24–9.
25. Uzun F, Erturk M, Cakmak HA, et al. Usefulness of the platelet-to-lymphocyte ratio in predicting long-term cardiovascular mortality in patients with peripheral arterial occlusive disease. Postepy Kardiol Interwencyjnej 2017;13:32–8.
26. Gary T, Pichler M, Belaj K, et al. Platelet-to-lymphocyte ratio: a novel marker for critical limb ischemia in peripheral arterial occlusive disease patients. PLoS One 2013;8:e67688.
27. Pizzimenti M, Riou M, Charles A-L, et al. The rise of mitochondria in peripheral arterial disease physiopathology: experimental and clinical data. J Clin Med 2019;8:2125.

Résultat II Ischémie critique chronique et sarcopénie : revue de la littérature

Objectifs : Le développement d'une sarcopénie est un facteur de risque pour les patients atteints d'artériopathie. Il est donc important d'identifier les mécanismes physiopathologiques sous-jacents, ainsi que les stratégies thérapeutiques potentielles pouvant améliorer la fonction musculaire.

Méthodes : Une revue systématique de la littérature a été réalisée en suivant les recommandations PRISMA.

Résultats : Au total, 79 publications ont été inclues dans la revue. L'analyse épidémiologique se base sur 18 études portant sur 2362 patients atteints d'artériopathie (31.39% [SD 7.6] de femmes, âgés de 72.42 [SD 2.8] ans en moyenne), la sarcopénie est présente chez 34.63% (SD 12.9) des patients. L'analyse des voies moléculaires impliquées est basée sur 5 études animales et 29 études cliniques. La force et la fonction musculaire sont altérées chez 1352 patients (26.49% [SD 17.3] de femmes, âgés de 67.67 [SD 5.1] ans en moyenne). Les fibres musculaires montrent des défauts histologiques chez 192 patients (9.2% [SD 11.2] de femmes, âgés de 64.3 [SD 1.0] ans en moyenne). Le stress oxydant est augmenté de 58.63% [SD 25.5] chez 69 patients (16.96% [SD 8.1] de femmes, âgés de 63.17 [1.4] ans en moyenne). Une mitochondriopathie est présente chez 153 patients (29.39% [SD 28.3] de femmes, âgés de 63.5 [SD 1.8] ans en moyenne). L'inflammation est augmentée de 15.58% [SD 7.4] chez 900 patients (40.77% [SD 3.7] de femmes, âgés de 74.9 [SD 2.8] ans en moyenne). Les voies de catabolisme et d'anabolisme musculaire sont altérées chez 51 patients (34.45% [SD 32.23] de femmes, âgés de 72.25 [SD 5.3] ans en moyenne). L'analyse des approches thérapeutiques est basée sur 7 études animales et 21 études cliniques. Au total, 884 patients ont suivi une thérapie par l'exercice et 18 ont reçu un traitement angiogénique (30.84% [SD 17.74] de femmes, âgés de 66.85 [SD 4.0] ans en moyenne).

Conclusions : Il est essentiel de sensibiliser à l'importance du diagnostic de la sarcopénie dans la pratique médicale quotidienne. La sarcopénie et l'artériopathie s'accompagnent d'un stress oxydatif, d'un dysfonctionnement mitochondrial, d'une inflammation et d'une altération des processus de catabolisme/anabolisme musculaire. A ce jour, outre la revascularisation, la

stratégie thérapeutique envisagée pour améliorer la fonction musculaire est l'exercice. Les mécanismes sous-jacents pourraient ouvrir la voie à de nouvelles avancées thérapeutiques.

REVIEW

Journal of Cachexia, Sarcopenia and Muscle 2020; **11**: 866–886
Published online 10 July 2020 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/jcsm.12587

Sarcopenia and peripheral arterial disease: a systematic review

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Abstract

Background Patients with lower extremity peripheral arterial disease (PAD) and sarcopenia are a population at risk requiring specific and targeted care. The aim of this review is to gather all relevant studies associating sarcopenia and PAD and to identify the underlying pathophysiological mechanisms as well as potential therapeutic strategies to improve skeletal muscle function.
Methods A systematic review was carried out following the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).
Results Data extraction allowed the evaluation of 140 publications; 87 met the inclusion criteria; of which 79 were included in the final review, reporting sufficient data for epidemiological and diagnostic criteria, mechanical analysis, and therapeutic approaches. Epidemiological analysis and diagnostic criteria were based on 18 studies following 2362 PAD patients [31.39% (SD 7.61) women], aged 72.42 (SD 2.84); sarcopenia was present in 34.63% (SD 12.86) of the patients. Mechanical and pathway analysis were based on five animal studies and 29 clinical reports, showing significantly altered muscle strength and function in 1352 PAD patients [26.49% (SD 17.32) women], aged 67.67 (SD 5.14) years; impaired muscle histology in 192 PAD patients (9.2% (SD 11.22) women), aged 64.3 (SD 0.99) years; +58.63% (SD 25.48) of oxidative stress in 69 PAD patients [16.96% (SD 8.10) women], aged 63.17 (SD 1.43) years; mitochondriopathy in 153 PAD patients [29.39% (SD 28.27) women], aged 63.50 (SD 1.83) years; +15.58% (SD 7.41) of inflammation in 900 PAD patients [40.77% (SD 3.71) women], aged 74.88 (SD 2.76) years; and altered signalling pathways in 51 PAD patients [34.45% (SD 32.23) women], aged 72.25 (SD 5.25) years. Therapeutic approaches analysis was based on seven animal studies and 21 clinical reports. In total, 884 patients followed an exercise therapy, and 18 received an angiogenesis treatment; 30.84% (SD 17.74) were women. Mean ages of patients studied were 66.85 (SD 3.96).
Conclusions Sarcopenia and lower extremity PAD have musculoskeletal consequences that directly impair patients' quality of life and prognosis. Although PAD is primarily a vascular disease, all etiological factors of sarcopenia identified so far are present in PAD. Indeed, both sarcopenia and PAD are accompanied by oxidative stress, skeletal muscle mitochondrial impairments, inflammation, inhibition of specific pathways regulating muscle synthesis or protection (i.e. IGF-1, RISK, and SAFE), and activation of molecules associated with muscle degradation. To date, besides revascularization, the best therapeutic strategy includes exercise, but approaches targeting the underlying mechanisms still deserve further studies.

Keywords Sarcopenia; Peripheral arterial disease; Pathological pathways; Oxidative stress; Inflammation; Mitochondrial function; Exercise training

Received: 29 July 2019; Revised: 31 January 2020; Accepted: 24 February 2020
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Introduction

Cardiovascular diseases are a major cause of death around the globe, with a prevalence gradually increasing as life expectancy rises.[1] Among them, lower extremity peripheral arterial disease (PAD) is defined by a reduction of or an obstruction to blood flow in the arteries, with symptoms ranging from intermittent claudication to critical limb ischemia (CLI), characterized by rest pain and/or ulcers.[2–4]

Peripheral arterial disease is commonly accompanied by musculoskeletal abnormalities including generalized loss of skeletal muscle mass, strength, and physical performance—also called sarcopenia.[5,6] Both PAD and sarcopenia can run in parallel, many patients with PAD being also diagnosed with a sarcopenic condition (and probably even more are undiagnosed). In addition to worsen the loss of muscle mass and function in a vicious circle, sarcopenia further aggravates patients' quality of life and prognosis.[7] It is therefore essential to have a better understanding on how PAD and sarcopenia may impair skeletal muscle.

This systematic review resulted from the need to raise awareness on the importance of diagnosing sarcopenia in daily practice, and to better understand the pathological mechanisms underlying sarcopenia and PAD that might open the way towards new therapeutic advances. Therefore, the aim of this article is to gather all the relevant studies associating sarcopenia and PAD. To this end, the proposed systematic review will answer the following questions:

1 Epidemiological data: is sarcopenia a rare condition in patients with PAD?
2 Diagnostic criteria: how to diagnose sarcopenia and is sarcopenia a factor of poor prognosis for patients with PAD?
3 Mechanical analysis: how does sarcopenia affect skeletal muscle?
4 Therapeutic approaches: can we reverse the sarcopenic condition in patients with PAD?

Methods

Systematic review of the literature

A systematic review was performed following previously published Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[8]

Eligibility criteria

Throughout the process of literature selection, clear inclusion and exclusion criteria were followed. Studies included were full text English or French publications without any chronological limit. All primary research studies reporting a link between sarcopenia and PAD were included. Studies not eligible for inclusion were reviews, letters, editorials, comments, book chapters, and studies focusing on other diseases. The main outcomes of interest were the presence of epidemiological data, mechanical data, and therapeutic data focusing on skeletal muscle aberrations and PAD. Studies that listed patient characteristics, assessment method of sarcopenia, duration of follow-up, and prognostic outcome were considered to have epidemiological significance. Studies that listed mechanistic pathways leading to muscular defects in PAD were considered to have mechanical significance. Studies that listed therapies targeting skeletal muscle for PAD patients were considered to have therapeutic significant.

Information sources and search strategy

The PubMed database was analysed with a combined strategy using the subject heading terms ('peripheral artery disease' OR 'peripheral arterial disease' OR 'critical limb ischemia' OR 'chronic limb-threatening ischemia') AND ('sarcopenia' OR 'muscle atrophy' OR 'amyotrophy' OR 'muscle strength' OR 'muscle loss' OR 'muscle dysfunction'). All titles and abstracts collected from the search strategy were screened for relevance. When a relevant article was found, full text articles were retrieved. Studies that did not meet the inclusion criteria were excluded. The publications of the reference lists of included studies were searched and scanned for other potentially relevant studies. The full text of all potentially relevant articles was obtained and reviewed for eligibility.

Study records and data items

Data were extracted using a standardized form. This was done in duplicate to increase accuracy and to reduce measurement bias. Data extracted included study characteristics (year of publication, study design, population, and parameters determined) and particularly skeletal muscle characteristics (type of muscle, measurement method of muscle mass and strength) and main results.

Results

A flowchart showing study selection is given in *Figure* 1. Data extraction led to the evaluation of 140 publications, of which 87 met the inclusion criteria, and 79 were included in the final review.[9–87] Of these, 18 gave sufficient data for epidemiological analysis and diagnostic criteria,[9–26] 33 gave sufficient data for mechanistical analysis,[27–59] and 28 gave sufficient data for therapeutic approaches.[60–87]

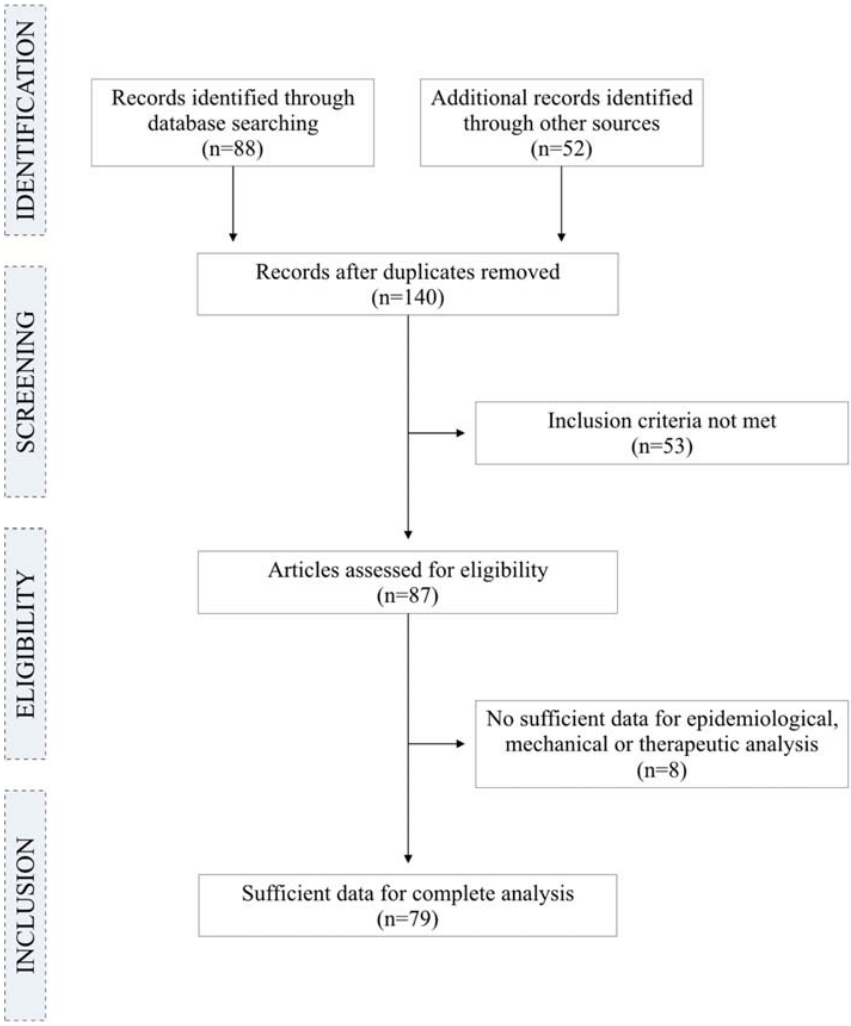

FIGURE 1 Flowchart of the systematic review.

1/Epidemiological data: Is sarcopenia a rare condition in patients with PAD?

Epidemiological analysis and diagnostic criteria were based on 17 studies following 2362 PAD patients; 31.39% (SD 7.61) were women. Mean age of patients studied was 72.42 (SD 2.84). Sarcopenia was present in 34.63% (SD 12.86) of the patients.

Sarcopenia is also described in conditions associated with PAD, such as metabolic syndrome. The metabolic syndrome —clustering of multiple metabolic abnormalities (i.e. diabetes mellitus, dyslipidemia, hypertension, or obesity)—is associated with an increased risk of cardiovascular morbidity and mortality and is highly prevalent in PAD patients.[9]

Interestingly, in a rat model of PAD, the presence of diabetes mellitus worsened the ischemia–reperfusion-induced skeletal muscle injury, as shown by a more severe decline in mitochondrial respiration and by enhanced levels of oxidative stress and apoptosis effectors in skeletal muscles.[10]

In humans, patients living with both PAD and diabetes mellitus displayed musculoskeletal and biomechanical changes in the lower limbs. Indeed, Bartolo *et al*. recently showed a significant increase in the electromyogram muscle amplitude of PAD and diabetic patients, characteristic of less efficient lower muscle contractions and probable early fatigue.[11]

Thus, skeletal muscle wasting is exacerbated in PAD patients presenting with a metabolic syndrome. Such patients represent a particularly fragile population that requires specific attention because of prominent atherosclerotic risk factors and poor outcomes. Moreover, the accumulation of skeletal muscle alterations might enhance the prevalence of sarcopenia—particularly hidden sarcopenia—in this fragile population. Indeed, it is important to note that a large

proportion of these patients might be obese and thus, might be at risk of developing sarcopenic visceral and/or subcutaneous obesity. Such potential hidden condition is resulting in a higher risk of adverse outcomes.[12]

2/Diagnostic criteria: How to diagnose sarcopenia and is sarcopenia a factor of poor prognosis for patients with PAD?

A/What is sarcopenia? Definition and diagnostic criteria

Sarcopenia is characterized by a decline in muscle mass and function with age or disease. It represents a significant burden for the patients, as it is associated with physical disability, increased hospitalization rates and mortality.[13] It is therefore essential for healthcare professionals to better recognize this condition. In this light and during the last decade, collaborative efforts were made all over the world, for example, Europe, America, and Asia in order to reach a consensus definition and diagnosis criteria for sarcopenia. To date, it appears accepted by the different working groups on sarcopenia that sarcopenia should be defined through three main criteria: (i) low muscle strength, (ii) low muscle quantity or quality, and (iii) low physical performance. Alone, Criterion 1 is considered the most reliable, and it should guide towards the diagnosis of sarcopenia. When combined, Criteria 1 and 2 account for the certainty of the diagnosis. Last, if all three criteria are met, sarcopenia is considered severe based on the link between low physical performance and poor prognosis.

Muscle strength assessment According to the European Working Group on Sarcopenia in Older People (EWGSOP), low muscle strength can be assessed with measures of grip strength, with cut-off points under 27 kg for men and 16 kg for women. The chair stand test can be used to measure impaired lower body strength (sarcopenia is suspected if the time required to complete five consecutive rises exceeds 15 s). Finally, isometric torque methods can be used to measure muscle extension/flexion power.

Muscle mass/quality assessment Low muscle quantity can be assessed by measurements of appendicular skeletal muscle mass (ASM) or skeletal muscle mass index (ASM/height^2) using bioelectrical impedance analysis (BIA) or dual X-ray absorptiometry (DXA). The reference values for low ASM are under 20 kg for men and 15 kg for women and under 7.0 kg/m^2 for men and 6.0 kg/m^2 for women for low skeletal muscle mass index. Magnetic resonance imaging (MRI) or computed tomography (CT) can also be used to appreciate muscle quantity; however, appropriate cut-offs values are not well defined for these measurements.

Physical performance assessment Low physical performance can be predicted by low gait speed (≤0.8 m/s) or low score on the short physical performance battery test (≤8 point).[14]

The data published show an important heterogeneity in the application of sarcopenic diagnostic criteria. Indeed, based on the articles in *Tables* 1 and 2, 12 articles are using a single-measurement approach (measure of muscle mass alone in six articles, muscle strength alone in five articles and physical performance alone in one article); nine articles are using two points of measure (commonly muscle strength and physical performance), and no articles are using all three criteria (muscle strength, muscle mass, and muscle function). Where muscle mass is used, four articles focused on lumbar muscles, one on leg muscles, and three on individual psoas muscle; and values are either unindexed in five articles, indexed to tibial length square in one article, height square in one article, or even to the adjacent vertebral body in one article. These disparities make it difficult to aggregate and compare the resulting data. Further, it is unlikely that a single muscle might be used as a sentinel for sarcopenia diagnostic. These data support the need of further studies to reach a consensus allowing clear and valid diagnostic criteria for sarcopenia in PAD patients.[15]

B/Sarcopenia in PAD: a factor of poor prognosis

Sarcopenia has been associated with multiple adverse events such as physical limitation, poor quality of life, and mortality and was shown to predict patients' prognosis and outcome following vascular procedures.[16] Similarly, recent data have revealed a link between sarcopenia, PAD and high comorbidity. In a 48-month longitudinal study, a reduction in calf muscle density, lower limb extension/flexion power, and hand grip strength was shown to be associated with an increase in mortality in 434 PAD patients.[17] Interestingly, this conclusion was also reached when only one parameter of sarcopenia was measured (muscle quantity[18] or muscle strength[19]). Indeed, in a retrospective study of 327 patients with PAD followed for up to 30 months, Sugai *et al*. found an independent link between low psoas muscle area and major adverse cardiovascular and limb events. Likewise, after a 9-month follow-up, Reeve *et al*. highlighted an association between lower grip strength and elevated comorbidity and cardiac risk in patients with vascular diseases, including PAD. Noteworthy, in a study realized on 410 patients with PAD followed for 60 months, poor leg extension/flexion power was reported to predict mortality in men but not in women.[20] This result might be explained by the larger proportion of men studied. Finally, a recent study following patients with PAD for 72 months showed no difference on overall survival between patients with high or low muscle mass, assessed through comparison of psoas-L4 vertebral index.[21] Together, these results indicate that muscle strength rather than muscle mass is a poor prognosis factor in PAD. This might be attributable to the fact that muscle mass

Table 1 Association between sarcopenia and poor outcomes in PAD patients

Reference	Patients population	Number of patients	Assessment method			Outcomes measured	Main results
			Muscle strength	Muscle mass/quality	Physical performance		
Shimazoe *et al.*, 2019, Ann Vasc Surg [26]	CLI	110	-	Skeletal muscle areas at the L3 level (CT)	Measures of basic aspects of activities related to self-care and mobility	3-year overall survival; amputation-free survival	Low activity of daily living was significantly associated with worse 3-year overall survival and amputation-free survival in patients with CLI and low muscle mass (defined as skeletal muscle area <114.0 cm^2 for men and <89.8 cm^2 for women).
Taniguchi *et al.*, 2019, Ann Vasc Dis [25]	CLI	75	-	Cross-sectional area of the psoas major muscles (CT)	-	Limb salvage and overall survival	Low muscle mass (21.4 ± 3.8 kg/m^2 in the sarcopenic group vs. 23.5 ± 3.1 kg/m^2 in the non-sarcopenic group) was associated with significantly lower limb salvage rates (73% vs. 100% at 2 years, $P < 0.05$) and overall survival rates (60% vs. 87% at 3 years, $P < 0.05$)
Morisaki *et al.*, 2019, Vascular [24]	CLI	127	-	Low skeletal muscle mass index (CT)	Non-ambulatory status	Overall survival	Low muscle mass (defined as skeletal muscle area <114.0 cm^2 for men and <89.8 cm^2 for women) was associated with significantly lower overall survival (89.7% in the CLI Frailty group vs. 60.5% in the CLI Non-frailty group at 2 years after revascularization, $P < 0.01$)
Reeve *et al.*, 2018, J Vasc Surg [19]	Vascular disease (AAA, carotid stenosis, PAD)	311	Dominant hand grip strength	-	- N-	Comorbidity, cardiac risk	Low muscle strength (19.7 ± 6.5 kg in the frail vs. 36.8 ± 10.3 kg in the non-frail patients) was associated with comorbidity (based on Charlson comorbidity index with 6.4 ± 2.2 points vs. 5.2 ± 2.2 points, $P < 0.0001$) and cardiac risk (based on revised cardiac risk index with 1.8 ± 0.8 vs. 1.5 ± 0.7, $P < 0.018$)
Sugai *et al.*, 2018, Circ J [18]	PAD	327	-	Psoas muscle value (CT)	-	Major adverse cardiovascular and limb events	Patients with major adverse cardiovascular and limb events had significantly lower mean psoas muscle value (41.0 ± 7.4 vs. 46.7 ± 5.7 Hounsfield unit, $P < 0.001$) than those without
Matsubara *et al.*, 2017, J Vasc Surg [23]	CLI	114	-	Vertebral body at the L3 level (CT)	-	Cardiovascular event-free survival	Low muscle mass (defined as skeletal muscle area <114.0 cm^2 for men and <89.8 cm^2 for women) was associated with lower cardiovascular event-free survival rates (43.1% for patients with sarcopenia vs. 91.2% without sarcopenia at 3 years, $P < 0.01$)

(*Continues*)

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DOI: 10.1002/jcsm.12587

Table 1 (continued)

Reference	Patients population	Number of patients	Assessment method			Outcomes measured	Main results
			Muscle strength	Muscle mass/quality	Physical performance		
Nyers *et al.*, 2017, J Vasc Surg [21]	PAD	188	-	Psoas-L4 verterbal index (Cross-sectional area of the bilateral psoas muscles and vertebral body at the L4 level) (CT)	-	Amputation-free survival	Muscle mass did not predict amputation-free survival (with a psoas-L4 vertebral index at 1.79 ± 0.55 for patients with 3 years amputation-free survival vs. 1.78 ± 0.57 for patients without 3 years amputation-free survival)
Matsubara *et al.*, 2015, J Vasc Surg [22]	CLI	64	-	Vertebral body at the L3 level (CT)	-	Overall survival	Low muscle mass (defined as skeletal muscle area <114.0 cm^2 for men and <89.8 cm^2 for women) was associated with lower survival rates (23.5% for patients with sarcopenia vs. 77.5% without sarcopenia at 5 years, $P < 0.001$)
McDermott *et al.*, 2012, J Am Coll Cardiol [17]	PAD	434	Knee extension/ Isometric knee extension/ Plantar flexion powerHand grip strength	Calf muscle density (CT)	-	Comorbidities and mortality	Lower calf muscle density was associated with higher cardiovascular disease mortality. Low plantar flexion strength, low baseline leg power and poor handgrip were associated with higher all-cause mortality (using proportional hazards analyses)
Singh *et al.*, 2010, J Vasc Surg [20]	PAD	410	Knee extension/ Isometric knee extension/Hip extension/Hip flexion power	-	-	Mortality	Low baseline strength for knee flexion/extension and hip extension were associated with higher all-cause mortality in men. Poorer strength for knee flexion and hip extension were associated with higher cardiovascular mortality in men (using proportional hazards analyses)

AAA, abdominal aortic aneurysm, CLI, critical limb ischemia; CT, computed tomography; F, female; M, male; PAD, peripheral artery disease.

