LIST OF PAPERS

This thesis is based on the present review and on selected work included in the listed papers.

Published or in manuscript form

I. **Rytter N**, Carter H, Piil P, Sørensen H, Ehlers T, Holmegaard F, Tuxen C, Jones H, Thijssen D, Gliemann L & Hellsten Y. Ischemic preconditioning improves microvascular endothelial function in remote vasculature by enhanced prostacyclin production. *In revesion, JAHA*.

II. Gliemann L*, **Rytter N***, Liu, Y, Tamariz-Elleman A & Hellsten Y. A high activity level is required for augmented muscle capillarization in aged women. *Submitted and under consideration, MSSE*.

* the authors contributed equally to the manuscript

Manuscripts in preparation

Due to major difficulties in recruitment of patients for Study III and IV, the initiation of the studies was substantially delayed and later affected by the Covid-19 health crisis. Consequently, some of the data and analysis is lacking and the manuscripts are therefore not finalised. Study V concerns proteome profiling of platelets collected in Study I, though a manuscript is being drafted but is not in a form which can be included separately. These three studies are presented in abbreviated versions as part of the study summaries. Publications for these three studies are planned and publication agreements exist.

III. **Rytter N**, Christoffersen L, Lawrence M, Olsen L, Gliemann L, Evans A, Kruuse C & Hellsten Y. The effect of two weeks of remote ischemic preconditioning on fractal dimension of incipient blood clots in stroke patients: a pilot trial.

IV. **Rytter N**, Lawrence M, Christoffersen L, Kruuse C, Hellsten Y & Evans A. Acute exercise adversely affects clot microstructure of incipient blood clots in stroke patients.

V. **Rytter N**, Beck H, Bjerre N, Carter H, Gliemann L, Rasmussen L & Hellsten Y. The effect of ischemic preconditioning on the proteome profile of platelets and platelet releasate in humans.

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TABLE OF CONTENTS

ABSTRACT

Microvascular dysfunction and specifically impairments in the function of vascular endothelium is intrinsic to cardiovascular events and a hallmark in the development of lifestyle related diseases. Several therapeutic strategies are known to positively influence the microvasculature locally, although their remote effects remain unresolved. Here, the innovative procedure of ischemic preconditioning was studied in conjunction with the proven approach of exercise. The objective of the present thesis was to study remote microvascular function, blood clot formation and intercellular crosstalk mediated by platelets in the human vasculature.

The studies of the present thesis included young healthy participants, aged women with different training histories and a group of patients recently diagnosed with stroke. This combination allowed for investigation of mechanisms of action related to remote functional adaptations and intercellular crosstalk mediated by platelets and provided a clinical perspective by elucidating the effects of remote ischemic preconditioning and acute exercise on the novel biomarker of clot microstructure, which indicates the severity of blood clot formation. By arterial infusion of acetylcholine, it was demonstrated that ischemic preconditioning acutely enhanced microvascular function in remote vascular tissue, an effect that was localised to the endothelial cells and most likely driven by a potentiation of the prostacyclin system. These novel findings suggest that improved endothelial function plays an important role in promoting cardiovascular health with remote ischemic preconditioning. In stroke patients, remote ischemic preconditioning as well as acute exercise were found to transiently alter clot microstructure negatively towards denser blood clots. The negative effect of remote ischemic preconditioning was not seen in regard to the long term effect, where a two week period of remote ischemic preconditioning applied daily had no effect on clot microstructure. These important findings, which are of significant clinical relevance, indicate that both remote ischemic preconditioning and acute exercise actually may increase the risk of severe blood clot formation in stroke patients, and that these therapeutic strategies may not be appropriate in all pathological conditions. At last, it was confirmed that platelets play a role as intercellular messengers in the vasculature and evidence for a functional role in angiogenesis is provided. Acute ischemic preconditioning also markedly altered the proteome profile of platelets and stimulation with an agonist induced release of a substantial number of proteins, suggesting that platelets may be involved in multiple signalling pathways.

