CLINICAL EFFECTS OF CROSS-SEX HORMONE THERAPY IN ADULT TRANS PERSONS

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CLINICAL EFFECTS OF CROSS-SEX HORMONE THERAPY IN ADULT TRANS PERSONS

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LIST OF ABBREVIATIONS

aBMD	Areal Bone Mineral Density
APC	Activated protein C
AR	Androgen Receptor
BMD	Bone Mineral Density
BMI	Body Mass Index
CA	Cyproterone acetate
CAPI	Computer Assisted Personal Interview
CASI	Computer Assisted Self Interview
CBG	Corticosteroid-Binding Globulin
CSH	Cross-sex Hormone
COC's	Combined Oral Contraceptives
CRP	C-Reactive Protein
CTX	C-terminal telopeptides of type 1 collagen
CV	Coefficient of Variation
CVD	Cardiovascular Disease
DHEA	Dehydroepiandrosterone
DHEAS	Dehydroepiandrosterone Sulfate
DHT	Dihydrotestosterone
DSM	Diagnostic and Statistical Manual
DXA	Dual energy X-ray Absorptiometry
E_2	Oestradiol
ECCA	Échelle d'evaluation Clinique des Cicatrices d'Acné
EE	Ethinyl Estradiol
ER	Estrogen Receptor
ERα	Estrogen Receptor alfa
ERβ	Estrogen Receptor beta
ERKO	Estrogen Receptor Knock-Out
EV	Estradiol valerate
FFA	Free Fatty Acids
FSH	Follicle Stimulating Hormone
FT	Free Testosterone
F&G	Ferriman and Gallwey
GA	Gender Ambivalence

GAGS	Global Acne Grading Scale
GI	Gender Incongruence
GID	Gender Identity Disorder
GD	Gender Dysphoria
GnRH	Gonadotropin Releasing Hormone
hCG	Human Chorion Gonadotrophin
HRT	Hormone Replacement Therapy
HSDD	Hypoactive Sexual Desire Disorder
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HDL	High-Density Lipoprotein
HSD	Hydroxysteroid Dehydrogenase
ICD	International Classification of Diseases
IGF1	Insulin-like Growth Factor
IGF1BP3	Insulin-like Growth Factor-Binding Protein 3
IL6	Interleukin 6
LC-MS	Liquid Chromatography-Mass Spectrometry
LDL	Low-Density Lipoprotein
LGB	Lesbian Gay Bisexual
LGBT	Lesbian Gay Bisexual Trans
LH	Luteinizing Hormone
LPL	Lipoprotein Lipase
MABP	Mean Arterial Blood Pressure
MI	Myocardial Infarction
PE	Pulmonary Embolism
pQCT	Peripheral Quantitative Computed Tomography
PSA	Prostate Specific Antigen
PSU	Pilosebaceous Unit
PTH	Parathyroid Hormone
P1NP	Protocollagen 1 Aminoterminal Propeptide
QOL	Quality Of Life
RANKL	Receptor Activator of the Nuclear Factor kB Ligand
RLE	Real Life Experience
SD	Standard Deviation
SDI	Sexual desire Inventory
SMI	Sexual Minority Individual
SRS	Sex Reassignment Surgery
SRT	Sex Reassignment Therapy

SHBG	Sex Hormone Binding Gobulin
T	Testosterone
TIA	Transient Ischemic Attack
VLDL	Very Low-Density Lipoprotein
VT	Venous Thrombosis
WHO	World Health Organisation
WHI	Women's Health Initiative
WHR	Waist-Hip Ratio
WPATH	World Professional Association of Transgender Heath

NEDERLANDSTALIGE SAMENVATTING

Genderdysforie wordt omgeschreven als het lijden dat een persoon ervaart ten gevolge van de incongruentie tussen enerzijds zijn/haar genderidentiteit -het gevoel man, vrouw of ongedetermineerd te zijn- en zijn/haar geboortegeslacht anderzijds. Om dit lijden te verminderen wordt vaak een medische behandeling ondergaan die bestaat uit een hormonale behandeling en chirurgische ingrepen.

