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Publications during the PhD which are not included in the thesis

Li, V.L., He, Y., Contrepois, K., Liu, H., Kim, J. T., Wiggenhorn, A. L., Tanzo, J. T., Tung, A. S., Lyu, X., Zushin, P. H., Jansen, R. S., Michael, B., Loh, K. Y., Yang, A. C., **Carl, C. S.**, Voldstedlund, C. T., Wei, W., Terrell, S. M., Moeller, B. C., Arthur, R. M., Wallis, G. A., Wetering, K. V. D., Stahl, A. Kiens, B., Richter, E. A. Banik, S. M., Snyder, M. P., Xu, Y., Long, J. Z. (2022). An exercise-inducible metabolite that suppresses feeding and obesity. **Nature.** 606, 785-790 https://doi.org/10.1038/s41586-022-04828-5

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). Exercise increases phosphorylation of the putative mTORC2 activity readout NDRG1 in human skeletal muscle. **American Journal of Physiology-Endocrinology and Metabolism**, 322(1). https://doi.org/10.1152/ajpendo.00389.2021

Fritzen, A. M., Domingo-Espín, J., Lundsgaard, A.-M., Kleinert, M., Israelsen, I., **Carl, C. S.**, Nicolaisen, T. S., Kjøbsted, R., Jeppesen, J. F., Wojtaszewski, J. F. P., Lagerstedt, J. O., & Kiens, B. (2020). ApoA-1 improves glucose tolerance by increasing glucose uptake into heart and skeletal muscle independently of AMPKα2. **Molecular Metabolism**, 35. https://doi.org/10.1016/j.molmet.2020.01.013

Lundsgaard, A.-M., Fritzen, A. M., Nicolaisen, T. S., **Carl, C. S.**, Sjøberg, K. A., Raun, S. H., Klein, A. B., Sanchez-Quant, E., Langer, J., Ørskov, C., Clemmensen, C., Tschöp, M. H., Richter, E. A., Kiens, B., & Kleinert, M. (2020). Glucometabolic consequences of acute and prolonged inhibition of fatty acid oxidation. **Journal of Lipid Research**, 61(1). https://doi.org/10.1194/jlr.RA119000177

Hansen, S. L., Bojsen-Møller, K. N., Lundsgaard, A.-M., Hendrich, F. L., Nilas, L., Sjøberg, K. A., Hingst, J. R., Serup, A. K., Olguín, C. H., **Carl, C. S.**, Wernblad, L. F., Henneberg, M., Lustrup, K. M., Hansen, C., Jensen, T. E., Madsbad, S., Wojtaszewski, J. F. P., Richter, E. A., & Kiens, B. (2020). Mechanisms underlying absent training-induced improvement in insulin action in lean, hyperandrogenic women with polycystic ovary syndrome. **Diabetes**, 69(11). https://doi.org/10.2337/db20-0062

Reddy, A., Bozi, L. H. M., Yaghi, O. K., Mills, E. L., Xiao, H., Nicholson, H. E., Paschini, M., Paulo, J. A., Garrity, R., Laznik-Bogoslavski, D., Ferreira, J. C. B., **Carl, C. S.**, Sjøberg, K. A., Wojtaszewski, J. F. P., Jeppesen, J. F., Kiens, B., Gygi, S. P., Richter, E. A., Mathis, D., & Chouchani, E. T. (2020). pH-Gated Succinate Secretion Regulates Muscle Remodeling in Response to Exercise. **Cell**, 183(1), 62-75.e17. https://doi.org/10.1016/j.cell.2020.08.039

Nicolaisen, T. S., Klein, A. B., Dmytriyeva, O., Lund, J., Ingerslev, L. R., Fritzen, A. M., **Carl, C. S.**, Lundsgaard, A. M., Frost, M., Ma, T., Schjerling, P., Gerhart-Hines, Z., Flamant, F., Gauthier, K., Larsen, S., Richter, E. A., Kiens, B., & Clemmensen, C. (2020). Thyroid hormone receptor α in skeletal muscle is essential for T3-mediated increase in energy expenditure. **FASEB Journal**, 34(11). https://doi.org/10.1096/fj.202001258RR

Larsen, I. S., Fritzen, A. M., **Carl, C. S.**, Agerholm, M., Damgaard, M. T. F., Holm, J. B., Marette, A., Nordkild, P., Kiens, B., Kristiansen, K., Wehkamp, J., & Jensen, B. A. H. (2019). Human Paneth cell α-defensin-5 treatment reverses dyslipidemia and improves glucoregulatory capacity in diet-induced obese mice. **American Journal of Physiology - Endocrinology and Metabolism**, 317(1). https://doi.org/10.1152/ajpendo.00019.2019