To conclude, the seminal findings of the present thesis provide evidence that ischemic preconditioning induces a potent stimulus which can lead to functional adaptations in remote microvasculature, namely vascular endothelium, and alter the expression of proteins in platelets. Importantly, the findings also imply that therapeutic strategies in pathological conditions, such as stroke, should be prescribed only on the basis of a thorough prior assessment of eligibility.

DANISH SUMMARY

Nedsat funktionsevne af det mikrovaskulære netværk, og især af de vaskulære endothelceller, er ofte en væsentlig del af hjertekar tilfælde og udviklingen af livsstilssygdomme. Flere behandlingsstrategier påvirker det mikrovaskulære netværk positivt, men deres systemiske effekter er ikke undersøgt. Heri undersøges en innovative procedure kendt som iskæmisk prækonditionering i sammenhold med en mere anerkendt strategi, nemlig fysisk aktivitet. Det overordnede formål med denne afhandling var at undersøge systemisk mikrovaskulære funktionsevne, blodpropdannelse og kommunikation mellem celler i kredsløbet.

Studierne i denne afhandling inkluderede unge raske forsøgspersoner, ældre kvinder med forskellige træningshistorik samt en gruppe patienter der alle for nyligt var diagnosticeret med apopleksi. Denne kombination tillod at undersøge underliggende mekanismer relateret til systemiske funktionelle adaptationer og celle-til-celle kommunikation induceret af blodplader. Desuden gav det afhandlingen et klinisk perspektiv ved at belyse de systemiske effekter af iskæmisk prækonditionering og fysisk aktivitet på en blodprops strukturelle opbygning, undersøgt ved en ny biomarkør som et udtryk for hvor farlig en blodprop er. Ved indgivelse af acetylkolin i et blodkar blev det påvist at iskæmisk prækonditionering forbedrer den mikrovaskulære funktionsevne systemisk, samt at denne effekt kan tilskrives et forbedret prostacyklin system i de vaskulære endothelceller. Disse vigtige fund indikerer at forbedret funktionsevne af de vaskulære endothelceller spiller en vigtig rolle i forbedret hjertekar sundhed induceret af iskæmisk prækonditionering. I apopleksi patienter blev det observeret at iskæmisk prækonditionering såvel som fysisk aktivitet akut og kortvarigt påvirkede den strukturelle opbygning af en blodprop negativt. Denne negative effekt blev ikke fundet ved langvarig brug af iskæmisk prækonditionering, demonstreret ved at to uger med daglig brug ikke ændrede på en blodprops struktur. Disse fund er højst relevante i et klinisk perspektiv, og indikerer at både iskæmisk prækonditionering og fysisk aktivitet rent faktisk kan øge risikoen for alvorlig blodpropdannelse i apopleksi patienter, hvorfor disse behandlingsstrategier måske slet ikke er tilrådelige i alle sygdomstilfælde. Til sidst blev det bekræftet at blodplader spiller en rolle i celle-til-celle kommunikation, at iskæmisk prækonditionering i udpræget grad påvirkede blodpladernes proteom, at stimulering af blodplader med en agonist inducerer frigivelse af adskillige proteiner og at blodplader har en funktionel betydning i kapillærvækst. Disse fund indikerer at blodplader muligvis er involveret i flere signaleringsveje.

Det kan konkluderes at de afgørende fund i denne afhandling bibringer evidens for at iskæmisk prækonditionering inducerer et stærkt stimulus som medfører funktionelle adaptationer systemisk i det mikrovaskulære netværk, især i de vaskulære endothelceller, samt ændrer på udtrykket af proteiner i blodpladerne. Af afgørende betydning, så indikerer disse fund også at visse behandlingsstrategier kun skal tilbydes på et nøje vurderet grundlag, givet den øgede risiko for alvorlig blodpropdannelse.