Deze hormonale behandeling heeft als voornaamste doel om de secundaire geslachtskenmerken van het gewenste geslacht te bekomen en deze van het geboortegeslacht te onderdrukken. De hormonale behandeling van de transvrouw, of man-naar-vrouw transseksuele persoon, bestaat meestal uit een combinatie van anti-androgenen, welke de effecten van het mannelijk geslachtshormoon testosteron onderdrukken, en vrouwelijke hormonen of oestrogenen, die de feminisatie induceren. Bij de transman of vrouw-naar-man transseksuele persoon wordt testosterontherapie gegeven om masculinisatie te bekomen en de menstruatie te onderdrukken.

Naast de effecten op de ontwikkeling van de geslachtsorganen en secundaire geslachtskenmerken, spelen geslachtshormonen ook een rol in tal van fysiologische processen in spier-, vet- en botweefsel alsook in de huid, het immuunsysteem en het cardiovasculair stelsel. Hierdoor wordt de veiligheid van de hormonale behandeling bij transpersonen soms in vraag gesteld. Tot op heden zijn de effecten van de hormonale behandeling op korte en lange termijn echter niet goed gekend mede door de zeldzaamheid van deze diagnose, een gebrek aan studies en grote variaties in behandelingsschema's tussen verschillende centra.

In de afgelopen jaren werd het belang van kwaliteit van leven van patiënten en de invloed van de behandeling op de kwaliteit van leven van patiënten steeds meer belangrijk in de research agenda. Deze informatie ontbrak echter nog in deze specifieke populatie.

De doelstellingen van deze thesis waren daarom tweeledig. Ten eerste was het de bedoeling om meer informatie te verwerven over de klinische effecten en bijwerkingen van cross-sex hormonale behandeling op korte en lange termijn, zowel bij transmannen als transvrouwen. Ten tweede wilden we de kwaliteit van leven van transpersonen nagaan na de hormonale behandeling en associaties onderzoeken tussen kwaliteit van leven en verschillende aspecten van de behandeling.

In **hoofdstuk 1** wordt de synthese en het werkingsmechanisme van geslachtshormonen en hun klinische effecten beschreven. Daarnaast worden ook de reeds gekende effecten en bijwerkingen van cross-sex hormonale behandeling bij transpersonen besproken. Tot slot werden in dit hoofdstuk de doelstellingen van dit werk geformuleerd, de bestudeerde populaties en gebruikte materialen en methodes toegelicht.

Het doel van **hoofdstuk 2.1** was om de klinische effecten en bijwerkingen van de hormonale therapie tijdens de eerste 12 behandelingsmaanden na te gaan aan de hand van een prospectieve multicentrische studie in 53 transvrouwen en 53 transmannen. De resultaten van deze studie toonden dat de gebruikte behandelingsschema's in transvrouwen met cyproteroneacetaat 50 mg in combinatie met oestrogenen (<45 jaar: oestradiol valeraat 4 mg per dag, ≥45 jaar: transdermale 17β oestradiol pleister 100 μg/24u) en in transmannen (3 maandelijkse injecties met testosteron undecanoaat 1000 mg) veilig en effectief waren. Tijdens deze studieperiode werden geen ernstige bijwerkingen zoals cardiovasculaire aandoeningen of sterfte waargenomen. Bij 5.6% van de transvrouwen werd een transiënte stijging van de leverenzymes opgemerkt en bij 15.7% een stijging van de prolactinespiegels (tweemaal hoger dan de bovenlimiet van de referentiewaarden). Tijdens de behandeling met anti-androgenen en oestrogenen werd een significante stijging van symptomen van borstgevoeligheid, warmteopwellingen, emotionaliteit en verlaagd seksueel verlangen gevonden. Transmannen hadden een klein risico voor het ontstaan van erythrocytose (3.8%) en transiënte stijging van de leverenzymes (1.9%) en rapporteerden beduidend meer symptomen van hoog seksueel verlangen, steminstabiliteit en clitorale pijn tijdens de testosterontherapie.

