Skeletal muscle mitochondrial clearance

Valorisation

Societal relevance

Chronic obstructive pulmonary disease (COPD) is a lung disease characterized by irreversible airflow limitation in the lungs, and is currently the third leading cause of death in the world (1). Currently, there are almost **600.000 COPD patients in the Netherlands alone** (2), and **over 250 million patients worldwide** (3). Although COPD is primarily a lung disease, patients often suffer from skeletal muscle dysfunction of which the two major underlying impairments include muscle wasting and the loss of mitochondrial quantity. These impairments may also contribute to the development of co-morbidities such as diabetes or cardiovascular disease (4). Extrapulmonary impairments and comorbidities contribute to an increased mortality, decreased quality of life, and increased healthcare costs (4-6). Prevention or reversion of skeletal muscle impairments can therefore **contribute to increasing quality of life and decreasing COPD-related healthcare costs.**

The loss of skeletal muscle mitochondrial quantity in COPD patients suggests that these patients have an altered balance between mitochondrial biogenesis and mitochondrial clearance. The role of impaired mitochondrial biogenesis regulation has been reported in several previous studies (7-9), but mitochondrial clearance has hardly been studied in muscle of COPD patients to date. Therefore, research aimed to unravel the role of skeletal muscle mitochondrial clearance is essential to understand the development of mitochondrial impairments in skeletal muscle of these patients (10).

In this thesis, we studied the regulation of mitochondrial clearance in skeletal muscle of COPD patients. Moreover, we applied *in vivo* and *in vitro* experimental models to study the effect of several isolated COPD-related factors that may affect skeletal muscle oxidative capacity (11, 12). Our results show that molecular signalling indicative of mitochondrial clearance is present in skeletal muscle of COPD patients who suffer from loss of muscle oxidative capacity. Moreover, systemic inflammation, muscle disuse, recovery of muscle disuse, and iron deficiency can individually contribute to the molecular regulation and dysregulation of skeletal muscle mitochondrial clearance. As other chronic diseases (*e.g.* congestive heart failure and type 2 diabetes) are also characterised by at least some of these factors, and suffer from skeletal muscle mitochondrial impairments, the work presented in this thesis may translate to these diseases as well. Therefore, we identified mitochondrial clearance as a **potential therapeutic target** in disorders characterized by loss of skeletal muscle mitochondrial capacity. Furthermore, as **prevention of mitochondrial**

dysfunction could be preferred over the inhibition of mitochondrial clearance *per se*, we propose that **lifestyle modifications**, as well as **nutritional or physical interventions** targeting the upstream effectors (such as systemic inflammation, muscle disuse, or iron status) could prove beneficial as mitochondrial-clearance limiting and mitochondrial content-preserving therapies for a broad range of patients.

Non-scientific audience

As the presence of many of the above-mentioned factors, *i.e.* muscle disuse and iron deficiency, can be prevented by a healthy lifestyle, it is essential that we increase patients' and healthcare professionals awareness of their benefits. For example, skeletal muscle mitochondrial health greatly benefits from physical activity and a healthy diet. Moreover, since loss of skeletal muscle oxidative capacity already presents itself in mild-to-moderate COPD patients (9), the prevention of loss of oxidative capacity should already start in subjects at high risk for- or in an early stage of COPD.

Innovation

As stated above, several previous studies have reported changes in regulation of mitochondrial biogenesis in skeletal muscle of patients with COPD, and similar results were reported for patients with congestive heart failure and type 2 diabetes as well (7-9, 13, 14). Interestingly, our study investigating mitochondrial clearance in skeletal muscle of COPD patients, is one of the first to report a comprehensive set of protein and mRNA expression levels of markers for mitochondrial breakdown and autophagy in combination with measurements for markers of mitochondrial quantity (10, 15). Therefore, our current results **underline the importance to study mitochondrial homeostasis in a comprehensive approach**, targeting multiple mechanisms of mitochondrial quantity regulation, even in the absence of changes in mitochondrial quantity.

The identification of mitochondria in extracellular vesicles secreted by skeletal muscle, as described in chapter 7, is likely to have a **high impact on our view of skeletal muscle mitochondrial homeostasis**. The metabolic phenotype of skeletal muscle has been a popular research topic for many years, and has been studied in relation to many different diseases. Our observation that mitochondria can be secreted by skeletal muscle cells provides a **novel paradigm**

in skeletal muscle cell plasticity research where regulation of mitochondrial homeostasis is currently still regarded as a single-cell and intra-cellular process (16).

Our data describing secretion of mitochondria-containing extracellular vesicles from muscle cells, in combination with previous studies reporting that muscle cells are able to secrete extracellular vesicles and non-muscular cells are able to secrete mitochondria via extracellular vesicles (17-20), suggests that skeletal muscle mitochondrial clearance is regulated at the inter-cellular level, providing interesting new targets for future oxidative capacity preserving therapies. Moreover, our work opens the door to a highly relevant research question: "Are skeletal muscle cells able to reintegrate functioning mitochondria from their extracellular environment?". Although **highly speculative**, this line of research could provide several important improvements to skeletal muscle oxidative capacity boosting therapies. For example, it might be opportunistic to explore possibilities to boost mitochondrial biogenesis and mitochondrial-containing vesicle secretion in non-skeletal muscle cells, which could subsequently be incorporated into skeletal muscle. In addition, it might eventually be possible to generate a therapy in which patients are treated with elsewhere created intact mitochondria-containing vesicles which are incorporated into skeletal muscles, thus improving their oxidative capacity, contributing to increased exercise capacity and quality of life.

