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List of studies:

This thesis is based on the following three studies that will be referred to as **Study I-III**.

Study I: Onslev J, Jensen J, Bangsbo J, Wojtaszewski J, Hostrup M. β2-agonist induces net leg glucose uptake and free fatty acid release at rest but not during exercise in young men. *J Clin Endocrinol Metab.* 2019;**104**(3):647-657.

Study II: Onslev J, Thomassen M, Wojtaszewski J, Bangsbo J, Hostrup M. Salbutamol Increases Leg Glucose Uptake and Metabolic Rate but not Muscle Glycogen Resynthesis in Recovery from Exercise. *J Clin Endocrinol Metab*. 2022;107(3):E1193–203

Study III: Onslev J, Fiorenza M, Thomassen M, Havelund J, Bangsbo J, Færgeman N, Wojtaszewski J, Hostrup M. Beta$_2$-adrenergic agonist attenuates insulin-stimulated muscle glucose uptake independent of fatty acid availability in insulin resistant men. *Draft*.

Summary

Beta$_2$-adrenoceptor agonists (beta$_2$-agonists) acutely elevate insulin-independent muscle glucose uptake in rodents and augment insulin sensitivity after chronic treatment in humans. With the development and future projection of the global prevalence of type II diabetes, the interest in leveraging pharmacological manipulation of the beta$_2$-adrenoceptor to treat type II diabetes has increased. However, reports in obese rodents demonstrate that beta$_2$-agonists acutely impair insulin and glucose tolerance, which, if translatable to humans, could significantly reduce the therapeutic potential of activating the beta$_2$-adrenoceptor in insulin resistant humans. Furthermore, the acute effects of beta$_2$-adrenoceptor agonists on muscle glucose uptake and metabolism and the underlying mechanism(s) both at basal conditions and during metabolic challenges such as exercise, recovery and insulin stimulation remains incompletely understood in humans. Thus, the purpose of the current PhD thesis is to delineate the impact of beta$_2$-agonist on acute glucometabolic regulation during different conditions to enable a better understanding of the therapeutic potential of beta$_2$-agonists including its' pitfalls. This purpose has been pursued in three different studies investigating the impact of beta$_2$-agonist on muscle glucose uptake and metabolism at the basal condition, during and in recovery from a single bout of exercise and during hyperinsulinemic isoglycemic conditions.

The results from the three studies demonstrate that beta$_2$-adrenoceptor agonist elevates basal muscle glucose uptake (**Study I)** in line with that observed in *in vitro* rodent studies, but that the elevation *in vivo* might be confounded by increased insulin secretion, which makes the impact of muscle beta$_2$-adrenergic signalling on basal muscle glucose uptake difficult to delineate. During exercise, beta$_2$-agonist decreased muscle glucose uptake (**Study II**), an effect that shifted to an increase in muscle glucose uptake in recovery from exercise (**Study II**). Lastly, beta$_2$-agonist markedly impaired insulin-stimulated muscle glucose uptake in insulin resistant subjects (**Study**

III). The muscle biopsies sampled throughout the studies suggest that beta$_2$-agonist primarily impairs exercise- and insulin-stimulated muscle glucose uptake through alterations in glycogen metabolism. Conversely, the increases in muscle glucose uptake at basal conditions and in recovery from exercise likely occurs due to increased glycolytic flux and elevated glucose transport which, perhaps, occurs in an insulin-independent manner.

Collectively, this thesis demonstrates that beta$_2$-agonist elicits some intriguing acute effects on muscle glucose uptake in humans as seen in **Study I** and **II** that potentially could be harnessed to combat insulin resistance and type II diabetes. However, **Study III** clearly highlights that beta$_2$-agonist markedly impairs insulin-stimulated muscle glucose uptake in insulin resistant subjects. Thus, administration of beta$_2$-agonist in people with type II diabetes could result in acutely aggravated postprandial hyperglycemia, which would severely reduce the therapeutic potential of beta$_2$-agonists in type II diabetes. How this translates to long term treatment with beta$_2$-agonists and whether or not it supresses the beneficial impact of beta$_2$-agonists effect on insulin sensitivity in people with type II diabetes should be examined in future studies.