In **hoofdstuk 2.1** maar vooral in **hoofdstuk 2.2** beschreven we de veranderingen in cardiovasculaire risicofactoren tijdens de cross-sex hormonale behandeling. We toonden aan dat deze behandeling bij transvrouwen enerzijds gunstige veranderingen in cardiovasculaire risicofactoren induceerde zoals een verbetering van het lipidenprofiel en het ontstaan van een gynecoïde lichaamsbouw (of 'peervorm' waarbij de vetmassa zich voornamelijk opstapelt ter hoogte van de dijen); maar anderzijds ook ongunstige veranderingen teweegbracht door een daling in de spiermassa en insulinegevoeligheid en een stijging van de vetmassa thv het abdomen. Net zoals bij transvrouwen, induceerde de hormonale behandeling bij transmannen behalve gunstige ook ongunstige veranderingen. Enerzijds werd er een daling in vetmassa en een stijging in spiermassa en insulinegevoeligheid geobserveerd maar anderzijds induceerde de testosterontherapie, geheel in tegenstelling tot de behandeling bij de transvrouw, een minder

gunstig lipidenprofiel en de zogenaamde androïde lichaamsbouw (of 'appelvorm' waarbij vetmassa zich voornamelijk ter hoogte van de buik opstapelt).

Dat een teveel aan androgenen of mannelijke hormonen bij vrouwen zorgt voor dermatologische bijwerkingen zoals hirsutisme, acne en androgene alopecie (ook gekend als 'klassieke mannelijke kaalheid') is gekend. Een van onze onderzoeksdoelen was dan ook de dermatologische effecten van de testosterontherapie na te gaan aangezien de gegevens hierover schaars waren (**hoofdstuk 2.3**). Zoals verwacht (en beoogd) observeerden we tijdens het eerste jaar van de testostosteronbehandeling een stijging in gezicht- en lichaamsbeharing zodat 80% van de transmannen hirsutisme hadden op basis van de Ferriman and Gallwey score, een frequent gebruikte evaluatiemethode. We vonden een relatief laag risico voor het ontwikkelen van androgene alopecie tijdens de eerste 12 behandelingsmaanden maar een hoog risico voor het ontstaan van acne. Deze acneletsels namen toe zowel op het gezicht als op de rug en borstkas zodat ongeveer de helft van de transmannen een lokale of orale acnebehandeling startte. Toch moet gezegd worden dat de meeste mannen slechts milde of matige acneletsels hadden en bij geen enkele man ernstige of zeer ernstige acneletsels werden vastgesteld.

Niet alleen de effecten van de testosteronbehandeling op de huid bij transmannen op korte termijn maar ook deze op lange termijn werden beschreven in **hoofdstuk 2.3** aan de hand van een studie bij 50 transmannen die een gemiddelde behandelingsduur van 10 jaar hadden. Deze studie toonde dat de gezicht- en lichaamsbeharing nog flink toeneemt tijdens de testosterontherapie en dat de tevredenheid van transmannen ook sterk geassocieerd was met deze toename in beharing. In tegenstelling tot de gezicht- en lichaamsbeharing nam de schedelbeharing verder af zodat bij ongeveer 64% van de transmannen androgene alopecie werd vastgesteld. De acne scores waren daarentegen veel lager dan deze tijdens het eerste jaar van de testosterontherapie.

In **hoofdstuk 2.4** en **hoofdstuk 2.5** werd aan de hand van drie verschillende studies de langetermijneffecten op de algemene gezondheid van transpersonen beschreven. De eerste twee studies werden uitgevoerd bij respectievelijk 50 transvrouwen en 50 transmannen, die gemiddeld 10 jaar met hormonale therapie werden behandeld. We observeerden dat 11,5% van de transvrouwen en 26,7% van de transmannen suprafysiologische hormonale waarden hadden tov de normale referentiewaarden van het gewenste geslacht, wat het risico op cardiovasculare aandoeningen kan vergroten. Anderzijds hadden 63% van de transvrouwen en 8.9% van de transmannen subfysiologische waarden tov de normale referentiewaarden, wat dan weer het risico verhoogt op bijwerkingen zoals gekend bij hypogonadale personen. Inderdaad, een belangrijke

bevinding van deze studie was dat ongeveer een vierde van de transvrouwen osteoporose had ter hoogte van de lumbale wervelzuil, heup of arm. Daarentegen werd bij transmannen geen osteoporose vastgesteld. Er werden geen hormoongerelateerde kankers genoteerd noch bij transvrouwen noch bij transmannen.

