Table of contents

List of studies

Study I: *Jessen S*, Reitelseder S, Kalsen A, Kreiberg M, Onslev J, Gad A, Ørtenblad N, Backer V, Holm L, Bangsbo J, Hostrup M. Beta$_2$-adrenergic agonist salbutamol augments hypertrophy in MHCIIa fibers and sprint mean power output but not muscle force during 11 weeks of resistance training in young men. *Journal of Applied Physiology*.

Study II: *Jessen S*, Onslev J, Lemminger A, Backer V, Bangsbo J, Hostrup M. Hypertrophic effect of inhaled beta$_2$-agonist with and without concurrent exercise training: A randomized controlled trial. *Scandinavian Journal of Medicine & Science in Sports*.

Study III: *Jessen S*, Bangsbo J, Hostrup M. Daily formoterol or terbutaline inhalation impairs aerobic performance and does not increase isometric muscle strength despite inducing muscle hypertrophy. *Draft*.

List of publications not included

I. Hostrup, M., Reitelseder, S., **Jessen, S**., Kalsen, A., Nyberg, M., Egelund, J., Kreiberg, M., Kristensen, C. M., Thomassen, M., Pilegaard, H., Backer, V., Jacobson, G. A., Holm, L., & Bangsbo, J. (2018). Beta$_2$-adrenoceptor agonist salbutamol increases protein turnover rates and alters signalling in skeletal muscle after resistance exercise in young men. J Physiol. doi:10.1113/JP275560

II. Lemminger, A. K., **Jessen, S.,** Habib, S., Onslev, J., Xu, S. F. S., Backer, V., Bangsbo, J., & Hostrup, M. (2019). Effect of beta$_2$-adrenergic agonist and resistance training on maximal oxygen uptake and muscle oxidative enzymes in men. Scand J Med Sci Sports. doi:10.1111/sms.13544

III. Hostrup, M., Jacobson, G. A., **Jessen, S**., & Lemminger, A. K. (2020). Anabolic and lipolytic actions of beta$_2$-agonists in humans and antidoping challenges. Drug Test Anal. doi:10.1002/dta.2728

IV. **Jessen, S.,** Becker, V., Rzeppa, S., Backer, V., Bengtsen, K., Hullstein, I., Dehnes, Y., & Hostrup, M. (2020). Pharmacokinetics of salmeterol and its main metabolite alpha-hydroxysalmeterol after acute and chronic dry powder inhalation in exercising endurance-trained men: Implications for doping control. Drug Test Anal. doi:10.1002/dta.2978

V. **Jessen, S.,** Solheim, S. A., Jacobson, G. A., Eibye, K., Bangsbo, J., Nordsborg, N. B., & Hostrup, M. (2020). Beta$_2$-adrenergic agonist clenbuterol increases energy expenditure and fat oxidation, and induces mTOR phosphorylation in skeletal muscle of young healthy men. Drug Test Anal. doi:10.1002/dta.2755

VI. Solheim, S. A., **Jessen, S**., Mørkeberg, J., Thevis, M., Dehnes, Y., Eibye, K., Hostrup, M., & Nordsborg, N. B. (2020). Single-dose administration of clenbuterol is detectable in dried blood spots. Drug Test Anal. doi:10.1002/dta.2872

VII. Eibye, K., Jacobson, G. A., Bengtsen, K., **Jessen, S**., Backer, V., Bangsbo, J., & Hostrup, M. (2020). Effect of one-week oral or inhaled salbutamol treatment with washout on repeated sprint performance in trained subjects. Transl Sports Med. doi:https://doi.org/10.1002/tsm2.210

VIII. Breenfeldt Andersen, A., Jacobson, G. A., Bejder, J., Premilovac, D., Richards, S. M., Rasmussen, J. J., **Jessen, S**., & Hostrup, M. (2021). An Abductive Inference Approach to Assess the Performance-Enhancing Effects of Drugs Included on the World Anti-Doping Agency Prohibited List. Sports Med. doi:10.1007/s40279-021-01450-9

IX. **Jessen, S**., Eibye, K., Christensen, P. M., Hostrup, M. & Bangsbo, J. (2021). No additive effect of acetaminophen when co-ingested with caffeine on cycling performance in well-trained young men. J Appl Physiol. doi:10.1152/japplphysiol.00108.2021.