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DOI: 10.1002/jcsm.12587

Table 2 Association between impaired muscle strength/function and PAD

Reference	Patients population	Number of patients/ controls	Assessment method			Main results
			Muscle strength	Muscle mass/ quality	Physical performance	
Kakihana *et al.*, 2017, J Vasc Surg [35]	PAD	16/10	-	-	7-m walkway embedded with a force plate test	PAD was associated with slower walk at self-selected walking speed (88.32 ± 15.15 cm/s for PAD patients vs. 126.04 ± 16.31 cm/s for controls, $P < 0.001$) and at fast walking speed (119.90 ± 21.07 cm/s vs. 162.01 ± 21.47 cm/s for controls, $P < 0.001$); lower cadence at self-selected walking speed (109.92 ± 12.17 step/min vs. 118.38 ± 7.28 steps/min, $P < 0.001$) and at fast walking speed (121.29 ± 11.39 steps/min vs. 135.11 ± 9.47 step/min, $P < 0.001$); and reduced peak hip flexor generation power at self-selected walking speed (0.50 ± 0.18 W/kg vs. 1.00 ± 0.22 W/kg, $P < 0.001$) and at fast walking speed (0.78 ± 0.27 W/kg vs. 1.40 ± 0.39 W/kg, $P < 0.001$)
Schieber *et al.*, 2017, J Vasc Surg [29]	PAD	94/16	Maximal isometric plantar flexion contractions of 10 s	-	-	PAD patients exhibited strength deficits, with impaired peak torque values (69.1 ± 28.7 N.m for claudicating patients vs. 98.2 ± 27.6 N.m for controls, $P < 0.01$)
Dziubek *et al.*, 2015, Maturitas [33]	CLI	85/50	Force-velocity parameters (peak torque, total work, average power) of the lower limb	-	6-min walk test	PAD was associated with lower 6-min walk distance (349.77 ± 65.08 m for PAD patients vs. 515.86 ± 96.39 for controls, $P < 0.0001$), lower mean walk speed (3.49 ± 0.65 km/h vs. 5.15 ± 0.96 km/h for controls, $P < 0.01$), and significantly lower values of force-velocity parameters (including peak torque, total work and average power of the knee joint) compared with the control group ($P < 0.005$)
Parmenter *et al.*, 2013, J Vasc Surg [34]	PAD	22/–	Maximum strength/endurance testing (hip extensors, hip abductors, quadriceps, hamstrings, plantar flexors, pectoral, upper back muscles)	-	6-min walk test	Greater severity of PAD was associated with reduced bilateral hip extensor strength ($r = 0.54$, $P = 0.007$), whole body strength ($r = 0.32$, $P = 0.05$), shorter distance to first stop during the 6-min walk test ($r = 0.38$, $P = 0.05$) and poorer single leg balance ($r = 0.44$, $P = 0.03$) (using univariate and stepwise multiple regression models)
Câmara *et al.*, 2012, Ann Vasc Surg [30]	PAD	20/9	Plantar flexion/ dorsiflexion movements, knee extension/ flexion movements	-	Plantar flexion/ dorsiflexion movements, knee extension/ flexion movements	PAD patients presented lower muscle strength in dorsiflexion (0.20 ± 0.10 N/m/kg for PAD patients vs. 0.29 ± 0.10 N/m/kg for controls, $P < 0.01$), plantar flexion (0.36 ± 0.20 N/m/kg vs. 0.53 ± 0.20 N/m/kg, $P < 0.01$) and knee flexion movements (0.50 ± 0.30 N/m/kg vs. 0.62 ± 0.10, $P = 0.04$). Also, PAD was associated with lower muscle endurance in dorsiflexion (8.0 ± 3.5 N/m/kg vs. 9.9 ± 6.6 N/m/kg, $P = 0.01$) and plantar flexion movements (20.0 ± 9.0 N/m/kg vs. 25.7 ± 10.7 N/m/kg, $P = 0.02$)
	PAD	30/32		-	-	

(*Continues*)

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Table 2 (continued)

Reference	Patients population	Number of patients/ controls	Assessment method			Main results
			Muscle strength	Muscle mass/ quality	Physical performance	
Wurdeman *et al.*, 2012, Gait Posture [31]			Joint moments and powers at early, mid and late stance (hip and knee and ankle joints)			PAD was associated with reduced peak hip power absorption in midstance (−0.788 ± 0.25 W/kg for PAD patients vs.−0.950 ± 0.27 W/kg for controls, $P = 0.017$), reduced peak knee power absorption in late stance (−0.729 ± 0.21 W/kg vs.−0.899 ± 0.33 W/kg for controls, $P = 0.02$), and reduced peak ankle power generation in late stance (2.677 ± 0.45 W/kg vs. 2.998 ± 0.60 W/kg, $P = 0.021$)
Koutakis *et al.*, 2010, J Vasc Surg [32]	PAD	20/16	Joint torques and powers at early, mid and late stance (hip, knee and ankle joints)	-	Ambulation on a walkway	PAD patients presented significantly reduced hip power generation in late stance (0.569 ± 0.18 W/kg for claudicating patients vs. 0.706 ± 0.24 W/kg for controls, $P = 0.03$), knee power absorption in late stance (−0.580 ± 0.25 W/kg vs. −0.882 ± 0.32 W/kg, $P = 0.0015$), and ankle power generation in late stance (2.178 ± 0.51 W/kg vs. 2.957 ± 0.69 W/kg, $P = 0.0001$) Also, PAD was associated with reduced gait velocity (1.09 ± 0.13 m/s for claudicating patients vs. 1.28 ± 0.13 m/s for controls, $P = 0.0007$) and stride length (1.27 ± 0.11 m vs. 1.47 ± 0.11 m for controls, $P < 0.001$)
Koutakis *et al.*, 2010, J Vasc Surg [36]	PAD	20/10	Joint torques and powers at early, mid and late stance (hip, knee and ankle joints)	-	-	PAD was associated with reduced knee power generation in early stance (0.26 ± 0.31 W/kg for claudicating patients vs. 0.62 ± 0.25 W/kg for controls, $P < 0.05$) and ankle power generation in late stance (2.05 ± 0.59 W/kg vs. 4.00 ± 0.88 W/kg for controls, $P < 0.05$)
Herman *et al.*, 2009, J Am Geriatr Soc [37]	PAD	374/−	Hip extension/flexion, knee extension/flexion strength Walking over a force platform	-	7-m walking speed test 6-min walk test Short physical performance battery	In women with PAD, weaker baseline hip and knee flexion strength were associated with faster average annual decline in usual-paced 4-m walking velocity (P trend < 0.001 and P trend = 0.02 respectively) and in short physical performance battery test (P trend = 0.019 and P trend = 0.01, respectively)
McDermott *et al.*, 2008, J Am Geriatr Soc [28]	PAD	424/271	Isometric knee extension/ plantar flexion strength Handgrip strength Knee extension power	-	6-min walk test 4-m walking velocity test	Lower arterial brachial index values were associated with lower plantar flexion strength (P trend = 0.04) and lower knee extension power (P trend < 0.001)
Kuo *et al.*, 2008, J Gerontol A Biol Sci Med Sci [38]	PAD	206/ 1592	Isokinetic dynamometer	-	20-ft timed walk test	PAD associated with weak leg force, low gait speed and functional dependence (based on multiple logistic regression analyses)

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DOI: 10.1002/jcsm.12587

measurements are extremely susceptible to bias, supporting the need for homogenized technic used, muscle analysed, and cut-off values of low muscle mass.

Sarcopenia was also linked with CLI and high mortality rates. Indeed, in 2015, and subsequently in 2017, Matsubara *et al*. reported that sarcopenia was associated with higher cardiovascular events and lower survival, in 64 and 114 patients suffering from CLI, respectively.[22,23] Recent papers confirmed this association in retrospective studies including patients with CLI, where low skeletal muscle mass was predictive of a worse overall survival[24–26] (*Table*1).

3/Mechanistical analysis: how does sarcopenia affect skeletal muscle?

Mechanistical analysis was based on five animal studies [10; 43–44; 48; 58] and 29 clinical reports. This allowed the analysis of the following:

i muscle strength and function,[27–38] based on 1352 PAD patients [26.49% (SD 17.32) women], aged 67.67 (SD 5.14) years;
ii muscle histology,[39–42] based on 192 PAD patients [9.2% (SD 11.22) women], aged 64.3 (SD 0.99) years;
iii oxidative stress,[43–47] based on 69 PAD patients [16.96% (SD 8.10) women], aged 63.17 (SD 1.43) years;
iv mitochondriopathy [43; 47–53], based on 153 PAD patients [29.39% (SD 28.27) women], aged 63.50 (SD 1.83) years;
v inflammation,[54–56] based on 900 PAD patients [40.77% (SD 3.71) women], aged 74.88 (SD 2.76) years;
vi signalling pathways [10; 57–59], based on 51 PAD patients [34.45% (SD 32.23) women], aged 72.25 (SD 5.25) years.

Muscle dysfunction in PAD: the missing link to better understand the common mechanisms of sarcopenia and PAD pathophysiology

Patients with lower extremity PAD present various skeletal muscle defects, such as weak baseline strength, functional decline, and abnormal muscle histology. Although sarcopenia is currently defined by its clinical manifestations, all etiological factors identified so far—including excessive oxidative stress production, skeletal muscle mitochondrial impairments, high inflammation, and altered muscle kinetic process—are present in PAD and emphasizing skeletal muscle injuries (*Figure* 2).

A/Impaired muscle strength and function

Peripheral arterial disease pathophysiology is characterized by metabolic and structural myopathic changes in skeletal muscles, responsible for the decline in strength and function.[27] Lower extremity ischemia was shown to specifically impair proximal and distal leg muscles. Indeed, in a study of 424 patients with PAD, plantar flexion and knee extension strength was significantly lower when compared with age-matched controls.[28] Similarly, analysis of strength and endurance of hip, knee, ankle, and plantar muscles of patients with and without PAD revealed an association between PAD and weaker leg muscles.[29–32] PAD physiopathology is thus characterized by a failure of specific muscles that are necessary for normal walking.

Accordingly, the loss of muscle strength appears to play a central role in the functional defects observed in patients. In the chronic stage of PAD, values of force-velocity parameters of the lower limbs and 6-min walk capacity were significantly lower compared with the control group.[33] Interestingly, the impairment in force and mobility was also seen in earlier stages of PAD, as shown by three independent studies. In the first study, 22 patients with PAD underwent 6-min walking speed testing, maximum strength/endurance testing of lower limb muscles, as well as performance-based testing of muscle function. Patients with PAD presented overall body disability, muscle weakness, and reduced physical performance.[34] The other two studies highlighted a gait impairment in patients with PAD, using a walkway embedded with a force plate.[35,36] Moreover, in a study of 374 patients with PAD, the correlation between poor strength/mobility and lower limb ischemia was found in women, but not men, probably because of their higher baseline strength.[37]

It is important to note that in a total of 11 studies associating low muscle strength and/or function and/or quality and PAD, none used the term sarcopenia. Yet a sarcopenic diagnosis represents a turning point for patients, families, and clinicians. Indeed, beyond aggravating patients' prognosis, sarcopenia constitutes a great burden, as it participates to the isolation and dependence of patients with PAD[38] (*Table*2).

B/Impaired muscle histology

The morphological and physiological consequences of the denervation–reinnervation process that occur in PAD were assessed in 26 patients with PAD based on *gastrocnemius* cross-sectional area values and peak treadmill walking time. Overall, PAD was characterized by a general decline in type II muscle fibres number and size, responsible for the decay in general muscle strength.[39] Subsequent studies focusing on myofibre morphometrics of PAD *gastrocnemius* revealed significant changes in muscle quality, including lower diameter and density, rounder myofibres, and a fast-to-slow switch resulting in a predominance of type I fibres, as demonstrated by cross-sectional area analysis.[40–42]

Thus, the genesis of sarcopenia in PAD patients might be explained by the progressive loss of motoneurons included in type II motor units, which remains uncompensated despite reinnervation of muscle fibres by adaptive sprouting.

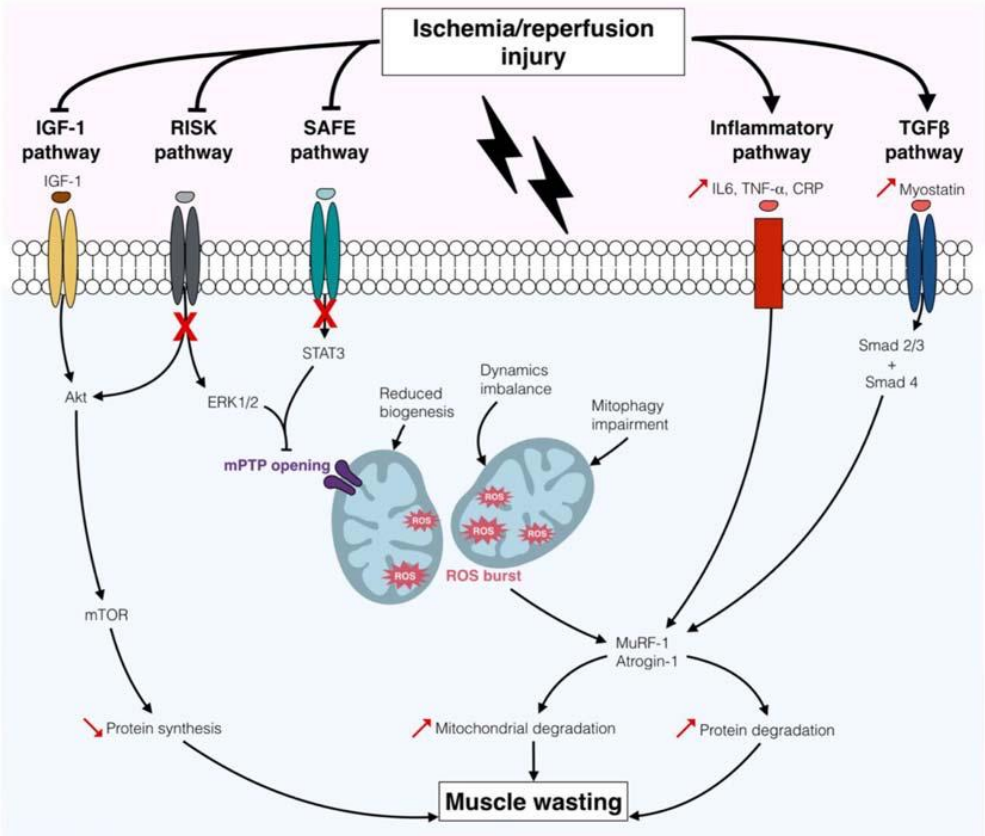

FIGURE 2 Major signalling pathways associated with sarcopenia and peripheral arterial disease (PAD). In the context of PAD, ischemia/reperfusion (I/R) injury induces a decrease in mitochondrial biogenesis, dynamics, and mitophagic activities, resulting in reactive oxygen species (ROS) burst and consecutive oxidative stress. Additionally, elevated levels of IL6, TNF-α, and CRP are responsible for the activation of the inflammatory pathway. Ultimately, I/R-induced oxidative stress and inflammation enhance the activity of the atrophy-related ubiquitin ligases MuRF-1 and atrogin-1 and the degradation of mitochondria and proteins. I/R is also associated with defective stimulation of the muscle synthesis PI3K/Akt/mTOR pathway, notably via lower activity of the IGF-1 and RISK pathways. Moreover, alteration of the protective pathways RISK and SAFE lead to persistent mitochondrial permeability transition pore (mPTP) opening, reduction in mitochondrial calcium retention capacity, and aggravation of mitochondrial dysfunction. Further, during I/R, myostatin overexpression results in enhanced activity of the muscle degradation pathway TGFβ.

C/Oxidative stress

An imbalance between pro-oxidant and antioxidant activities (also called oxidative stress) is responsible for reactive oxygen species (ROS) accumulation, mitochondrial respiratory chain dysfunctions, and oxidative damage of DNA. In PAD, ischemic lesions generate extensive oxidative stress. Accordingly, murine models of PAD displayed reduced antioxidant enzymes mRNA levels, increased ROS production,[43] increased oxidative stress, and impaired mitochondrial respiration, notably with reduced electron transport chain complexes I, III, and IV activities in ischemic muscles.[44]

Similarly, human studies reported altered antioxidant enzyme activities, reduced electron transport chain complexes I, III, and IV activities and impaired mitochondrial respiration in patients with PAD.[45] PAD was also associated with significant oxidative stress and ROS production.[46] Interestingly, the extend of oxidative damage in PAD *gastrocnemius* was shown to be associated with advanced disease stage and lower myofibre cross-sectional area.[47]

Taken together, these data suggest the pathological implication of excessive oxidative stress in myofibres damage and PAD.

D/Mitochondriopathy

With disease and/or advancing age, mitochondrial dysfunctions accumulate, thus disrupting vital mitochondrial-dependent activities.[47] Accordingly, studies in murine models of CLI revealed mitochondrial DNA damages in ischemic aged muscles, lower mitochondrial respiration, declined mitochondrial biogenesis, impaired calcium retention capacity and muscle atrophy, and muscle contractile deficits.[43,48]

Human investigations regarding mitochondrial dysfunction in PAD showed an increase in skeletal muscle mitochondrial DNA injuries[49] and abnormal mitochondrial respiratory activity of ischemic muscles[50] compared with controls. Interestingly, Koutakis *et al*. investigated the potential role of the muscle specific intermediate filament desmin in PAD pathophysiology and linked abnormal desmin accumulation with low mitochondrial respiration.[51] Moreover, immunohistochemical analysis of muscle biopsies revealed accumulation of microtubule-associated protein light chain 3 (LC3)—an autophagic marker—in the area depleted of mitochondria in PAD myofibres, thus suggesting an association between PAD and aberrant mitophagy process.[52] Last, a recent work has stressed a unique and severe mitochondriopathy touching patients with CLI. Indeed, besides the reduced mitochondrial oxidative capacity and respiratory activity also observed in patients with PAD, CLI was shown to be characterized by deficits in permeabilized myofibre mitochondrial function and decreased abundance of mitochondria-associated mRNAs and proteins.[53]

Overall, mitochondriopathy is thought to be a major contributor to sarcopenia and PAD, notably with significant disruption of mitochondrial biogenesis, dynamics, and mitophagy.

E/Inflammation

Systemic inflammation is considered an important pathophysiological mechanism in PAD and likely contributes to skeletal muscle wasting. Accordingly, several vascular inflammatory markers such as IL6, IL1 receptor antagonist, fibrinogen, and CRP were found elevated in PAD patients compared with controls subjects.[54] Further studies revealed an association between higher levels of these markers of inflammation and poorer 6-min walk distance and overall performance (combining walking speed, balance, and chair rises exercises),[55] lower calf strength, and more adverse calf muscle characteristics.[56]

It is therefore possible that sarcopenia in PAD patients finds its origin in altered inflammatory process, likely mediated by the dysregulation of multiple cytokine factors.

F/Impaired signalling pathways

IGF-1 synthesis pathway The IGF-1/PI3K/Akt/mTOR signalling pathway is a key player in muscle growth, stimulating protein synthesis, and satellite cell proliferation in muscle, all together while simultaneously suppressing pathways responsible for protein degradation. Regarding the exact role played by this synthesis pathway in PAD, data are scarce. In 2004, Tuomisto *et al*. reported an up-regulation of the anabolic factors IGF-1 and IGF-2 in atrophic and regenerating ischemic myocytes of patients with CLI.[57] The IGF system could promote skeletal muscle survival, regeneration and angiogenesis under chronic ischemia, notably via the VEGF pathway.

RISK and SAFE protective pathways The RISK and SAFE pathways play essential roles in the reduction of ischemia/reperfusion (I/R) injuries, as they participate to muscle regeneration and mitochondrial integrity. Though this phenomenon is well documented in the field of cardiology, very little is known during PAD. In our rat model of PAD exposed to 3 h of ischemia followed by 2 h of reperfusion, the RISK and SAFE pathways were inefficiently activated, leading to mitochondrial dysfunctions, increased oxidative stress and apoptosis.[10]

Impaired muscle degradation pathways Several members of the TGFβ superfamily play a key role in protein degradation, among them, myostatin is well known for the extreme hyper muscularity of myostatin knock-out mice and conversely, for the muscle atrophy of mice overexpressing myostatin. In mice models of PAD, silencing of myostatin led to *gastrocnemius* hypertrophy and improved running performance.[58]

In humans, very little is known about the role of the TGFβ superfamily in PAD pathology. A recent research conducted by Ha *et al*. showed that TGFβ1 expression increased with advancing PAD severity.[59] Overall, myostatin is thought to be an important factor in the pathophysiology of sarcopenia and PAD.

Overall, evidence seem to indicate an imbalance between protein synthesis and degradation leading to reduced or impaired anabolic pathway and, in a broader sense, impaired muscle function and strength in PAD.

4/Therapeutic approaches: can we reverse the sarcopenic condition in patients with PAD?

Therapeutic analysis was based on seven animal studies [61–63; 83–86] and 21 clinical reports, Among the clinical reports, 19 were observational trial studies [64–68; 70–82; 87], and two were prospective studies [60; 69]. In total, 884 patients followed an exercise therapy, and 18 received an angiogenesis treatment; 30.84% (SD 17.74) were women. Mean age of patients studied was 66.85 (SD 3.96).

Treating sarcopenia in PAD

Lower limb revascularization surgery is the treatment of choice for patients suffering from CLI, enabling blood flow restoration and limb salvage, while reversing some sarcopenic features. Indeed, in a prospective study following 18 patients with CLI, surgical revascularization improved muscle strength, 6-min walk distance, bodily pain and overall quality of life.[60] However, these muscular parameters are not currently tested in routine clinical practice, and therefore, we do not know whether ischemia-related muscle impairment are normalized or whether sequela remain. Nevertheless, additional therapeutic approaches like exercise training or angiogenesis therapies seems useful to further sustain

Table 3 Effects of exercise on sarcopenia associated with PAD in experimental and clinical studies

Reference	Population	Number studied (symptomatic/controls)	Exercise therapy		Outcomes measured			Main results
			Exercise program	Duration	Muscle strength	Muscle mass/quality	Physical performance	
Nagase *et al.*, 2017, PLoS One [63]	Mice, PAD	6/4	Treadmill training	2 weeks (twice a week)	-	Quantitative analysis of mRNA levels	-	Treadmill training significantly reduced the mRNA expression of skeletal muscle regeneration markers ($P < 0.05$) compared with the non-exercised PAD group
Lejay *et al.*, 2017, Front Physiol [61]	Mice, CLI	10/10	Treadmill training	3 weeks (5 times per week)	-	Histological analysis	Functional score	Treadmill training reduced tissue damage (with a score of 1.9 for the exercised group vs. 4.0 for the non-exercised group at day 30, $P < 0.01$), enhanced muscle function (with a score of 1.4 for the exercised group vs. 2.8 for the non-exercised group at day 30, $P < 0.01$), stimulated mitochondrial biogenesis and anti-oxidant defences
Hain *et al.*, 2011, Am J Physiol Regul Integr Comp Physiol [62]	Rats, PAD	Ns	Electrical stimulation causing repeated muscle contractions and mimicking exercise	5 days	-	Fibre cross-sectional area	-	Repeated cycles of muscle contraction decreased the mean fibre cross-sectional area by 35% (1834 ± 219.9 μm^2 in the exercised group vs. 2834 ± 132.5 μm^2 in the non-exercised group, $P < 0.05$)
Schieber *et al.*, 2019, J Vasc Surg [80]	Human, PAD	47/–	Supervised walking exercise	6 months (3 times per week)	Plantar flexor strength	-	Walking distance, gait biomechanics	Supervised walking exercise improved muscle strength, walking distance and gait biomechanics
Vun *et al.*, 2016, J Vasc Surg [73]	Human, PAD	36/–	Supervised treadmill exercise program	12 weeks (twice a week)	-	Whole-body dual-energy X-ray absorptiometry	Pain-free walking distance 6-min walking distance	Supervised treadmill exercise improved pain-free walking distance (213 ± 93 m after 12 weeks vs. 165 ± 78 m at baseline, $P = 0.001$) and 6-min walk distance (421 ± 68 m after 12 weeks vs. 395 ± 78 m at baseline, $P = 0.004$)
Gardner *et al.*, 2014, J Am Heart Assoc [67]	Human, PAD	60/–	Step-monitored home walking to mild-to-moderate claudication pain	12 weeks (3 times per week)	-	-	6-min walking distance Walking speed	Home walking exercise improved 6-min walk distance (372 ± 119 m after the 12-week test vs. 328 ± 108 m at

(*Continues*)

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DOI: 10.1002/jcsm.12587

Table 3 (continued)

Reference	Population	Number studied (symptomatic/ controls)	Exercise therapy		Outcomes measured			Main results
			Exercise program	Duration	Muscle strength	Muscle mass/quality	Physical performance	
								pre-test, $P < 0.001$), peak walking time (490 ± 350 s vs. 380 ± 274 s at pre-test, $P < 0.001$), and daily ambulatory activity notably with improvement in average cadence (11.8 ± 3.0 strides/ min vs. 11.1 ± 2.7 strides/min, $P < 0.01$)
Januszek *et al.*, 2014, J Cardiol [75]	Human, PAD	67/–	Supervised treadmill training	12 weeks (3 times per week)	-	-	Maximal walking time	Treadmill training improved maximal walking time (+90%, $P < 0.001$) and flow-mediated dilatation (+43%, $P < 0.001$) in PAD patients in comparison to baseline
Pilz *et al.*, 2014, Wien Klin Wochenschr [82]	Human, PAD	42/–	Supervised exercise training on strength (couch pedal ergometer work on lower legs) and endurance (walk sessions)	6 months (twice a week)	Pushing power Pulling power Tip-toe standing power	-	Pain-free walking distance Walking-speed	Combined exercise program improved walking distance (568.9 ± 461.5 m after 6 months vs. 446.3 ± 276.6 m at baseline, $P < 0.05$), walking speed (4.39 ± 1.08 km/h vs. 4.17 ± 0.85 km/h at baseline, $P < 0.05$), pushing power (662.4 ± 530.4 J vs. 348.6 ± 270.3 J, $P < 0.01$), pulling power (96.4 ± 51.5 J vs. 58.7 ± 37.7 J, $P < 0.0001$), and tiptoe standing power (83.5 ± 48.6 repetitions vs. 49 ± 21.5 repetitions, $P < 0.0001$)
		52/–		12 months (twice a week)				Combined exercise program further improved walking distance (647.8 ± 496.3 m after 12 months vs. 500.2 ± 427.9 m at baseline, $P < 0.001$), walking speed (4.53 ± 0.80 km/h vs. 4.03 ± 0.90 km/h at baseline, $P < 0.0001$), pushing power (637.8 ± 407.1 J vs. 337.2 ± 232.9 J, $P < 0.001$), pulling power (97.5 ± 59.8 J vs. 55.6 ± 38.8 J, $P < 0.0001$), and

(*Continues*)

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Table 3 (continued)

Reference	Population	Number studied (symptomatic/controls)	Exercise therapy		Outcomes measured			Main results
			Exercise program	Duration	Muscle strength	Muscle mass/quality	Physical performance	
								tiptoe standing power (84.9 ± 69.3 repetitions vs. 39.8 ± 15.3 repetitions, $P < 0.0001$)
Parmenter *et al.*, 2013, J Am Geriatr Soc [81]	Human, PAD	7/–	High-intensity progressive resistance training (weight lifting)	6 months (3 times per week)	-	-	6-min walking distance	Progressive resistance training increased 6-min walking distance (381.8 ± 151.6 m after 24 weeks of training vs. 321.9 ± 109.1 m at baseline, $P = 0.02$)
Mosti *et al.*, 2011, Scand J Med Sci Sports [71]	Human, PAD	10/–	Leg press maximal strength training and plantar flexion endurance training	8 weeks (3 times per week)	Leg press maximal force Rate of force development	-	Plantar flexion endurance	Exercise training improved muscle strength, notably with increased rates of force development (3675 ± 1315 N/s post-test vs. 1943 ± 1027 N/s pre-test, $P < 0.01$) and leg press maximal strength (152 ± 33 kg vs. 110 ± 24 kg, $P < 0.01$); but also walking distance (1203 ± 451 m vs. 1099 ± 463 m, $P < 0.01$)
Cousin *et al.*, 2011, Ann Phys Rehabil Med [69]	Human, PAD	31/–	Walking sessions, selective muscle strengthening, general physical exercise	4 weeks (5 days per week)	Ankle plantar and dorsal flexors strength Concentric contractions at the angular velocity of 30°/s, 120°/s and 180°/s for muscle fatigue	-	Walking distance on a treadmill < 400 m	Rehabilitation program improved walking distance (977.4 ± 854.2 m upon completing the program vs. 282.4 ± 239.8 m at baseline, $P < 0.0001$)
Saetre *et al.*, Angiology, 2011 [66]	Human, PAD	29/–	Supervised exercise training	8 weeks (twice a week)	-	Quantitative analysis of plasma inflammatory levels	Pain-free walking distance, maximal walking distance	Exercise training reduced the plasma levels of E-selectin (45.5 before training to 40.4 ng/ml after training, $P = 0.013$) and ICAM-1 (342.0 to 298 ng/ml) in PAD patients. Both walking distance increased after exercise training ($P < 0.01$)
Wang *et al.*, 2010, Scand J Med Sci Sports [70]	Human, PAD	10/–	Maximal strength training (dynamic leg press)	8 weeks (3 times per week)	Leg press force Rate of force development	-	Walking economy test	Maximal strength training improved rates of force development (2901 ± 1848 N/s after the 8-week training