Kleinert, M., Clemmensen, C., Sjøberg, K. A., **Carl, C. S.**, Jeppesen, J. F., Wojtaszewski, J. F. P., Kiens, B., & Richter, E. A. (2018). Exercise increases circulating GDF15 in humans. **Molecular Metabolism**, 9. https://doi.org/10.1016/j.molmet.2017.12.016

Abstract

Exercise is a powerful stimulus to increase energy metabolism, especially in the exercising muscles. If repeated, exercise training can treat and prevent numerous chronic conditions like obesity and metabolic disorders. When exercise is initiated, many biological processes are regulated, and a plethora of signaling pathways are affected, primarily via the rapid regulation of protein phosphorylation. Many factors contribute to the cellular response following exercise, including exercise intensity, but a thorough investigation of the regulated phosphoproteome following different exercise intensities has not been performed.

In recovery from an acute bout of exercise, insulin sensitivity is enhanced in the previously active muscles. However, in the recovery period from whole-body exercise, the concentration of plasma FAs increases if no food is ingested, and the concomitant increase in plasma FA concentration may dampen the insulin-sensitizing effect post-exercise. This regulation is suggested to take place by the pyruvate dehydrogenase complex (PDC), which controls the conversion of pyruvate derived from glycolysis to acetyl-CoA. Dichloroacetate (DCA), a pharmacological activator of PDC, was used to elucidate whether PDC plays an important regulatory role in post-exercise insulin sensitivity.

Thus, this thesis has a dual aim. First, to elucidate the phosphoproteome regulated following three different exercise modalities (endurance, sprint, and resistance). Second, to investigate the role of PDC in lipid-induced decreased insulin sensitivity post-exercise.

In study I, a comparative analysis of the regulated phosphoproteome acutely after or three hours in recovery from endurance, sprint, or resistance exercise, identified modality-specific regulation, especially in recovery where 1, 2904, and 687 phosphorylation sites were regulated in endurance, sprint, and resistance, respectively. Immediately post-exercise, 420 phosphosites were regulated in all three modalities, which comprised *the canonical exercise phosphoproteome.* The phosphoproteins containing the most regulated phosphosites were implicated in acetyl-CoA and glycogen metabolism. However, only ~6% of the canonical phosphoproteome have been functionally characterized.

In study II, one-legged knee extension exercise was used to investigate whether the exercise-induced increase in insulin-stimulated glucose uptake in skeletal muscle may be inhibited due to a high plasma FA concentration post-exercise and if so, to elucidate the mechanisms behind it. It was revealed that insulin sensitivity was decreased in a previously exercised leg when plasma FA concentration was raised to levels as observed following whole-body exercise. Furthermore, it was shown by the use of the pharmacological PDC-activator DCA that PDC plays an important regulatory role in post-exercise skeletal muscle insulin sensitivity.

In conclusion, protein phosphorylation is regulated in a modality-specific and temporal manner, and only a small fraction of the molecular events have been characterized. By using a comparative approach of three different exercise modalities and pathway and kinome analysis, a ranking of important phosphorylation sites for future experiments has been performed. Further, PDC activation was shown to prevent lipid-induced insulin resistance post-exercise, presenting PDC as an important link between lipid and glucose metabolism and a regulator of skeletal muscle insulin sensitivity post-exercise.

Resumé

Ved fysisk aktivitet øges energiomsætningen, specielt i de arbejdende muskler, og hvis træningen bliver gentaget over en længere periode vil dette kunne behandle samt forebygge adskillige kroniske tilstande som fedme og stofskiftesygdomme. Når motion påbegyndes, bliver mange biologiske processer reguleret, og et stort antal molekylære signaleringsveje påvirkes, primært ved fosforylering af proteiner. Mange faktorer bidrager til det specifikke cellulære svar efter fysisk aktivitet inklusiv træningsintensitet, men en dybdegående undersøgelse af det regulerede fosforproteom efter forskellige træningsintensiteter er ikke blevet lavet.

Efter fysisk aktivitet er blevet udført, vil de tidligere arbejdende muskler have en øget følsomhed for insulin. Men i perioden efter et helkropsarbejde vil koncentrationen af fedtsyrer (FA) i plasma stige, og denne stigning i plasma-FA-koncentrationen dæmper muligvis den øgede insulinfølsomhed, som er opnået efter arbejdet. Denne regulering menes at finde sted ved proteinkomplekset pyruvat dehydrogenase (PDC), som kontrollerer omdannelsen af pyruvat, der kommer fra glykolysen, til acetyl-CoA. Om PDC er hæmmet af den forhøjede fedtoxidation efter et akut arbejde, eller om den fedt-inducerede nedsatte insulinfølsomhed kan ændres ved farmakologisk at aktivere PDC i muskler, vides i øjeblikket ikke. Dichlororacetat (DCA), der er en farmakologisk aktivator af PDC, blev benyttet til at klarlægge om PDC udgør en vigtig rolle i den øgede insulinfølsomhed efter et akut arbejde.

