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# Metabolic and functional changes in transgender individuals following cross-sex hormone treatment: Design and methods of the GEnder Dysphoria Treatment in Sweden (GETS) study

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ABSTRACT

*Background:* Although the divergent male and female differentiation depends on key genes, many biological differences seen in men and women are driven by relative differences in estrogen and testosterone levels. Gender dysphoria denotes the distress that gender incongruence with the assigned sex at birth may cause. Gender-affirming treatment includes medical intervention such as inhibition of endogenous sex hormones and subsequent replacement with cross-sex hormones. The aim of this study is to investigate consequences of an altered sex hormone profile on different tissues and metabolic risk factors. By studying subjects undergoing gender-affirming medical intervention with sex hormones, we have the unique opportunity to distinguish between genetic and hormonal effects.

*Methods:* The study is a single center observational cohort study conducted in Stockholm, Sweden. The subjects are examined at four time points; before initiation of treatment, after endogenous sex hormone inhibition, and three and eleven months following sex hormone treatment. Examinations include blood samples, skeletal muscle-, adipose- and skin tissue biopsies, arteriography, echocardiography, carotid Doppler examination, whole body MRI, CT of muscle and measurements of muscle strength.

*Results:* The primary outcome measure is transcriptomic and epigenomic changes in skeletal muscle. Secondary outcome measures include transcriptomic and epigenomic changes associated with metabolism in adipose and skin, muscle strength, fat cell size and ability to release fatty acids from adipose tissue, cardiovascular function, and body composition.

*Conclusions:* This study will provide novel information on the role of sex hormone treatment in skeletal muscle, adipose and skin, and its relation to cardiovascular and metabolic disease.

*Abbreviations:* ANOVA, Andrology Sexual Medicine and Transgender Medicine at the Karolinska University Hospital; BSA, body surface area; CFR, coronary flow velocity reserve; GETS, GEnder Dysphoria Treatment in Sweden; GnRH, Gonadotropin releasing hormone; HOMA-IR, Homeostatic model assessment of insulin resistance; PBMC, peripheral blood mononuclear cells; TAPSE, right ventricular tricuspid annulus; TTE, transthoracic echocardiography

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## 1. Introduction

The effects of sex on different biological mechanisms is a recurrent question in medical research and it is obvious that sex and gender have great impact on epidemiology, risk, clinical manifestations and course of disease [1]. Many of the biological differences seen in men and women in skeletal muscle, adipose, and cardiovascular risk factors are driven by relative differences in estrogen and testosterone levels, even though the primary divergent embryo and fetal development depend on the chromosome constitution and key genes [2–4]. Differences that are particularly significant but not well known are 1) regulation of skeletal muscle mass and adipose tissue, 2) metabolic changes in the regulation of glucose homeostasis and lipid metabolism, and 3) regulation of vascular function and structural effects on the heart and arteries. Traditionally, human studies investigating sex differences have compared men and women. However, it is very difficult to match subjects and avoid confounders in this comparison since individual differences are vast at genetic levels, as are environmental exposures during development in early and later life. Subjects exposed to both estrogen and testosterone at different time points would be ideal to compare the effects of sex-hormones in comparison to constitutional gene expression profiles.

Gender dysphoria denotes the distress that gender incongruence with the assigned sex at birth may cause. Gender-affirming treatment aims to align the body with the gender identity and includes treatment with designated sex hormones and may also include surgery to change primary and secondary sex characteristics, voice therapy, and hair removal, and could be either masculinizing or feminizing. The GETS study described here is designed to investigate the effects of altered sex hormone pattern on skeletal muscle, adipose, skin, heart, blood vessels and metabolic risk factors in subjects with gender dysphoria undergoing cross sex-hormone treatment. Both transgender men and women are studied. The primary outcome of the GETS study is transcriptomic and epigenomic changes in skeletal muscle. Secondary outcome measures include transcriptomic and epigenomic changes associated with metabolism in adipose and skin, muscle strength, fat cell size and ability to release fatty acids from adipose tissue, cardiovascular function, and body composition.