(*Continues*)

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Table 3 (continued)

Reference	Population	Number studied (symptomatic/controls)	Exercise therapy		Outcomes measured			Main results
			Exercise program	Duration	Muscle strength	Muscle mass/quality	Physical performance	
								program vs. 1368 ± 893 N/s in the control period, $P < 0.05$), maximal strength (148 ± 33 kg vs. 114 ± 25 kg) and walking time to exhaustion (1095 ± 426 s vs. 1009 ± 448 s, $P < 0.05$)
McDermott *et al.*, 2009, JAMA [78]	Human, PAD	156/–	Supervised treadmill walking training *vs.*resistance training	24 weeks (3 times per week)	-	-	6-min walk performance, short physical performance battery, treadmill walking performance, walking impairment questionnaire, overall physical functioning score	Supervised treadmill walking training improved 6-min walk performance (by 35.9 m, $P < 0.001$), maximal treadmill walking time (by 3.44 min, $P < 0.001$) and overall quality of life ($P = 0.02$) compared to untrained controls. Resistance training increased maximal treadmill walking time (by 1.90 min, $P = 0.009$), stair climbing ($P = 0.03$) and overall quality of life ($P = 0.04$)
Wang *et al.*, 2006, Clin J Sport Med [72]	Human, PAD	17/–	Supervised treadmill walking training	12 weeks (3 times per week)	Calf-muscle strength and endurance	-	Walking capacity	Supervised treadmill-walking program improved peak torque at 30 degrees/s (175 ± 40 N/m post-training vs. 159 ± 32 N/m at pre-training, $P < 0.01$), mean peak force (358 ± 87 N vs. 314 ± 68 N, $P < 0.001$), and mean power (80 ± 26 W vs. 66 ± 19 W, $P < 0.001$). This training program also increased pain-free walking time (382 ± 261 s vs. 137 ± 70 s, $P < 0.001$) and maximal walking time (696 ± 191 s vs. 314 ± 138 s, $P < 0.001$)
Signorelli *et al.*, 2003, Vasc Med [65]	Human, PAD	20/20	Treadmill test	1 session	-	Quantitative analysis of plasma inflammatory levels	-	One treadmill exercise session increased plasma levels of ICAM-1 (317 ± 4 at rest to 421 ± 10 ng/ml after exercise), VCAM-1 (485 ± 14 to 576 ± 16), TNF-α (14 ± 3 to

(*Continues*)

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Table 3 (continued)

Reference	Population	Number studied (symptomatic/controls)	Exercise therapy		Outcomes measured			Main results
			Exercise program	Duration	Muscle strength	Muscle mass/quality	Physical performance	
								27 ± 5) and IL6 (12 ± 1 to 16 ± 2) in PAD patients
McGuigan *et al.*, 2001, J Gerontol A Biol Sci Med Sci [68]	Human, PAD	11/–	Progressive resistance training	6 months (3 times per week)	Leg press strength Calf press strength	Biopsies from gastrocnemius muscles	-	Progressive resistance training improved the 10-repetition maximum loading leg (by 155%) and calf (by 126%) press strength in the trained subjects, at 24 weeks Training also increased type I (3442 ± 981 μm^2 after training vs. 2695 ± 867 μm^2 at pre-training, $P < 0.05$) and type II muscle fibre area (4273 ± 1113 μm^2 vs. 3 421 ± 1148 μm^2, $P < 0.05$)
Brevetti *et al.*, 2001, Clin Hemorheol Microcirc [64]	Human, PAD	21/18	Maximally tolerated treadmill exercise	1 session	-	Quantitative analysis of plasma inflammatory levels	-	One treadmill exercise session increased plasma levels of ICAM-1 (285 ± 15 at rest to 317 ± 16 ng/ml after exercise, $P < 0.01$) and VCAM-1 (671 ± 45 to 751 ± 47 ng/ml, $P < 0.05$) in PAD patients, while no modifications were observed in controls
Gardner *et al.*, 2000, J Gerontol [79]	Human, PAD	63/–	Supervised walking exercise	6 months (3 times per week)	-	-	Walking economy	Exercise training improved walking economy by 10% ($P < 0.05$) compared with the untrained group
Hiatt *et al.*, 1996, J Appl Physiol [74]	Human, PAD	26/–	Treadmill walking exercise	12 weeks (3 times per week)	-	Biopsies from gastrocnemius muscles	Peak exercise performance	Treadmill training was associated with improved exercise performance despite increased denervated fibres (7.6 ± 5.4 before exercise to 15.6 ± 7.5% after exercise, $P < 0.05$)
Regensteiner *et al.*, 1996, J Vasc Surg [77]	Human, PAD	29/–	Supervised treadmill walking training	12 weeks (3 h per week) or 24 weeks (3 h per week)	-	-	Functional status (questionnaires on walking ability, habitual physical activity level, and physical/social functioning, well-being, overall	Exercise training improved functional status and monitored activity level ($P < 0.05$) after 12 weeks and to a greater extend after 24 weeks

(*Continues*)

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Table 3 (continued)

Reference	Population	Number studied (symptomatic/ controls)	Exercise therapy		Outcomes measured			Main results
			Exercise program	Duration	Muscle strength	Muscle mass/quality	Physical performance	
							health); monitored activity levels	
Hiatt *et al.*, 1994, Circulation [76]	Human, PAD	29/–	Supervised treadmill walking training *vs.*strength training (resistive training of five muscle groups of each leg)	12 weeks (3 h per week) or 24 weeks (3 h per week)	-	-	Peak exercise performance	Patients in the 12 weeks treadmill training program had higher increase in peak walking time and higher improvement in peak oxygen consumption and onset of claudication pain compared with patients in the strength training program; with further improvements over 24 weeks of training

CLI, critical limb ischemia; Ns, not specified; PAD, peripheral artery disease.

functional muscular improvement and to preserve patients' quality of life.

A/Exercise training

Molecular and cellular effects In a mouse model of chronic CLI, moderate exercise consisting in a 3-week treadmill training up to five times per week enhanced mitochondrial biogenesis and antioxidant enzymes mRNA levels, restored mitochondrial respiration and calcium retention capacity and reduced tissue damages, while generating low amount of oxidative stress.[61] On the other hand, Hain *et al*. showed that repeated cycles of electrical stimulation (mimicking exercise) resulted in increased NF-κB activity and muscle fibre atrophy.[62] Further, 2-week treadmill exercise was shown to have consequences on skeletal muscle mRNA expression in PAD models, notably by down-regulating skeletal muscle regeneration markers.[63] These results might indicate that exercise intensity could be associated with adverse effects on skeletal muscle function and might also underscore the beneficial systemic effects of exercise.

In humans, studies show a significant increase of the inflammatory markers ICAM-1, VCAM-1, TNF-α, and IL6 directly following one treadmill exercise.[64,65] However, a reduction in the inflammatory process was shown in PAD patients following either a 8-week supervised training program[66] or a 12-week homebased exercise training.[67] Last, 6 to 12 months of progressive resistance training was shown to increase type I and type II skeletal muscle fibre areas and capillary density.[68]

Functional and vascular effects In humans, the impact of exercise on PAD was assessed in prospective studies following training sessions for 4 weeks up to 12 months.

4-week program After a 4-week rehabilitation program consisting in walking exercises, selective muscle strengthening, and sports, patients suffering from PAD showed significant improvement in walking distance.[69]

8-week program Patients following 8 weeks of maximal strength training alone,[70] or combined with plantar flexion endurance training,[71] presented increased leg strength, force development, and walking performance.

12-week program A 12-week homebased exercise training was shown to positively influence vascular function (microcirculation of the lower extremities measured by calf muscle haemoglobin oxygen saturation) and endurance (6-min walk distance, peak walking time, and daily ambulatory activity) in PAD patients.[67]

The effects of a 12-week treadmill-walking program on PAD were analysed in several independent studies. Notably Wang *et al*. demonstrated the importance of this training program in improving endurance (walking capacity) and strength (peak force, peak torques in plantar flexion) in

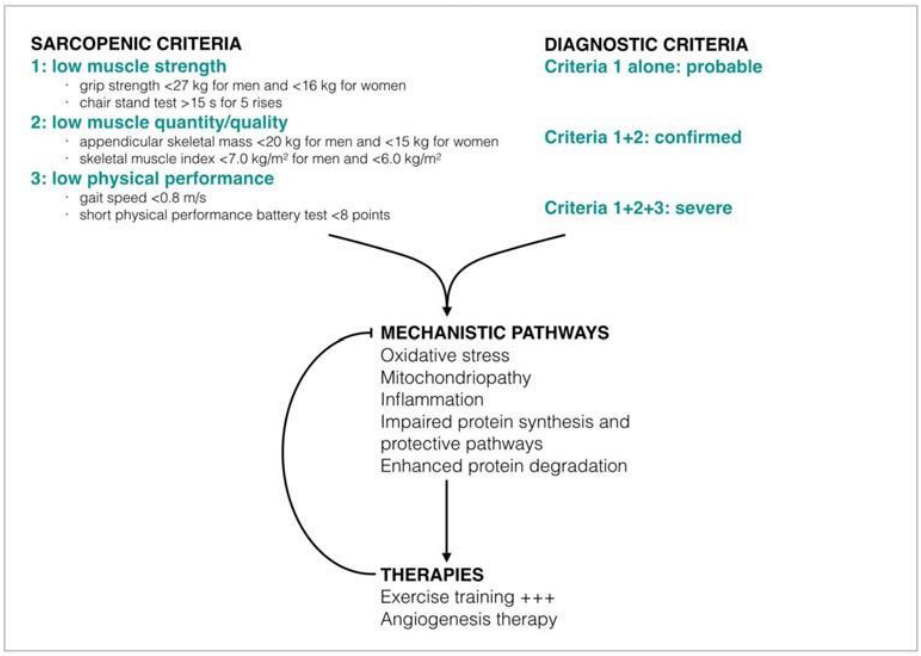

FIGURE 3 Sarcopenia and PAD: Diagnostic criteria, mechanistic pathways, and current therapies.

patients with PAD.[72] The improvements in endurance (pain-free walking distance, 6-min walking distance, and peak exercise performance) were accompanied by a large decline in bilateral thigh lean mass[73] or by a significant increase in the number of denervated calf muscle fibres.[74] Here, regions remote to the ischemic lesion are mostly affected by a decline in muscle mass and muscle quality, possibly caused by sensory and motor nerve dysfunction during exercise. Further, this training program was shown to improve both muscular function (i.e. increased pain-free walking distance) and endothelial function (i.e. increased flow-mediated dilatation) in PAD patients.[75] Interestingly, a 12-week treadmill walking exercise was found more effective than a 12-week strength training program in term of exercise performance.[76]

12-week vs. 24-week program A supervised treadmill walking exercise program was shown to improve exercise performance and overall functional status after 12 weeks, with continued improvement after 24 weeks.[76,77]

24-week program The effects of two training program were assessed in patients suffering from PAD. Improvements in 6-min walk and treadmill walking performance, brachial artery flow-mediated dilatation, and overall quality of life were observed in the group following supervised treadmill training; whereas climbing ability, treadmill performance, and quality of life were ameliorated in the group following resistance training, when compared with controls.[78]

6-month to 12-month program Two studies demonstrated the functional and vascular benefits of a 6-month supervised walking exercise in PAD. Indeed, Andrew *et al*. observed improvements in peripheral circulation, walking economy, and cardiopulmonary function[79]; while Schieber *et al*. observed improvements in walking distance, muscle strength, and gait biomechanics, as well as overall quality of life.[80] Moreover, in medium to longer term (i.e. 6 to 12 months follow-up), progressive resistance training was shown to have beneficial effects on walking ability[81] and muscle strength.[82]

In brief, exercise induces (i) molecular adaptations, notably with modifications of the transcript levels of mitochondrial biogenesis, antioxidant, oxidative phosphorylation enzymes, and reduction of the inflammatory process; (ii) cellular adaptations with enhanced mitochondrial plasticity, muscle fibre areas, and capillarization; and (iii) functional and vascular adaptations with enhanced functional status and improved microvascular circulation of the lower extremities in PAD (*Table* 3). Regular physical activity is highly recommended to prevent or reduce the onset of adverse effects occurring with PAD.

Angiogenesis PAD and CLI are characterized by vascular dysfunction, reduced microvascular flow and altered angiogenesis process. On this basis, therapeutic angiogenesis represents a promising approach in the restoration of blood flow and treatment of ischemic lesions. In mouse models of CLI, angiogenic therapy consisting in bone marrow cells injections resulted in reduced limb necrosis and muscle

impairment, enhanced *gastrocnemius* and quadriceps muscle mass, and blood flow regeneration, compared with untreated ischemic animals.[83–85] Interestingly, in a murine hindlimb ischemic model, injections of donepezil—an anti-Alzheimer drug—upregulated angiomyogenesis factors (VEGF, HIF-1α, and Akt) and reduced skeletal muscle atrophy.[86]

Data in humans did not confirm the potential benefice of angiogenesis therapy in PAD, notably showing similar exercise performance and survival rates.[87]

Concluding remarks and perspectives

With more than 200 million individuals affected worldwide, lower extremity PAD is a major issue for public healthcare. The morbidity and mortality rates are alarmingly high, especially in patients also presenting with sarcopenia. The mechanistic link between sarcopenia and PAD remains to be investigated but likely involves oxidative stress, mitochondrial dysfunction, inflammation and impaired muscle synthesis, and degradation pathways. Although difficult, the diagnosis of sarcopenia is crucial for PAD patients' care, as it determines prognosis, quality of life, and possible treatments. Indeed, targeting the muscular defects through exercise training could reverse the sarcopenic features observed in patients suffering from PAD and thus, ameliorate their quality of life and overall prognosis. Further, although therapeutic data are largely contradictory, complementary pharmacologic strategies focused on muscle mitochondrial dysfunction through oxidative stress, inflammation, and/or angiogenesis modulation should be further investigated in view of their potential usefulness as new innovative therapeutic approaches against sarcopenia (*Figure* 3).

Ethical statement

The authors certify that they comply with the ethical guidelines for authorship and publishing in the *Journal of Cachexia, Sarcopenia and Muscle*.[88]

Conflict of interest

None declared.

References

1. MendisS, Puska P, Norrving B. Global atlas on cardiovascular disease prevention and control. *Global atlas on cardiovascular disease prevention and control* Published Online First: 2011.https://www.cabdirect.org/cabdirect/abstract/20123402600 (accessed 10 Dec 2018).
2. NorgrenL, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FGR. Inter-society consensus for the management of peripheral arterial disease (TASC II). *Eur J Vasc Endovasc Surg* 2007;**33**:S1–S75.
3. AboyansV, Ricco JB, Bartelink MLE, Björck M, Brodmann M, Cohnert T, et al. Editor's Choice - 2017 ESC Guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS). *Eur J Vasc Endovasc Surg* 2018;**55**:305–368.
4. ConteMS, Bradbury AW, Kolh P, White JV, Dick F, Fitridge R, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia. *J Vasc Surg* 2019;**69**:3S–125S.e40.
5. McDermottMM, Guralnik JM, Ferrucci L, Tian L, Pearce WH, Hoff F, et al. Physical activity, walking exercise, and calf skeletal muscle characteristics in patients with peripheral arterial disease. *J Vasc Surg* 2007;**46**:87–93.
6. Cruz-JentoftAJ, Sayer AA. Sarcopenia. *Lancet* 2019;**393**:2636–2646.
7. MolnarAO, Eddeen AB, Ducharme R, Garg AX, Harel Z, McCallum MK, et al. Association of computed tomographic leg muscle characteristics with lower limb and cardiovascular events in patients with peripheral artery disease. *J Am Heart Assoc* 2018;**7**:e009943.
8. MoherD. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med* 2009;**151**:264.
9. JudeEB, Oyibo SO, Chalmers N, Boulton AJ. Peripheral arterial disease in diabetic and nondiabetic patients: a comparison of severity and outcome. *Diabetes Care* 2001;**24**:1433–1437.
10. PottecherJ, Adamopoulos C, Lejay A, Bouitbir J, Charles AL, Meyer A, et al. Diabetes worsens skeletal muscle mitochondrial function, oxidative stress, and apoptosis after lower-limb ischemia-reperfusion: implication of the RISK and SAFE pathways? *Front Physiol* 2018;**9**:579.
11. BartoloE, Thorne CS, Gatt A, Formosa C. The influence of peripheral arterial disease on lower limb surface myoelectric signals in patients living with type II diabetes mellitus. *Gait Posture* 2019;**73**:228–232.
12. PernaS, Spadaccini D, Rondanelli M. Sarcopenic obesity: time to target the phenotypes. *J Cachexia Sarcopenia Muscle* 2019;**10**:710–711.
13. Cruz-JentoftAJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, et al. Sarcopenia: European consensus on definition and diagnosis: report of the European working group on sarcopenia in older people. *Age Ageing* 2010;**39**:412–423.
14. Cruz-JentoftAJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T, et al. Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;**48**:16–31.
15. BaracosVE. Psoas as a sentinel muscle for sarcopenia: a flawed premise. *J Cachexia Sarcopenia Muscle* 2017;**8**:527–528.
16. HaleAL, Twomey K, Ewing JA, Langan EM III, Cull DL, Gray BH. Impact of sarcopenia on long-term mortality following endovascular aneurysm repair. *Vasc Med* 2016;**21**:217–222.
17. McDermottMM, Liu K, Tian L, Guralnik JM, Criqui MH, Liao Y, et al. Calf muscle characteristics, strength measures, and mortality in peripheral arterial disease: a longitudinal study. *J Am Coll Cardiol* 2012;**59**:1159–1167.
18. SugaiT, Watanabe T, Otaki Y, Goto J, Watanabe K, Toshima T, et al. Decreased psoas muscle computed tomography value predicts poor outcome in peripheral artery disease. *Circ J* 2018;**82**:3069–3075.
19. ReeveTE IV, Ur R, Craven TE, Kaan JH, Goldman MP, Edwards MS, et al. Grip strength measurement for frailty assessment in patients with vascular disease and associations with comorbidity, cardiac risk, and sarcopenia. *J Vasc Surg* 2018;**67**:1512–1520.
20. SinghN, Liu K, Tian L, Criqui MH, Guralnik JM, Ferrucci L, et al. Leg strength predicts mortality in men but not in women with peripheral arterial disease. *J Vasc Surg* 2010;**52**:624–631.

21. NyersES, Brothers TE. Perioperative psoas to lumbar vertebral index does not successfully predict amputation-free survival after lower extremity revascularization. *J Vasc Surg* 2017;**66**:1820–1825.
22. MatsubaraY, Matsumoto T, Aoyagi Y, Tanaka S, Okadome J, Morisaki K, et al. Sarcopenia is a prognostic factor for overall survival in patients with critical limb ischemia. *J Vasc Surg* 2015;**61**:945–950.
23. MatsubaraY, Matsumoto T, Inoue K, Matsuda D, Yoshiga R, Yoshiya K, et al. Sarcopenia is a risk factor for cardiovascular events experienced by patients with critical limb ischemia. *J Vasc Surg* 2017;**65**:1390–1397.
24. MorisakiK, Furuyama T, Matsubara Y, Inoue K, Kurose S, Yoshino S, et al. External validation of CLI Frailty Index and assessment of predictive value of modified CLI Frailty Index for patients with critical limb ischemia undergoing infrainguinal revascularization. *Vascular* 2019;1708538119836005, https://doi.org/10.1177/1708538119836005
25. TaniguchiR, Deguchi J, Hashimoto T, Sato O. Sarcopenia as a possible negative predictor of limb salvage in patients with chronic limb-threatening ischemia. *Ann Vasc Dis* 2019;**12**:194–199.
26. ShimazoeH, Mii S, Koyanagi Y, Ishida M. Impact of low activity of daily living on the prognosis of patients with critical limb ischemia and sarcopenia. *Ann Vasc Surg* 2019;**61**:156–164.
27. MyersSA, Applequist BC, Huisinga JM, Pipinos II, Johanning JM. Gait kinematics and kinetics are affected more by peripheral arterial disease than by age. *J Rehabil Res Dev* 2016;**53**:229–238.
28. McDermottMM, Tian L, Ferrucci L, Liu K, Guralnik JM, Liao Y, et al. Associations between lower extremity ischemia, upper and lower extremity strength, and functional impairment with peripheral arterial disease. *J Am Geriatr Soc* 2008;**56**:724–729.
29. SchieberMN, Hasenkamp RM, Pipinos II, Johanning JM, Stergiou N, DeSpiegelaere HK, et al. Muscle strength and control characteristics are altered by peripheral artery disease. *J Vasc Surg* 2017;**66**:178–186.e12.
30. CâmaraLC, Ritti-Dias RM, Menêses AL, Greve JMDA, Jacob Filho W, Santarém JM, et al. Isokinetic strength and endurance in proximal and distal muscles in patients with peripheral artery disease. *Ann Vasc Surg* 2012;**26**:1114–1119.
31. WurdemanSR, Koutakis P, Myers SA, Johanning JM, Pipinos II, Stergiou N. Patients with peripheral arterial disease exhibit reduced joint powers compared to velocity-matched controls. *Gait Posture* 2012;**36**:506–509.
32. KoutakisP, Johanning JM, Haynatzki GR, Myers SA, Stergiou N, Longo GM, et al. Abnormal joint powers before and after the onset of claudication symptoms. *J Vasc Surg* 2010;**52**:340–347.
33. DziubekW, Bulińska K, Stefańska M, Woźniewski M, Kropielnicka K, Jasiński T, et al. Peripheral arterial disease decreases muscle torque and functional walking capacity in elderly. *Maturitas* 2015;**81**:480–486.
34. ParmenterBJ, Raymond J, Dinnen PJ, Lusby RJ, Singh MAF. Preliminary evidence that low ankle-brachial index is associated with reduced bilateral hip extensor strength and functional mobility in peripheral arterial disease. *J Vasc Surg* 2013;**57**:963–973.e1.
35. KakihanaT, Ito O, Sekiguchi Y, Ito D, Goto H, Akamatsu D, et al. Hip flexor muscle dysfunction during walking at self-selected and fast speed in patients with aortoiliac peripheral arterial disease. *J Vasc Surg* 2017;**66**:523–532.
36. KoutakisP, Pipinos II, Myers SA, Stergiou N, Lynch TG, Johanning JM. Joint torques and powers are reduced during ambulation for both limbs in patients with unilateral claudication. *J Vasc Surg* 2010;**51**:80–88.
37. HermanSD, Liu K, Tian L, Guralnik JM, Ferrucci L, Criqui MH, et al. Baseline lower extremity strength and subsequent decline in functional performance at 6-year follow-up in persons with lower extremity peripheral arterial disease. *J Am Geriatr Soc* 2009;**57**:2246–2252.
38. KuoH-K, Yu Y-H. The relation of peripheral arterial disease to leg force, gait speed, and functional dependence among older adults. *J Gerontol A Biol Sci Med Sci* 2008;**63**:384–390.
39. RegensteinerJG, Wolfel EE, Brass E, Carry MR, Ringel SP, Hargarten ME, et al. Chronic changes in skeletal muscle histology and function in peripheral arterial disease. *Circulation* 1993;**87**:413–421.
40. KoutakisP, Myers SA, Cluff K, Ha DM, Haynatzki G, McComb RD, et al. Abnormal myofiber morphology and limb dysfunction in claudication. *J Surg Res* 2015;**196**:172–179.
41. KingS, Vanicek N, O'Brien TD. Dynamic muscle quality of the plantar flexors is impaired in claudicant patients with peripheral arterial disease and associated with poorer walking endurance. *J Vasc Surg* 2015;**62**:689–697.
42. KingSL, Vanicek N, O'Brien TD. Gastrocnemius muscle architecture and achilles tendon properties influence walking distance in claudicants with peripheral arterial disease. *Muscle Nerve* 2016;**53**:733–741.
43. LejayA, Choquet P, Thaveau F, Singh F, Schlagowski A, Charles AL, et al. A new murine model of sustainable and durable chronic critical limb ischemia fairly mimicking human pathology. *Eur J Vasc Endovasc Surg* 2015;**49**:205–212.
44. PipinosII, Swanson SA, Zhu Z, Nella AA, Weiss DJ, Gutti TL, et al. Chronically ischemic mouse skeletal muscle exhibits myopathy in association with mitochondrial dysfunction and oxidative damage. *Am J Physiol Regul Integr Comp Physiol* 2008;**295**:R290–R296.
45. PipinosII, Judge AR, Zhu Z, Selsby JT, Swanson SA, Johanning JM, et al. Mitochondrial defects and oxidative damage in patients with peripheral arterial disease. *Free Radic Biol Med* 2006;**41**:262–269.
46. HartCR, Layec G, Trinity JD, Kwon OS, Zhao J, Reese VR, et al. Increased skeletal muscle mitochondrial free radical production in peripheral arterial disease despite preserved mitochondrial respiratory capacity. *Exp Physiol* 2018;**103**:838–850.
47. WeissDJ, Casale GP, Koutakis P, Nella AA, Swanson SA, Zhu Z, et al. Oxidative damage and myofiber degeneration in the gastrocnemius of patients with peripheral arterial disease. *J Transl Med* 2013;**11**:230–210.
48. SchmidtCA, Ryan TE, Lin CT, Inigo MM, Green TD, Brault JJ, et al. Diminished force production and mitochondrial respiratory deficits are strain-dependent myopathies of subacute limb ischemia. *J Vasc Surg* 2017;**65**:1504–1514.e11.
49. BhatHK, Hiatt WR, Hoppel CL, Brass EP. Skeletal muscle mitochondrial DNA injury in patients with unilateral peripheral arterial disease. *Circulation* 1999;**99**:807–812.
50. PipinosII, Sharov VG, Shepard AD, Anagnostopoulos PV, Katsamouris A, Todor A, et al. Abnormal mitochondrial respiration in skeletal muscle in patients with peripheral arterial disease. *J Vasc Surg* 2003;**38**:827–832.
51. KoutakisP, Miserlis D, Myers SA, Kim JKS, Zhu Z, Papoutsi E, 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.
52. WhiteSH, McDermott MM, Sufit RL, Kosmac K, Bugg AW, Gonzalez-Freire M, 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**:284–215.
53. RyanTE, Yamaguchi DJ, Schmidt CA, Zeczycki TN, Shaikh SR, Brophy P, et al. Extensive skeletal muscle cell mitochondriopathy distinguishes critical limb ischemia patients from claudicants. *JCI Insight* 2018;**3**:https://www.ncbi.nlm.nih.gov/pubmed/30385731
54. McDermottMM, Guralnik JM, Corsi A, Albay M, Macchi C, Bandinelli S, et al. Patterns of inflammation associated with peripheral arterial disease: The InCHIANTI study. *Am Heart J* 2005;**150**:276–281.
55. McDermottMM, Greenland P, Green D, Guralnik JM, Criqui MH, Liu K, et al. D-dimer, inflammatory markers, and lower extremity functioning in patients with and without peripheral arterial disease. *Circulation* 2003;**107**:3191–3198.
56. McDermottMM, Ferrucci L, Guralnik JM, Tian L, Green D, Liu K, et al. 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.
57. TuomistoTT, Rissanen TT, Vajanto I, Korkeela A, Rutanen J, Ylä-Herttuala S. HIF-VEGF-VEGFR-2, TNF-alpha and IGF