INTRODUCTION

Sufficient delivery of oxygen and nutrients is vital for all cells and thereby also for intact organ function in the human body. In healthy individuals, the cardiovascular system facilitates this important function securing adequate blood flow and perfusion of tissue and organs. The smaller arteries and arterioles are responsible for the regulation of vascular tone, which involves a complex integration of various stimuli in the endothelial cells that line the luminal surface of the blood vessels. This paradigm is termed microvascular function and is fundamental for intact organ function. Eventually, the arterioles terminate in the capillary network, which is the primary site for exchange of oxygen and nutrients and thus likewise essential in maintaining vascular homeostasis.

Impaired microvascular function is a hallmark of cardiovascular disease, including stroke. Impairments in microvascular function and the capillary network may lead to malperfusion and insufficient oxygen supply to tissue and organs. Moreover, impairments in vascular endothelium may also promote severe blood clot formation, which can render tissue and organs ischemic. Consequently, it is of significant clinical relevance to maintain microvascular function and prevent the formation of severe blood clots, given the close association with cardiovascular events and premature death.

A vast amount of evidence supports the beneficial role of exercise training in maintaining microvascular function and the capillary network. Intriguingly, the benefits of exercise training do not seem to be confined only to the contracting muscle and its surroundings, but also to remote vascular tissue. Remote ischemic preconditioning is the novel phenomenon whereby brief repeated bouts of ischemia and reperfusion applied in a distinct vascular tissue or organ confer global protection, rendering remote vascular tissue and organs resistant to ischemia reperfusion injury. To what extent exercise and ischemic preconditioning acutely leads to functional adaptations in remote microvascular tissue has yet to be resolved.

The beneficial and protective effect of ischemic preconditioning on vascular tissue has been found to be transferable, indicating that the peripheral signal is of humoral origin. Likely candidates mediating this remote effect could be circulating blood cells, extracellular vesicles or molecules freely dissolved in plasma. Of interest, platelets are known to carry and release signalling molecules with vasoactive properties and their involvement in the maintenance of microvascular function and capillary growth has been well described. Thus, in the present thesis a key focus was on platelets as intercellular messengers in the human vasculature.

Objective of the present thesis

The objective of the present thesis was to examine the functional adaptations induced acutely by ischemic preconditioning and exercise in remote microvasculature, and to identify mechanisms of intercellular crosstalk with a specific focus on the role of platelets as messengers in the human vasculature.

Hypotheses

The overall hypotheses of the present thesis were:

- Ischemic preconditioning and exercise enhance microvascular function acutely in remote vascular tissue.

- Acute and long term remote ischemic preconditioning reduces the risk of severe blood clot formation, whereas acute exercise transiently increases the risk of forming severe blood clots.

- Platelets carry and release signalling molecules and are significantly involved in ischemic preconditioning and angiogenesis.

Specific study aims and hypotheses

Study I

Aim. To determine the acute effect of ischemic preconditioning and hand-grip exercise on microvascular function in remote vascular tissue.

Hypothesis. Ischemic preconditioning and hand-grip exercise induces potent stimuli leading to acute enhancement in vasodilatory responsiveness to intra-arterial acetylcholine in remote vascular tissue.

Study II

Aim. To elucidate if lifelong exercise training in aged women determines the angiogenic potential of platelets, herein circulating levels of VEGF and the proliferative effect of platelet releasate.

Hypothesis. The angiogenic potential of platelets are increased in very active women, as demonstrated by higher VEGF levels in platelets and an increased proliferative effect of platelet releaste.

Study III

Aim. To determine the effect of acute and long term remote ischemic preconditioning on clot microstructure in patients recently diagnosed with stroke.

Hypothesis. Both acute and long term remote ischemic preconditioning positively influences clot microstructure.

Study IV

Aim. To determine changes in clot microstructure acutely after cycling exercise in stroke patients.

Hypothesis. A single session of cycling exercise adversely affects clot microstructure in patients recently diagnosed with stroke.

Study V

Aim. To confirm that platelets carry and release signalling molecules and determine whether ischemic preconditioning changes the proteome profile of platelets.

Hypothesis. Platelets releases a substantial number of signalling molecules upon activation and ischemic preconditioning induces acute changes in the proteome profile of platelets.