Göteborg, 2023

# New sensitive and specific methods for sex steroid measurements in rodent serum and tissues

Akademisk avhandling

Som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet kommer att offentligen försvaras i Hjärtats aula, Vita stråket 12, den 19 december 2023, klockan 09.00

av **Maria Nilsson**

Fakultetsopponent: Dr. Andrea Romano, Associate Professor, University of Maastricht, The Netherlands

**Avhandlingen baseras på följande delarbeten**

I. Nilsson ME*, Vandenput L*, Tivesten Å, Norlén A-K, Lagerquist MK, Windahl SH, Börjesson AE, Farman HH, Poutanen M, Benrick A, Maliqueo M, Stener-Victorin E, Ryberg H*, Ohlsson C*. **Measurement of a comprehensive sex steroid profile in rodent serum by high-sensitive gas chromatography-tandem mass spectrometry.** Endocrinology, 2015; 156(7):2492-2502 *contributed equally

II. Colldén H*, Nilsson ME*, Norlén A-K, Landin A, Windahl SH, Wu J, Gustafsson KL, Poutanen M, Ryberg H, Vandenput L*, Ohlsson C*. **Comprehensive sex steroid profiling in multiple tissues reveals novel insights in sex steroid distribution in male mice.** Endocrinology, 2022; 163(3):bqac001 *contributed equally

III. Nilsson ME, Colldén H, Norlén A-K, Landin A, Windahl SH, Wu J, Sjögren K, Palsdottir V, Ryberg H, Poutanen M, Vandenput L, Ohlsson C. **The effect of high-fat diet-induced obesity on tissue levels of sex steroids in male mice.** Manuscript

**SAHLGRENSKA AKADEMIN**
**INSTITUTIONEN FÖR MEDICIN**

GÖTEBORGS UNIVERSITET

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# New sensitive and specific methods for sex steroid measurements in rodent serum and tissues

**Maria Nilsson**

Avdelningen för invärtesmedicin och klinisk nutrition, Institutionen för medicin, Sahlgrenska akademin, Göteborgs universitet, Sverige, 2023.

**Abstract**

Accurate measurement of sex steroids (androgens, estrogens, and progesterone) is essential in research and clinical settings for common diseases including osteoporosis, prostate cancer, breast cancer, and cardiovascular diseases. Sex steroids are not only produced in the gonads and adrenals but may also be locally synthesized in peripheral target tissues. As many conventional immunoassay-based methods proved to be inaccurate, especially in the lower concentration range, the first aim of this thesis was to develop and validate a highly sensitive and specific gas chromatography-tandem mass spectrometry technique for sex steroid profiling in rodent serum and tissues. In addition, to increase the understanding of the local sex steroid metabolism, we aimed to use this assay to determine the sex steroid levels in multiple rodent tissues and assess the impact of castration and obesity on these levels. We first successfully developed and validated a highly sensitive method for the analysis of a broad panel of sex steroids (testosterone (T), dihydrotestosterone (DHT), estradiol, estrone, dehydroepiandrosterone, androstenedione, and progesterone) in both serum and tissues of rodents. Using this assay, we accurately measured a comprehensive sex steroid profile in serum of female rats and mice according to estrous cycle phase. Next, we developed the first detailed atlas of the levels of a broad panel of sex steroids in multiple tissues in both gonadal intact and castrated mice. This comprehensive atlas can be used by the research community as a reference database to compare sex steroid levels in different tissues. The majority of sex steroids in male mice was found within white adipose tissue, which could possibly act as a buffer for circulating sex steroids. Also, progesterone was the most abundant sex steroid found in castrated male mice. We observed that high-fat diet-induced obesity reduced the DHT/T ratio, reflecting 5α-reductase activity, in muscle and seminal vesicles of male mice but increased DHT levels in the liver. Finally, obesity reduced progesterone levels in adipose tissue. In conclusion, our highly sensitive assay allows mapping of sex steroid levels in serum and peripheral target tissues and can as such contribute to the understanding and potential development of new treatments involved in the regulation of sex steroid action in sex steroid-dependent diseases.

**Keywords:** sex steroids, mass spectrometry, rodents

ISBN: 978-91-8069-453-7 (TRYCK) http://hdl.handle.net/2077/78555
ISBN: 978-91-8069-454-4 (PDF)