pathways are upregulated in critical human skeletal muscle ischemia as studied with DNA array. *Atherosclerosis* 2004;**174**:111–120.
58. SugoT, Terada M, Oikawa T, Miyata K, Nishimura S, Kenjo E, et al. Development of antibody-siRNA conjugate targeted to cardiac and skeletal muscles. *J Control Release* 2016;**237**:1–13.
59. HaDM, Carpenter LC, Koutakis P, Swanson SA, Zhu Z, Hanna M, et al. Transforming growth factor-beta 1 produced by vascular smooth muscle cells predicts fibrosis in the gastrocnemius of patients with peripheral artery disease. *J Transl Med* 2016;**14**:39.
60. LandryGJ, Esmonde NO, Lewis JR, Azarbal AF, Liem TK, Mitchell EL, et al. Objective measurement of lower extremity function and quality of life after surgical revascularization for critical lower extremity ischemia. *J Vasc Surg* 2014;**60**:136–142.
61. LejayA, Laverny G, Paradis S, Schlagowski AI, Charles AL, Singh F, et al. Moderate Exercise Allows for shorter Recovery Time in Critical Limb Ischemia. *Front Physiol* 2017;**8**:523.
62. HainBA, Dodd SL, Judge AR. IκBα degradation is necessary for skeletal muscle atrophy associated with contractile claudication. *Am J Physiol Regul Integr Comp Physiol* 2011;**300**:R595–R604.
63. NagaseH, Yao S, Ikeda S. Acute and chronic effects of exercise on mRNA expression in the skeletal muscle of two mouse models of peripheral artery disease. *PLoS ONE* 2017;**12**:e0182456, https://doi.org/10.1371/journal.pone.0182456
64. BrevettiG, De Caterina M, Martone VD, Ungaro B, Corrado F, Silvestro A, et al. Exercise increases soluble adhesion molecules ICAM-1 and VCAM-1 in patients with intermittent claudication. *Clin Hemorheol Microcirc* 2001;**24**:193–199.
65. SignorelliSS, Mazzarino MC, Pino LD, Malaponte G, Porto C, Pennisi G, et al. High circulating levels of cytokines (IL-6 and TNFalpha), adhesion molecules (VCAM-1 and ICAM-1) and selectins in patients with peripheral arterial disease at rest and after a treadmill test. *Vasc Med* 2003;**8**:15–19.
66. SaetreT, Enoksen E, Lyberg T, Stranden E, Jørgensen JJ, Sundhagen JO, et al. Supervised exercise training reduces plasma levels of the endothelial inflammatory markers E-selectin and ICAM-I in patients with peripheral arterial disease. *Angiology* 2011;**62**:301–305.
67. GardnerAW, Parker DE, Montgomery PS, Blevins SM. Step-monitored home exercise improves ambulation, vascular function, and inflammation in symptomatic patients with peripheral artery disease: a randomized controlled trial. *J Am Heart Assoc* 2014;**3**:e001107, https://doi.org/10.1161/JAHA.114.001107
68. McGuiganMR, Bronks R, Newton RU, Sharman MJ, Graham JC, Cody DV, et al. 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**:B302–B310.
69. CousinA, Popielarz S, Wieczorek V, Tiffreau V, Mounier-Vehier C, Thevenon A. Impact of a rehabilitation program on muscular strength and endurance in peripheral arterial occlusive disease patients. *Ann Phys Rehabil Med* 2011;**54**:429–442.
70. WangE, Helgerud J, Loe H, Indseth K, Kaehler N, Hoff J. Maximal strength training improves walking performance in peripheral arterial disease patients. *Scand J Med Sci Sports* 2010;**20**:764–770.
71. MostiMP, Wang E, Wiggen ØN, Helgerud J, Hoff J. Concurrent strength and endurance training improves physical capacity in patients with peripheral arterial disease. *Scand J Med Sci Sports* 2011;**21**:e308–e314.
72. WangJ, Zhou S, Bronks R, Graham J, Myers S. Effects of supervised treadmill-walking training on strength and endurance of the calf muscles of individuals with peripheral arterial disease. *Clin J Sport Med* 2006;**16**:397–400.
73. VunSV, Miller MD, Delaney CL, Allan RB, Spark JI. The effect of supervised exercise therapy for intermittent claudication on lower limb lean mass. *J Vasc Surg* 2016;**64**:1763–1769.
74. HiattWR, 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.
75. JanuszekR, Mika P, Konik A, Petriczek T, Nowobilski R, Niżankowski R. Effect of treadmill training on endothelial function and walking abilities in patients with peripheral arterial disease. *J Cardiol* 2014;**64**:145–151.
76. HiattWR, Wolfel EE, Meier RH, Regensteiner JG. Superiority of treadmill walking exercise versus strength training for patients with peripheral arterial disease. Implications for the mechanism of the training response. *Circulation* 1994;**90**:1866–1874.
77. RegensteinerJG, Steiner JF, Hiatt WR. Exercise training improves functional status in patients with peripheral arterial disease. *J Vasc Surg* 1996;**23**:104–115.
78. McDermottMM, Ades P, Guralnik JM, Dyer A, Ferrucci L, Liu K, 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.
79. GardnerAW, Katzel LI, Sorkin JD, Killewich LA, Ryan A, Flinn WR, et al. Improved functional outcomes following exercise rehabilitation in patients with intermittent claudication. *J Gerontol A Biol Sci Med Sci* 2000;**55**:M570–M577.
80. Schieber, M.N., Pipinos, I.I., Johanning, J. M., Casale, G.P., Williams, M.A., DeSpiegelaere, H.K., Senderling, B. and Myers, S.A.Supervised walking exercise therapy improves gait biomechanics in patients with peripheral artery disease. *J Vasc Surg* Published Online First: 20 August 2019. doi:, **71**, 575, 583
81. ParmenterBJ, Raymond J, Dinnen P, Lusby RJ, Fiatarone Singh MA. High-intensity progressive resistance training improves flat-ground walking in older adults with symptomatic peripheral arterial disease. *J Am Geriatr Soc* 2013;**61**:1964–1970.
82. PilzM, Kandioler-Honetz E, Wenkstetten-Holub A, Doerrscheidt W, Mueller R, Kurz RW. Evaluation of 6- and 12-month supervised exercise training on strength and endurance parameters in patients with peripheral arterial disease. *Wien Klin Wochenschr* 2014;**126**:383–389.
83. ReisPEO, de Carvalho LP, Yasumura E, Silva FHD, Garcia BC, Beutel A, et al. Impact of angiogenic therapy in the treatment of critical lower limb ischemia in an animal model. *Vasc Endovascular Surg* 2014;**48**:207–216.
84. LiuQ, Chen Z, Terry T, McNatt JM, Willerson JT, Zoldhelyi P. Intra-arterial transplantation of adult bone marrow cells restores blood flow and regenerates skeletal muscle in ischemic limbs. *Vasc Endovascular Surg* 2009;**43**:433–443.
85. Da CunhaFF, Martins L, Martin PKM, Stilhano RS, Gamero EJP, Han SW. Comparison of treatments of peripheral arterial disease with mesenchymal stromal cells and mesenchymal stromal cells modified with granulocyte and macrophage colony-stimulating factor. *Cytotherapy* 2013;**15**:820–829.
86. NoguchiT, Kakinuma Y, Arikawa M, Okazaki K, Hoshino E, Iiyama T, et al. Donepezil can improve ischemic muscle atrophy by activating angiomyogenic properties of satellite cells. *Circ J* 2014;**78**:2317–2324.
87. RajagopalanS, Trachtenberg J, Mohler E, Olin J, McBride S, Pak R, et al. Phase I study of direct administration of a replication deficient adenovirus vector containing the vascular endothelial growth factor cDNA (CI-1023) to patients with claudication. *Am J Cardiol* 2002;**90**:512–516.
88. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the Journal of Cachexia, Sarcopenia and Muscle: update 2019. *J Cachexia Sarcopenia Muscle* 2019;**10**:1143–1145.

Résultat III Effets d'un protocole d'exercice court et de faible intensité sur la sarcopénie dans un modèle murin d'ischémie critique chronique

Objectifs : L'objectif de cette étude est de déterminer les mécanismes causatifs de la sarcopénie associée à l'ICC dans un modèle murin, et d'étudier les effets de l'exercice de faible intensité sur la sarcopénie.

Méthodes : Trois groupes de souris ont été étudiés : SHAM (n=10), ICC (ligatures séquentielles de l'artère fémorale superficielle droite et de l'artère iliaque commune droite, n=14), et ICC+exercice (ligatures séquentielles + une semaine d'exercice, n=10). La sarcopénie a été évaluée par le test de suspension pour la force musculaire et le poids du quadriceps pour la masse musculaire. La structure histologique des muscles comprenait la taille des fibres (coloration à l'hématoxyline éosine), la taille des fibres par type de fibre (immunofluorescence) et la fibrose (coloration au trichrome de Masson). L'analyse mécanistique a été étudiée par western blot, avec l'analyse des voies de renouvellement des protéines et d'inflammation.

Résultats : Les souris ICC ont développé une sarcopénie : diminution de 35,7% de la force musculaire et de 46% de la masse musculaire du quadriceps par rapport aux souris SHAM ($p<0,01$). La sarcopénie était caractérisée par une atrophie des fibres : diminution de 30,7% de la taille des fibres et de la fibrose : augmentation de 7,6% de la fraction volumique de collagène ($p<0,01$ et $p<0,05$ respectivement, par rapport aux souris SHAM). L'analyse par western blot a montré une augmentation de la dégradation protéique : augmentation de 2 fois du niveau d'expression de MuRF1, et de l'inflammation : augmentation de 7 fois du niveau d'expression de TNFα dans les muscles sarcopéniques ($p<0,05$ par rapport aux souris SHAM).
L'exercice a restauré la force musculaire par rapport aux souris ICC, mais pas la masse musculaire. L'exercice a augmenté la taille globale des fibres musculaire, et en particulier la taille des fibres IIB et IIBX, et a diminué la fraction volumique de collagène.

Conclusions : La physiopathologie de la sarcopénie dans l'ICC implique une fibrose étendue, une dégradation accrue des protéines et une inflammation. L'exercice de faible intensité a inversé l'atrophie des fibres de type II, les altérations de la force musculaire et de la motricité chez la souris, réduisant ainsi la fibrose musculaire. Cela suggère que l'exercice de faible intensité pourrait permettre d'autres améliorations fonctionnelles dans le cadre de l'ICC et pourrait diminuer les effets délétères de la sarcopénie associée.

Effect of short-term low intensity exercise training on sarcopenia in a mouse model of critical limb threatening ischemia

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Wordcount: 4031 words

Category of the manuscript: Original article

Running head: exercise, sarcopenia and limb threatening ischemia

What this paper adds

Critical limb threatening ischemia associated to sarcopenia has bad prognosis. Revascularization, when feasible, is the gold standard. However, adjunctive therapies need to be developed in order to improve the outcome in these high-risk patients. This experimental study helps to better understand the physiopathology of sarcopenia in the setting of CLTI, highlighting the role of fibrosis, protein degradation and inflammation. Moreover, short-term and low-intensity exercise reverses muscle strength alterations in CLTI mice, by reducing muscle fibrosis. These mechanistic data further support the usefulness of adapted exercise or low-intensity muscle stimulation in CLTI patients.

Abstract

Objectives: The purpose of this study is to determine the causative mechanisms of sarcopenia associated to critical limb threatening ischemia (CLTI) in a murine model, and to investigate the effects of low-intensity exercise on sarcopenia.

Methods: Three groups of mice were studied: SHAM (n=10), CLTI (sequential ligatures of the right superficial femoral artery, and the right common iliac artery, n=14), and CLTI+exercise (sequential ligatures + 1 week of exercise, n=10). Sarcopenia was assessed using the hanging test for muscle strength, and quadriceps weight for muscle mass. Histological structure of the muscles included fiber size (hematoxylin eosin staining), fiber size per fiber type (immunofluorescence), and fibrosis (Masson trichrome staining). Mechanistical analysis was studied by western blots focusing on protein turnover and inflammatory pathways.

Results: CLTI mice developed sarcopenia: decrease of 35.7% for muscle strength and 46% for quadriceps muscle mass as compared to SHAM mice ($p<.01$). Sarcopenia was characterized by fiber atrophy: decrease of 30.7% for fiber size and fibrosis: increase of 7.6% collagen volume fraction ($p<.01$ and $p<.05$ respectively, as compared to SHAM mice). Western blot analysis showed higher protein degradation with a 2-fold increase in MuRF1 expression level, and higher inflammation with a 7-fold increase in TNFα expression level in sarcopenic muscles ($p<.05$ as compared to SHAM mice).

Exercise restored muscle strength as compared to CLTI mice, but not muscle mass. Exercise increased overall muscle fiber size, and particularly type IIB and IIBX fiber size, and decreased collagen volume fraction.

Conclusions: Physiopathology of sarcopenia in CLTI involves extended fibrosis, increased protein degradation and inflammation. Low-intensity exercise reversed type II fiber atrophy, muscle strength and motricity alterations in mice, reducing muscle fibrosis. This suggests that low-intensity exercise might allow further functional improvements in the setting of CLTI, and might decrease the deleterious effects of associated sarcopenia.

Keywords: Sarcopenia, muscle, ischemia, peripheral arterial disease, vascular disease

Introduction

Sarcopenia is defined by: 1) low muscle strength, 2) low muscle quantity or quality, and 3) low physical performance.[1,2] About 35% of critical limb threatening ischemia (CLTI) patients present sarcopenia, and sarcopenia associated to CLTI has been shown to increase the risk of limb loss and major cardiovascular events, suggesting that muscle wasting might progress as arterial function declines.[3–7]

The pathophysiology of sarcopenia in the setting of CLTI is still however not well understood. It might involve fibrosis, inflammation, and an imbalance between muscle protein synthesis and degradation.[2,8]

Revascularization is the treatment of choice in CLTI patients. However, adjunctive therapies or protocols should be developed in order to improve the outcome of these patients, especially when sarcopenia is associated to CLTI. As exercise has been shown to reduce muscle fibrosis, it might have protective effects in CLTI patients presenting with sarcopenia.[9,10]

The purpose of this study was to determine the mechanisms of sarcopenia associated to CLTI in a murine model, and to investigate whether the likely protective effects of exercise could modulate pathways involved in CLTI-associated sarcopenia.

Materials and methods

1) Animals

The study was approved by the Ethics Committee for Animal Research of the university (#2018100217187326) and the criteria for the care and use of laboratory animals in research were followed. 34 Swiss mice (male, 8 weeks old, weighting 35-40 grams, Charles River) were randomized into three groups: SHAM (n=10); CLTI (CLTI obtained by sequential ligation of the right superficial femoral artery and collaterals, and of the right common iliac artery[11], n=14) and CLTI+exercise (CLTI + 1 week of exercise, n=10). Study protocol is described in **Figure 1**.

2) Critical limb threatening ischemia model

For CLTI and CLTI+exercise groups, surgery was performed under general anesthesia with isoflurane. Right limb ischemia was induced in both groups by a two steps procedure. The first step consisted of ligation of the right femoral artery (performed midway between the superficial epigastric artery and bifurcation of the popliteal and saphenous arteries) and of its collaterals.

The second step was performed 4 days later and consisted of the ligation of the right common iliac artery 0.5 cm distal to its origin.[11] The SHAM group underwent surgical exposure of the right femoral artery and collaterals, and exposure of the right common iliac artery 4 days later but no ligature was performed.

3) Exercise protocol

The CLTI+exercise group was trained one time per day over a 1-week period, starting at day 7, when CLTI was known to be effective.[11] Exercise was performed on a motorized treadmill (Rodent Treadmill NG 47300, Ugo Basile). Each session started with a warm-up phase of 2 min (10° incline, at 9 m/min), followed by 5 min of acceleration (10°, to go from 9 m/min to 15 m/min), and either 25 minutes of running at constant speed (10°, at 15 m/min) for day 7 and 8; 40 minutes for day 9 and 10; or 55 minutes for day 11, 12 and 13.

4) Hanging test

At day 15, muscle strength was evaluated in all animals using the hanging test. Mice were suspended on a cage lid 40 cm above a surface with bedding. The time of fall was recorded over a maximum period of 60 s. The test was performed three times for each mouse, with 10 min of rest between each test, and average performance was calculated for each animal.

5) Footprint assay

At day 16, gait was evaluated in all animals using the footprint assay. Hind and fore paws were painted with black and red non-toxic waterproof ink respectively. Animals were allowed to walk on a white paper along a corridor (60 cm x 5 cm x 10 cm) toward a dark goal box. The footprint patterns made on the paper lining were scored for hindlimb stride length (average distance between three consecutive steps), base width (average lateral distance between three consecutive steps, the base width was determined by measuring the perpendicular distance of a given step to a line connecting its opposite preceding and succeeding steps), and overlap (average distance between hind and fore paws across three consecutive steps) **(Figure S1)**.

6) Tissue collection

At day 17, mice were anesthetized and blood was collected by cardiac puncture. Then quadriceps muscles from right limbs of animals were dissected and weighted. Samples for histology were embedded in optimal cutting temperature (OCT) compound, immersed in liquid

nitrogen and stored at -80°C. Samples for western blotting were frozen in liquid nitrogen and stored at -80°C.

7) Histology

For histological analysis, a cryostat microtome was used to obtain 10 µm thick sections of quadriceps which were mounted onto glass slides.

Hematoxylin and eosin To assess fiber size – also called MinFeret – sections were stained with hematoxylin and eosin (H&E) using routine protocols (n=5 random animals per group). Sections were examined under Apotome (Zeiss). All fibers per individual muscle section were manually analyzed using ImageJ software.

Fiber type immunofluorescence To assess fiber distribution and MinFeret per fiber type, immunostaining protocol was performed (n=5 random animals per group). The quadriceps sections were blocked with 3% bovine serum albumin (BSA) + phosphate-buffered saline (PBS). Sections were incubated with primary antibodies of mouse anti-type I (1:50, DSHB #BA-D5), mouse anti-type IIa (1:50, DSHB #SC-71) and mouse anti-type IIb (1:50, DSHB #BF-F3) overnight at 4°C. After 3x5' wash in PBS under agitation, sections were incubated with secondary antibodies as follows: goat anti-mouse IgG2b Cy3 (1:100, JIR for detecting #BA-D5), goat anti-mouse IgG1 Alexa 488 (1:100, JIR for detecting #SC-71), goat anti-mouse IgM DyLight 405 (1:100, JIR for detecting #BF-F3), and Wheat Germ Aglutinin Alexa 647 (1:200, ThermoFisher). Sections were washed 3x5' in PBS under agitation, and mounted with FluorSave Reagent (Merck). Sections were examined under Nanozoomer 2HT slide scanner (Hamamatsu). All fibers per individual muscle section were manually analyzed using ImageJ software.

Masson trichrome To assess the extent of fibrosis, sections were stained with Masson trichrome using routine protocols (n=5 random animals per group). Sections were examined under Apotome (Zeiss). The collagen volume fraction corresponding to the ratio of blue dye area to red dye area was analyzed using a macro developed in Fiji.

8) Western blot

Western blot analysis investigated protein degradation (MAFbx and MuRF1), protein synthesis (mTOR, Akt and their downstream effectors rpS6 and 4EBP1), and inflammation (TNFα). Quadriceps samples were homogenized in 10 volumes of lysis buffer [50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 100 mM NaF, 5 mM Na_3VO_4, 40 mM β-glycerophosphate, protease inhibitor mixture, 1% Triton and 1% sodium dodecyl sulfate

(SDS)], and centrifuged at 10 000 g for 10 minutes at 4°C. Twenty micrograms of protein extracts were loaded into 5-15% SDS-polyacrylamide gels, and transferred onto nitrocellulose or PVDF membranes (iBlot 2 Dry Blotting System, Invitrogen). The membranes were blocked for 1 hour at room temperature with 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, and 0.1% Tween 20 (TBS-T) containing 5% skimmed milk. The membranes were incubated overnight at 4°C with primary antibodies for protein degradation: anti-MAFbx (Santa Cruz, 1:200 sc-166806), anti-MuRF1 (Santa Cruz, 1:200 sc-398608); protein synthesis: anti-4E-BP1 (1:1000, Cell Signaling #2855S and #9644S), anti-Akt (1:1000, Cell Signaling #4060S and #9272S), anti-mTOR (Cell Signaling, 1:1000 #2971S and #2983S), anti-S6 Ribosomal Protein (Cell Signaling, 1:1000 #5364S and #2217S); and for inflammation: anti-TNFα (Santa Cruz, 1:200 sc-52746). The membranes were washed three times with TBS-T, and incubated with anti-rabbit (Cell Signaling, 1:4000 #7074S) or anti-mouse (Cell Signaling, 1:4000 #7076S) secondary antibodies at room temperature for 1 hour. The blots were revealed using a Pierce ECL kit (Thermo Scientific), and proteins were visualized by enhanced chemiluminescence (iBright 1500 Imaging System, Invitrogen). Ponceau S was used as the loading control.

9) Data analysis

Statistical analysis was performed with GraphPad Prism 8 (GraphPad Software, Inc). Normality was assessed using Shapiro Wilk test. For normally distributed data, statistical differences were determined using a one-way ANOVA test, with or without Welch's correction, followed by Tukey or Dunnett's post test. For other data, non-parametric Krustal-Wallis test followed by Dunn's post test were performed. Data are expressed as means and standard errors or numbers and percentage. $p<.05$ was considered as indicative of statistical significance.

Results

1) Mechanisms of sarcopenia include muscle fiber atrophy, fibrosis, protein degradation and inflammation

Functional parameters analysis Muscle strength was significantly lower in CLTI mice compared with SHAM mice (-35.7%, p<.01) **(Figure 2A) (Figure S2)**. Quadriceps weight was significantly lower in ischemic limbs of CLTI mice compared with limbs of SHAM mice (-46.0%, p<.01) **(Figure 2B)**. Footprint analysis revealed similar stride length, base width and

overlap between SHAM and CLTI mice. CLTI mice displayed gait abnormalities: 36% of them were unable to put their ischemic paws down, thus walking on three limbs **(Figure 2C-2E)**.

Muscle fiber size analysis H&E staining revealed that CLTI muscles presented with smaller muscle fibers compared with SHAM muscles (-30.7% size, p<.01) **(Figure 3A-B)**. In CLTI muscles, fiber size distribution was shifted toward smaller fibers with a peak in MinFeret at 25 to 30 µm. In SHAM muscles, peak MinFeret was at 50 to 55 µm **(Figure 3C)**. Immunofluorescence staining revealed no statistical difference in fiber type distribution between SHAM and CLTI muscles **(Figure 3D)**. CLTI muscles presented with smaller type IIA, IIAX, IIB, IIBX and IIX fibers compared with SHAM muscles **(Figure 3E-I)**. There was no statistical difference in MinFeret of type I and type I/IIA fibers between SHAM and CLTI mice (data not shown).

Mechanistical analysis Masson Trichrome staining revealed that CLTI muscles presented with increased collagen volume fraction compared with SHAM muscles (+7.6%, p<.05) **(Figure 4A-B)**. Western blot analysis showed that MuRF1 expression was increased by 2-fold in CLTI muscles compared to SHAM muscles (p<.05) **(Figure 4C-D)**. The activities of mTOR, Akt and their downstream effectors rpS6 and 4EBP1 (estimated with the ratio of phosphorylated and total protein expression) were unchanged between SHAM and CLTI mice **(Figure 4E-F)**. TNFα expression was increased by 7-fold in CLTI muscles compared to SHAM muscles (p<.05) **(Figure 4G-H) (Figure S3)**.

2) <u>Low-intensity exercise reversed muscle fiber atrophy and fibrosis, resulting in muscle strength and motricity gain</u>

Functional parameters analysis Exercise enabled mice to gain muscle strength (+40.5% compared with CLTI mice, p<.05); to a level that is similar to SHAM mice **(Figure 2A) (Figure S2)**. Exercise only slightly increased quadriceps weight, but this failed to reach statistical significance **(Figure 2B)**. Exercise increased stride length compared to both SHAM (+15.3%, p<.05) and CLTI mice (+22.6%, p<.01), decreased base width compared to SHAM (-21.5%, p<.01), and decreased overlap compared to CLTI (-42.0%, p<.05). Exercise reversed these gait abnormalities since all CLTI+exercise mice walked on four limbs **(Figure 2C-E)**.

