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The PhD thesis is based on four studies referred to in the thesis by their roman numerals as “Study I-IV”. The four completed studies resulted in four scientific papers of which one is published, one is in press and two are in manuscript form.


II. **Baasch-Skytte T.**, Gunnarsson TP., Fiorenza M. and Bangsbo J. 2020 - Skeletal muscle proteins important for work capacity are altered with type 2 diabetes – effect of 10-20-30 training. Physiological Reports, in press.


Summary

Exercise is a cornerstone in the treatment of type 2 diabetes (T2D). Moderate-intensity continuous training (MICT) lowers glycated hemoglobin (HbA$_1$c), reduces fat mass and increases maximal oxygen consumption ($\dot{V}O_2$max) in T2D patients. In recent years, high-intensity interval training (HIIT) has increased in popularity in the healthy population but also in cardiometabolic patient groups such as T2D to improve glycemic control. Few studies have investigated the effect of HIIT in a non-clinical setting, thus the reported results may not reflect the effects of HIIT in a “real-life” setting. HIIT promotes a number of adaptations in the skeletal muscles resembling those of endurance exercise in both healthy subjects and patient groups. However, the skeletal muscle adaptations in T2D patients following HIIT compared to MICT are not clear.

T2D is characterized by a decreased insulin sensitivity, which is a complex interplay of proteins and signaling pathways. Exercise and diet improves insulin sensitivity but the underlying mechanisms are not clear. The skeletal muscle is the primary site for insulin-mediated glucose disposal, thus playing a central role in insulin sensitivity and glucose metabolism. In recent cell culture- and animal studies treatment with beta2-agonists increased insulin sensitivity and glucose tolerance. Beta2-agonists have profound systemic effects such as inducing hypertrophy in humans. Thus, the peripheral effects of chronic beta2-agonist treatment are an emerging field, specifically lacking human studies on the effect of chronic beta2-agonist treatment on the whole-body insulin sensitivity.

The present PhD thesis is based on 4 peer-reviewed studies in the cross-field between clinical and classical exercise physiology comparing the effects of different training modalities on glycemic control and skeletal muscle adaptations, the effect of beta2-agonists on whole-body insulin sensitivity and performance-related manipulation of the antioxidant defense system and ion handling.

Study I compared the efficacy of HIIT vs. traditional MICT conducted in a non-clinical setting, in men with T2D. HIIT was superior to MICT in lowering HbA$_1$c and similar in increasing $\dot{V}O_2$max as well reducing total fat mass, despite a 42% lower training time commitment. These results are of clinical relevance as a lower HbA$_1$c and higher $\dot{V}O_2$max decreases the mortality.

Study II investigated whether men with T2D exhibited lower expression of skeletal muscle proteins regulating cellular processes important for the exercise capacity compared to age- and
body composition matched non-diabetic men. Further, whether HIIT promotes adaptations in proteins related to exercise capacity. The men with T2D exhibited a lower content of the antioxidant protein SOD1 and electron transport chain (ETC) complex V and higher protein content in ETC complex IV, mitochondrial fusion/fission and ion handling proteins than the controls. HIIT increased the content of proteins related to the antioxidant defense system, ETC and ion handling. In addition, HIIT improved exercise capacity but not $\dot{V}O_2$max in the men with T2D.

**Study III** investigated the effect of nicorandil intake, an ATP$_{\text{sensitive}}$ potassium channel (Kir6.2) opener with an antioxidant effect, on the exercise capacity and K$^+$ handling in young overweight insulin resistant subjects compared with the effect of nicorandil intake in healthy lean subjects on exercise capacity. Interstitial K$^+$ was assessed by the method of microdialysis in the overweight insulin resistant subjects, and leg oxygen consumption was calculated from measurements of femoral arterial blood flow and blood a-v difference. Nicorandil increased exercise capacity in the overweight insulin resistant subjects and decreased exercise capacity in the healthy lean subjects. Interestingly, nicorandil did not affect K$^+$ handling in the overweight insulin resistant subjects during exercise, suggesting that the nicorandil related improvement in exercise capacity was related to the potential antioxidant effects of nicorandil.

**Study IV** investigated the effect of daily inhalation of the beta$_2$-agonist terbutaline on whole-body insulin sensitivity during a 2 h hyperinsulinemic-euglycemic clamp in healthy lean men. Four weeks of daily terbutaline inhalation increased whole-body insulin sensitivity by 27% along with an increase in fat-free mass of 1.1 kg. Treatment with terbutaline did not affect expression of muscle GLUT4 and hexokinase II or adipose tissue GLUT4. Thus, the beta$_2$-induced increase in whole-body insulin sensitivity was associated with skeletal muscle hypertrophy in healthy lean men.