Denne Ph.d.-afhandling har dermed et todelt formål. Først, at belyse det regulerede fosforproteom i muskler efter tre forskellige træningsmodaliteter (udholdenhed, sprint og styrkearbejde). Dernæst, at undersøge PDC's rolle i fedtinduceret nedsat insulinfølsomhed efter et akut arbejde.

I studie 1 identificerede en komparativ analyse af det regulerede fosforproteome lige efter, eller tre timer efter, henholdsvis udholdenhed, sprint eller styrkearbejde en modalitetsspecifik regulering. Dette sås specielt efter tre timers restitution, hvor 1, 2904 og 687 fosforyleringer var reguleret efter hhv. udholdenhed, sprint og styrkearbejde. Lige efter arbejdets ophør var 420 fosforyleringer reguleret i alle tre modaliteter, og disse udgjorde *det kanoniske arbejdsfosforproteom*. Fosforproteinerne med de mest regulerede fosforyleringer var involveret i acetyl-CoA og glykogenmetabolisme. Det er dog kun ~6% af det kanoniske arbejdsfosforproteom, der tidligere er blevet funktionelt karakteriseret.

I studie 2 blev et-bens knæekstensionsarbejde brugt til at undersøge om den øgede insulinstimulerede glukoseoptagelse i skeletmuskler efter et akut arbejde kunne blive hæmmet af en højere plasma-FA-koncentration efter arbejdet, og hvis det var tilfældet, at klarlægge mekanismerne bag. Det blev her

fundet, at insulinfølsomheden var nedsat i et tidligere arbejdende ben, når plasma-FA-koncentrationen blev hævet til niveauer, normalt observeret efter et helkropsarbejde. Ydermere blev det vist, ved at benytte den farmakologiske PDC aktivator DCA, at PDC spiller en vigtig regulatorisk rolle i den øgede insulinfølsomhed i skeletmuskler efter et akut arbejde.

Det kan konkluderes at proteinfosforylering er reguleret på en modalitetsspecifik og tidsmæssig måde efter fysisk aktivitet, og kun en lille del af de molekylære mekanismer er indtil nu blevet karakteriseret. Ved at benytte en komparativ tilgang af tre træningsmodaliteter med forskellig intensitet samt analyser af signaleringsveje og kinaser, har det været muligt at rangere forskellige fosforyleringer til fremtidige eksperimenter. Yderligere blev det fundet, at aktivering af PDC kunne forhindre den nedsatte insulinfølsomhed efter et arbejde når plasma-FA-niveauerne var forhøjede. Dette viser at PDC spiller en vigtig rolle mellem fedt- og sukkerstofskiftet for den øgede insulinfølsomhed i skeletmuskulaturen efter et akut arbejde.

Enclosed manuscripts

The present PhD thesis includes two manuscripts listed below. The first study has been published in Cell Metabolism, and the second study is ready for submission. The studies will be referred to as study I and II throughout this thesis. The manuscripts are included in their full length at the end of this thesis.

Study I:

Ronnie Blazev*, **Christian S. Carl***, Yaan-Kit Ng*, Jeffrey Molendijk, Christian T. Voldstedlund, Yuanyuan Zhao, Di Xiao, Andrew J. Kueh, Paula M. Miotto, Vanessa R. Haynes, Justin P. Hardee, Jin D. Chung, James W. McNamara, Hongwei Qian, Paul Gregorevic, Jonathan S. Oakhill, Marco J. Herold, Thomas E. Jensen, Leszek Lisowski, Gordon S. Lynch, Garron T. Dodd, Matthew J. Watt, Pengyi Yang, Bente Kiens, Erik A. Richter, Benjamin L. Parker. **Phosphoproteomics of three exercise modalities identifies canonical exercise signaling and C18ORF25 as an AMPK substrate regulating skeletal muscle function.** *Cell Metabolism, July 2022.* DOI: https://doi.org/10.1016/j.cmet.2022.07.003

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Study II:

Christian S. Carl, Marie M. Jensen, Kim A. Sjøberg, Dumitru Constantin-Teodosiu, Ian R. Hill, Rasmus Kjøbsted, Paul L. Greenhaff, Jørgen F.P. Wojtaszewski, Erik A. Richter, Andreas M. Fritzen, and Bente Kiens**. Pharmacological activation of pyruvate dehydrogenase complex counteracts lipid-induced inhibition of insulin-stimulated glucose uptake in human skeletal muscle during recovery from acute exercise.** *Ready for submission*