Muscle fiber size analysis H&E staining revealed that exercise increased fibers size (+26.4% size compared with CLTI, p<.05) to a level that is similar to SHAM mice **(Figure 3A-B)**. Fiber size distribution increased with exercise with a peak MinFeret at 40-45 µm. In CLTI muscles, peak MinFeret was at 25-30 µm **(Figure 3C)**. Immunofluorescence staining revealed no statistical difference in fiber type distribution between CLTI+exercise and CLTI mice **(Figure**

3D). Exercise increased type IIB and IIBX (+36.7% and +35.9% size respectively, compared with CLTI, p<.05); to a size that is similar to SHAM mice fibers **(Figure 3G-H)**. There was no statistical difference in MinFeret of type I and type I/IIA fibers between CLTI+exercise and CLTI mice (data not shown).

Mechanistical analysis Masson staining revealed that exercise decreased collagen volume fraction (-6.5% compared with CLTI, p=.06), to a percentage that is similar to SHAM mice **(Figure 4A-B)**. Western blot analysis showed that protein degradation levels were unchanged between CLTI+exercise and CLTI mice **(Figure 4C-D)**. The activities of mTOR, Akt and their downstream effectors rpS6 and 4EBP1 (estimated with the ratio of phosphorylated and total protein expression) were unchanged between CLTI+exercise and CLTI mice **(Figure 3E-F) (Figure S3)**. Exercise only slightly reduced inflammation level, but this failed to reach statistical significance **(Figure 4G-H) (Figure S3) (Figure 5)**.

Discussion

The key findings of this study are that sarcopenia in CLTI mice is associated to fiber muscle atrophy, extended fibrosis, inflammation and impaired walking capacity. Low-intensity and short-term exercise could reduce fiber atrophy and fibrosis and restore muscle strength together with walking ability.

The first objective of this study was to better understand the physiopathology of sarcopenia in CLTI. The histological analysis we performed highlighted the role of extensive muscle fiber atrophy, particularly of type II fibers. This result is in accordance with a previous study investigating the effects of peripheral arterial disease (PAD) on gastrocnemius histology.[12] PAD was responsible for the decline in type II muscle fiber size, that was consistent with a decline in muscle strength.[12] The western blot analysis we then performed showed that muscle protein degradation was increased, but muscle protein synthesis was unchanged. This imbalance could explain the decline in type II fibers size, and consequently in muscle strength. Finally, the role of muscle fibrosis was confirmed, with large accumulation of collagen, and a high-state inflammation within the affected muscles. This is consistent with studies highlighting the link between inflammation, fibrosis and PAD progression.[8,13–16] Fibrosis leads to muscle stiffness, decreased muscle movement capacity, and thereby lowered exercise capacity.

Accordingly, high protein degradation and inflammation might have disrupted muscle fiber histology, resulting in smaller and weaker fibers, leading to muscle fibrosis and sarcopenia in our model of CLTI.

The second objective of this study was to investigate if and how low-intensity and short-term exercise can provide protective effects against sarcopenia in CLTI. Low-intensity exercise training reversed muscle strength loss, and increased muscle function, but failed to improve muscle mass, suggesting that the loss of muscle mass is not correlated to a loss of muscle strength.[17] It is likely that the increase in muscle strength does not come from muscle growth, but rather primarily neural growth. Indeed, one study including 26 men investigated the effect of high- versus low-load training on muscle mass, muscle strength and neural adaptations.[18] After the training protocols, both high- and low-load exercise training groups had similar muscle mass gains, but the group following high-load training had greater muscle strength and neural adaptations.[18]

In our study, mechanisms of exercised-induced muscle protection involve increased muscle fiber size and reduced fibrosis. Low-intensity exercise training reversed the fiber atrophy, with +26.4% fiber size as compared to CLTI mice. Particularly, exercise reversed type IIB and IIBX fiber size. Our results are consistent with another study investigating the effects of resistance training on gastrocnemius histology of PAD patients. Resistance training was shown to increase type II fibers size and muscle strength.[19] In our study, we also showed that low-intensity exercise increased muscle protein degradation, and did not affect muscle protein synthesis. Thus, exercise is beneficial to restore muscle function and histology, but these improvements cannot be attributable to an increase in protein turnover. We hypothesize that exercise induces neural adaptations (i.e. by stimulating motor cortex, spinal cord or motor neurons), increasing type II fiber size, and subsequently muscle strength.[20–23] The mechanisms potentially involved remain to be further investigated. Low-intensity exercise training also reversed muscle fibrosis, greatly limiting collagen accumulation within the affected muscles. Interestingly, the lack of fibrosis after exercise might indicate that the muscle was correctly regenerated. This hypothesis is further supported by the fiber size improvements seen in skeletal muscle histology. Reducing skeletal muscle fibrosis might have enhanced muscle strength.

From a clinical point of view, this study could help in developing adjunctive procedures or protocols that would improve the outcome of CLTI patients presenting with sarcopenia. Revascularization surgery is the treatment of choice, but treatment should not be limited to

surgery, and it is important to find adjuncts procedures targeting the underlying mechanisms responsible for skeletal muscle wasting in order to improve the outcomes.[24] Exercise represents an effective treatment, but it must be adapted to every patient, since many of them are unable to perform heavy exercises due to poor health conditions (comorbidities, pain, ulcers). Accordingly, a short-term and low-intensity protocol could be proposed in these patients and may allow beneficial effects. Moreover, low-intensity exercise could be performed using muscle electrical stimulation in patients that are unable to perform supervised exercise.

This study presents several limitations. It is an experimental study and the mechanisms involved in our murine model may not be totally the same than those involved in humans, although published data demonstrated similarities between mice and humans in CLTI.[2] Moreover, the intensity and length of the exercise protocol would vary in humans. Finally, the neural pathway that could be involved in the way of protection might also be interesting to investigate.

Conclusion

In conclusion, CLTI associated to sarcopenia demonstrated a worse prognosis in humans.[2] Even if revascularization is the treatment of choice in such patients, we investigated the underlying mechanisms, in order to develop adjunct protocols that could improve the outcome. Accordingly, a simple and short exercise protocol reversed type II fiber atrophy, decreased fibrosis, and reversed muscle strength and motricity alterations in mice. These mechanistic data further support the usefulness of low-intensity exercise protocols in CLTI patients presenting with sarcopenia, along with revascularization procedures.

Declaration of interest

None.

References

1. Cruz-Jentoft AJ, Bahat G, Bauer J, Boirie Y, Bruyère O, Cederholm T *et al.* Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 2019;**48**:16–31.
2. Pizzimenti M, Meyer A, Charles A-L, Giannini M, Chakfé N, Lejay A *et al.* Sarcopenia and peripheral arterial disease: a systematic review. *J Cachexia Sarcopenia Muscle* 2020;**11**:866–886.
3. Matsubara Y, Matsumoto T, Aoyagi Y, Tanaka S, Okadome J, Morisaki K *et al.* Sarcopenia is a prognostic factor for overall survival in patients with critical limb ischemia. *Journal of Vascular Surgery* 2015;**61**:945–950.
4. Matsubara Y, Matsumoto T, Inoue K, Matsuda D, Yoshiga R, Yoshiya K *et al.* Sarcopenia is a risk factor for cardiovascular events experienced by patients with critical limb ischemia. *Journal of Vascular Surgery* 2017;**65**:1390–1397.
5. Morisaki K, Furuyama T, Matsubara Y, Inoue K, Kurose S, Yoshino S *et al.* External validation of CLI Frailty Index and assessment of predictive value of modified CLI Frailty Index for patients with critical limb ischemia undergoing infrainguinal revascularization. *Vascular* 2019;1708538119836005.
6. Taniguchi R, Deguchi J, Hashimoto T, Sato O. Sarcopenia as a Possible Negative Predictor of Limb Salvage in Patients with Chronic Limb-Threatening Ischemia. *Ann Vasc Dis* 2019;**12**:194–199.
7. Shimazoe H, Mii S, Koyanagi Y, Ishida M. Impact of Low Activity of Daily Living on the Prognosis of Patients with Critical Limb Ischemia and Sarcopenia. *Ann Vasc Surg* 2019;**61**:156–164.
8. Cong G, Cui X, Ferrari R, Pipinos II, Casale GP, Chattopadhyay A *et al.* Fibrosis Distinguishes Critical Limb Ischemia Patients from Claudicants in a Transcriptomic and Histologic Analysis. *J Clin Med* 2020;**9**.
9. Nader GA, Dastmalchi M, Alexanderson H, Grundtman C, Gernapudi R, Esbjörnsson M *et al.* A longitudinal, integrated, clinical, histological and mRNA profiling study of resistance exercise in myositis. *Mol Med* 2010;**16**:455–464.
10. Amani M, Rahmati M, Fathi M, Ahmadvand H. Reduce Muscle Fibrosis through Exercise via NRG1/ErbB2 Modification in Diabetic Rats. *Journal of Diabetes Research* 2020;**2020**:e6053161.
11. Lejay A, Choquet P, Thaveau F, Singh F, Schlagowski A, Charles AL *et al.* A new murine model of sustainable and durable chronic critical limb ischemia fairly mimicking human pathology. *Eur J Vasc Endovasc Surg* 2015;**49**:205–12.
12. Regensteiner JG, Wolfel EE, Brass EP, Carry MR, Ringel SP, Hargarten ME *et al.* Chronic changes in skeletal muscle histology and function in peripheral arterial disease. *Circulation* 1993;**87**:413–421.
13. Pottecher J, Guillot M, Belaidi E, Charles A-L, Lejay A, Gharib A *et al.* Cyclosporine A normalizes mitochondrial coupling, reactive oxygen species production, and inflammation and partially restores skeletal muscle maximal oxidative capacity in experimental aortic cross-clamping. *J Vasc Surg* 2013;**57**:1100-1108.e2.
14. Pizzimenti M, Riou M, Charles A-L, Talha S, Meyer A, Andres E *et al.* The Rise of Mitochondria in Peripheral Arterial Disease Physiopathology: Experimental and Clinical Data. *J Clin Med* 2019;**8**.
15. Ha DM, Carpenter LC, Koutakis P, Swanson SA, Zhu Z, Hanna M *et al.* Transforming growth factor-beta 1 produced by vascular smooth muscle cells predicts fibrosis in the gastrocnemius of patients with peripheral artery disease. *J Transl Med* 2016;**14**:39.
16. Casanegra A, Stoner J, Tafur A, Pereira H, Rathbun S, Gardner A. Differences in

Galectin-3, A Biomarker of Fibrosis, Between Participants with Peripheral Artery Disease and Participants with Normal Ankle-Brachial Index. *Vasc Med* 2016;**21**:437–444.
17. Reggiani C, Schiaffino S. Muscle hypertrophy and muscle strength: dependent or independent variables? A provocative review. *European Journal of Translational Myology* 2020;**30**.
18. Jenkins NDM, Miramonti AA, Hill EC, Smith CM, Cochrane-Snyman KC, Housh TJ *et al.* Greater Neural Adaptations following High- vs. Low-Load Resistance Training. *Front Physiol* 2017;**8**.
19. McGuigan MR, Bronks R, Newton RU, Sharman MJ, Graham JC, Cody DV *et al.* 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**:B302-310.
20. Leardini-Tristao M, Charles A-L, Lejay A, Pizzimenti M, Meyer A, Estato V *et al.* Beneficial Effect of Exercise on Cognitive Function during Peripheral Arterial Disease: Potential Involvement of Myokines and Microglial Anti-Inflammatory Phenotype Enhancement. *J Clin Med* 2019;**8**.
21. Perrey S. Promoting Motor Function by Exercising the Brain. *Brain Sci* 2013;**3**:101–122.
22. Aagaard P, Suetta C, Caserotti P, Magnusson SP, Kjaer M. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. *Scand J Med Sci Sports* 2010;**20**:49–64.
23. Del Vecchio A, Casolo A, Negro F, Scorcelletti M, Bazzucchi I, Enoka R *et al.* The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding. *J Physiol* 2019;**597**:1873–1887.
24. Conte MS, Bradbury AW, Kolh P, White JV, Dick F, Fitridge R *et al.* Global Vascular Guidelines on the Management of Chronic Limb-Threatening Ischemia. *Eur J Vasc Endovasc Surg* 2019;**58**:S1-S109.e33.

Figures

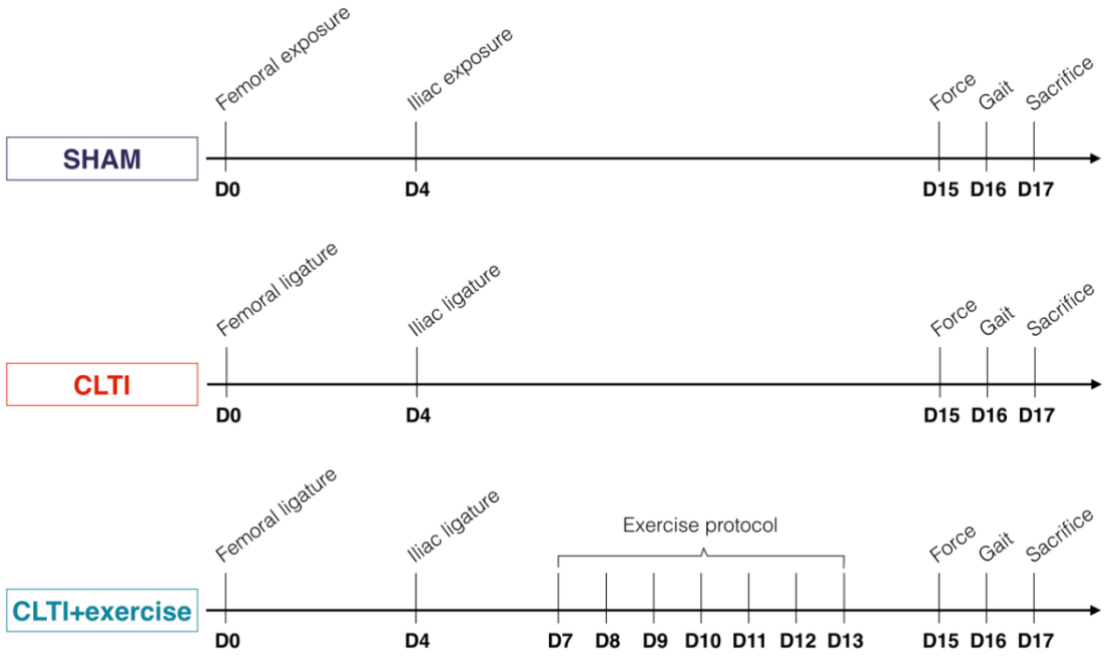
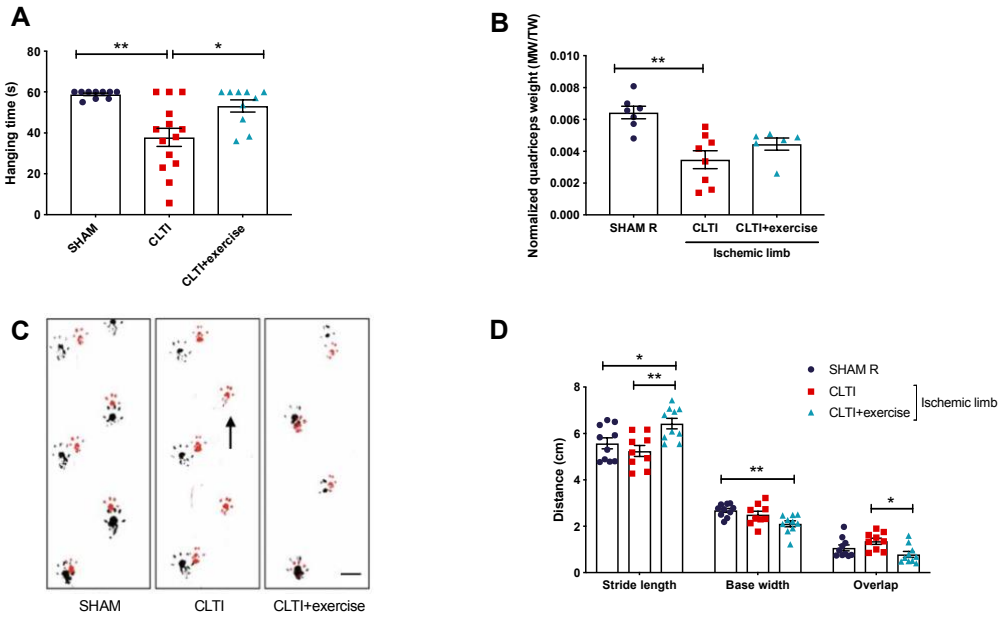


Figure 1. Description of the study protocol
CLTI: critical limb threatening ischemia group; CLTI+exercise: critical limb threatening ischemia + exercise group; D: day



E

Gait characteristics	SHAM	CLTI	CLTI+exercise
Walk on 4 limbs	**10** (100%)	**9** (64%)	**10** (100%)
Walk on 3 limbs	**0** (0%)	**5** (36%)	**0** (0%)

Figure 2. Study of functional parameters: muscle strength, muscle mass and motricity
(A) Muscle strength of SHAM, CLTI and CLTI+exercise mice measured by hanging test. **(B)** Measurements of quadriceps muscle masses. **(C-E)** Gait parameters evaluated using the footprint assay. (**C**) Representative illustrations of footprint results; forelimbs were marked with red ink, hindpaws with black ink. The arrow represents the missing limb print. Scale bar, 1 cm. (**D**) Measurements of hindlimb stride length, hindlimb base width and overlap between hind and forelimbs. (**E**) Summary of motricity parameters. Results are expressed as means and standard errors or numbers (%). *p<0.05; **p<0.01. For figures A, C, D and E, SHAM n=10 mice; CLTI n=14; CLTI+exercise n=10. For figure B, SHAM n=7, CLTI n=8, CLTI+exercise n=6. CLTI: critical limb threatening ischemia; CLTI+exercise: critical limb threatening ischemia + exercise; MW: muscle weight; SHAM R: SHAM right limb; TW: total weight

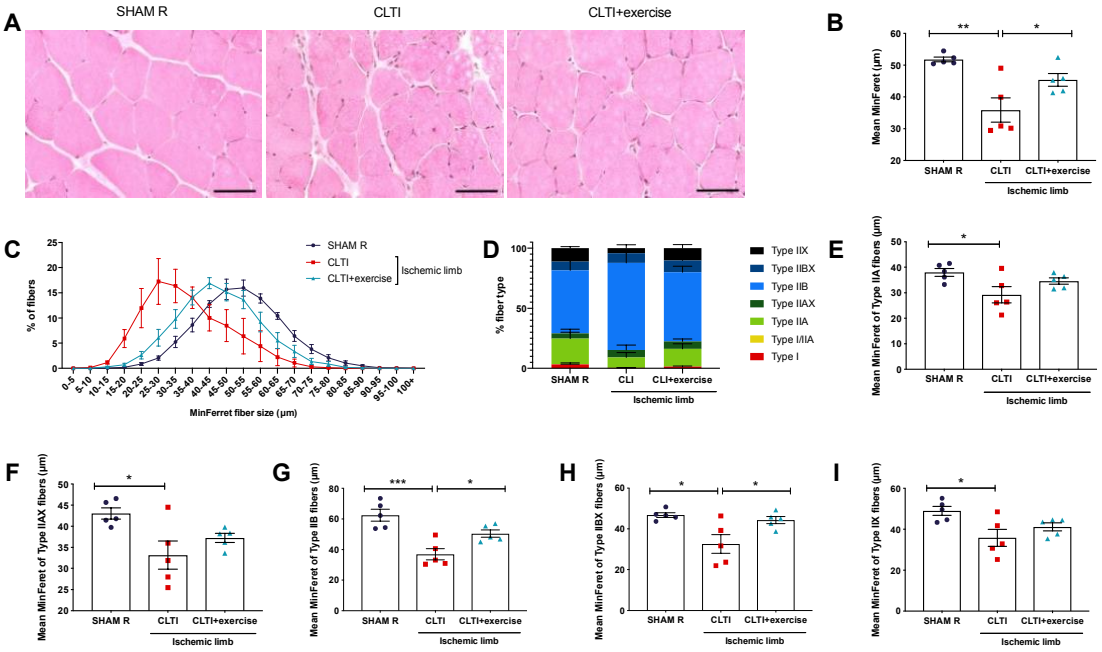


Figure 3. Study of histological parameters: fiber atrophy and fiber atrophy per fiber type
(A) Representative examples of transversal quadriceps muscle sections stained with hematoxylin and eosin (H&E). Scale bars, 50 µm. **(B)** Mean Minferet of quadriceps fibers. **(C)** MinFeret of quadriceps fibers grouped into 5 µm intervals. **(D)** Fiber type distribution stained by immunofluorescence. **(E-I)** Mean MinFeret of type IIA, IIAX, IIB, IIBX and IIX fibers. Results are expressed as means and standard errors. *p<0.05; **p<0.01; ***p<0.001. n=5 mice per group. CLTI: critical limb threatening ischemia; CLTI+exercise: critical limb threatening ischemia + exercise; SHAM R: SHAM right limb

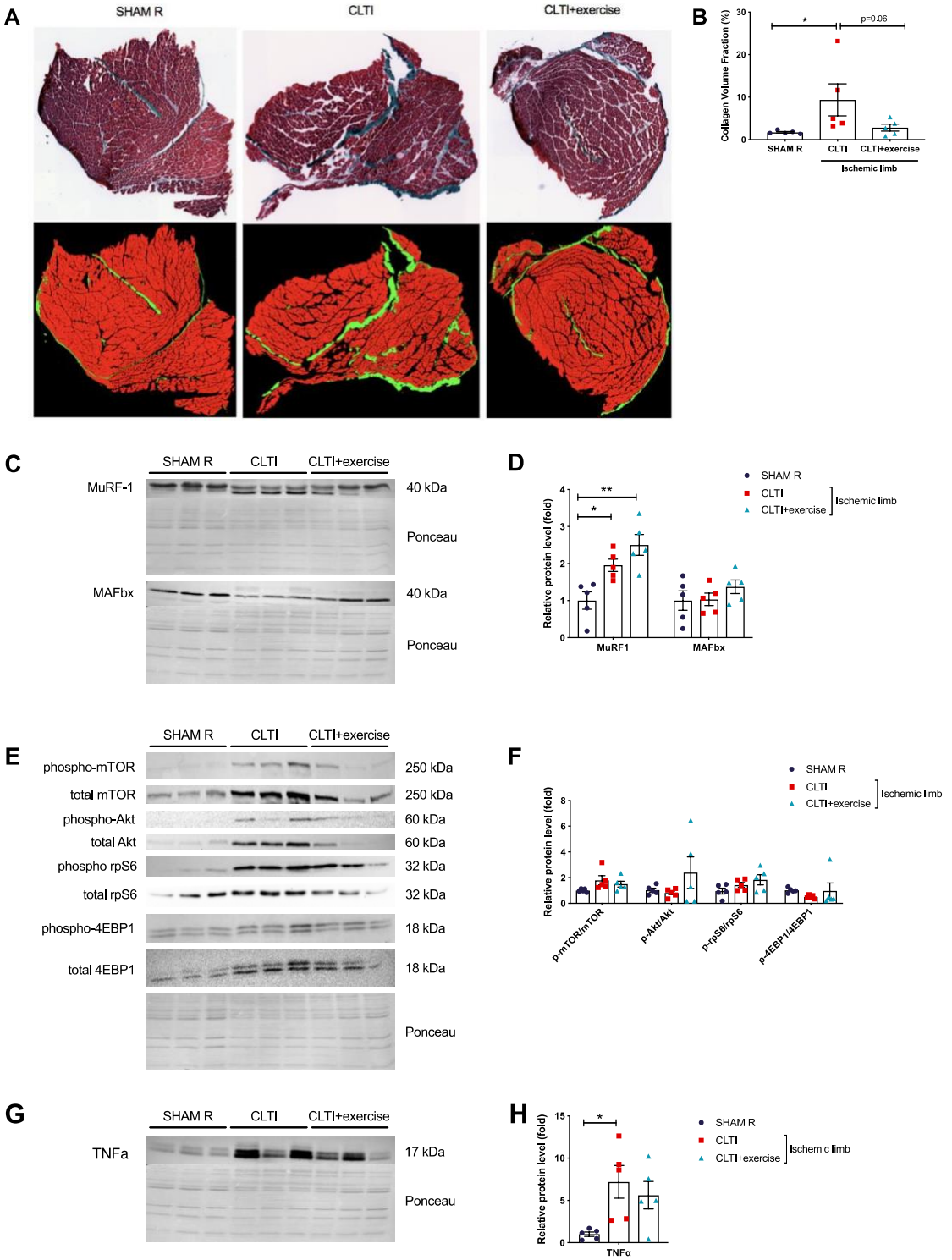


Figure 4. Study of the mechanisms involved: fibrosis, protein turnover and inflammation **(A)** Representative examples of transversal whole quadriceps muscle sections stained with Masson trichrome (top), and the segmented images representing fibrosis in green, muscle fibers in red, and background in black and generated using a macro developed in Fiji (bottom). **(B)** Mean Collagen Volume Fraction of quadriceps fibers. **(C-D)** Western blots from quadriceps muscles probed with MuRF1 and MAFbx antibodies. **(E-F)** Western blots from quadriceps

muscles probed with mTOR, Akt, rpS6 and 4EBP1 phosphorylated and total antibodies. **(G-H)** Western blots from quadriceps muscles probed with TNFα antibody. Results are expressed as means and standard errors. *p<0.05; **p<0.01. n=5 mice per group. CLTI: critical limb threatening ischemia; CLTI+exercise: critical limb threatening ischemia + exercise; SHAM R: SHAM right limb

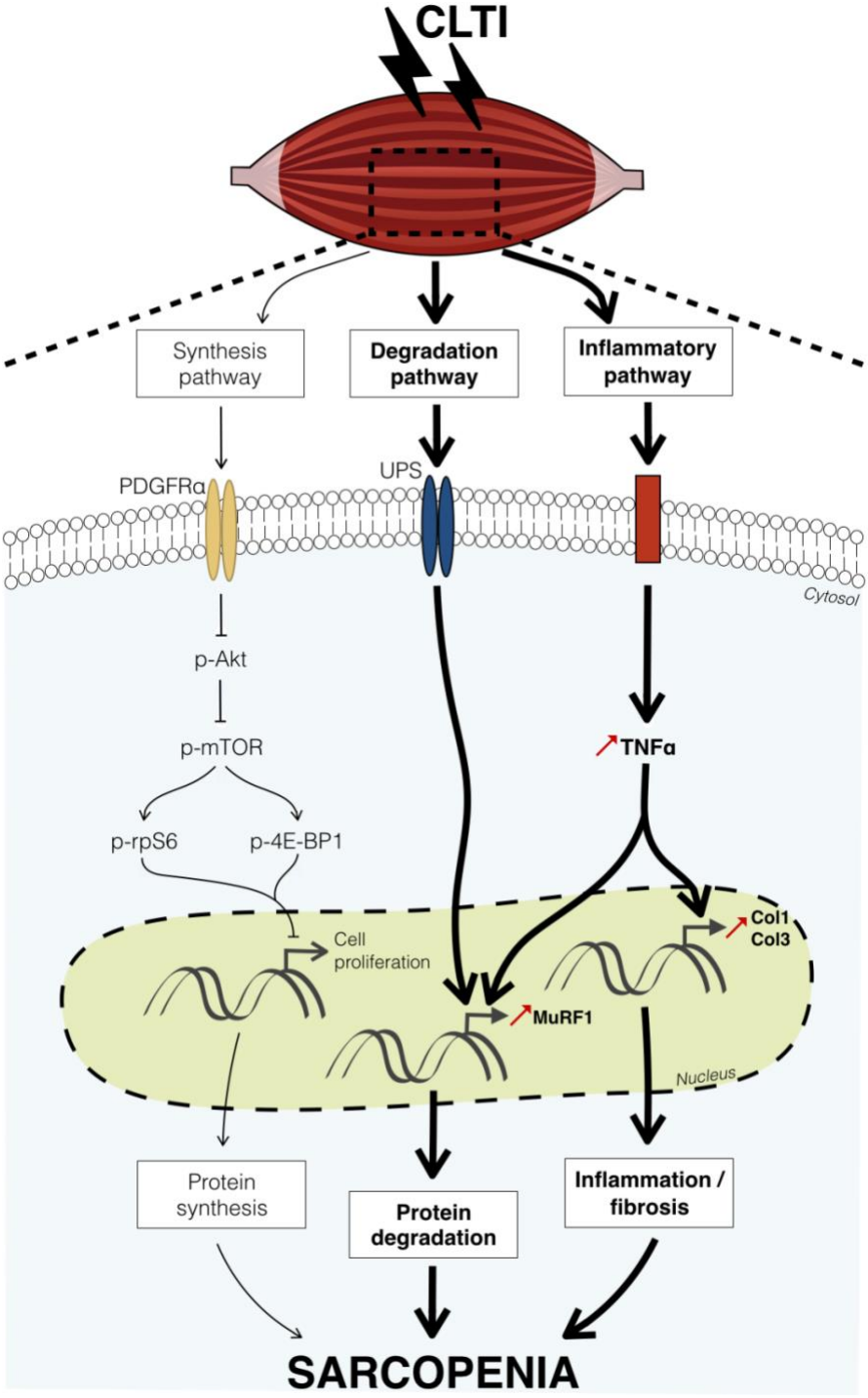


Figure 5. Major signaling pathways associated with sarcopenia and critical limb threatening ischemia (CLTI)

In the context of CLTI, ischemic injuries activate the degradation pathway, with increased level of the ubiquitin ligase MuRF1. Elevated level of TNFα activates the inflammatory pathway, and further enhances the activity of MuRF1. Inflammation also leads to increase collagen production and fibrosis. The imbalance between protein synthesis and degradation, the inflammation and extensive fibrosis thus lead to the genesis of sarcopenia in CLTI.