In summary, HIIT was more efficient than MICT to improve glycemic control in type 2 diabetes patients in a non-clinical setting and thus, an efficient training modality in promoting health-related outcomes in type 2 diabetes patients. Furthermore, type 2 diabetes patients exhibit lower antioxidant protein- but higher ion channel protein expression compared to non-diabetic men. In overweight insulin resistant men, the antioxidant defense system may affect the exercise capacity, which was suggested to be improved by the antioxidant effect exerted by nicorandil.
intake. Lastly, treatment with daily inhalation of terbutaline increased whole-body insulin sensitivity in healthy lean men, which was associated with muscle hypertrophy.

**Resumé**

Motion anses som essentielt i behandlingen af type 2 diabetes (T2D). Træning ved moderat intensitet (MICT) kan sænke langtidsblodsukkeret (HbA1c), reducere fedtmassen og øge den maksimale iltoptagelse (VO\textsubscript{2}max) hos patienter med T2D. De seneste år har høj-intens interval træning (HIIT) opnået øget opmærksomhed, både hos raske populationer men også i populationer med kroniske sygdomme, herunder T2D. Dog har kun få studier undersøgt effekten af HIIT i et ikke-klinisk miljø, hvorfor resultater fra andre studier muligvis ikke afspejler effekten af HIIT i et ikke-klinisk miljø. HIIT resulterer i en bred vifte af adaptationer i skeletmusklerne ligesom udholdenhedstræning, dog er skeletmuskuladaptionerne ved HIIT sammenligning med MICT hos patienter med T2D stadig uklares.

T2D karakteriseres ved nedsat insulin sensitivitet, hvilket består af et komplekst samspil af mange proteiner og signaleringsveje. Motion og diæt kan øge insulin sensitiviteten, men mekanismerne bag er ikke fuldt afklarede. Skeletmusklerne er den primære aftager for sukkeroptag, og har derfor en vigtig rolle for insulin sensitivitet og glukose metabolismen. I de seneste år har celle- og dyrestudier vist at beta\textsubscript{2}-agonister, anvendt til astma patienter, positivt påvirker glukose metabolismen. Beta\textsubscript{2}-agonister har en bred systemisk effekt, bl.a. ved at øge muskelmassen i mennesker. Derved er de perifere effekter af beta\textsubscript{2}-agonister et voksende emne, der især mangler humane studier der undersøger effekten af beta\textsubscript{2}-agonist indtag på insulin sensitiviteten.

Indeværende PhD afhandling undersøgte områder inden for T2D, med fokus på trænings intensitet, skeletmuskul adaptationer til intens- og moderat-intensitetstræning, effekten af manipulering med antioxidant systemet og ion håndtering på præstationsevnen. Derudover undersøgte PhD afhandlingen effekten af beta\textsubscript{2}-agonist indtag på insulin sensitiviteten.

**Studie I** sammenlignede effektiviteten af HIIT, udført efter 10-20-30 træningsprincippet med traditionel MICT hos mænd med T2D i et ikke-klinisk miljø. 10-20-30 træning var mere effektiv end MICT til at sænke HbA\textsubscript{1c}, og lige så effektiv som MICT til at øge VO\textsubscript{2}max og sænke fedtmassen, på trods af et 42% lavere tidsforbrug. Disse resultater er klinisk relevante da en lavere HbA\textsubscript{1c} og højere VO\textsubscript{2}max reducerer dødeligheden.
Studie II undersøgte, hvorvidt mænd med T2D, udtrykte en lavere ekspression af skeletmuskelproteiner involveret i cellulære processer vigtige for præstationsevnen, sammenlignet med alders og kropskompositions matchede mænd uden T2D. Skeletmuskelproteiner blev bestemt ved Western blotting metoden fra hvile muskelbiopsier. Mændene med T2D udtrykte et lavere indhold af antioxidant proteinet SOD1 og elektron transportkæde (ETK) kompleks V. Derudover udtrykte mænd med T2D højere protein indhold af ETK kompleks IV, mitokondriel fission/fusion og ion håndteringsproteiner sammenlignet med mænd uden T2D. 10-20-30 træning øgede mængden af proteiner involveret i antioxidant systemet, ETK og ion håndtering sammen med en øget udholdenhedspræstation, uden stigning i Vo2max.