Abstract

Inhaled beta$_2$-adrenoceptor agonists (beta$_2$-agonists) are used extensively in the treatment of asthma and exercise-induced bronchoconstriction, which is highly prevalent among elite athletes. Consequently, elite athletes have a high use of beta$_2$-agonists. Because of rising suspicions of performance enhancing effects, restrictions toward the use of beta$_2$-agonists were introduced in the early 1970's and still remain today. While the scientific basis for the performance enhancing effects with acute high-dose inhaled beta$_2$-agonists is well-established, limited knowledge exists on the effects of chronic administration of beta$_2$-agonists within the limits of the current anti-doping regulations. Specifically, there is reason to believe that beta$_2$-agonists can increase lean mass and sprinting ability in humans, but at present this notion is largely based on data from rodents. In the present thesis, three randomized, double-blinded, placebo controlled intervention studies were conducted to evaluate the potential of beta$_2$-agonists to induce increases in lean mass in humans and to evaluate the effects on exercise performance.

In **Study I**, 26 moderately trained, healthy, male participants (maximal oxygen consumption ($\dot{V}O_{2max}$): ≈50 mL/min/kg bw) received either placebo (n=13) or oral salbutamol (n=13) daily and completed an 11-week resistance training intervention (3 sessions weekly). Before and after the intervention, we collected a muscle biopsy from m. vastus lateralis and evaluated 10-s maximal sprinting ability on a bike ergometer and isometric and dynamic maximal torque of the quadriceps muscle. In **Study II**, 67 moderately trained male participants ($\dot{V}O_{2max}$: ≈50 mL/min/kg bw) received either placebo (n=29) or inhaled terbutaline (4 mg once daily; n=38) for 4 weeks. Participants were additionally allocated to one of three interventions, where they either a) maintained their habitual physical activity lifestyle ("no structured training; n=23), b) performed endurance training on indoor spinning bikes three times weekly ("endurance training"; n=21), or c) performed whole-body resistance training three times weekly ("resistance training"; n=23). Before and after the intervention, we assessed lean mass by dual-energy X-ray absorptiometry (DXA), $\dot{V}O_{2max}$, and 30-s maximal sprinting ability. In **Study III**, 61 well-trained, healthy participants (31 male, $\dot{V}O_{2max}$: ≈60 mL/min/kg/bw; 30 female, $\dot{V}O_{2max}$: ≈55 mL/min/kg bw) received either placebo (n=20), inhaled formoterol (24 µg twice daily; n=21), or inhaled terbutaline (2 mg twice daily; n=20) for 6 weeks. Participants did not complete a training intervention but maintained their own high volume of training. Before and after the intervention, as well as every second week, we assessed lean mass by DXA scanning, quadriceps isometric maximal contraction torque, $\dot{V}O_{2max}$, and sprinting ability.

In **Study I** we observed that high-dose oral beta$_2$-agonist administration during an 11-week resistance training intervention increased muscle fiber cross sectional area more than placebo, and that this was preferentially related to increased hypertrophy of myosin heavy chain IIa fibers. However, there were no improvements with salbutamol in isometric and dynamic muscle strength of the quadriceps muscle compared to placebo, and only mean power output was augmented with salbutamol during maximal sprinting whereas peak power output was unchanged. In **Study II**, we observed that anabolic effects are also achievable with high-dose inhalation of beta$_2$-agonist terbutaline in a dose that is permitted by current anti-doping regulations with a therapeutic use exemption. In addition, the anabolic effect of terbutaline was evident in the "resistance training" intervention – in line with the observations from **Study I** – but also in the "no structured training" intervention. However, in the "endurance training" intervention, the anabolic effect of terbutaline was blunted. Lastly, inhaled terbutaline had no effect on anaerobic sprinting ability. In **Study III**, we observed an anabolic effect of twice-daily beta$_2$-agonist inhalation of both formoterol and terbutaline, respectively, although less so than with the once-daily administration regimen of terbutaline employed in **Study II**. However, once again there were no apparent performance enhancements on muscle strength or anaerobic sprinting ability. Conversely, formoterol lowered $\dot{V}O_{2max}$ and both formoterol and terbutaline decreased markers of aerobic exercise performance.

