Aim of the studies

Aim of study 1:

To investigate whether acute exercise-induced increase in insulin-stimulated glucose uptake in muscle is affected by training status.

To explore this, nine lean, healthy males performed one-legged knee-extensor exercise at the same relative intensity before and after 12 weeks of exercise training. Insulin-stimulated glucose uptake in the prior exercised and rested leg was measured during a euglycemic hyperinsulinemic clamp (EHC) by the leg balance technique. Muscle biopsies were obtained in both legs prior to and at the end of the EHC.

We hypothesized that exercise training would diminish the ability of a single bout of exercise to enhance insulin-stimulated glucose uptake. We predicted that this was associated with decreased AMPK $\alpha_2\beta_2\gamma_3$ expression/activation and thus lesser exercise- and insulin-induced TBC1D4 signaling after training.

Aim of study 2:

To investigate whether an acute bout of one-legged exercise to local exhaustion affects insulin action in non-exercised muscle and other tissues.

To elucidate this, eight young and healthy men underwent an EHC on two separate days; one day with prior one-legged knee-extensor exercise until local exhaustion and on another day without exercise. The two experimental days were performed in randomized order and separated by minimum 10 days. Whole-body, skeletal muscle and liver insulin action were measured during EHC by the leg balance technique and infusion of labelled glucose, respectively. Muscle biopsies were obtained prior to and at the end of the EHC.

We hypothesized that in response to an acute bout of one-legged knee-extensor exercise to local exhaustion, the prior exercised muscle would display enhanced insulin-stimulated glucose uptake while insulin-stimulated glucose uptake would be reduced in non-exercise muscle.

Aim of study 3:

To explore fiber type-specific adaptations to exercise training.

To elucidate this, we developed a workflow, which enabled proteomic analysis of fiber type-specific pools obtained before and after 12 weeks of training. Muscle biopsies from five subjects from study 1, obtained in the rested leg prior to insulin stimulation before and after training, were used for this study. To increase the depth of the proteome, we used a sequential multi-enzyme digestion strategy (LysC and Trypsin) and measured the proteome of human primary muscle cells (myoblast and

myotubes) together with type I and type II skeletal muscle fiber pools in MaxQuant under identical chromatographic conditions. Peptides were measured using LC-MS instrumentation consisting of an Easy nanoflow HPLC system coupled via a nanoelectrospray ion source to a Q Exactive HF mass spectrometer. MS spectra were acquired in a linear quadrupole Orbitrap analyzer. Raw MS files were analyzed using MaxQuant.

This study was not hypothesis-driven, although we generally hypothesized that some proteins would respond differently to training between type I and type II muscle fibers.