Studie III undersøgte effekten af nicorandil, en ATP sensitiv kalium kanal (Kir6.2) åbner med en antioxidant effekt, på præstationsevnen samt K⁺ håndtering i unge overvægtige insulin resistente mænd og sammenlignede effekten på præstationsevnen ved indtag af nicorandil i unge raske moderat trænede mænd. Interstitielt K⁺ blev målt ved mikrodialyse og benets iltoptagelse blev estimeret ved måling af blodgennemstrømning og arterielle-venøse blodprøver i de overvægtige mænd. Nicorandil øgede præstationsevnen hos de overvægtige insulin resistente mænd, men reducerede præstationsevnen hos de raske moderat trænede mænd. Nicorandil påvirkede ikke K⁺ håndtering hos de overvægtige insulin resistente mænd, hvilket tyder på at nicorandils antioxidantende effekt har øget præstationsevnen hos de overvægtige insulin resistente mænd.

Studie IV undersøgte effekten af daglig inhalering af beta2-agonisten terbutalin på helkrops insulin sensitivitet under en 2 timers hyperinsulinemic euglycemic clamp hos raske unge mænd. Fire ugers daglig inhalering af terbutalin øgede helkrops insulin sensitiviteten med 27% og den fedtfri masse med 1.1 kg. Terbutalin behandling ændrede ikke skeletmuskelp indehold af GLUT4 og hexokinase II eller GLUT4 i fedtvæv. Derved associeres den øgede insulin sensitivitet med forøgelsen i muskelmassen induceret af beta2-agonisten terbutalin i raske unge mænd.

Høj-intens interval træning tæt på maksimal intensitet viste sig mere effektiv end moderat-intensitets træning til at forbedre sundhedsparametre hos mænd med T2D i et ikke-klinisk miljø. Derudover blev vigtigheden af antioxidant forsvaretsystemet belyst samt dets effekt på præstationsevnen hos overvægtige insulin resistente mænd. Behandling med daglig inhalering af beta2-agonisten terbutalin øgede helkrops insulin sensitiviteten, hvilket blev associeret med en forøgelse af muskelmassen.
1 – Introduction

Exercise is a cornerstone in the treatment of type 2 diabetes (T2D). In recent years high-intensity interval training (HIIT) has become a popular exercise modality and has been reported superior to moderate-intensity continuous training (MICT) in improving exercise capacity in both untrained (Fiorenza et al., 2018, Toennesen et al., 2018a) and trained subjects (Iaia et al., 2008, Bangsbo et al., 2009, Iaia and Bangsbo, 2010, Gunnarsson and Bangsbo, 2012). In addition, HIIT is increasing in popularity within various patient groups including T2D patients, where HIIT recently was proposed superior to MICT in improving health-related outcomes (Grace et al., 2017, De Nardi et al., 2018, Liu et al., 2019). Several studies have investigated the effects of HIIT on health-related outcomes in T2D patients (Backx et al., 2011, Little et al., 2011, Terada et al., 2013, Hollekim-Strand et al., 2014, Stoa et al., 2017, Winding et al., 2018), and HIIT has become one of the top fitness trends in the society. HIIT covers a broad range of exercise intensities, but the most common type of HIIT is aerobic intervals (Wormgoor et al., 2017). In recent years, exercise conducted as intervals near maximal intensity, consisting of shorter sprints (<90 s), termed speed endurance training (SET) (Iaia and Bangsbo, 2010) or sprint interval training (SIT) (Weston et al., 2014), has been reported effective in improving performance in well-trained subjects (Iaia et al., 2008, Bangsbo et al., 2009, Iaia and Bangsbo, 2010, Gunnarsson and Bangsbo, 2012). Where HIIT is characterized by a high aerobic load (MacInnis and Gibala, 2017), SET/SIT is characterized by a high anaerobic load. Despite SET/SIT effectively improves performance and skeletal muscle adaptations in healthy trained individuals (Thomassen et al., 2010, Thomassen et al., 2016), only few studies have conducted this type of training with T2D patients, reporting conflicting findings on changes in glycemic control (Revdal et al., 2016, Ruffino et al., 2017, Sjoros et al., 2018, Banitalebi et al., 2019). It is noteworthy, that the protocols improving glycemic control in patients with T2D consisted of repeated 30 s sprints, which is extremely demanding even for elite athletes, thus this exercise modality may not be suitable for T2D patients in a non-clinical setting, considering the importance of exercise adherence. 10-20-30 training is an established exercise modality that consists of repeated 10 s sprints preceded by 30 and 20 s of low- and moderate-intensity exercise, inducing a high aerobic- and anaerobic load (Gunnarsson and Bangsbo, 2012, Fiorenza et al., 2018). 10-20-30 training effectively lowered blood pressure and fat mass, and increased fat-free mass and maximum oxygen uptake (\(\dot{V}O_2\text{max}\)) in hypertensive and asthmatic patients (Fiorenza et al., 2018, Toennesen et al., 2018b, Toennesen et al., 2018a). 10-20-30 training includes short sprints compared to SET/SIT (10 vs 30 seconds), and is reported as
motivating by different patient groups why it may be considered suitable for T2D patients in a non-clinical setting.