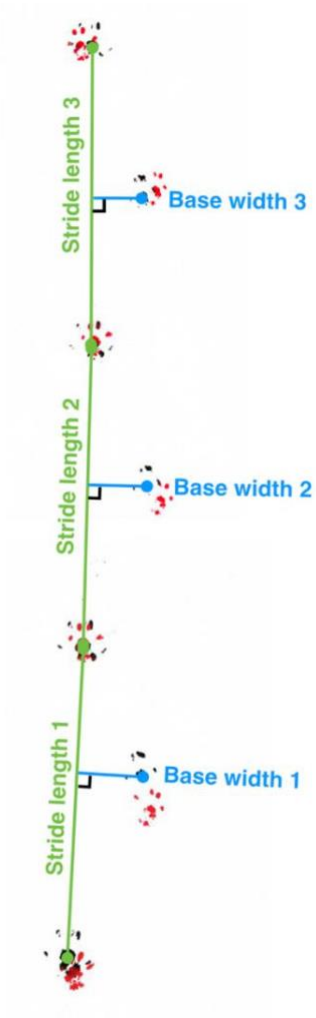


Figure S1. Visual explanation of stride length and base width measurement in the footprint assay

Hindlimb stride length is the average distance between three consecutive steps; base width is the average lateral distance between three consecutive steps, the base width was determined by measuring the perpendicular distance of a given step to a line connecting its opposite preceding and succeeding steps.

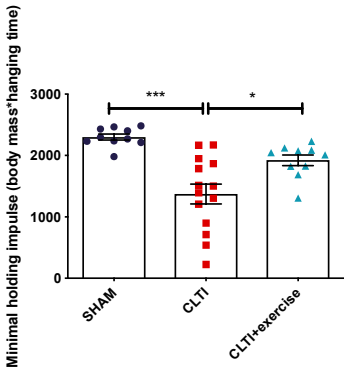


Figure S2. Minimal holding impulse

Hanging test corrected by mice weight. Results are expressed as means and standard errors. *p<0.05; ***p<0.001. SHAM n=10 mice; CLTI n=14; CLTI+exercise n=10. CLTI: critical limb threatening ischemia; CLTI+exercise: critical limb threatening ischemia + exercise; SHAM R: SHAM right limb

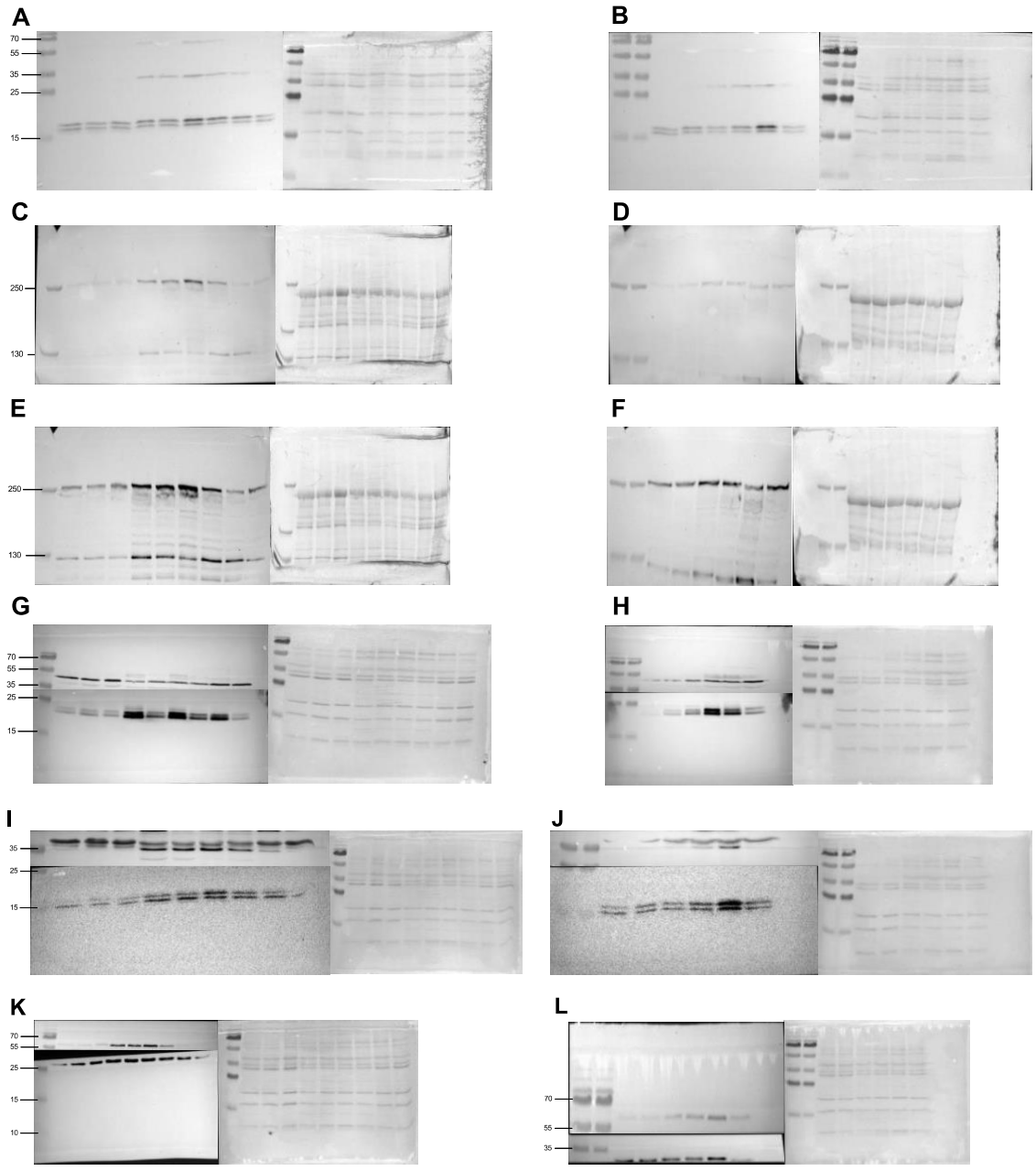


Figure S3. Western blot entire membranes and their ponceau
(A-B) Western blots from quadriceps muscles probed with phospho-Akt, phospho-rpS6 and phospho-4EBP1 antibodies, and the ponceau. **(A)** n=3 per group. **(B)** n=2 per group.
(C-D) Western blots from quadriceps muscles probed with phospho-mTOR, and the ponceau. **(C)** n=3 per group. **(D)** n=2 per group.
(E-F) Western blots from quadriceps muscles probed with total mTOR, and the ponceau. **(E)** n=3 per group. **(F)** n=2 per group.
(G-H) Western blots from quadriceps muscles probed with MAFbx and TNFα, and the ponceau. **(G)** n=3 per group. **(H)** n=2 per group.

(I-J) Western blots from quadriceps muscles probed with MuRF1 and total 4EBP1, and the ponceau. **(I)** n=3 per group. **(J)** n=2 per group.
(K-L) Western blots from quadriceps muscles probed with total Akt and total rpS6, and the ponceau. **(K)** n=3 per group. **(L)** n=2 per group.

DISCUSSION

Discussion

1 L'inflammation comme mécanisme commun à la sarcopénie et à l'ischémie critique chronique : son rôle comme marqueur de sarcopénie

Bien qu'essentiel pour les soins des patients, le diagnostic de sarcopénie est souvent négligé dans l'ICC. À ce jour, une grande majorité des patients souffrant d'ICC sont examinés par angioscanner préopératoire, ce qui pourrait être un moyen de procéder à une évaluation systématique et automatisée de la sarcopénie. Néanmoins, l'analyse par angioscanner peut être difficile à standardiser et peut être considérée comme longue. C'est pourquoi il est important de trouver des marqueurs sarcopéniques adaptés à la pratique quotidienne. L'inflammation est un pathomécanisme commun à la sarcopénie et à l'ICC. Nous avons donc émis l'hypothèse que le rapport inflammatoire plaquettes/lymphocytes (RPL) pourrait être un marqueur prédictif rapide de sarcopénie, comme cela a été démontré en oncologie, en chirurgie digestive ou chez les personnes âgées (A. Babber et al. 2020; Kitano et al. 2019; Liaw et al. 2017).

Dans notre étude, nous avons montré que les patients atteints de sarcopénie - diagnostiqués uniquement sur la base d'une masse musculaire faible - présentaient un RPL plus élevé que les patients non sarcopéniques. Ce résultat est en accord avec les niveaux d'inflammation plus élevés observés dans cette population, et mis en évidence tout au long de ce travail de thèse. Cependant, le RPL ne peut être à lui seul utilisé comme marqueur de sarcopénie chez les patients atteints d'ICC du fait de sa faible sensibilité, limitant son utilisation en pratique clinique courante.

Nous avons également mis en évidence que la sarcopénie était associée à un mauvais pronostic en termes de morbidité post-opératoire. Ce résultat est cohérent avec de précédents travaux montrant que la sarcopénie était associée à un taux élevé d'événements cardiovasculaire (Matsubara et al. 2017) et à une survie plus faible chez les patients en ICC (Matsubara et al. 2015). De plus, plusieurs études ont mis en évidence qu'un RPL élevé était associé à un mauvais pronostic en termes de sauvetage de membre chez les patients souffrants d'ICC après une procédure de revascularisation, et chez les patients pour lesquels la chirurgie n'était pas possible (Huang et al. 2019; Taşoğlu et al. 2014; Wang et al. 2017). D'autres études ont montré qu'un RPL élevé était associé à une augmentation des événements cardiovasculaires et de la mortalité chez les patients en ICC (Gary et al. 2013; Uzun et al. 2017). Le RPL serait donc associé à la

progression et au mauvais pronostic des patients souffrant d'ICC, et pourrait aider à identifier un sous-groupe de patients à haut risque.

En conclusion, notre étude montre que le PLR est un test de dépistage simple qui pourrait aider à identifier des sous-groupes de patients qui nécessiteraient des traitements personnalisés ou des stratégies thérapeutiques spécifiques, comme les patients sarcopéniques.

2 Physiopathologie de la sarcopénie dans l'ischémie critique chronique : atrophie musculaire et implication de la fibrose, du catabolisme protéique et de l'inflammation musculaire

Lorsqu'elle est associée à l'ICC, la sarcopénie altère la qualité de vie et le pronostic des patients. Dans ce contexte, nous avons cherché à déterminer les voies de signalisation impliquées dans la genèse de cette sarcopénie dans un modèle murin d'ICC, de façon à ouvrir de nouvelles voies thérapeutiques. Ce modèle est induit par des ligatures séquentielles de l'artère fémorale superficielle droite et de l'artère iliaque commune droite. Deux semaines après l'induction de l'ICC, les souris développent une sarcopénie : avec une faiblesse musculaire, une diminution de la masse musculaire et une altération de la démarche. Ce modèle remplit donc les critères diagnostics de sarcopénie déterminés par les différents groupes de travail (L.-K. Chen et al. 2016; Cruz-Jentoft et al. 2019; Morley et al. 2011), permettant ainsi l'étude des mécanismes impliqués.

Dans le cadre de notre étude, nous avons pu montrer l'importance de la fibrose, du catabolisme protéique et de l'inflammation dans l'atrophie des fibres musculaires et la genèse de la sarcopénie dans l'ICC.

Atrophie musculaire

L'atrophie des fibres d'un muscle a un impact direct sur la force de ce muscle (McPhee et al. 2018). Dans notre étude expérimentale, nous avons observé une atrophie des fibres musculaires, et particulièrement des fibres de type II, sans changement de distribution par type de fibre ou de nombre de fibres totales. Nos résultats sont cohérents avec d'autres travaux de la littérature montrant une diminution de la taille des fibres musculaires de type II chez des patients en artériopathie (Regensteiner et al. 1993). De par les propriétés des fibres de type II, il n'est pas étonnant d'observer que leur atrophie résulte en une faiblesse musculaire. De manière intéressante, les fibres de type II semblent plus affectées par l'ischémie que les fibres de type I : cette susceptibilité pourrait s'expliquer par un pouvoir antioxydant moins important dans les fibres de type II par rapport aux fibres de type I (Charles et al. 2017).

Fibrose

La fibrose altère sévèrement les propriétés fonctionnelles et structurelles des tissus musculaires squelettiques (Mahdy 2019). Dans le cadre de l'étude mécanistique, nous avons observé chez

les souris une augmentation de la fibrose musculaire suite à l'ICC, en accord avec de récents travaux de la littérature montrant le lien entre la fibrose et la progression de l'artériopathie (Casanegra et al. 2016; Cong et al. 2020; Ha et al. 2016; Mietus et al. 2020). En réponse à l'hypoxie tissulaire, les voies pro-fibrotiques comme TGFβ et VEGF sont activées au niveau des micro-vaisseaux (Ha et al. 2016), entraînant un dépôt anormal de collagène au niveau des tissus lésés (Bersini et al. 2018; Braga, Agudelo, and Camara 2015).

Catabolisme protéique

L'homéostasie des protéines musculaires repose sur un équilibre dynamique entre leurs taux respectifs de synthèse et de dégradation. Notre étude expérimentale nous a permis d'observer une augmentation du catabolisme protéique musculaire caractérisée par une augmentation du processus de dégradation, sans ralentissement du processus de synthèse des protéines. On pouvait s'attendre à ce que la synthèse des protéines musculaires soit ralentie. Cependant, le muscle étant fragilisé par l'ICC, il est possible qu'il ait besoin de quantités plus élevées de protéines pour stimuler de manière efficace la synthèse des protéines musculaires. Ce phénomène, aussi appelé 'résistance anabolique' est très bien décrit dans le muscle âgé (Breen and Phillips 2011; Fry and Rasmussen 2011).

Inflammation

L'inflammation joue un rôle important dans l'atteinte musculaire. Dans le cadre de notre étude, nous avons montré que l'inflammation est fortement augmentée dans le muscle suite à l'ICC, ce qui est cohérent avec plusieurs travaux de la littérature (Mary M. McDermott et al. 2007; Mary McGrae McDermott et al. 2003, 2005). Un bon nombre des mécanismes impliqués dans la genèse de la sarcopénie interfèrent avec des médiateurs inflammatoires, comme la fibrose et le catabolisme protéique (Dalle, Rossmeislova, and Koppo 2017). En effet, l'ICC provoque une inflammation locale, fragilisant le réseau vasculaire et musculaire alentour. L'inflammation va venir stimuler la dégradation des protéines musculaires : les fibres musculaires sont alors soumises à des cycles fréquents de dégradation et d'auto-renouvellement (Costamagna et al. 2015). En conséquence, il existe une inflammation persistante dans le muscle qui favorise encore la formation de fibrose dans un cercle vicieux (Y.-W. Chen et al. 2005; Serrano and Muñoz-Cánoves 2010).

Autres voies potentiellement impactées

Deux autres voies potentiellement importantes dans la genèse de la sarcopénie ont été identifiées dans la revue scientifique : le stress oxydant et la mitochondriopathie. Ces voies avaient déjà fait l'objet d'études sur notre modèle expérimental, et seront donc discutées brièvement ici.

Le stress oxydant entraîne des dommages musculaires aussi bien sur la structure que sur la fonction du muscle (Bellanti, Buglio, and Vendemiale 2020). Les études précédentes ont mis en évidence une augmentation du stress oxydant à la suite de l'ICC (A. Lejay et al. 2015, 2017; Anne Lejay et al. 2019). Stress oxydant, inflammation et catabolisme protéique sont étroitement liés : le stress oxydant va venir léser le muscle, déclenchant une cascade d'inflammation et de dégradation protéique (Meng and Yu 2010). Il est donc possible que le stress oxydant participe à la genèse de la sarcopénie.

La mitochondriopathie s'associe à des défauts musculaires structurels et fonctionnels. Les études précédentes ont mis en évidence une altération de la respiration mitochondriale et de l'ouverture du pore de transition de perméabilité mitochondriale à la suite de l'ICC (A. Lejay et al. 2015, 2017; Anne Lejay et al. 2019). Le stress oxydant va induire une accumulation de dommage de l'ADN mitochondrial, ce qui entraîne la synthèse de composants de la chaîne de transport d'électrons dysfonctionnels, une respiration mitochondriale anormale, et la génération de RLO dans un cercle vicieux (Anne Lejay et al. 2014). Cela suggère que la mitochondriopathie serait potentiellement impliquée dans la pathogenèse de la sarcopénie (Figure 8).

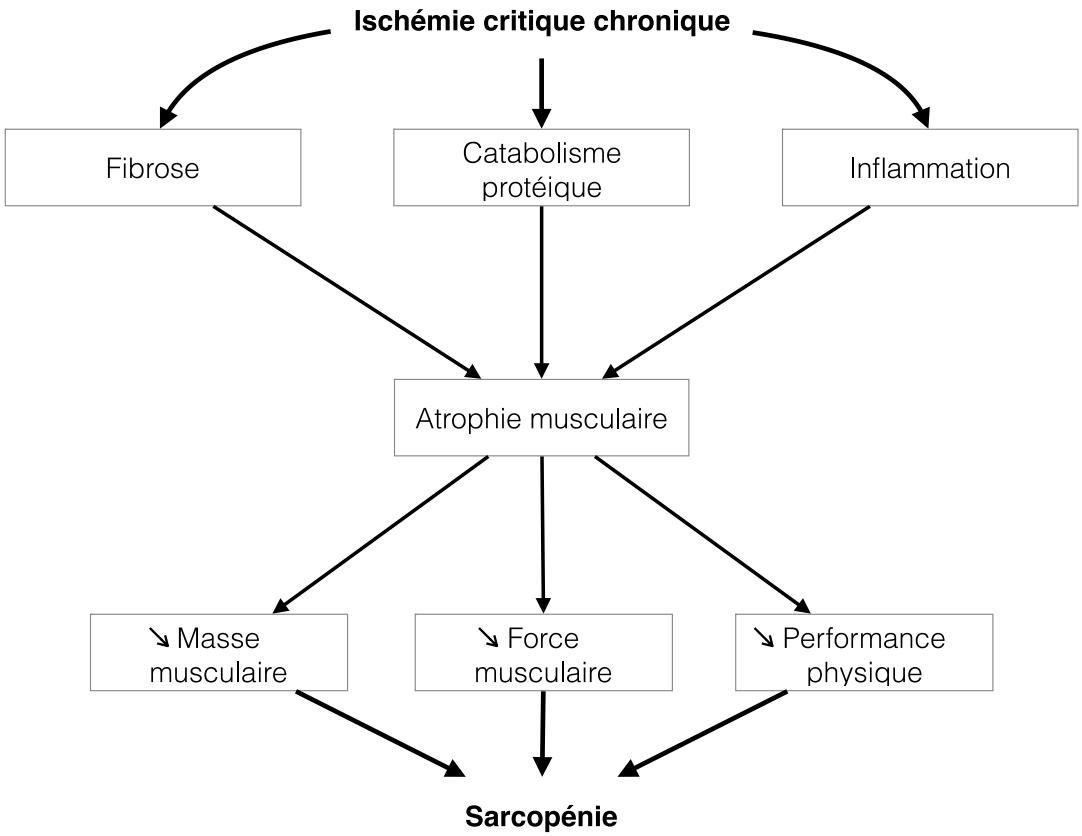


Figure 8 : implication de la fibrose, du catabolisme protéique et de l'inflammation dans l'atrophie musculaire et la genèse de la sarcopénie associée à l'ischémie critique chronique

3 Voies thérapeutiques pour lutter contre la sarcopénie dans l'ischémie critique chronique

Dans le cadre de notre étude, nous avons pu montrer qu'un protocole d'exercice court et de faible intensité permettait de réduire l'atrophie et la fibrose musculaire, et de restaurer la force et la capacité de marche des souris, mais pas la masse musculaire.

3.1 L'exercice

Il existe plusieurs types d'exercice :

- En endurance, qui se caractérise par des contractions répétées et de faible intensité pendant une période prolongée sans fatigue, comme la course à pied, le cyclisme ou la natation. Ce type d'entraînement est connu pour provoquer un changement dans la distribution des fibres, qui passent de majoritairement rapides, à majoritairement lentes, sans changement dans la taille des fibres.
- En résistance, qui implique des contractions à basse fréquence et à haute intensité contre une résistance externe, comme la musculation ou l'haltérophilie. Ce type d'entraînement ne modifie pas la distribution par type de fibre, mais provoque une hypertrophie des fibres musculaires.

Dans notre étude, bien que notre protocole s'apparente plutôt à un entraînement en endurance, nous avons observé tous les phénotypes d'un exercice en résistance. En effet, notre étude montre que l'exercice ne change pas la distribution par type de fibre, et restaure l'architecture musculaire en augmentant la taille des fibres, et particulièrement des fibres de type IIB et IIBX dans l'ICC. Il est possible que notre protocole d'exercice se rapproche plus d'un exercice en résistance, puisque les souris courent à une vitesse basse, en côte (sur un tapis de course incliné), et durant une période courte. Il s'agitait en quelque sorte de 'séances de musculation pour coureur'. En effet, nos résultats sont cohérents avec plusieurs études cliniques qui ont montré que l'entraînement en résistance permettait d'augmenter la taille des fibres de type II, et de lutter contre la faiblesse musculaire liée à l'âge (Kosek et al. 2006; Verdijk et al. 2009). L'entraînement en résistance stimulerait les cellules souches musculaires : les cellules satellites. Après prolifération et différenciation, ces cellules satellites fusionneraient avec les fibres musculaires existantes, ajoutant de nouveaux myonoyaux qui participeraient à la fonction ribosomique, et donc à l'hypertrophie des fibres musculaires (Petrella et al. 2006, 2008; Yin, Price, and Rudnicki 2013). Des études plus récentes suggèrent cependant que les adaptations à l'exercice se produisent de manière fibre-dépendante : si la relation entre cellules satellites,

nouveaux myonoyaux et hypertrophie semble validée dans les fibres de type I, elle est beaucoup moins évidente pour les fibres de type II. En effet, l'hypertrophie des fibres de type II observée après l'entraînement en résistance ne s'accompagne pas d'ajout de nouveaux myonoyaux (Fry and Rasmussen 2011; Moro et al. 2020). Il est possible que les myonoyaux existants des fibres de type II soient capables de soutenir une activité transcriptionnelle accrue pour maintenir l'hypertrophie induite par l'exercice (Kirby et al. 2016).