In hoofdstuk 2.1 en 2.2 beschreven we veranderingen in cardiovasculaire risicofactoren tijdens de eerste 12 maanden van de hormonale behandeling. De langetermijneffecten van de hormonale behandeling op het cardiovasculair risicoprofiel werden gerapporteerd in hoofdstuk 2.4. We observeerden dat transvrouwen en transmannen een gelijkaardige prevalentie van cardiovasculaire risicofactoren zoals obesitas, roken, hoge bloeddruk en verhoogde cholesterolspiegel hadden na een gemiddelde behandelingsduur van 10 jaar. Tevens hadden transpersonen een vergelijkbaar cardiovasculair risicoprofiel als beschreven in de algemene populatie, afgezien van een hoger aantal rokers in de transpopulatie. Desondanks vonden we in deze studie dat transvrouwen, maar niet transmannen, een opmerkelijk hogere prevalentie hadden van cardiovasculaire aandoeningen (12% versus 0%). Ook in deze studies werden geen verhoogd risico voor hormoongerelateerde kankers vastgesteld.

Omdat deze onderzoekspopulaties eerder klein waren, initieerden we de TransBel studie, een cross-sectionele multicentrische vragenlijststudie met als doel om de morbiditeit van de hormonale behandeling bij transpersonen beter in kaart te brengen. De recrutering in andere centra is momenteel nog lopende en daarom werden in dit proefschrift enkel de gegevens van ons eigen centrum besproken. Een totale populatie van 352 transpersonen werd geïncludeerd, gemiddeld 7,4 jaar behandeld met hormonale therapie. We bevestigden de resultaten van de klinische studies die geen verhoogd risico op hormoongerelateerde kankers aantoonden maar wel een verhoogd risico op cardiovasculaire ziekten bij transvrouwen doen vermoeden. Zo hadden respectievelijk 5,1% en 3,7% van de transvrouwen een veneuze trombose of cardiovasculaire aandoening doorgemaakt tijdens de hormonale behandeling. De voornaamste risicofactoren voor cardiovasculaire ziekte in deze studie waren oudere leeftijd en aanwezigheid van gekende cardiovasculaire risicofactoren met als voornaamste roken.

Afgezien van deze fysieke veranderingen toonden we in **hoofdstuk 3** en **hoofstuk 4** dat cross-sex hormonale behandeling ook een belangrijke invloed heeft op het seksueel functioneren en de kwaliteit van leven van transpersonen. We hebben reeds vermeld dat transvrouwen een lager seksueel verlangen en transmannen een hoger seksueel verlangen rapporteerden in onze prospectieve studie. Dit werd bevestigd in de TransBel studie aangezien 62,4% van de transvrouwen retrospectief een daling- en 71,0% van de transmannen een stijging in seksueel

verlangen rapporteerden (**hoofdstuk 3.1**). Bovendien vermeldde 73% van de transvrouwen dat ze zelden of nooit spontaan en responsief seksueel verlangen ervaren. Een derde van deze vrouwen (en dus 22% van de gehele groep van transvrouwen) gaf aan hierdoor persoonlijk of relationeel lijden te ervaren, een aantal dat hoger ligt dan beschreven in de algemene populatie. In tegenstelling tot transvrouwen, werd bij transmannen een gelijkaardige prevalentie gevonden van deze stoornis van hypoactief seksueel verlangen als in de algemene populatie. Bovendien gaf een kleine groep transmannen (3,6%) aan dat zij persoonlijk of relationeel lijden hebben ervaren door een teveel aan seksueel verlangen.