In T2D patients, the exercise capacity and \( \dot{V}O_2 \text{max} \) are lower than in healthy counterparts (Regensteiner \textit{et al.}, 1998, Baldi \textit{et al.}, 2003, Fang \textit{et al.}, 2005). These impairments may relate to both central (cardiovascular) and peripheral (muscular) factors (Baldi \textit{et al.}, 2003, Reusch \textit{et al.}, 2013). Determinants of the endurance exercise capacity are \( \dot{V}O_2 \text{max} \), lactate threshold and work efficiency (Bassett and Howley, 2000, McLaughlin \textit{et al.}, 2010). Where \( \dot{V}O_2 \text{max} \) is a central parameter limited by blood- and stroke volume together with the oxygen carrying capacity of the blood, lactate threshold and work efficiency are determined by peripheral skeletal muscular factors. Mitochondrial protein content, enzyme activity and the antioxidant defense system are among some of the determinants for the lactate threshold and work efficiency (Bassett and Howley, 2000, Joyner and Coyle, 2008) and ion handling are critical for the short-term intense work capacity (Hostrup and Bangsbo, 2017). Interestingly, skeletal muscle of T2D patients is characterized by increased oxidative damage, defined as increased levels of reactive oxygen species (ROS) (Pan \textit{et al.}, 2010, Aouacheri \textit{et al.}, 2015, Odegaard \textit{et al.}, 2016), mitochondrial dysfunction (Lowell and Shulman, 2005, Rovira-Llopis \textit{et al.}, 2017) and possibly impaired ion handling (Juel \textit{et al.}, 2004, Severino \textit{et al.}, 2018), all of which may negatively impact exercise capacity.

Induction of diabetes in rats causes alterations of the antioxidant enzyme activity of catalase and SOD1 which increases in heart-, pancreatic- and kidney tissue and decreases in liver tissue. The increased activity of catalase and SOD1 in heart-, pancreatic- and kidney tissue could suggest of a diabetes-induced increase in ROS production in these tissues (Wohaieb and Godin, 1987). On the other hand, antioxidant enzyme activity in plasma was lower in T2D patients compared to healthy subjects (Ramakrishna and Jailkhani, 2008, Pan \textit{et al.}, 2010). Results are conflicting between animal- and human studies and it is unclear if T2D affect protein content related to the antioxidant defense system in human skeletal muscle.

Mitochondria are among the main sources of ROS production in skeletal muscle (Szendroedi \textit{et al.}, 2011). Increased ROS may damage mitochondrial structural components, leading to mitochondrial dysfunction and ultimately exacerbating mitochondrial ROS production. Mitochondria are dynamic organelles and turnover is critical for the maintenance of a functional mitochondrial pool (Diaz and Moraes, 2008). Mitochondrial fusion- and fission cycles are central for remodeling and, thus the function of mitochondria. MFN2 and DRP1 are key regulators of
mitochondrial fusion and fission, respectively (Hall et al., 2014). Fusion is restoration of defective mitochondrial organelles by formation of a network of mitochondria, and fission is segregation of damaged mitochondria from this network. There was no difference in muscle MFN2 and DRP1 between healthy lean subjects and obese or T2D patients (Gundersen et al., 2020), but muscle MFN2 protein content was lower in obese compared to lean subjects (Bach et al., 2003). However, in both studies the subjects were not matched for body composition which may confound the results (DeLuise et al., 1982). Thus, while reduced antioxidant defenses and mitochondrial dysfunction appear to play a critical role in the impaired exercise capacity in T2D patients, it is unknown whether this is associated with alterations in muscle proteins regulating redox homeostasis and mitochondrial function compared to healthy counterparts.

