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PART 1

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Activity of lipoprotein lipase in skeletal muscle – different regulation by whole body exercise and in situ *contractions*

Summary

Understanding of how metabolism is regulated in the healthy individual is, despite extensive research, far from complete. Skeletal muscle represents 30-40 % of total body mass and plays a major role in whole body homeostasis. It is well established that sex differences exist in skeletal muscle, both at a morphologic and metabolic level. The underlying cause of this sexual dimorphism in whole body metabolism remains uncertain. To address whether the sex difference in whole body metabolism difference originate from a sex difference intrinsic to the muscle cells, human muscle cell cultures (myotubes) were used as a model in study 1. Satellite cells were isolated from muscle biopsies obtained from vastus lateralis from male and female donors, matched according to age, fitness level (VO_2peak/kg LBM) and training history. In the present thesis it is demonstrated that 18 hrs of AICAR stimulation increased lipid oxidation to a greater extent in myotubes from female compared with male donors. These findings suggest, that sex differences in regulation of lipid metabolism observed *in vivo* might be due to differences inherent to the muscle cell. Conversely, glucose handling at the level of glucose uptake and incorporation into glycogen both in the basal state and after insulin stimulation, did not differ between myotubes from male and female donors. The higher insulin sensitivity that is observed in women *in vivo* must be explained by factors not genetically inherent to the muscle cells.

Triacylglycerol (TG) is transported in the blood bound to chylomicrons and VLDL, and delivered to the peripheral tissue for either storage or oxidation. Lipoprotein lipase is the rate limiting enzyme for hydrolysis of TG and therefore represents an important regulator of delivery of fatty acids to the muscle cell. Energy demand increases several fold in exercising skeletal muscle compared with rest, and especially glycogen depleting exercise has been shown to lead to an increase in the activity of LPL in skeletal muscle (mLPL). In the present thesis, exercise was used as a way of increasing the activity of mLPL to elucidate the possible mechanisms regulating this activity. In rats, it was demonstrated in study 2 that in response to prolonged whole body swimming exercise muscle glycogen was depleted and mLPL activity was increased. A role for the 5'- AMP regulated protein kinase (AMPK) could not be ruled out based on these findings. Therefore, a third study was carried out comparing mLPL activity in genetically engineered mice lacking the AMPK α2 subunit (AMPK α2 KO) and their wild type littermates. While this study was inconclusive as to the role of AMPK for exercise induced mLPL activity, it was demonstrated that AMPK α2 was dispensable for basal activation of mLPL.

Resumé

Forståelsen af, hvordan stofskiftet reguleres i det raske individ er, på trods af omfattende forskning, langt fra komplet. Skeletmuskulaturen udgør 30-40 % af kroppens samlede masse og spiller en stor rolle i homeostasen på helkropsniveau. Det er velkendt, at der findes kønsforskelle i skelet-muskulaturen, både på det morfologiske og metaboliske niveau, men de underliggende årsager til disse kønsforskelle er stadig uklare. For at undersøge, om forskellen skyldes en kønsforskel iboende i muskelcellen, blev humane muskel cellekulturer (myotuber) anvendt som model i studie 1. Satellitceller blev isoleret fra biopsier fra vastus lateralis fra mandlige og kvindelige donorer, som var matchet ud fra alder, træningsstatus (VO_2peak/kg LBM) og træningshistorie. I nærværende afhandling er det påvist, at 18 timers AICAR stimulering øgede lipidoxidationen i et større omfang i myotuber fra kvindelige sammenlignet med mandlige donorer. Dette fund indikerer, at kønsforskelle i reguleringen af lipidmetabolismen observeret *in vivo* kan skyldes forskelle iboende i muskelcellen. Omvendt blev der for glukose-metabolismen ikke fundet nogen forskel mellem myotuber fra kvinder og mænd, hverken på optagelse af glukose eller på inkorporeringen af glukose til glykogen. Dette var tilfældet uanset om der måltes i basal- eller insulinstimuleret tilstand, hvilket indikerer at den højere insulin følsomhed, der observeres i kvinder sammenlignet med mænd *in vivo*, må forklares af faktorer, der ikke er genetisk indbygget i muskelcellen.

Triacylglycerol (TG) transporteres i blodet bundet til chylomikroner og VLDL. Herfra leveres TG til de perifere væv, hvor til enten lagring eller oxidation. Lipoprotein lipase (LPL) er det hastigheds-begrænsende enzym for hydrolysen af TG og repræsenterer derfor en vigtig regulator af levering af fedtsyrer til bl.a. muskelcellerne. Energikravet stiger flerfold i skelet muskulaturen i forbindelse med arbejde og specielt efter glykogennedbrydende arbejde, har aktiviteten af muskel LPL (mLPL) vist sig at være øget. I nærværende afhandling blev akut arbejde brugt til at inducere en stigning i mLPL aktiviteten, for yderligere at kunne belyse de mulige reguleringsmekanismer. I studie 2 blev det i rotter demonstreret, at indholdet af muskel glykogen var nedsat og mLPL aktiviteten øget i skeletmuskulaturen efter langvarigt svømmearbejde. Det kunne, baseret på disse resultater, ikke udelukkes at 5'-AMP reguleret protein kinase (AMPK) kunne spille en rolle i reguleringen af mLPL efter akut arbejde. Derfor udførtes et tredje studie, hvor aktiveringen af mLPL blev sammenlignet i genetisk modificerede mus uden AMPK α2 (AMPK α2 KO) og vild type kuldfæller. På baggrund af dette studie kunne det dog ikke konkluderes om AMPK α2 var nødvendig for en arbejdsinduceret stigning i mLPL aktivitet, men at AMPK α2 ikke var nødvendig for den basale aktivering af mLPL.

List of studies

Study 1

Mette L. B. Christiansen, Signe Bech, Lubna Al-Khalili, Anna Krook and Bente Kiens

Lipid and glucose metabolism: Effects of sex in human myotubes from matched young donors

Manuscript in preparation

Study 2

Mette L. B. Christiansen, Jacob Jeppesen, Louise D. Høeg and Bente Kiens

Activity of lipoprotein lipase in skeletal muscle – different regulation by whole body exercise and *in situ* contractions

Manuscript in preparation

Study 3

Mette L. B. Christiansen, Andreas M. Fritzen, Jacob Jeppesen and Bente Kiens

Is AMPK involved in regulation of LPL activity in skeletal muscle?

Introduction

During this PhD project three studies were conducted aimed at elucidating:

- The contribution of sex differences intrinsic to the skeletal muscle cell to the sexual dimorphism observed in human whole body metabolism, this was addressed in study 1.
- The mechanisms regulating LPL activity in response to acute exercise and in post exercise recovery with emphasis on the role of muscle glycogen and AMPK; this was addressed in study 2 and 3.

Based on the results from study 1 and study 2, two manuscripts were prepared, these are included in the present thesis. In the present thesis the results are presented and discussed in relation to the existing literature in the field. The results from study 3 are not presented in a manuscript form, but included in part 2 of the thesis.