Table of contents

1. Abstract

Type 2 diabetes is a major global health concern, with skeletal muscle insulin resistance being an early and important hallmark. In healthy muscle, insulin stimulates glucose transporter 4 (GLUT4) translocation to the sarcolemma and T-tubules to enable glucose uptake. This process is impaired in insulin resistance, and the precise trafficking defects underlying this impairment remain poorly defined, particularly in adult skeletal muscle. Moreover, methodological limitations have hindered not only the direct visualization of endogenous GLUT4 in its native compartments within mature muscle fibers, but also the progression of our understanding of GLUT4 trafficking defects in health and disease. To overcome some of these challenges, this PhD project aimed to develop imaging strategies to investigate GLUT4 translocation and subcellular localization in skeletal muscle, with particular focus on application to human muscle and with future potential for solving outstanding questions in insulin resistance.

In Study 1, we established and validated an antibody-based microscopy method targeting exofacial GLUT4 epitopes to detect endogenous GLUT4 translocation in rodent and human skeletal muscle. Using this approach, we confirmed insulin- and exercise-stimulated GLUT4 translocation in human muscle, as well as impaired response in insulin-resistant muscle, providing a powerful tool for studying endogenous GLUT4.

In Study 2, we applied proximity ligation assay (PLA) to resolve compartment-specific GLUT4 localization in rodent and human muscle. Our findings suggest that insulin resistance is associated with reduced GLUT4 storage vesicle content, while insulin, exercise, and AMPK activation promoted GLUT4 proximity to T-tubules. Notably, we demonstrated that TBC1D4 is required for GLUT4 translocation to the T-tubule system, highlighting a previously underappreciated role for this signaling node in regulating compartmentalized GLUT4 trafficking.

A third study developed a correlative light and electron microscopy (CLEM) workflow in human skeletal muscle and mature cardiomyocytes to achieve ultrastructural mapping of GLUT4 subcellular regions of interest. This method enabled visualization of GLUT4-positive vesicles in both perinuclear and peripheral regions at nanoscale resolution, offering a platform for future ultrastructural studies of protein localization in adult muscle.

In conclusion, this PhD project demonstrates that endogenous GLUT4 translocation to the sarcolemma and T-tubules can be directly detected with high resolution in adult skeletal muscle, revealing impairments in insulin-resistant muscle and providing alternatives to prior indirect methods. The findings suggest that insulin resistance is associated with GLUT4 mislocalization, as captured by our compartment-specific detection strategies, while the establishment of an optimized CLEM workflow enables the resolution of GLUT4 localization at the nanoscale level in mature muscle. Together, these advances lay a foundation for future mechanistic insights into GLUT4 dynamics in adult muscle across the health-disease spectrum.

2. Resumé

Type 2-diabetes udgør en alvorlig global sundhedsudfordring, hvor insulinresistens i skeletmuskulaturen er et tidligt og centralt kendetegn. I rask muskel stimulerer insulin translokation af glukosetransporteren GLUT4 til sarkolemma og T-tubuli, hvilket muliggør glukoseoptag. Denne proces er nedsat ved insulinresistens, men de præcise defekter i GLUT4-trafikken, der ligger til grund herfor, er fortsat dårligt belyst - særligt i moden skeletmuskel. Derudover har metodemæssige begrænsninger ikke blot hæmmet den direkte visualisering af endogent GLUT4 i dets naturlige kontekst i modne muskelfibre, men også bremset fremskridt i vores forståelse af GLUT4 dynamikker i både sundhed og sygdom. For at imødegå disse udfordringer havde dette ph.d.-projekt til formål at udvikle billeddannelsesmetoder til at undersøge GLUT4-translokation og subcellulær lokalisering i skeletmuskel med særligt fokus på human muskel og med potentiale for at adressere centrale ubesvarede spørgsmål inden for insulinresistens.

I Studie 1 etablerede og validerede vi en antistofbaseret mikroskopimetode, der retter sig mod ekstracellulære epitoper på GLUT4, for at detektere endogen GLUT4-translokation i både gnaver- og human skeletmuskel. Ved hjælp af denne metode kunne vi bekræfte, at insulin og fysisk aktivitet stimulerer GLUT4-translokation i moden muskel, mens responsen var nedsat i insulinresistent muskel. Metoden udgør dermed et værdifuldt værktøj til studier af endogent GLUT4 i moden muskel.

I Studie 2 anvendte vi proximity ligation assay (PLA) til at kortlægge GLUT4-lokalisering i specifikke subcellulære områder i gnaver- og menneskemuskel. Vores resultater viste, at insulinresistens er forbundet med reduceret indhold af GLUT4-lagringsvesikler, mens insulin, akut arbejde og AMPK-aktivering fremmer GLUT4-nærhed til T-tubuli-områder. Desuden demonstrerede vi, at TBC1D4 er nødvendig for GLUT4-translokation til T-tubuli-systemet, hvilket fremhæver en tidligere undervurderet rolle for denne signaleringskomponent i reguleringen af GLUT4-trafikken.

I det tredje studie udviklede vi en metode til korrelativ lys- og elektronmikroskopi (CLEM) i modne hjerteceller med henblik på ultrastrukturel kortlægning af GLUT4-kompartmenter. Ved hjælp af denne metode lykkedes det os at visualisere GLUT4-positive vesikler i både perinukleære og perifere områder, hvilket giver nanoskalaoversigt af GLUT4-lokalisering og skaber et fundament for fremtidige ultrastrukturelle studier i skeletmuskel.