## 2. Methods

### 2.1. Study design

This study is designed as a single center observational cohort study.

### 2.2. Recruitment

The study population consists of individuals that have been referred to ANOVA, Andrology Sexual Medicine and Transgender Medicine at the Karolinska University Hospital, Stockholm, Sweden for evaluation of gender dysphoria and who have been accepted to start gender-affirming medical intervention. These individuals diagnosed with gender dysphoria are assessed for eligibility (see Table 1 for inclusion and exclusion criteria). If eligible, they are provided a brief oral and written presentation of the study background and practical implications. A total of 40 patients (20 transgender men and 20 transgender women) are planned to be recruited.

### 2.3. Informed consent

The subjects are informed that their participation in the study is completely voluntary and that they could withdraw their consent to participate at any time without the need of explanation. They are also informed that their decision to participate or not, or the withdrawal of consent to participate, would not in any way change their treatment. Oral and written informed consent is obtained from all subjects. The regional ethical review board in Stockholm, Sweden, approved the study (Dnr 2014/409-31/4).

**Table 1**
Inclusion/exclusion criteria.

| Inclusion criteria |
|---|
| Age 20 - < = 40 years |
| Individuals with Gender Dysphoria that have been accepted for cross-sex hormone treatment |
| Willingness to participate in the study |
| **Exclusion criteria** |
| Already started with any hormone therapy |
| Ongoing infectious disease |
| Treatment with warfarin or other anticoagulants |
| History of cardiovascular disease |
| Type 1 diabetes |
| Other serious psychiatric or somatic morbidity |
| Alcohol or drug dependency |
| Language difficulties |

### 2.4. Overview of study design

An overview of the study time-line is shown in Fig. 1. Examinations are conducted at four time points: (1) before treatment initiation, (2) four weeks after initiated gonadal hormonal down regulation but before hormone replacement, (3) three months after hormone replacement therapy, and (4) eleven months after hormone replacement therapy. Each time point is divided into two examination days (Mondays and Thursdays). On the first day, the participant comes to the laboratory in the morning after an overnight fast. After at least 5 min of rest, blood pressure is measured and blood samples are collected. After administration of local anesthesia, tissue samples from skeletal muscle, adipose and skin are collected. Subsequently, after a 15 min rest, arterial stiffness is measured with an arteriograph. On the second day, muscle strength is evaluated using isokinetic dynamometry. After a 15 min rest, a transthoracic echocardiography (TTE) is performed for estimation of chamber dimensions as well as ventricular and valvular function, and coronary flow velocity reserve (CFR) is assessed in the left anterior descending coronary artery by pulsed Doppler, followed by a carotid Doppler examination. On the first and last time points of the study, the participants undergo a CT muscle scan followed by whole body MRI. These investigations are performed on a separate day before the biopsies.

### 2.5. Medical treatment

Endocrine therapy in order to reverse the endocrine environment from male to female and vice versa is initiated with injection of a Gonadotropin releasing hormone (GnRH) antagonist (Degarelix 240 mg sc). This results in immediate reduction in gonadotropin secretion and brings sex hormone levels (estradiol and testosterone) to castrate levels within 24 h and for the duration of the washout period of 4 weeks. Continued cross-hormone treatment is started after post castration assessments have been made. Transgender men (former called female-to-male) are treated with testosterone injections (testosterone undecanoate 1000 mg i.m.) with the two first injections given with a 6 week interval and thereafter one injection every tenth week, with dose adjustments in order to maintain androgen levels within the normal adult male reference range. Further gonadotropin suppression is maintained with GnRH-analogue administered i.m. every third months. Transgender women (former called male-to-female) are also given GnRH-analogue treatment to maintain suppression of the gonadal axis. Estradiol therapy is administered with either transdermal therapy or i.m. injections (estradiol polyphosphate) both aiming to produce serum