Intense exercise i.e. exercise at or above the intensity associated with \( \dot{V}O_2 \text{max} \), induces major ion perturbations, which may impair force production of skeletal muscle (Cairns and Lindinger, 2008). The ion handling capacity of skeletal muscle play a major role in fatigue development and is a critical determinant of exercise capacity during intense exercise (Allen et al., 2008, Hostrup and Bangsbo, 2017). Intense exercise induces profound \( Na^+ \) and \( K^+ \) perturbations caused in part by the ATP-sensitive potassium channel (Kir6.2) and counteracted by the \( Na^+/K^+ \)-pumps. Opening of Kir6.2 channels causes an outward \( K^+ \) flux, increasing interstitial \( K^+ \) accumulation and decreasing cell excitability (Clausen, 2003), which may negatively impact muscle fiber recruitment. \( Na^+/K^+ \)-pump expression has been associated with improved exercise capacity (Bangsbo et al., 2009, Iaia and Bangsbo, 2010, Thomassen et al., 2010) and animal studies indicate that diabetes decreases muscle protein expression as well as activity of \( Na^+/K^+ \)-pumps (Kjeldsen et al., 1987, Nishida et al., 1992, Schmidt et al., 1994), whereas human data are inconsistent, reporting lower (Schmidt et al., 1994), higher (Djurhuus et al., 2001) or similar (Dela et al., 2004) expression of \( Na^+/K^+ \)-pump subunits in T2D patients compared to healthy counterparts. Taken together, expression of ion handling proteins in T2D patients compared to healthy controls are inconsistent and it is unclear if the exercise capacity is associated with aberrant expression of proteins related to ion handling.

Beta2-adrenoceptor agonists are commonly used for treatment of obstructive respiratory diseases via the inhaled route of administration (Tesse et al., 2018). Beta2-agonists have a profound systemic effect (Hostrup et al., 2018, Jessen et al., 2018) although the main site of function of inhaled beta2-agonists is locally in the airways. In recent years, the peripheral effects of chronic beta2-agonist treatment has become an emerging field with investigations on the effects of chronic
beta2-agonists treatment on body composition, energy balance and exercise performance (Holgate et al., 1980, Le Panse et al., 2006, Genc et al., 2012, Hostrup et al., 2015, Jessen et al., 2018, Lemminger et al., 2019) in humans. Beta2-agonists exert skeletal muscle hypertrophy in humans (Hostrup et al., 2015, Jessen et al., 2018) even when administered in close-to-therapeutic dosages. Since skeletal muscle is the primary site for insulin mediated glucose disposal (DeFronzo et al., 1981), it is interesting that chronic beta2-agonist treatment improve insulin sensitivity and glucose metabolism in rodents (Torgan et al., 1993, Jacob et al., 1999, Pan et al., 2001, Sato et al., 2014, Kalinovich et al., 2020). However, the chronic effects of beta2-agonist treatment on insulin sensitivity in humans are limited to one study (Scheidegger et al., 1984) showing increased insulin sensitivity with chronic beta2-agonist treatment. However, the latter study was confounded as no wash-out period was included before assessment of insulin sensitivity during a hyperinsulinemic-euglycemic clamp, thus the acute effects of the administered beta2-agonists may have affected the glucose metabolism and uptake positively as shown in healthy lean men (Onslev et al., 2019).

The present PhD thesis investigated I) the effect of exercise intensity on glycemic control and skeletal muscle adaptations in patients with T2D II) the performance-related effect of manipulating the antioxidant defense system in young overweight insulin resistant men, and III) the effects of beta2-agonist treatment on whole-body insulin sensitivity in healthy young men. The thesis includes 4 scientific papers conducted from 2017-2020 during the PhD and aimed at answering the following questions:

1) Is exercise training conducted at near maximal intensity more efficient than moderate-intensity exercise training in improving health-related outcomes in T2D patients, and is it applicable in a non-clinical setting? (study I+II)

2) What are the skeletal muscle adaptations to exercise training, conducted at near maximal intensity compared to moderate-intensity, in relation to glucose metabolism, ion handling, mitochondrial function and antioxidant defense system in T2D patients? (study I+II)

3) How does the antioxidant defense system affect the exercise capacity in overweight insulin resistant subjects compared to healthy lean subjects? (study III)

4) Does chronic administration of a beta2-agonist increase whole-body insulin sensitivity in young healthy men? (study IV)