In conclusion, chronic beta$_2$-agonist administration in humans is anabolic and this effect is achievable with inhalation within doses permitted by the current anti-doping regulations. The effects of beta$_2$-agonists on lean mass appear related to a drug class effect as in the present thesis beta$_2$-agonists salbutamol, formoterol, and terbutaline all increased lean mass. Despite increasing lean mass, beta$_2$-agonists do not appear to improve muscle strength or anaerobic exercise performance in healthy individuals. On the contrary, chronic beta$_2$-agonist administration may in fact be detrimental to aerobic exercise performance. These results indicate that within the current anti-doping regulations, there is very limited potential to enhance exercise performance with chronic beta$_2$-agonist treatment.

Dansk resumé

Inhalerede beta$_2$-adrenoceptor agonister (beta$_2$-agonister) er anvendt i vid udstrækning blandt eliteatleter som behandling mod astma og anstrengelsesudløst bronkiekonstriktion, som er meget prævalent i denne population. Som følge af en stigende mistanke om præstationsfremmende effekter indførtes i starten af 1970'erne gennemgående restriktioner for indtaget af beta$_2$-agonister. I de efterfølgende år blev det i kontrollerede studier bekræftet at akut indtag af inhalerede beta$_2$-agonister i høje doser kunne medføre præstationsfremmende effekter. Derimod er det endnu sparsommeligt belyst hvorvidt det er muligt at opnå en præstationsfremmende effekt ved kronisk (dvs. dagligt i flere uger) indtag af beta$_2$-agonister i doser som holder sig inden for grænsen af de tilladte doser i henhold til de gældende anti-doping regler. Nærmere bestemt forefindes der i dyrestudier videnskabelig evidens som sandsynliggør at beta$_2$-agonister kan inducere skeletmuskelhypertrofi og øge sprintevnen i mennesker. Indeværende afhandling er baseret på tre randomiserede, dobbeltblindede, placebokontrollerede interventionsstudier med det formål at undersøge hvorvidt det er muligt at opnå skeletmuskelhypertrofi med beta$_2$-agonister og hvorvidt dette påvirker præstationsevnen.

I **Studie I** inkluderede vi 26 raske, moderat trænede, mandlige forsøgspersoner (maksimalt iltoptag ($\dot{V}O_{2max}$): ≈50 mL/min/kg) som dagligt indtog enten placebo (n=13) eller oral salbutamol (n=13) og gennemførte en 11-ugers styrketræningsintervention (3 træninger per uge). Før og efter interventionen indsamlede vi en muskelbiopsi fra m. vastus lateralis og undersøgte desuden maksimal sprintevne under en 10 sekunders test på cykelergometer og isometrisk såvel som dynamisk muskelstyrke af quadricepsmusklen. I **Studie II** inkluderede vi 67 raske, moderat trænede, mandlige forsøgspersoner ($\dot{V}O_{2max}$: ≈50 mL/min/kg), som dagligt indtog enten placebo (n=29) eller inhaleret terbutalin (4 mg én gang dagligt; n=38) i 4 uger. Forsøgsdeltagerne blev desuden inddelt i én af tre interventioner som bestod i enten: a) vedligehold af deres daglige habituelle fysisk-aktivitetsniveau ("ingen struktureret træning", n=23), b) udholdenhedstræning på indendørs spinningscykler tre gange ugentligt ("udholdenhedstræning", n=21), eller helkropsstyrketræning tre gange ugentligt ("styrketræning", n=23). Før og efter interventionen bestemte vi fedtfri masse ved dual-energy X-ray absorptiometry (DXA) skanning, $\dot{V}O_{2max}$, og sprintevne under en 30 sekunders maksimal test på cykelergometer. I **Studie III** inkluderede vi 61 veltrænede, raske forsøgsdeltagere (31 mænd, $\dot{V}O_{2max}$: ≈60 mL/min/kg; 30 kvinder, $\dot{V}O_{2max}$: ≈55 mL/min/kg) som modtog enten placebo (n=20), inhaleret formoterol, (24 µg to gange dagligt; n=21) eller inhaleret terbutalin (2 mg to gange dagligt, n=20) i 6 uger. Forsøgsdeltagere udførte ingen træningsintervention, men vedligeholdt derimod deres vanlige høje træningsvolumen. Før og efter interventionen, samt hver 2. uge under interventionen, bestemte