Dans notre étude, nous avons pu montrer que l'exercice de faible intensité inversait la fibrose musculaire, limitant considérablement l'accumulation de collagène dans les muscles lésés. Les bénéfices de l'exercice pour contrer la fibrose restent cependant très controversés. En effet, il est possible d'induire une fibrose sur des souris présentant une dystrophie ou une myopathie inflammatoire, grâce à un protocole d'exercice chronique (3 entraînements par semaine pendant 1 mois sur tapis de course à une vitesse de 12 mètres / minute (Pessina et al. 2014) ; ou un entraînement quotidien pendant 2 semaines sur un tapis de course à une vitesse de 17 mètres / minute (Saito et al. 2020)). Ces modèles développent une fibrose déclenchant une accumulation rapide de tissu fibreux qui se maintient pendant une période prolongée, avec des conséquences négatives sur la fonction musculaire. Saito et al. ont mis en évidence que dans un contexte de myopathie inflammatoire, un exercice chronique empêche les progéniteurs fibro-adipogéniques (FAP) d'entrer en sénescence. Les FAP acquièrent donc un phénotype anti-apoptotique et pro-fibrotique, rendant la régénération du muscle impossible (Saito et al. 2020). Nous pouvons donc faire l'hypothèse qu'un exercice de faible intensité - comme utilisé dans notre étude - est plus adapté dans un contexte musculaire fragilisé comme avec l'ICC (Boppart et al. 2013). Il serait intéressant d'étudier l'état apoptotique (par analyse de l'expression protéiques de Bax, Bak, caspase-9) et de sénescence (par analyse de l'expression génique de différents facteurs de sénescence comme *Cdkn2a*, *Trp53*, *P21*) des muscles des souris en ICC afin de confirmer si un changement vers un état pro-apoptotique et anti-fibrotique s'effectue, ce qui pourrait expliquer le recul de la fibrose observé dans notre modèle.

Dans le cadre de notre étude, nous avons montré que l'exercice de faible intensité augmentait la dégradation des protéines musculaires, sans modification de la synthèse des protéines musculaires. La dégradation des protéines musculaires est un phénomène bien connu en réponse à l'exercice en résistance (Tipton, Hamilton, and Gallagher 2018). Il est probable que cette dégradation cible des protéines endommagées, et de ce fait, joue un rôle important dans le processus adaptatif de remodelage et de renouvellement des protéines musculaires.

Notre étude montre que l'inflammation médiée par TNFα n'est pas réduite avec l'exercice. Cela est en accord avec ce qui a été publié dans la littérature (Monteiro-Junior et al. 2018). Cependant, le rôle précis de l'exercice sur TNFα reste inconnu. Nous pouvons faire l'hypothèse qu'au départ, l'exercice induit des lésions musculaires et une réponse inflammatoire majeure, qui sont fondamentales pour la réparation et la régénération correcte du muscle. Il est également possible qu'une fois la période d'adaptation à l'exercice passée, l'intensité de l'inflammation diminue.

Il est intéressant de noter que dans notre étude, l'exercice de faible intensité n'a pas permis d'inverser la perte de masse musculaire, malgré une restauration de la force. Ce résultat étonnant suggère que le gain de force musculaire pourrait venir d'un gain de qualité musculaire (avec l'amélioration de la fibrose notamment), plutôt que de quantité musculaire. De plus, il est possible que l'exercice induise des adaptations neuronales importantes en stimulant le cortex moteur, la moelle épinière ou les motoneurones, et participe au remodelage musculaire (Škarabot et al. 2021). Il serait intéressant d'étudier l'implication de la voie neuronale dans le gain de force musculaire dans l'ICC.

D'un point de vue clinique, cette étude pourrait aider à développer des procédures ou des protocoles d'appoint qui amélioreraient les résultats des patients en ICC présentant une sarcopénie. La chirurgie de revascularisation est le traitement de choix, mais le traitement ne doit pas se limiter à la chirurgie, et il est important de trouver des procédures d'appoint ciblant les mécanismes sous-jacents responsables de l'atrophie musculaire squelettique afin d'améliorer les résultats. L'exercice représente un traitement efficace, mais il doit être adapté à chaque patient, car nombre d'entre eux sont incapables d'effectuer des exercices lourds en raison d'un mauvais état de santé (comorbidités, douleurs, ulcères). En conséquence, un protocole de courte durée et de faible intensité pourrait être proposé chez ces patients et pourrait être bénéfique.

3.2 Perspectives : autres traitements sans exercice

L'exercice physique de faible intensité est bénéfique pour inverser la sarcopénie associée à l'ICC. Cependant, certains patients sont incapables d'effectuer des exercices en raison d'un mauvais état de santé (comorbidités, douleurs, ulcères). Pour ces patients, il est important de proposer des traitements alternatifs qui tiennent compte de leurs difficultés physiques. Dans ce

contexte, plusieurs approches comme des inhibiteurs de fibrose, de l'électrostimulation ou encore un programme nutritionnel adapté pourraient représenter des pistes thérapeutiques prometteuses.

Inhibiteur de fibrose

La fibrose apparaît comme un des mécanismes causatifs de la sarcopénie associée à l'ICC, qui, lorsqu'elle est réduite (par un protocole d'exercice, comme dans notre étude expérimentale), permet d'inverser la sarcopénie. Les inhibiteurs de fibrose apparaissent donc comme une cible thérapeutique d'intérêt. A notre connaissance, il n'existe aucune étude de l'effet de l'inhibition de la fibrose sur la fonction musculaire dans le contexte de l'ICC.

L'inhibition de la fibrose passe principalement par le ciblage de la voie de signalisation de TGFβ. Plusieurs composés réduisent les niveaux de TGFβ : des médicaments (losartan, suramine, décorine…) ou des anticorps neutralisants TGFβ (Mahdy 2019). Chez l'animal, ces agents anti-fibrotiques améliorent la fonction musculaire, en réduisant la fibrose et en augmentant la régénération musculaire sur des modèles de sarcopénie ou de myopathies (Burks et al. 2011; Cohn et al. 2007). Chez l'homme, il n'existe qu'une étude de cas, qui montre une amélioration de la fonction musculaire après traitement anti-fibrotique (Gharaibeh et al. 2012).

Electrostimulation

La stimulation électrique utilise de courtes impulsions électriques transmises par des électrodes placées à la surface de la peau pour générer une contraction musculaire. Elle apparaît comme une approche simple, peu coûteuse, non invasive et pouvant surmonter les limites de l'entraînement physique pour les patients atteints d'ICC associée à une sarcopénie (Adarsh Babber et al. 2016). Cependant, ses effets sur la fonction musculaire dans le cadre de l'ICC restent encore incertains.

Chez les rongeurs, la stimulation électrique permet d'améliorer l'état ischémique des rongeurs en augmentant les paramètres fonctionnels, cellulaires/endothéliaux et hémodynamiques (Brown et al. 2005; Kelsall et al. 2004). Cependant, une stimulation trop intense (6 h par jour) ou trop faible (1 Hz) n'a pas réussi à améliorer ces paramètres, et a même aggravé la fatigue musculaire (Hudlicka et al. 1994; Shen et al. 2009).

Chez l'homme, la stimulation électrique semble améliorer la capacité de marche et les paramètres hémodynamiques des patients en ICC (Mifsud and Cassar 2015; Yilmaz et al. 2017). Il est intéressant de noter que la stimulation électrique a montré de meilleurs résultats en termes d'état fonctionnel et de paramètres hémodynamiques lorsqu'elle est couplée à des programmes d'exercices supervisés, soulignant que la stimulation électrique pourrait être considérée comme une thérapie d'appoint (A. Babber et al. 2020; Presern-Strukelj and Poredos 2002).

D'un point de vue mécanistique, la stimulation électrique pourrait influencer positivement la sarcopénie associée à l'ICC. En effet, la stimulation électrique active tous les types de fibres musculaires simultanément et entraîne une hypertrophie et un recrutement accru des fibres de type I et II (Bickel, Gregory, and Dean 2011; Tsutaki et al. 2013). La stimulation électrique permet également de réduire l'accumulation de fibrose musculaire (Honda et al. 2021). De plus, en augmentant le débit sanguin et la disponibilité en oxygène dans les muscles ischémiques, la stimulation électrique déclenche une synthèse des protéines musculaires et de l'angiogenèse (Guo et al. 2021). Ainsi, nous pouvons faire l'hypothèse que la stimulation électrique pourrait améliorer la fatigue, la force et les performances musculaires dans le contexte de l'ICC.

La principale limitation de la stimulation électrique est le manque de protocoles standardisés. Or, les effets de la stimulation dépendent des caractéristiques des patients, notamment de leur symptomatologie et de leurs comorbidités. Idéalement, chaque protocole de stimulation doit être adapté à chaque patient, en reconnaissant qu'une stimulation d'intensité trop faible et/ou de courte durée peut ne pas être utile et qu'une stimulation d'intensité trop élevée et/ou de durée plus longue peut être délétère. Ainsi, la meilleure stratégie de stimulation doit préciser la fréquence et l'intensité de stimulation, qui doivent être basées sur l'amélioration de la qualité de vie des patients et des paramètres fonctionnels et hémodynamiques (Doucet, Lam, and Griffin 2012).

Nutrition

Les patients atteints d'ICC sont particulièrement vulnérables sur le plan nutritionnel, ce qui pourrait aggraver encore la perte de masse et de force musculaire (Salomon du Mont et al. 2017; Sieber 2019). Il serait intéressant d'étudier les effets d'une thérapie nutritionnelle ciblée sur la sarcopénie associée à l'ICC.

Si les études semblent formelles sur les effets négatifs d'une malnutrition sur le muscle squelettique, le rôle de la nutrition dans la prévention et le traitement de la sarcopénie reste très mal connu. De plus, chaque individu présente un schéma unique de carence en micronutriments et de besoins nutritionnels. La supplémentation devrait donc être adaptée pour répondre aux besoins uniques de chaque patient et ne peut pas être généralisée. Les micronutriments devraient être évalués dans les échantillons de sérum et complétés au besoin. Les besoins en protéines et en énergie varient en fonction de la présence ou non de comorbidités ou d'ulcères, ou du fait que le patient subisse une intervention opératoire ou basée sur l'exercice, et devraient donc être adaptés en conséquence.

CONCLUSION ET PERSPECTIVES

Conclusion et perspectives

Chez l'Homme, nous avons identifié le RPL comme marqueur simple et rapide de sarcopénie chez les patients atteints d'ICC, ces patients devant alors être considérés à haut-risque chirurgical, du fait de leur mauvais pronostic post-opératoire. Cependant, sa faible sensibilité limite son utilisation en routine.

L'étude de la sarcopénie sur notre modèle établi d'ICC nous a permis de mieux comprendre les mécanismes physiopathologiques sous-jacents, à savoir une fibrose musculaire, une altération des voies de dégradation des protéines musculaires, ainsi qu'une augmentation des paramètres inflammatoires. L'exercice de faible intensité est une approche thérapeutique protectrice qui permet de réduire l'atrophie et la fibrose musculaire, et de restaurer la force et la capacité de marche dans notre modèle murin.

Il serait intéressant de mettre en place chez l'Homme un protocole d'exercice de faible intensité et de courte durée, de manière complémentaire à la revascularisation, pour étudier si un tel protocole peut améliorer voire reverser les effets délétères de la sarcopénie chez les patients atteints d'ICC. Cependant, l'entraînement physique est difficile à généraliser, de nombreux patients étant incapables de faire de l'exercice en raison de mauvaises conditions de santé (comorbidités, douleurs, troubles trophiques des membres inférieurs). Dans ce contexte, des inhibiteurs de fibrose, un protocole de stimulation électrique des membres inférieurs associés à des compléments nutritionnels pourraient représenter des approches complémentaires.

Bibliographie

Aboyans, Victor et al. 2018. “Editor’s Choice - 2017 ESC Guidelines on the Diagnosis and Treatment of Peripheral Arterial Diseases, in Collaboration with the European Society for Vascular Surgery (ESVS).” *European Journal of Vascular and Endovascular Surgery: The Official Journal of the European Society for Vascular Surgery* 55(3): 305–68.

Altun, Mikael et al. 2010. “Muscle Wasting in Aged, Sarcopenic Rats Is Associated with Enhanced Activity of the Ubiquitin Proteasome Pathway.” *The Journal of Biological Chemistry* 285(51): 39597–608.

Antunes, Ana C., Daniela A. Araújo, Manuel T. Veríssimo, and Teresa F. Amaral. 2017. “Sarcopenia and Hospitalisation Costs in Older Adults: A Cross-Sectional Study.” *Nutrition & Dietetics: The Journal of the Dietitians Association of Australia* 74(1): 46–50.

Arango-Lopera, V. E. et al. 2013. “Mortality as an Adverse Outcome of Sarcopenia.” *The Journal of Nutrition, Health & Aging* 17(3): 259–62.

Babber, A. et al. 2020. “Effect of Footplate Neuromuscular Electrical Stimulation on Functional and Quality-of-Life Parameters in Patients with Peripheral Artery Disease: Pilot, and Subsequent Randomized Clinical Trial.” *The British Journal of Surgery* 107(4): 355–63.

Babber, Adarsh, Raveena Ravikumar, Katherine Williams, and Alun H. Davies. 2016. “FT06. Neuromuscular Electrical Stimulation in the Management of Intermittent Claudication: A ‘Stimulating’ Prospect.” *Journal of Vascular Surgery* 63(6): 16S-17S.

Bachettini, Nathalia Perleberg et al. 2020. “Sarcopenia as a Mortality Predictor in Community-Dwelling Older Adults: A Comparison of the Diagnostic Criteria of the European Working Group on Sarcopenia in Older People.” *European Journal of Clinical Nutrition* 74(4): 573–80.

Batsis, John A. et al. 2013. “Variation in the Prevalence of Sarcopenia and Sarcopenic Obesity in Older Adults Associated with Different Research Definitions: Dual-Energy X-Ray Absorptiometry Data from the National Health and Nutrition Examination Survey 1999-2004.” *Journal of the American Geriatrics Society* 61(6): 974–80.

Beaudart, Charlotte et al. 2019. “Malnutrition as a Strong Predictor of the Onset of Sarcopenia.” *Nutrients* 11(12): 2883.

Beavers, Kristen M. et al. 2013. “Associations between Body Composition and Gait-Speed Decline: Results from the Health, Aging, and Body Composition Study.” *The American Journal of Clinical Nutrition* 97(3): 552–60.

Becker, Clemens et al. 2015. “Myostatin Antibody (LY2495655) in Older Weak Fallers: A Proof-of-Concept, Randomised, Phase 2 Trial.” *The Lancet. Diabetes & Endocrinology* 3(12): 948–57.

Bellanti, Francesco, Aurelio Lo Buglio, and Gianluigi Vendemiale. 2020. "Chapter 9 - Oxidative Stress and Sarcopenia." In *Aging (Second Edition)*, eds. Victor R. Preedy and Vinood B. Patel. Academic Press, 95–103. https://www.sciencedirect.com/science/article/pii/B9780128186985000092 (August 20, 2021).

Benz, Elizabeth et al. 2019. "Sarcopenia in COPD: A Systematic Review and Meta-Analysis." *European Respiratory Review* 28(154). https://err.ersjournals.com/content/28/154/190049 (July 20, 2021).

Bersini, S. et al. 2018. "Tackling Muscle Fibrosis: From Molecular Mechanisms to next Generation Engineered Models to Predict Drug Delivery." *Advanced Drug Delivery Reviews* 129: 64–77.

Bickel, C. Scott, Chris M. Gregory, and Jesse C. Dean. 2011. "Motor Unit Recruitment during Neuromuscular Electrical Stimulation: A Critical Appraisal." *European Journal of Applied Physiology* 111(10): 2399–2407.

Bloemberg, Darin, and Joe Quadrilatero. 2012. "Rapid Determination of Myosin Heavy Chain Expression in Rat, Mouse, and Human Skeletal Muscle Using Multicolor Immunofluorescence Analysis." *PloS One* 7(4): e35273.

Boppart, Marni D., Michael De Lisio, Kai Zou, and Heather D. Huntsman. 2013. "Defining a Role for Non-Satellite Stem Cells in the Regulation of Muscle Repair Following Exercise." *Frontiers in Physiology* 4: 310.

Borba, Victoria Zeghbi Cochenski, Tatiana Lemos Costa, Carolina Aguiar Moreira, and Cesar Luiz Boguszewski. 2019. "MECHANISMS OF ENDOCRINE DISEASE: Sarcopenia in Endocrine and Non-Endocrine Disorders." *European Journal of Endocrinology* 180(5): R185–99.

Brack, Andrew S. et al. 2007. "Increased Wnt Signaling during Aging Alters Muscle Stem Cell Fate and Increases Fibrosis." *Science (New York, N.Y.)* 317(5839): 807–10.

Braga, Tarcio Teodoro, Juan Sebastian Henao Agudelo, and Niels Olsen Saraiva Camara. 2015. "Macrophages During the Fibrotic Process: M2 as Friend and Foe." *Frontiers in Immunology* 6: 602.

Breen, Leigh, and Stuart M. Phillips. 2011. "Skeletal Muscle Protein Metabolism in the Elderly: Interventions to Counteract the 'anabolic Resistance' of Ageing." *Nutrition & Metabolism* 8(1): 68.

Brown, M. D. et al. 2005. "A New Model of Peripheral Arterial Disease: Sustained Impairment of Nutritive Microcirculation and Its Recovery by Chronic Electrical Stimulation." *Microcirculation (New York, N.Y.: 1994)* 12(4): 373–81.

Bruyère, Olivier et al. 2019. "The Health Economics Burden of Sarcopenia: A Systematic Review." *Maturitas* 119: 61–69.

Burks, Tyesha N. et al. 2011. "Losartan Restores Skeletal Muscle Remodeling and Protects Against Disuse Atrophy in Sarcopenia." *Science translational medicine* 3(82): 82ra37.

Cai, Dongqing et al. 2004. “Ubiquitin Expression Is Up-Regulated in Human and Rat Skeletal Muscles during Aging.” *Archives of Biochemistry and Biophysics* 425(1): 42–50.

Calderón, Juan C., Pura Bolaños, and Carlo Caputo. 2014. “The Excitation–Contraction Coupling Mechanism in Skeletal Muscle.” *Biophysical Reviews* 6(1): 133–60.

Campbell, M. J., A. J. McComas, and F. Petito. 1973. “Physiological Changes in Ageing Muscles.” *Journal of Neurology, Neurosurgery, and Psychiatry* 36(2): 174–82.

Casanegra, AI et al. 2016. “Differences in Galectin-3, A Biomarker of Fibrosis, Between Participants with Peripheral Artery Disease and Participants with Normal Ankle-Brachial Index.” *Vascular medicine (London, England)* 21(5): 437–44.

Ceafalan, Laura Cristina et al. 2018. “Skeletal Muscle Regeneration Involves Macrophage-Myoblast Bonding.” *Cell Adhesion & Migration* 12(3): 228–35.

Chakravarthy, M. V., B. S. Davis, and F. W. Booth. 2000. “IGF-I Restores Satellite Cell Proliferative Potential in Immobilized Old Skeletal Muscle.” *Journal of Applied Physiology (Bethesda, Md.: 1985)* 89(4): 1365–79.

Charles, Anne-Laure et al. 2017. “Muscles Susceptibility to Ischemia-Reperfusion Injuries Depends on Fiber Type Specific Antioxidant Level.” *Frontiers in Physiology* 8: 52.

Chen, Liang-Kung et al. 2014. “Sarcopenia in Asia: Consensus Report of the Asian Working Group for Sarcopenia.” *Journal of the American Medical Directors Association* 15(2): 95–101.

———. 2016. “Recent Advances in Sarcopenia Research in Asia: 2016 Update From the Asian Working Group for Sarcopenia.” *Journal of the American Medical Directors Association* 17(8): 767.e1-7.

Chen, Y.-W. et al. 2005. “Early Onset of Inflammation and Later Involvement of TGFbeta in Duchenne Muscular Dystrophy.” *Neurology* 65(6): 826–34.

Cohn, Ronald D. et al. 2007. “Angiotensin II Type 1 Receptor Blockade Attenuates TGF-Beta-Induced Failure of Muscle Regeneration in Multiple Myopathic States.” *Nature Medicine* 13(2): 204–10.

Cong, Guangzhi et al. 2020. “Fibrosis Distinguishes Critical Limb Ischemia Patients from Claudicants in a Transcriptomic and Histologic Analysis.” *Journal of Clinical Medicine* 9(12). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7763090/ (March 19, 2021).

Costamagna, Domiziana, Paola Costelli, Maurilio Sampaolesi, and Fabio Penna. 2015. “Role of Inflammation in Muscle Homeostasis and Myogenesis.” *Mediators of Inflammation* 2015: e805172.

Couffinhal, T. et al. 1998. “Mouse Model of Angiogenesis.” *The American Journal of Pathology* 152(6): 1667–79.

Cruz-Jentoft, Alfonso J. et al. 2010. “Sarcopenia: European Consensus on Definition and Diagnosis.” *Age and Ageing* 39(4): 412–23.

———. 2019. “Sarcopenia: Revised European Consensus on Definition and Diagnosis.” *Age and Ageing* 48(1): 16–31.

Curcio, Francesco et al. 2020. “Sarcopenia and Heart Failure.” *Nutrients* 12(1): 211.

Dalle, Sebastiaan, Lenka Rossmeislova, and Katrien Koppo. 2017. “The Role of Inflammation in Age-Related Sarcopenia.” *Frontiers in Physiology* 8: 1045.

Doucet, Barbara M., Amy Lam, and Lisa Griffin. 2012. “Neuromuscular Electrical Stimulation for Skeletal Muscle Function.” *The Yale Journal of Biology and Medicine* 85(2): 201–15.

Duff, Steve, Michael S. Mafilios, Prajakta Bhounsule, and James T. Hasegawa. 2019. “The Burden of Critical Limb Ischemia: A Review of Recent Literature.” *Vascular Health and Risk Management* 15: 187–208.

Emdin, Connor A. et al. 2015. “Usual Blood Pressure, Peripheral Arterial Disease, and Vascular Risk: Cohort Study of 4.2 Million Adults.” *BMJ (Clinical research ed.)* 351: h4865.

Evans, William J. 2010. “Skeletal Muscle Loss: Cachexia, Sarcopenia, and Inactivity.” *The American Journal of Clinical Nutrition* 91(4): 1123S-1127S.

Farber, A., and R. T. Eberhardt. 2016. “The Current State of Critical Limb Ischemia: A Systematic Review.” *JAMA Surg* 151(11): 1070–77.

Frontera, Walter R., and Julien Ochala. 2015. “Skeletal Muscle: A Brief Review of Structure and Function.” *Calcified Tissue International* 96(3): 183–95.

Fry, Christopher S., and Blake B. Rasmussen. 2011. “Skeletal Muscle Protein Balance and Metabolism in the Elderly.” *Current Aging Science* 4(3): 260–68.

Ganapathy, Aravinda, and Jeri W. Nieves. 2020. “Nutrition and Sarcopenia—What Do We Know?” *Nutrients* 12(6): 1755.

Gardner, Andrew W., Donald E. Parker, Polly S. Montgomery, and Steve M. Blevins. 2014. “Step-Monitored Home Exercise Improves Ambulation, Vascular Function, and Inflammation in Symptomatic Patients with Peripheral Artery Disease: A Randomized Controlled Trial.” *Journal of the American Heart Association* 3(5): e001107.

Gary, Thomas et al. 2013. “Platelet-to-Lymphocyte Ratio: A Novel Marker for Critical Limb Ischemia in Peripheral Arterial Occlusive Disease Patients.” *PloS One* 8(7): e67688.

Gharaibeh, Burhan et al. 2012. “Biological Approaches to Improve Skeletal Muscle Healing after Injury and Disease.” *Birth defects research. Part C, Embryo today : reviews* 96(1): 82–94.

Giallauria, Francesco, Antonio Cittadini, Neil Andrew Smart, and Carlo Vigorito. 2016. “Resistance Training and Sarcopenia.” *Monaldi Archives for Chest Disease = Archivio Monaldi Per Le Malattie Del Torace* 84(1–2): 738.

Goates, S. et al. 2019. “Economic Impact of Hospitalizations in US Adults with Sarcopenia.” *The Journal of Frailty & Aging* 8(2): 93–99.

Goodpaster, Bret H. et al. 2006. “The Loss of Skeletal Muscle Strength, Mass, and Quality in Older Adults: The Health, Aging and Body Composition Study.” *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 61(10): 1059–64.

Guo, Yuxiao, Bethan E Phillips, Philip J Atherton, and Mathew Piasecki. 2021. “Molecular and Neural Adaptations to Neuromuscular Electrical Stimulation; Implications for Ageing Muscle.” *Mechanisms of Ageing and Development* 193: 111402.

Guralnik, J. M. et al. 1994. “A Short Physical Performance Battery Assessing Lower Extremity Function: Association with Self-Reported Disability and Prediction of Mortality and Nursing Home Admission.” *Journal of Gerontology* 49(2): M85-94.

Ha, Duy M. et al. 2016. “Transforming Growth Factor-Beta 1 Produced by Vascular Smooth Muscle Cells Predicts Fibrosis in the Gastrocnemius of Patients with Peripheral Artery Disease.” *Journal of Translational Medicine* 14: 39.

von Haehling, Stephan, John E. Morley, Andrew J. S. Coats, and Stefan D. Anker. 2017. “Ethical Guidelines for Publishing in the Journal of Cachexia, Sarcopenia and Muscle: Update 2017.” *Journal of Cachexia, Sarcopenia and Muscle* 8(6): 1081–83.

Hellingman, A. A. et al. 2010. “Variations in Surgical Procedures for Hind Limb Ischaemia Mouse Models Result in Differences in Collateral Formation.” *European Journal of Vascular and Endovascular Surgery* 40(6): 796–803.

Honda, Yuichiro et al. 2021. “Effect of Belt Electrode-Skeletal Muscle Electrical Stimulation on Immobilization-Induced Muscle Fibrosis.” *PLOS ONE* 16(5): e0244120.

Howard, Dominic P. J. et al. 2015. “Population-Based Study of Incidence, Risk Factors, Outcome, and Prognosis of Ischemic Peripheral Arterial Events: Implications for Prevention.” *Circulation* 132(19): 1805–15.

Huang, Hsuan-Li et al. 2019. “Risk Stratification for Low Extremity Amputation in Critical Limb Ischemia Patients Who Have Undergone Endovascular Revascularization: A Survival Tree Analysis.” *Medicine* 98(33): e16809.

Hudlicka, O., M. D. Brown, S. Egginton, and J. M. Dawson. 1994. “Effect of Long-Term Electrical Stimulation on Vascular Supply and Fatigue in Chronically Ischemic Muscles.” *Journal of Applied Physiology (Bethesda, Md.: 1985)* 77(3): 1317–24.

Hunter, Sandra K., Hugo M. Pereira, and Kevin G. Keenan. 2016. “The Aging Neuromuscular System and Motor Performance.” *Journal of Applied Physiology* 121(4): 982–95.

