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Abstract

Remaining physically active during the entire life span can reduce the risk of developing metabolic diseases and increase quality of life. The beneficial effects of a high physical activity level are found throughout the body. During intense physical activity, skeletal muscle contractions induce a profound array of molecular signaling within the muscle fibers. By illuminating these molecular signaling events, the basis for development of pharmacological therapeutics aiming at initiating the same molecular mechanisms in skeletal muscle would be made and could presumably prevent or treat metabolic diseases. AMP-activated protein kinase (AMPK), a ubiquitously expressed heterotrimeric protein kinase, is activated in human skeletal muscle during exercise and regulates central processes within muscle metabolism. AMPK exists in many forms, but the exercise-responsive AMPKα2β2γ3 variant is only found in skeletal muscle. In this thesis, the aim was to investigate the role of AMPKγ3 and the AMPK heterotrimer that includes it for the regulation of skeletal muscle glucose metabolism, insulin action and AMPK activity.

Through studies of isolated muscle from AMPKγ3 knockout (KO) mice, it was shown that pharmacological activation of AMPKα2β2γ3 is sufficient to increase muscle glucose uptake. However, in contrast to our hypothesis, heterotrimers carrying AMPKγ1 also regulates glucose uptake in skeletal muscle. Contractions induced by electrical stimulation of mouse muscles *in situ* were only sufficient to increase insulin-stimulated glucose uptake in skeletal muscle from wild-type (WT) and not AMPKγ3 KO mice. Hence, AMPKγ3 is necessary for the regulation of skeletal muscle insulin sensitivity induced by prior contractions. The effect of a mutation in AMPKγ3 on skeletal muscle glycogen levels and AMPK activity was investigated in muscle from human and mouse carriers. In contrast to our hypothesis, glycogen levels were comparable between genotypes in both species, while AMPKα2β2γ3 activation was decreased in the carriers compared to control/WT.

Collectively, this thesis has illuminated important regulatory functions of AMPKα2β2γ3 in skeletal muscle metabolism. The conclusions of the thesis as a whole indicate that direct activation of this kinase by pharmacological means would presumably improve the metabolic health of skeletal muscle in several ways including the regulation of glucose uptake and insulin sensitivity.

Resumé

Ved at bibeholde et højt niveau af fysisk aktivitet over hele livet kan risikoen for udvikling af metaboliske sygdomme reduceres, hvilket medfører en øget livskvalitet. De gavnlige virkninger af fysisk aktivitet ses i alle dele af kroppen. Når skeletmuskler kontraheres, under intens fysisk aktivitet, igangsættes en bred vifte af molekylære signalering i muskelfiberen. Ved at belyse og beskrive disse molekylære reaktioner der forløber under fysisk aktivitet, vil man danne grundlaget for udvikling af farmakologiske behandlinger der igangsætter de samme molekylære reaktioner i skeletmuskler forhåbentlig bedring af den metaboliske sundhed til følge.

AMP-aktiveret protein kinase (AMPK), en heterotrimerisk proteinkinase der udtrykkes i alle kroppens celler, aktiveres i human muskulatur under fysisk aktivitet. AMPK findes i mange isoformer, men AMPKα2β2γ3, der kun findes i skeletmuskulatur, aktiveres i særligt høj grad under muskelkontraktioner. Denne afhandling undersøger vigtigheden af AMPKγ3 for reguleringen af glukosemetabolisme, virkningen af insulin samt AMPK-aktivitet generelt i skeletmuskulatur.

Ved at studere isolerede muskler fra AMPKγ3 knockout (KO) mus, blev det demonstreret at farmakologisk aktivering af AMPKα2β2γ3 kan øge skeletmusklers glukoseoptagelse. I modsætning til vores hypotese synes denne evne ikke at være isoleret til denne kinase, da AMPKγ1-komplekser også kan regulere glukoseoptagelsen.

Som følge af kontraktioner af skeletmuskler i mus *in situ* øges insulinfølsomheden kun i skeletmuskler fra vildtype (WT) mus og ikke i muskler fra AMPKγ3 KO mus. Dette indikerer at AMPKγ3 er nødvendig for reguleringen af musklers insulinfølsomhed under muskelkontraktioner.

Effekten af en mutation i AMPKγ3 på musklernes glykogennvieauer samt aktiviteten af AMPK generelt blev undersøgt i muskler fra mennesker samt mus. I modsætning til vores hypotese, viste det sig at glykogenniveauerne var uændrede mens aktiviteten af AMPKα2β2γ3 var nedsat i bærere af mutationen sammenlignet med kontrol/WT-niveauer.

Denne afhandling har således belyst den regulatoriske funktion af AMPKα2β2γ3 for skeletmusklers metabolisme. Afhandlingens konklusioner indikerer at direkte aktivering med farmakologiske præparater højest sandsynligt vil forbedre den metaboliske sundhed i mennesker og at dette bl.a. vil være grundet regulering af skeletmusklers glukoseoptagelse og insulinfølsomhed.

List of manuscripts

The current PhD thesis is based on three original manuscripts, one published and two ready for submission. Furthermore, references are made to unpublished observations from the Wojtaszewski group.

The manuscripts will be referred to as Study 1-3 throughout the thesis.

1. **Nicolas O. Jørgensen***, Rasmus Kjøbsted, Magnus R. Larsen, Jesper B. Birk, Nicoline R. Andersen, Bina Albuquerque, Peter Schjerling, Russell Miller, Christian K. Pehmøller, Jørgen F. P. Wojtaszewski. *Direct small molecule ADaM-site AMPK activators reveal an AMPKγ3-independent mechanism for blood glucose lowering.* Published in Molecular Metabolism, 2021, 51, 1-13.

2. **Nicolas O. Eskesen**, Rasmus Kjøbsted, Kohei Kido, Nicoline R. Andersen, Jesper B. Birk, Russell Miller, Christian K. Pehmøller, Jørgen F. P. Wojtaszewski. *AMPKα2β2γ3 activation is necessary for contraction-induced insulin sensitization of skeletal muscle.* Ready for submission.

3. **Nicolas O. Eskesen**, Rasmus Kjøbsted, Jesper B. Birk, Nicolas S. Henriksen, Nicoline R. Andersen, Stine Ringholm, Henriette Pilegaard, Russell Miller, Christian K. Pehmøller, Jørgen F. P. Wojtaszewski. *AMPKγ3 R225W KI mutation disrupts basal activity as well as AICAR- and contraction-induced activation of AMPKα2β2γ3 in skeletal muscle.* Ready for submission.

*Changed last name, due to marriage in 2021.