vi fedtfri masse ved DXA-skanning, maksimal isometrisk styrke af quadricepsmusklen, $\dot{V}O_{2max}$, og sprintevne under en 30 sekunders maksimal test på cykelergometer.

I **Studie I** observerede vi at højdosis oral beta$_2$-agonist indtag under en 11-ugers styrketræningsintervention øgede muskelfibertværsnitsareal mere end placebo, og at dette var fortrinsvis relateret til hypertrofi af myosin heavy chain IIa fibre. Imidlertid observerede vi ingen forbedringer med salbutamol i isometrisk og dynamisk muskelstyrke sammenlignet med placebo, og kun middeleffektudvikling var forbedret med salbutamol under sprinttesten, mens maksimal effektudvikling var uændret sammenlignet med placebo. I **Studie II** observerede vi at anabole effekter også kan opnås med højdosis inhalation af beta$_2$-agonisten terbutalin i en dosis der er inden for de fastsatte grænseværdier i henhold til de nuværende dopingregler ved besiddelse af en "therapeutic use exemption". Den anabole effekt forekom i "styrketræning"-interventionen – hvilket er i overensstemmelse med **Studie I** – men også i "ingen struktureret træning"-interventionen. Derimod var der ingen stigning i fedtfri masse i "udholdenhedstræning"-interventionen. Slutteligt observerede vi ingen fremgang i sprintevne med inhaleret terbutalin. I **Studie III** observerede vi en anabol effekt af to-gange-daglig beta$_2$-agonist inhalation med både formoterol eller terbutalin, som dog var mindre end stigningen observeret med én-gang-dagligt administrering af terbutalin som i **Studie II**. Endnu engang var der ingen præstationsfremgang i muskelstyrke eller sprintevne med hverken formoterol eller terbutalin. Tværtimod var der en forringelse i $\dot{V}O_{2max}$ med formoterol, og både formoterol og terbutalin forringede markører for aerob præstationsevne.

Afslutningsvis konkluderes det at kronisk indtag af beta$_2$-agonist i mennesker har en anabol effekt, og at denne effekt er synlig selv ved indtag i doser inden for dem fastsat i de nuværende anti-doping regler. Effekterne af beta$_2$-agonist-indtag på fedtfri masse ser ud til at være relateret til en klasseeffekt, da alle de anvendte beta$_2$-agonister (salbutamol, terbutalin og formoterol) i indeværende afhandling inducerede stigninger i fedtfri masse. På trods af disse stigninger i fedtfri masse øger beta$_2$-agonister tilsyneladende hverken muskelstyrke eller sprintevne i raske personer. Tværtimod ser kronisk beta$_2$-agonist indtag ud til at være uhensigtsmæssig for den aerobe præstationsevne. Disse resultater viser at der inden for de nuværende anti-dopingregler er meget begrænset mulighed for at opnå præstationsfremmende effekter med kronisk beta$_2$-agonist-indtag.