Sammenfattende viser dette ph.d.-projekt, at endogen GLUT4-translokation til sarkolemma og T-tubuli kan detekteres direkte og med høj opløsning i voksen skeletmuskel, hvorved vi påviser defekter i insulinresistent muskel og tilbyder alternative værktøjer til tidligere indirekte metoder. Vores resultater antyder, at insulinresistens er forbundet med GLUT4-mislokalisering, som opfanget med vores specifikke detektionsstrategier, og at etableringen af en optimeret CLEM-metode muliggør visualisering af GLUT4-trafikdefekter på nanoskalaniveau i moden muskulatur. Samlet set lægger disse fremskridt grundlaget for fremtidige mekanistiske indsigter i GLUT4-dynamik i moden muskel på tværs af sundhed og sygdom.

3. Enclosed manuscripts

This thesis is based on the two manuscripts listed below, along with additional observations throughout. A third, ongoing study focuses on establishing an optimized correlative light-electron microscopy workflow for adult muscle and is presented prior to the manuscripts at the end of this thesis.

Study 1:

Kaspar W. Persson[1], Casper Fjeldsøe[1], Lukas W. Frandsen[1], Jonas B. Roland[1], SeongEun Kwak[2], Haiyan Wang[2], Christian T. Voldstedlund[1], Magnus R. Leandersson[1], Carol Witzcak[3], Jørgen F.P. Wojtaszewski[1] Erik A. Richter[1], Gregory D. Cartee[2], Thomas E. Jensen[1]

Exofacial epitope-specific antibodies detect GLUT4 translocation in adult skeletal muscle

Manuscript prepared for submission

Study 2:

Kaspar W. Persson[1], Lukas W. Frandsen[1], Casper Fjeldsøe[1], SeongEun Kwak[2], Haiyan Wang[2], Jonas B. Roland[1], Joelina S. Lienau[1], Christian T. Voldstedlund[1], Roberto Meneses-Valdés[1], Samantha Gallero[1], Tianjiao Li[1], Nicolai S. Henriksen[1], Carol Witzcak[3], Erik A. Richter[1], Gregory D. Cartee[2], Thomas E. Jensen[1]

High-resolution proximity ligation detects TBC1D4-dependent GLUT4 translocation to T-tubules and suggests decreased GLUT4 storage vesicle content in insulin-resistant skeletal muscle

Manuscript prepared for submission

4. Publications not included in the thesis

*indicates shared first authorships

Muscle-Specific AXIN1 and 2 double knockout does not alter AMPK/mTORC1 signaling or glucose metabolism. **Persson, K. W.,** Meneses-Valdés, R., Andersen, N. R., Pedersen, F. S., Gallero, S., Hesselager, S. A., Henriquez-Olguin, C., Jensen, TE. *Revision in Journal of Physiology*

mTOR Ser1261 is an AMPK-dependent phosphosite in mouse and human skeletal muscle not required for mTORC2 activity. Li, J., Madsen, A. B., Knudsen, J. R., Henriquez-Olguin, C., **Persson, K. W.**, Li, Z., Raun, S. H., Li, T., Kiens, B., Wojtaszewski, J. F. P., Richter, E. A., Nogara, L., Blaauw, B., Ogasawara, R., & Jensen, T. E. (2025). *FASEB journal*, *39*(2), e70277. https://doi.org/10.1096/fj.202402064R

Revisiting insulin-stimulated hydrogen peroxide dynamics reveals a cytosolic reductive shift in skeletal. Henríquez-Olguín, C., Gallero, S., Reddy, A., **Persson, K. W.**, Schlabs, F. L., Voldstedlund, C. T., Valentinaviciute, G., Meneses-Valdés, R., Sigvardsen, C. M., Kiens, B., Chouchani, E. T., Richter, E. A., & Jensen, T. E. (2025). muscle. *Redox biology*, *82*, 103607. https://doi.org/10.1016/j.redox.2025.103607

Reducing the mitochondrial oxidative burden alleviates lipid-induced muscle insulin resistance in humans. Fiorenza, M., Onslev, J., Henríquez-Olguín, C., **Persson, K. W.,** Hesselager, S. A., Jensen, T. E., Wojtaszewski, J. F. P., Hostrup, M., & Bangsbo, J. (2024). *Science advances*, *10*(44), eadq4461. https://doi.org/10.1126/sciadv.adq4461

Unresolved questions in the regulation of skeletal muscle insulin action by reactive oxygen species. Gallero, S.*, **Persson, K. W.***, & Henríquez-Olguín, C. (2024). *FEBS letters*, *598*(17), 2145–2159. https://doi.org/10.1002/1873-3468.14937

Microtubule-mediated GLUT4 trafficking is disrupted in insulin-resistant skeletal muscle. Knudsen, J. R., **Persson, K. W.**, Henriquez-Olguin, C., Li, Z., Di Leo, N., Hesselager, S. A., Raun, S. H., Hingst, J. R., Trouillon, R., Wohlwend, M., Wojtaszewski, J. F. P., Gijs, M. A. M., & Jensen, T. E. (2023). *eLife*, *12*, e83338. https://doi.org/10.7554/eLife.83338

Exercise increases phosphorylation of the putative mTORC2 activity readout NDRG1 in human skeletal muscle. Knudsen, J. R., **Persson, K. W.,** Meister, J., Carl, C. S., Raun, S. H., Andersen, N. R., Sylow, L., Kiens, B., Jensen, T. E., Richter, E. A., & Kleinert, M. (2022). *American journal of physiology. Endocrinology and metabolism*, *322*(1), E63–E73. https://doi.org/10.1152/ajpendo.00389.2021