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Abstract

Decrements in mitochondrial function and insulin sensitivity play primary roles in the decline in muscle function during aging and obesity. Exercise training (ET) opposes these deleterious effects by improving mitochondrial energetics and enhancing insulin action. Our understanding of the adaptive responses in skeletal muscle to ET has advanced due to various omic approaches; yet, the functional consequences of many players involved in these processes are not defined. In the present PhD study, I aimed to uncover new potential regulators of ET-mediated health benefits that were previously identified (1) to be regulated by ET: i) SRA stem-loop interacting RNA-binding protein (SLIRP) and ii) Traf2- and Nck interacting protein kinase (TNIK). In addition, mechanisms of actions of SLIRP and TNIK in skeletal muscle biology and the adaptive response to ET were evaluated.

We integrated exercise or diet-induced obesity studies of transgenic mice, *Drosophila melanogaster*, and humans with a multitude of metabolic and biochemical analyses to functionally characterize the role of SLIRP and TNIK in mitochondrial function and metabolic regulation. SLIRP was identified as a pivotal regulator of mitochondrial structure and function in skeletal muscle **(Study I)**, and TNIK as a crucial regulator of glucose and lipid metabolism on a whole-body level **(Study II)**. Genetic ablation of SLIRP led to defects in muscle mitochondrial structure, respiration, mitochondrial mRNA pools in mice, and reduced life span in flies. Remarkably, voluntary ET by wheel running counteracted these mitochondrial defects elicited by the loss of SLIRP in mice. ET robustly upregulated SLIRP content in human skeletal muscle across various exercise modalities **(Study I)**. Global *Tnik* knockout (KO) increased spontaneous physical activity and protected mice against diet-induced obesity, peripheral insulin resistance, and hepatic steatosis **(Study II)**. The obesity-resistant phenotype of *Tnik* KO mice was primarily conferred by elevated physical activity, which enhanced muscle and fat insulin action and improved substrate handling. According to the Type 2 Diabetes knowledge portal, TNIK variants were strongly associated with body mass index, fasting glucose, and type 2 diabetes **(Study II)**.

The insights derived from my PhD project outline the complexity of adaptive responses of skeletal muscle and non-skeletal-muscle tissues, including liver and adipose tissue, in response to ET. My findings underscore ET as a frontline strategy against mitochondrial and metabolism-associated diseases and further suggest TNIK as a potential pharmacological target to inspire the conception of new therapeutic strategies against metabolism-associated diseases in obesity.

Resumé (Dansk)

Dysfunktionelle mitokondrier og nedsat insulinfølsomhed medvirker til en reduceret muskelfunktion hos ældre og overvægtige individer. Fysisk aktivitet (FA) modvirker disse skadelige virkninger ved at forbedre mitokondriefunktionen samt øge insulinfølsomhed. Vores forståelse af de molekylære ændringer, der finder sted efter FA er blevet bedre i takt med udviklingen af nye teknikker; dog er funktionen af en del af de proteiner, der indgår i mitokondrierne ikke kendt på nuværende tidspunkt. I dette ph.d.-projekt var formålet at beskrive funktionen af ikke-karakteriserede mitokondrielle proteiner involveret i de gavnlige sundhedsmæssige virkninger af FA. Vi undersøgte derfor funktionen af to proteiner, som allerede var vist reguleret af FA, men hvor funktionen var ukendt: i) SRA stam-loop interagerende RNA-bindende protein (SLIRP) og ii) Traf2 - og Nck-interagerende proteinkinase (TNIK). Ved hjælp af træningsstudier og diæt-inducerede fedmestudier af transgene mus, Drosophila melanogaster (bananfluer) og mennesker, karakteriserede vi rollen af SLIRP og TNIK i mitokondriel funktion og metabolisk sundhed. SLIRP blev identificeret som en vigtig regulator af mitokondriel struktur og funktion i skeletmuskulatur **(Studie I)**, hvor TNIK regulerede glukose og lipidmetabolisme på helkropsniveau **(Studie II)**. Global *Slirp* knockout (KO) i mus førte til defekter i den mitokondrielle struktur, nedsat respiration, samt reduceret mitokondrielle mRNA-pools i muskler hos mus, hvor global *Slirp* KO i fluer reducerede deres levetid. FA forhindrede disse mitokondrielle defekter i *Slirp* KO mus. Vi fandt endvidere at FA, på tværs af forskellige træningsmodaliteter i mennesker og mus, øgede proteinekspressionen af SLIRP i muskler **(Studie I)**. Global *Tnik* KO førte til øget spontan FA og beskyttede musene mod diætinduceret fedme, perifer insulinresistens og fedtophobning i leveren (steatose). Den fedme-resistente fænotype af *Tnik* KO-mus skyldes sandsynligt den øgede spontane FA, hvilket førte til en forbedret håndtering af substrater samt øget respons på insulin i fedt- og muskelvæv. Ifølge databasen *Type 2 Diabetes knowledge portal* var flere TNIK-varianter stærkt forbundet med BMI, fasteglukose og type 2-diabetes **(Studie II)**.

Samlet giver resultaterne fra mit ph.d.-projekt ny indsigt i kompleksiteten af de adaptationer som sker i både skeletmuskulaturen samt lever og fedtvæv, som tilpasning til øget FA og fremhæver betydningen og funktion af to indtil nu ukarakteriserede proteiner. Mine resultater fremhæver FA som en vigtig del at behandlingen mod sygdomme der er associeret med metaboliske og mitokondrielle dysfunktioner. Ydermere antyder vores data, at TNIK er et farmakologisk mål som måske kan bruges i fremtidens behandlingsstrategier mod fedme og metabolisk syndrom.

Publication list during the PhD

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Study I: **Pham, T. C. P.** et al. The mitochondrial mRNA stabilizing protein, SLIRP, controls mitochondrial function in skeletal muscle via mechanisms that can be circumvented by exercise. 2022. (In manuscript form, to be submitted to PNAS in October 2022)

Study II: **Pham, T. C. P.** et al. Cross-species comparative analysis identifies TNIK as a regulator of glucose and lipid metabolism in obesity. 2022. (In manuscript form, to be submitted to Nature Metabolism in September 2022)

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Co-author

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