Since the pathways of mitochondrial homeostasis are greatly preserved in different tissues, it is likely that the fundamental knowledge concerning the initiation of mitochondria-containing vesicular excretion could be applied to other research disciplines as well. Especially studies on metabolic active tissues (*e.g.* cardiac muscle, liver, or brain) might benefit from knowledge obtained in the current thesis.

In conclusion, this thesis provides new insights in the etiology of chronic disease-driven muscle mitochondrial impairments and the putative role of mitophagy and mitochondrial clearance via vesicles secretion herein. These insights do not directly lead to new treatments at this moment, but are potentially important in generation of novel therapies aimed at muscle mitochondrial impairments in COPD and other chronic diseases.

References

1. WHO. Fact sheets: The top 10 causes of death 2018 2018 [Available from: http://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.
2. Volksgezondhiedszorg. Prevalentie en nieuwe gevallen van COPD 2018 [Available from: https://www.volksgezondheidenzorg.info/onderwerp/copd/cijfers-context/huidige-situatie#node-prevalentie-en-nieuwe-gevallen-van-copd.
3. WHO. Chronic obstructive pulmonary disease (COPD) 2018 [Available from: http://www.who.int/news-room/fact-sheets/detail/chronic-obstructive-pulmonary-disease-(copd).
4. Maltais F, Decramer M, Casaburi R, Barreiro E, Burelle Y, Debigare R, et al. An official American Thoracic Society/European Respiratory Society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2014;189(9):e15-62.
5. Mannino DM, Higuchi K, Yu TC, Zhou H, Li Y, Tian H, et al. Economic Burden of COPD in the Presence of Comorbidities. Chest. 2015;148(1):138-50.
6. Jansson SA, Backman H, Ronmark E, Lundback B, Lindberg A. Hospitalization Due to Co-Morbid Conditions is the Main Cost Driver Among Subjects With COPD-A Report From the Population-Based OLIN COPD Study. Copd. 2015;12(4):381-9.
7. Konokhova Y, Spendiff S, Jagoe RT, Aare S, Kapchinsky S, MacMillan NJ, et al. Failed upregulation of TFAM protein and mitochondrial DNA in oxidatively deficient fibers of chronic obstructive pulmonary disease locomotor muscle. Skeletal muscle. 2016;6:10.
8. Remels AH, Schrauwen P, Broekhuizen R, Willems J, Kersten S, Gosker HR, et al. Peroxisome proliferator-activated receptor expression is reduced in skeletal muscle in COPD. The European respiratory journal. 2007;30(2):245-52.
9. van den Borst B, Slot IG, Hellwig VA, Vosse BA, Kelders MC, Barreiro E, et al. Loss of quadriceps muscle oxidative phenotype and decreased endurance in patients with mild-to-moderate COPD. Journal of applied physiology. 2013;114(9):1319-28.
10. Guo Y, Gosker HR, Schols AM, Kapchinsky S, Bourbeau J, Sandri M, et al. Autophagy in locomotor muscles of patients with chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2013;188(11):1313-20.
11. Gosker HR, Lencer NH, Franssen FM, van der Vusse GJ, Wouters EF, Schols AM. Striking similarities in systemic factors contributing to decreased exercise capacity in patients with severe chronic heart failure or COPD. Chest. 2003;123(5):1416-24.
12. Gosker HR, Wouters EF, van der Vusse GJ, Schols AM. Skeletal muscle dysfunction in chronic obstructive pulmonary disease and chronic heart failure: underlying mechanisms and therapy perspectives. The American journal of clinical nutrition. 2000;71(5):1033-47.
13. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nature genetics. 2003;34(3):267-73.
14. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proceedings of the National Academy of Sciences of the United States of America. 2003;100(14):8466-71.

15. Kruse R, Vind BF, Petersson SJ, Kristensen JM, Hojlund K. Markers of autophagy are adapted to hyperglycaemia in skeletal muscle in type 2 diabetes. Diabetologia. 2015;58(9):2087-95.
16. Romanello V, Sandri M. Mitochondrial Quality Control and Muscle Mass Maintenance. Frontiers in physiology. 2015;6:422.
17. Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, et al. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nature communications. 2015;6:8472.
18. Falchi AM, Sogos V, Saba F, Piras M, Congiu T, Piludu M. Astrocytes shed large membrane vesicles that contain mitochondria, lipid droplets and ATP. Histochemistry and cell biology. 2013;139(2):221-31.
19. Forterre A, Jalabert A, Berger E, Baudet M, Chikh K, Errazuriz E, et al. Proteomic analysis of C2C12 myoblast and myotube exosome-like vesicles: a new paradigm for myoblast-myotube cross talk? PloS one. 2014;9(1):e84153.
20. Kim S, Lee MJ, Choi JY, Park DH, Kwak HB, Moon S, et al. Roles of Exosome-Like Vesicles Released from Inflammatory C2C12 Myotubes: Regulation of Myocyte Differentiation and Myokine Expression. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2018;48(5):1829-42.