Joosten, Michel M. et al. 2012. “Associations between Conventional Cardiovascular Risk Factors and Risk of Peripheral Artery Disease in Men.” *JAMA* 308(16): 1660–67.

Kelsall, C. J. et al. 2004. “Arteriolar Endothelial Dysfunction Is Restored in Ischaemic Muscles by Chronic Electrical Stimulation.” *Journal of Vascular Research* 41(3): 241–51.

Kirby, Tyler J. et al. 2016. “Myonuclear Transcription Is Responsive to Mechanical Load and DNA Content but Uncoupled from Cell Size during Hypertrophy.” *Molecular Biology of the Cell* 27(5): 788–98.

Kitano, Yuki et al. 2019. “Sarcopenia Affects Systemic and Local Immune System and Impacts Postoperative Outcome in Patients with Extrahepatic Cholangiocarcinoma.” *World Journal of Surgery* 43(9): 2271–80.

Koopman, René, and Luc J. C. van Loon. 2009. “Aging, Exercise, and Muscle Protein Metabolism.” *Journal of Applied Physiology (Bethesda, Md.: 1985)* 106(6): 2040–48.

Kosek, David J. et al. 2006. “Efficacy of 3 Days/Wk Resistance Training on Myofiber Hypertrophy and Myogenic Mechanisms in Young vs. Older Adults.” *Journal of Applied Physiology (Bethesda, Md.: 1985)* 101(2): 531–44.

Landry, Gregory J. et al. 2014. “Objective Measurement of Lower Extremity Function and Quality of Life after Surgical Revascularization for Critical Lower Extremity Ischemia.” *Journal of Vascular Surgery* 60(1): 136–42.

Larsson, L. 1978. “Morphological and Functional Characteristics of the Ageing Skeletal Muscle in Man. A Cross-Sectional Study.” *Acta Physiologica Scandinavica. Supplementum* 457: 1–36.

Law, Timothy D., Leatha A. Clark, and Brian C. Clark. 2016. “Resistance Exercise to Prevent and Manage Sarcopenia and Dynapenia.” *Annual review of gerontology & geriatrics* 36(1): 205–28.

Lejay, A. et al. 2015. “A New Murine Model of Sustainable and Durable Chronic Critical Limb Ischemia Fairly Mimicking Human Pathology.” *Eur J Vasc Endovasc Surg* 49(2): 205–12.

———. 2017. “Moderate Exercise Allows for Shorter Recovery Time in Critical Limb Ischemia.” *Front Physiol* 8: 523.

Lejay, Anne et al. 2014. “Mitochondria: Mitochondrial Participation in Ischemia-Reperfusion Injury in Skeletal Muscle.” *The International Journal of Biochemistry & Cell Biology* 50: 101–5.

———. 2019. “Critical Limb Ischemia Exacerbates Mitochondrial Dysfunction in ApoE-/- Mice Compared to ApoE+/+ Mice, but N-Acetyl Cysteine Still Confers Protection.” *Eur J Vasc Endovasc Surg*.

Liaw, Fang-Yih et al. 2017. “Higher Platelet-to-Lymphocyte Ratio Increased the Risk of Sarcopenia in the Community-Dwelling Older Adults.” *Scientific Reports* 7(1): 16609.

Lieber, Richard L., and Samuel R. Ward. 2013. “Cellular Mechanisms of Tissue Fibrosis. 4. Structural and Functional Consequences of Skeletal Muscle Fibrosis.” *American Journal of Physiology. Cell Physiology* 305(3): C241-252.

Lilja, Erika et al. 2021. “The Impact of Diabetes Mellitus on Major Amputation among Patients with Chronic Limb Threatening Ischemia Undergoing Elective Endovascular

Therapy- a Nationwide Propensity Score Adjusted Analysis." *Journal of Diabetes and its Complications* 35(2): 107675.

Limbourg, Anne et al. 2009. "Evaluation of Postnatal Arteriogenesis and Angiogenesis in a Mouse Model of Hind-Limb Ischemia." *Nature Protocols* 4(12): 1737–48.

Lotfi, Shamim et al. 2013. "Towards a More Relevant Hind Limb Model of Muscle Ischaemia." *Atherosclerosis* 227(1): 1–8.

Luengo-Fernandez, Ramon et al. 2018. "Hospital and Institutionalisation Care Costs after Limb and Visceral Ischaemia Benchmarked Against Stroke: Long-Term Results of a Population Based Cohort Study." *European Journal of Vascular and Endovascular Surgery* 56(2): 271–81.

Mahdy, Mohamed A. A. 2019. "Skeletal Muscle Fibrosis: An Overview." *Cell and Tissue Research* 375(3): 575–88.

Mallinson, Joanne E., and Andrew J. Murton. 2013. "Mechanisms Responsible for Disuse Muscle Atrophy: Potential Role of Protein Provision and Exercise as Countermeasures." *Nutrition (Burbank, Los Angeles County, Calif.)* 29(1): 22–28.

Malmstedt, Jonas et al. 2008. "Outcome after Leg Bypass Surgery for Critical Limb Ischemia Is Poor in Patients with Diabetes: A Population-Based Cohort Study." *Diabetes Care* 31(5): 887–92.

Malmstrom, Theodore K. et al. 2016. "SARC-F: A Symptom Score to Predict Persons with Sarcopenia at Risk for Poor Functional Outcomes." *Journal of Cachexia, Sarcopenia and Muscle* 7(1): 28–36.

Mann, Christopher J. et al. 2011. "Aberrant Repair and Fibrosis Development in Skeletal Muscle." *Skeletal Muscle* 1(1): 21.

Marcus, Robin L., Diana I. Brixner, Sameer Ghate, and Paul LaStayo. 2012. "Fat Modulates the Relationship between Sarcopenia and Physical Function in Nonobese Older Adults." *Current Gerontology and Geriatrics Research* 2012: 216185.

Martel, Gregory F. et al. 2006. "Age and Sex Affect Human Muscle Fibre Adaptations to Heavy-Resistance Strength Training." *Experimental Physiology* 91(2): 457–64.

Martinez-Aguilar, Esther et al. 2017. "Reduced High-Density Lipoprotein Cholesterol: A Valuable, Independent Prognostic Marker in Peripheral Arterial Disease." *Journal of Vascular Surgery* 66(5): 1527-1533.e1.

Masaki, Ichiro et al. 2002. "Angiogenic Gene Therapy for Experimental Critical Limb Ischemia: Acceleration of Limb Loss by Overexpression of Vascular Endothelial Growth Factor 165 but Not of Fibroblast Growth Factor-2." *Circulation Research* 90(9): 966–73.

Matsubara, Yutaka et al. 2015. "Sarcopenia Is a Prognostic Factor for Overall Survival in Patients with Critical Limb Ischemia." *Journal of Vascular Surgery* 61(4): 945–50.

———. 2017. “Sarcopenia Is a Risk Factor for Cardiovascular Events Experienced by Patients with Critical Limb Ischemia.” *Journal of Vascular Surgery* 65(5): 1390–97.

McCuller, Christopher, Rishita Jessu, and Avery L. Callahan. 2021. “Physiology, Skeletal Muscle.” In *StatPearls*, Treasure Island (FL): StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK537139/ (August 13, 2021).

McDermott, Mary M. et al. 2007. “Elevated Levels of Inflammation, d-Dimer, and Homocysteine Are Associated with Adverse Calf Muscle Characteristics and Reduced Calf Strength in Peripheral Arterial Disease.” *Journal of the American College of Cardiology* 50(9): 897–905.

———. 2009. “Treadmill Exercise and Resistance Training in Patients with Peripheral Arterial Disease with and without Intermittent Claudication: A Randomized Controlled Trial.” *JAMA* 301(2): 165–74.

McDermott, Mary McGrae et al. 2003. “D-Dimer, Inflammatory Markers, and Lower Extremity Functioning in Patients with and without Peripheral Arterial Disease.” *Circulation* 107(25): 3191–98.

———. 2005. “Patterns of Inflammation Associated with Peripheral Arterial Disease: The InCHIANTI Study.” *American Heart Journal* 150(2): 276–81.

McPhee, Jamie S et al. 2018. “The Contributions of Fiber Atrophy, Fiber Loss, In Situ Specific Force, and Voluntary Activation to Weakness in Sarcopenia.” *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 73(10): 1287–94.

Meng, Si-Jin, and Long-Jiang Yu. 2010. “Oxidative Stress, Molecular Inflammation and Sarcopenia.” *International Journal of Molecular Sciences* 11(4): 1509–26.

Metter, E. J. et al. 1999. “Muscle Quality and Age: Cross-Sectional and Longitudinal Comparisons.” *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 54(5): B207-218.

Mietus, Constance J. et al. 2020. “Abnormal Microvascular Architecture, Fibrosis, and Pericyte Characteristics in the Calf Muscle of Peripheral Artery Disease Patients with Claudication and Critical Limb Ischemia.” *Journal of Clinical Medicine* 9(8): 2575.

Mifsud, Maximilian, and Kevin Cassar. 2015. “The Use of Transcutaneous Electrical Stimulation of the Calf in Patients Undergoing Infrainguinal Bypass Surgery.” *Annals of Vascular Surgery* 29(8): 1524–32.

Mitchell, Robert G. et al. 2007. “Increased Levels of Apoptosis in Gastrocnemius Skeletal Muscle in Patients with Peripheral Arterial Disease.” *Vascular Medicine (London, England)* 12(4): 285–90.

Monteiro-Junior, Renato Sobral et al. 2018. “Effect of Exercise on Inflammatory Profile of Older Persons: Systematic Review and Meta-Analyses.” *Journal of Physical Activity & Health* 15(1): 64–71.

Morisaki, Koichi et al. 2017. “Prognostic Factor of the Two-Year Mortality after Revascularization in Patients with Critical Limb Ischemia.” *Vascular* 25(2): 123–29.

Morley, John E. et al. 2011. “Sarcopenia With Limited Mobility: An International Consensus.” *Journal of the American Medical Directors Association* 12(6): 403–9.

Moro, Tatiana et al. 2020. “Resistance Exercise Training Promotes Fiber Type-Specific Myonuclear Adaptations in Older Adults.” *Journal of Applied Physiology* 128(4): 795–804.

Mustapha, Jihad A. et al. 2018. “Determinants of Long-Term Outcomes and Costs in the Management of Critical Limb Ischemia: A Population-Based Cohort Study.” *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease* 7(16): e009724.

Nilwik, Rachel et al. 2013. “The Decline in Skeletal Muscle Mass with Aging Is Mainly Attributed to a Reduction in Type II Muscle Fiber Size.” *Experimental Gerontology* 48(5): 492–98.

Norgren, L. et al. 2007. “Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II).” *Eur J Vasc Endovasc Surg* 33 Suppl 1: S1-75.

Novak, Margaret L., and Timothy J. Koh. 2013. “Macrophage Phenotypes during Tissue Repair.” *Journal of Leukocyte Biology* 93(6): 875–81.

de Palma, Luigi, Mario Marinelli, Matteo Pavan, and Alessandro Orazi. 2008. “Ubiquitin Ligases MuRF1 and MAFbx in Human Skeletal Muscle Atrophy.” *Joint, Bone, Spine: Revue Du Rhumatisme* 75(1): 53–57.

Paradis, Stéphanie et al. 2019. “Aging Exacerbates Ischemia-Reperfusion-Induced Mitochondrial Respiration Impairment in Skeletal Muscle.” *Antioxidants (Basel, Switzerland)* 8(6).

Payne, Anthony Michael et al. 2009. “Role of Ca2+, Membrane Excitability, and Ca2+ Stores in Failing Muscle Contraction with Aging.” *Experimental gerontology* 44(4): 261–73.

Pedersen, Bente K., and Mark A. Febbraio. 2012. “Muscles, Exercise and Obesity: Skeletal Muscle as a Secretory Organ.” *Nature Reviews Endocrinology* 8(8): 457–65.

Peng, Tao-Chun et al. 2020. “Sarcopenia and Cognitive Impairment: A Systematic Review and Meta-Analysis.” *Clinical Nutrition (Edinburgh, Scotland)* 39(9): 2695–2701.

Pessina, Patrizia et al. 2014. “Novel and Optimized Strategies for Inducing Fibrosis in Vivo: Focus on Duchenne Muscular Dystrophy.” *Skeletal Muscle* 4: 7.

Petrella, John K. et al. 2006. “Efficacy of Myonuclear Addition May Explain Differential Myofiber Growth among Resistance-Trained Young and Older Men and Women.” *American Journal of Physiology. Endocrinology and Metabolism* 291(5): E937-946.

———. 2008. “Potent Myofiber Hypertrophy during Resistance Training in Humans Is Associated with Satellite Cell-Mediated Myonuclear Addition: A Cluster Analysis.” *Journal of Applied Physiology (Bethesda, Md.: 1985)* 104(6): 1736–42.

Pillon, Nicolas J., Philip J. Bilan, Lisbeth N. Fink, and Amira Klip. 2013. “Cross-Talk between Skeletal Muscle and Immune Cells: Muscle-Derived Mediators and

Metabolic Implications." *American Journal of Physiology. Endocrinology and Metabolism* 304(5): E453-465.

Pottecher, Julien et al. 2018. "Diabetes Worsens Skeletal Muscle Mitochondrial Function, Oxidative Stress, and Apoptosis After Lower-Limb Ischemia-Reperfusion: Implication of the RISK and SAFE Pathways?" *Frontiers in Physiology* 9: 579.

Presern-Strukelj, Metka, and Pavel Poredos. 2002. "The Influence of Electrostimulation on the Circulation of the Remaining Leg in Patients with One-Sided Amputation." *Angiology* 53(3): 329–35.

Regensteiner, J. G. et al. 1993. "Chronic Changes in Skeletal Muscle Histology and Function in Peripheral Arterial Disease." *Circulation* 87(2): 413–21.

Reinecke, Holger et al. 2015. "Peripheral Arterial Disease and Critical Limb Ischaemia: Still Poor Outcomes and Lack of Guideline Adherence." *European Heart Journal* 36(15): 932–38.

Russ, David W., Kimberly Gregg-Cornell, Matthew J. Conaway, and Brian C. Clark. 2012. "Evolving Concepts on the Age-Related Changes in 'Muscle Quality.'" *Journal of Cachexia, Sarcopenia and Muscle* 3(2): 95–109.

Sacheck, Jennifer M., Akira Ohtsuka, S. Christine McLary, and Alfred L. Goldberg. 2004. "IGF-I Stimulates Muscle Growth by Suppressing Protein Breakdown and Expression of Atrophy-Related Ubiquitin Ligases, Atrogin-1 and MuRF1." *American Journal of Physiology. Endocrinology and Metabolism* 287(4): E591-601.

Saito, Yuki et al. 2020. "Exercise Enhances Skeletal Muscle Regeneration by Promoting Senescence in Fibro-Adipogenic Progenitors." *Nature Communications* 11(1): 889.

Salomon du Mont, Lucie et al. 2017. "Impact of Nutritional State on Critical Limb Ischemia Early Outcomes (DENUCRITICC Study)." *Annals of Vascular Surgery* 45: 10–15.

dos Santos, Leandro et al. 2017. "Sarcopenia and Physical Independence in Older Adults: The Independent and Synergic Role of Muscle Mass and Muscle Function." *Journal of Cachexia, Sarcopenia and Muscle* 8(2): 245–50.

Schiaffino, Stefano, and Carlo Reggiani. 2011. "Fiber Types in Mammalian Skeletal Muscles." *Physiological Reviews* 91(4): 1447–1531.

Schieber, Molly N. et al. 2019. "Supervised Walking Exercise Therapy Improves Gait Biomechanics in Patients with Peripheral Artery Disease." *Journal of Vascular Surgery*.

Scisciola, Lucia et al. 2021. "Sarcopenia and Cognitive Function: Role of Myokines in Muscle Brain Cross-Talk." *Life* 11(2): 173.

Sepúlveda-Loyola, Walter et al. 2020. "Diagnosis, Prevalence, and Clinical Impact of Sarcopenia in COPD: A Systematic Review and Meta-Analysis." *Journal of Cachexia, Sarcopenia and Muscle* 11(5): 1164–76.

Serrano, Antonio L., and Pura Muñoz-Cánoves. 2010. "Regulation and Dysregulation of Fibrosis in Skeletal Muscle." *Experimental Cell Research* 316(18): 3050–58.

Shafiee, Gita et al. 2017. "Prevalence of Sarcopenia in the World: A Systematic Review and Meta- Analysis of General Population Studies." *Journal of Diabetes and Metabolic Disorders* 16: 21.

Shen, Mei, Jing Gao, Jianan Li, and Juan Su. 2009. "Effect of Stimulation Frequency on Angiogenesis and Gene Expression in Ischemic Skeletal Muscle of Rabbit." *Canadian Journal of Physiology and Pharmacology* 87(5): 396–401.

Shimazoe, Hirofumi, Shinsuke Mii, Yasuhiro Koyanagi, and Masaru Ishida. 2019. "Impact of Low Activity of Daily Living on the Prognosis of Patients with Critical Limb Ischemia and Sarcopenia." *Annals of Vascular Surgery* 61: 156–64.

Shono, Jun-ichi et al. 2013. "Preliminary Time-Course Study of Antiinflammatory Macrophage Infiltration in Crush-Injured Skeletal Muscle." *Animal Science Journal = Nihon Chikusan Gakkaiho* 84(11): 744–50.

Sieber, Cornel C. 2019. "Malnutrition and Sarcopenia." *Aging Clinical and Experimental Research* 31(6): 793–98.

Simon, F. et al. 2018. "Pathophysiology of Chronic Limb Ischemia." *Gefasschirurgie* 23(Suppl 1): 13–18.

Škarabot, Jakob et al. 2021. "The Knowns and Unknowns of Neural Adaptations to Resistance Training." *European Journal of Applied Physiology* 121(3): 675–85.

Snijders, Tim, Lex B. Verdijk, and Luc J. C. van Loon. 2009. "The Impact of Sarcopenia and Exercise Training on Skeletal Muscle Satellite Cells." *Ageing Research Reviews* 8(4): 328–38.

Soga, Yoshimitsu et al. 2014. "Two-Year Life Expectancy in Patients with Critical Limb Ischemia." *JACC. Cardiovascular interventions* 7(12): 1444–49.

Son, Jyung Mean, and Changhan Lee. 2021. "Aging: All Roads Lead to Mitochondria." *Seminars in Cell & Developmental Biology* 116: 160–68.

Steffl, Michal, Jan Sima, Kate Shiells, and Iva Holmerova. 2017. "The Increase in Health Care Costs Associated with Muscle Weakness in Older People without Long-Term Illnesses in the Czech Republic: Results from the Survey of Health, Ageing and Retirement in Europe (SHARE)." *Clinical Interventions in Aging* 12: 2003–7.

Svanberg, E. et al. 1996. "Role of Insulin and IGF-I in Activation of Muscle Protein Synthesis after Oral Feeding." *The American Journal of Physiology* 270(4 Pt 1): E614-620.

Tang, Gale L. et al. 2005. "The Effect of Gradual or Acute Arterial Occlusion on Skeletal Muscle Blood Flow, Arteriogenesis, and Inflammation in Rat Hindlimb Ischemia." *Journal of Vascular Surgery* 41(2): 312–20.

Taniguchi, Ryosuke, Juno Deguchi, Takuya Hashimoto, and Osamu Sato. 2019. "Sarcopenia as a Possible Negative Predictor of Limb Salvage in Patients with Chronic Limb-Threatening Ischemia." *Annals of Vascular Diseases* 12(2): 194–99.

Tanner, Ruth E. et al. 2015. "Age-Related Differences in Lean Mass, Protein Synthesis and Skeletal Muscle Markers of Proteolysis after Bed Rest and Exercise Rehabilitation." *The Journal of Physiology* 593(18): 4259–73.

Taşoğlu, İrfan et al. 2014. "Neutrophil-Lymphocyte Ratio and the Platelet-Lymphocyte Ratio Predict the Limb Survival in Critical Limb Ischemia." *Clinical and Applied Thrombosis/Hemostasis: Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis* 20(6): 645–50.

Tipton, Kevin D., D. Lee Hamilton, and Iain J. Gallagher. 2018. "Assessing the Role of Muscle Protein Breakdown in Response to Nutrition and Exercise in Humans." *Sports Medicine (Auckland, N.z.)* 48(Suppl 1): 53–64.

Tsutaki, Arata et al. 2013. "Effect of Intermittent Low-Frequency Electrical Stimulation on the Rat Gastrocnemius Muscle." *BioMed Research International* 2013: e480620.

Uzun, Fatih et al. 2017. "Usefulness of the Platelet-to-Lymphocyte Ratio in Predicting Long-Term Cardiovascular Mortality in Patients with Peripheral Arterial Occlusive Disease." *Postepy W Kardiologii Interwencyjnej = Advances in Interventional Cardiology* 13(1): 32–38.

Verdijk, Lex B. et al. 2009. "Skeletal Muscle Hypertrophy Following Resistance Training Is Accompanied by a Fiber Type–Specific Increase in Satellite Cell Content in Elderly Men." *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 64A(3): 332–39.

Virkkunen, Jyrki et al. 2004. "Diabetes as an Independent Risk Factor for Early Postoperative Complications in Critical Limb Ischemia." *Journal of Vascular Surgery* 40(4): 761–67.

Vun, Simon Vui et al. 2016. "The Effect of Supervised Exercise Therapy for Intermittent Claudication on Lower Limb Lean Mass." *Journal of Vascular Surgery* 64(6): 1763–69.

Wang, Qi et al. 2017. "Neutrophil-to-Lymphocyte Ratio Is Effective Prognostic Indicator for Post-Amputation Patients with Critical Limb Ischemia." *Saudi Medical Journal* 38(1): 24–29.

Waters, Richard E., Ronald L. Terjung, Kevin G. Peters, and Brian H. Annex. 2004. "Preclinical Models of Human Peripheral Arterial Occlusive Disease: Implications for Investigation of Therapeutic Agents." *Journal of Applied Physiology (Bethesda, Md.: 1985)* 97(2): 773–80.

Wei, Yuda et al. 2016. "Prevention of Muscle Wasting by CRISPR/Cas9-Mediated Disruption of Myostatin In Vivo." *Molecular Therapy* 24(11): 1889–91.

Yeung, Suey S.Y. et al. 2019. “Sarcopenia and Its Association with Falls and Fractures in Older Adults: A Systematic Review and Meta-analysis.” *Journal of Cachexia, Sarcopenia and Muscle* 10(3): 485–500.

Yilmaz, Seyhan et al. 2017. “Augmentation of Arterial Blood Velocity with Electrostimulation in Patients with Critical Limb Ischemia Unsuitable for Revascularization.” *Vascular* 25(2): 137–41.

Yin, Hang, Feodor Price, and Michael A. Rudnicki. 2013. “Satellite Cells and the Muscle Stem Cell Niche.” *Physiological Reviews* 93(1): 23–67.

Zhang, Xiaoming et al. 2020. “Falls among Older Adults with Sarcopenia Dwelling in Nursing Home or Community: A Meta-Analysis.” *Clinical Nutrition* 39(1): 33–39.

Zhang, Yan et al. 2021. “Sarcopenia in Heart Failure: A Systematic Review and Meta-Analysis.” *ESC Heart Failure* 8(2): 1007–17.

Zoico, Elena et al. 2010. “Adipose Tissue Infiltration in Skeletal Muscle of Healthy Elderly Men: Relationships With Body Composition, Insulin Resistance, and Inflammation at the Systemic and Tissue Level.” *The Journals of Gerontology: Series A* 65A(3): 295–99.

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Physiopathologie et traitement de la sarcopénie au cours de l'ischémie critique chronique des membres inférieurs

Résumé

La sarcopénie est caractérisée par une diminution de la force et de la masse musculaire, parfois associée à une faible performance physique. Elle peut toucher les patients atteints d'ischémie critique chronique (ICC) mais est souvent peu diagnostiquée, voire négligée, puisque les lésions liées à l'ICC sont au premier plan. Néanmoins, la sarcopénie associée à l'ICC est un facteur de mauvais pronostic, que ce soit en termes de survie ou de sauvetage de membre. Chez l'Homme, nous avons identifié le ratio inflammatoire plaquettes/lymphocytes comme marqueur simple et rapide de sarcopénie chez les patients atteints d'ICC, ces patients devant alors être considérés à haut-risque chirurgical, du fait de leur mauvais pronostic post-opératoire. Cependant, sa faible sensibilité limite son utilisation en routine.

L'étude de la sarcopénie dans notre modèle murin d'ICC nous a permis de mieux comprendre les mécanismes physiopathologiques sous-jacents, à savoir une fibrose musculaire, une altération des voies de dégradation des protéines musculaires, ainsi qu'une augmentation des paramètres inflammatoires, et de mettre en place un protocole d'exercice visant à protéger le muscle squelettique sarcopénique chez la souris. L'exercice de faible intensité est ainsi une approche thérapeutique protectrice permettant de réduire l'atrophie et la fibrose musculaire, et de restaurer la force et la capacité de marche dans notre modèle murin. Il serait intéressant d'étudier si un protocole d'exercice de faible intensité chez l'Homme pourrait améliorer voire reverser les effets délétères de la sarcopénie.

Mots-clés : Ischémie critique chronique, sarcopénie, muscle, fibrose, exercice

Abstract

Sarcopenia is characterized by a decrease in muscle strength and mass, sometimes associated with low physical performance. It can affect patients with chronic limb-threatening ischemia (CLTI) but it is often undiagnosed or even overlooked, since CLTI-related lesions are prominent. Nevertheless, sarcopenia associated with CLTI is a factor of poor prognosis, either in terms of survival or limb salvage. In humans, we have identified the inflammatory platelet/lymphocyte ratio as a simple and rapid marker of sarcopenia in patients with CLTI, these patients should then be considered at high surgical risk, due to their poor postoperative prognosis. However, its low sensitivity limits its routine use.

The study of sarcopenia in our mouse model of CLTI allowed us to better understand the underlying pathophysiological mechanisms, namely muscle fibrosis, alteration of muscle protein degradation pathways, as well as increase in inflammatory parameters, and to set up an exercise protocol aimed at protecting sarcopenic skeletal muscles in mice. Low-intensity exercise is thus a protective therapeutic approach to reduce muscle atrophy and fibrosis, and to restore strength and walking ability in our model. It would be interesting to study whether a low-intensity exercise regimen in humans could improve or even reverse the deleterious effects of sarcopenia.

Keywords: chronic limb-threatening ischemia, sarcopenia, muscle, fibrosis, exercise