Testosterone related proteins and Disease

**Introduction** - The current method to measure testosterone has a poor correlation to the activity of the hormone. Testosterone deficiency in males, e.g. hypogonadism, is associated with serious diseases, such as cardiovascular disease, diabetes type two, and increased premature mortality. However, little is known about how the hormone relates to disease. Therefore, there is a great need to both measure the activity of testosterone more accurately and to learn more about the pathophysiology of the comorbidities.

**Aim/method** - The aim of the thesis is to identify markers of testosterone activity by proteomics, and also investigate how testosterone relates to diseases. Our designed human model (n=30) was utilized to develop the assay and is based on young healthy males who underwent a treatment plan resulting in modulation of testosterone and gonadotropins, allowing for paired analysis (n=90). Plasma was analyzed by mass spectrometry and bioinformatic tools were utilized in order to identify proteins that are related to the activity or level of testosterone. The samples were initially analyzed by Data Dependent Analysis (DDA) pools (3 by 3 for each time point). Four identified candidate proteins were verified in individual samples, and three of the candidate proteins were further verified in a cohort of patients with hypogonadism (n=35). We also performed a DDA experiment with individual samples from the human model. The proteins were then further analyzed and a targeted multiple reaction monitoring (MRM) assay with isotopically labeled standards was created and applied to patients with fertility issues (n=85). Currently, we are preparing samples from a cohort of patients with cardiovascular disease.

**Preliminary results** - Based on our model, we identified 16 proteins that are markers of testosterone and developed an MRM-assay. We have analyzed the assay in a cohort of patients with fertility issues and found that it performed well for some of the markers.

**Significance/meaning** - The significance of our findings is that it will potentially lead to a new way of measuring testosterone activity in the clinic, which would aid in diagnostics and treatment. We will also have novel mechanistic findings relating testosterone to disease.