Dansk resumé

Beta$_2$-adrenoceptor agonist (beta$_2$-agonist) øger akut insulinafhængig glukoseoptagelse i muskulaturen i gnavere og forbedrer insulinfølsomhed efter kronisk behandling i mennesker. På grund af udviklingen og fremskrivningen af den globale prevalens af type II diabetes er interessen for at udnytte farmaologisk manipulation af beta$_2$-adrenoceptoren til behandling af type II diabetes steget. Der er dog studier i overvægtige gnavere der viser, at beta$_2$-adrenoceptor agonist akut sænker både insulin- og glukosetolerancen, hvilket, hvis det også er gældende i mennesker, vil hæmme det terapeutiske potentiale ved aktiveringen af beta$_2$-adrenoceptoren i insulin resistente mennesker. Desuden er de akutte effekter af beta$_2$-adrenoceptor agonisten på muskelglukosemetabolismen og de underliggende mekanismer både i hvile og ved metaboliske udfordringer såsom ved fysisk arbejde, restitution fra fysisk arbejde og insulinstimulering ikke fuldt ud kortlagt. Derfor er formålet med denne PhD-afhandling at undersøge den akutte virkning af beta$_2$-agonist på den glukometaboliske regulering og glukoseoptagelsen i muskulaturen under forskellige betingelser for at muliggøre en bedre forståelse og fortolkning af beta$_2$-adrenoceptor agonistens terapeutiske potentiale i type 2 diabetes, inklusive bivirkninger. Dette er i PhD'en blevet gennemført i tre forskellige studier, der har undersøgt beta$_2$-agonistens betydning for den muskulære glukoseoptagelse under basale betingelser, under og i restitution fra fysisk arbejder samt ved insulinstimulering.

Resultaterne fra de tre studier viser at beta$_2$-agonist øger den basale glukoseoptagelse i muskulaturen (**Study I**) ligesom det er observeret i *in vitro* studier, men at øgningen i **Study I** kan være confoundet af øget insulinsekretion, hvilket gør det svært at fortolke betydningen af beta$_2$-adrenerg signalering på den basale glukoseoptagelse i muskulaturen. Under fysisk arbejde sænker beta$_2$-agonist optagelsen af glukose i muskulaturen (**Study II**), en effekt der skrifter til en øget optagelse af glukose i muskulaturen under restitution fra det fysiske arbejde. Slutteligt hæmmer beta$_2$-agonisten den insulinstimulerede glukoseoptagelse i muskulaturen markant (**Study III).**

Biopsierne der blev indsamlet igennem de tre studier tyder på at beta$_2$-agonister primært hæmmer arbejds- og insulinstimuleret muskelglukoseoptagelse igennem forandringer i glykogenmetabolismen, hvorimod øgningen i den basale muskelglukoseoptagelse og ved restitution fra fysisk arbejde sandsynligvis skyldes øget glykolytisk flux og glukosetransport, der måske faciliteres igennem en insulinuafhængig mekanisme.

Alt i alt så viser denne afhandling at administration af beta$_2$-agonist medfører nogle interessante effekter på glukoseoptagelse og -metabolisme i muskulaturen som vist i **Study I** og **Study II**, der potentielt vil kunne udnyttes i den fremtidige behandling af type II diabetes. Resultaterne fra **Study III** fremhæver dog også, at beta$_2$-agonisten markant hæmmer den insulinstimulerede glukoseoptagelse i muskulaturen i insulinresistente mennesker, hvilket betyder at behandling med beta$_2$-agonister hos mennesker med type II diabetes kan medføre forværring i postprandiel hyperglykæmi. Dette reducerer det terapeutiske potentiale af beta$_2$-agonister væsentligt. Hvilken betydning disse fund har for længerevarende behandling med beta$_2$-agonist og om de nyfundne negative effekter opvejes af de kendte positive effekter på insulinfølsomheden vides ikke og bør undersøges i fremtidige studier.