LIST OF STUDIES

I. Study I: **Hansen C**, Olsen K, Pilegaard H, Bangsbo J, Gliemann L, Hellsten Y. High metabolic substrate load induces mitochondrial dysfunction in rat skeletal muscle microvascular endothelial cells. *Physiol Rep*. 2021;9:1–17.

II. Study II: Hansen C, Møller S, Wickham KA, Gliemann L, Hellsten Y. Treatment of microvascular endothelial cells with the mitochondria targeted antioxidant MitoQ enhances nitric oxide bioavailability and metabolism. 2021; *(In manuscript form, to be submitted mid december 2021)*

III. Study III: Møller, S.*, **Hansen, C. C.***, Ehlers, T. S., Tamariz-Ellemann, A., Tolborg, S. á R., Kurell, M. E., Pérez-Gómez, J., Patrzalek, S. S., Maulitz, C., Hellsten, Y., & Gliemann, L. Exercise training lowers arterial blood pressure independently of Pannexin-1 in men with essential hypertension. 2021. (*In review with Medicine & Science in Sports & Exercise*)

IV. Study IV: **Hansen C**, Møller S, Ehlers T, Wickham KA, Bangsbo J, Gliemann L, Hellsten Y. Redox balance in human skeletal muscle-derived endothelial cells - effect of exercise training. 2021 *(Free Radical Biology and Medicin, minor revision being undertaken)*

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ABSTRACT

Cardiovascular disease is highly prevalent and associated with the highest mortality rate in the Western world. Impaired function of endothelial cells, located on the inside of all blood vessels, is one of the main causes of cardiovascular disease. It has been proposed, that individuals with cardiovascular disease have impaired mitochondrial function and increased mitochondrial formation of reactive oxygen species (ROS), but evidence for this proposition is lacking. Enhanced ROS formation in endothelial cells is critical, as ROS readily react with and inactivate nitric oxide (NO), a key player in the control of vascular tone. Enhanced knowledge of mitochondrial function and ROS formation in cardiovascular disease is important for the molecular understanding of endothelial dysfunction and for the development of novel treatment strategies in cardiovascular disease. This thesis focuses on hypertension and addresses the role of mitochondria in endothelial dysfunction with a specific focus on redox balance, NO bioavailability and metabolism in skeletal muscle microvascular endothelial cells. The studies of this thesis include data on primary endothelial cells isolated from skeletal muscle of both rats and humans, as well as data from an *in-vivo* human study, in which the impact of regular exercise training was assessed. Moreover, to assess the role of mitochondria derived ROS for endothelial phenotype and function, cells were subjected to chronic treatment with the mitochondrial antioxidant, Mitoquinone mesylate (MitoQ).

Main findings in this thesis were that hypertension was associated with elevated mitochondrial ROS (***Study II*** and ***Study IV***) with an associated lower availability of NO in skeletal muscle microvascular endothelial cells (***Study II***). The higher ROS production from the mitochondria in human endothelial cells in patients with hypertension correlated with endothelial function determined *in vivo* by acetylcholine infusion (***Study III***). Finally, there was an improved redox balance and potential for prostaglandin synthesis with exercise training and with MitoQ treatment. These interventions were also associated with a reduction in glycolytic metabolism in the endothelial cells.

In conclusion, this thesis has for the first time established that, in hypertension, skeletal muscle derived microvascular endothelial cells present a higher mitochondrial ROS formation, lower antioxidant levels and a consequent lower NO bioavailability. Exercise training and treatment with MitoQ can ameliorate this redox imbalance. Future studies should provide further insight into the specific molecular signalling pathways underpinning these regulatory mechanisms.

DANISH RESUMÉ

Hjertekarsygdomme er den mest udbredte dødelige sygdom i den vestlige verden. En af hovedårsagerne til hjertekarsygdomme er nedsat funktion i cellerne placeret på indersiden af blodårerne, kaldet endothelcellerne. Funktionen af de mikrovasklære endothelceller bestemmes ved indgivelse af acetylkolin, et stof, der fører til endothelafhængig vasodilation. Det er foreslået, men endnu ikke vist, at mitokondriernes funktion nedsættes og en øget mængde frie radikaler udskilles ved forhøjet blodtryk, hvilket nedsætter signaleringsmolekylet nitrogenoxid, der har betydning for den vaskulære tone, og deraf forværre sygdomstilstanden. En forståelse af mitokondriernes funktion og en øget mængde af frie radikaler i endothelcelledysfunktion er vigtig, da den molekylære indsigt bidrager til potentielle nye behandlingsmuligheder i forhøjet blodtryk. Denne afhandling omhandler derfor forhøjet blodtryk samt en forståelse af mitokondriernes rolle til endothelcelledysfunktion med specifikt fokus på redoxbalance, nitrogenoxid biotilgængelighed og metabolisme i skeletmuskel mikrovaskulære endothelceller.

Nærværende studier viser at forhøjet blodtryk var associeret med forhøjet mitokondrielle frie radikaler (***Studie II*** and ***Studie IV***) og en konsekvent lavere biotilgængelighed af nitrogenoxid i skeletmuskel mikrovaskulære endothelceller (***Studie II***). Den højere produktion af frie radikaler fra mitokondrierne fundet i humane endothelceller fra patienter med forhøjet blodtryk var sammenlignelig med endothelfunktionen fundet ved acetylkolin infusion *in-vivo* i de samme patienter (***Studie III***). Afslutningsvis, fandt vi et forbedret potentiale for prostaglandin syntese og redox tilstand med MitoQ behandling sammenlignelig med effekterne ved højintenst træning, hvilket var associeret til en reducering i glykolytisk metbaolisme i mikrovaskulære endothelceller.

Sammenholdt er det vist for første gang i nærværende afhandling, at mikrovaskulær endothelcelledysfunktion fra skeletmuskel, fundet i forbindelse forhøjet blodtryk, leder til en øget produktion af frie radikaler fra mitokondrierne, der tilmed resulterede i en lavere biotilgængelighed af nitrogenoxid. Træning og behandling med MitoQ anitoxidant kan forbedre denne redox ubalance i mikrovaskulære endothelceller. Den specifikke molekylære signaleringsvej mellem disse reguleringsmekanismer, skal fremtidige studier dog belyse.

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