In een vorige studie van onze onderzoeksgroep werd geen verband aangetoond tussen concentraties in het bloed circulerende geslachtshormonen en seksueel verlangen bij transvrouwen. In **hoofdstuk 3.2** rapporteerden we deze potentiële associaties bij transmannen. In overeenstemming met de resultaten bij transvrouwen, werden geen directe associaties gevonden tussen (vrij) testosteron concentraties en solitair of dyadisch seksueel verlangen (respectievelijk het verlangen van een individu naar seksueel contact met zichzelf en het verlangen naar seksuele activiteiten met een partner). Maar we vonden wel een associatie tussen LH spiegels en solitair seksueel verlangen. Transmannen die hogere LH waarden hadden, wat een suboptimale testosterontherapie suggereert, hadden minder seksueel verlangen in vergelijking met transmannen met lagere LH waarden. Transmannen met lagere LH waarden rapporteerden ook een hogere nood te hebben aan seksuele activiteiten en meer last te hebben van een teveel aan seksueel verlangen.

In **hoofdstuk 4** werd de kwaliteit van leven van transpersonen geëvalueerd en vergeleken met een voor leeftijd en geslacht gepaarde controlepopulatie, die gerecruteerd was uit een groot Vlaams populatieonderzoek over seksuele gezondheid (de Sexpert-studie). Deze resultaten toonden dat transpersonen een lagere kwaliteit van leven hadden in vergelijking met deze controlepopulatie, ook na correctie voor belangrijke determinanten zoals leeftijd en socio-economische status. Zowel fysiek functioneren als mentaal functioneren scoorden significant lager bij transpersonen, maar het absoluut verschil was veel groter voor mentaal welzijn in vergelijking met fysiek welzijn. Ten tweede, werden de voornaamste determinanten van kwaliteit van leven bij transpersonen onderzocht. Wij vonden dat hormonale tevredenheid en werkstatus onafhankelijke predictoren waren van kwaliteit van leven zowel bij transvrouwen als bij transmannen. Het hebben van kinderen en het ondergaan hebben van de vaginoplastie waren additionele onafhankelijke negatieve predictoren van fysiek functioneren bij de transvrouw. Deze laatste associatie verdween echter wanneer we transvrouwen die recent geopereerd waren

excudeerden. Daarentegen was het ondergaan hebben van een gezichtsveranderende operatie een positieve determinant van mentaal welzijn bij de transvrouw. Bij transmannen was het hebben van kinderen een positieve determinant van mentaal welzijn.

Tot slot worden in **hoofdstuk 5** de belangrijkste bevindingen van deze verschillende studies samengevat en besproken, gevolgd door een vermelding van aantal mogelijke toekomstige onderzoeksvragen.

Samenvattend hebben we in dit proefschrift aangetoond dat onze huidige hormonale behandeling bij transpersonen effectief en relatief veilig is op korte termijn. Anderzijds toonden we aan dat langdurige hormonale behandeling mogelijk het risico kan verhogen op cardiovasculaire ziekte, vooral bij transvrouwen. Naast deze effecten op de fysieke gezondheid van transpersonen, observeerden we ook belangrijke effecten van de hormonale behandeling op het seksueel verlangen en kwaliteit van leven van transpersonen.

SUMMARY

Gender dysphoria is described as a condition in which a person experiences a discrepancy between the sex assigned at birth and the gender they identify with, leading to extensive personal distress. To conform to the other sex, trans persons often undergo treatment which may include hormonal therapy and surgery.

The main goals of cross-sex hormone therapy are to acquire the secondary sex characteristics of the desired sex and to reduce those of the natal sex. Trans women or male-to-female transsexual persons usually receive a combination of anti-androgen and estrogen therapy to induce feminization whereas trans men or female-to-male transsexual persons receive testosterone treatment to induce virilisation and suppress menstruation.

Besides its effects on secondary sex characteristics and reproductive tissue development, sex steroids are also involved in many other physiological processes in muscle, fat, bone, skin, larynx, immune system, hematopoietic cells and cardiovascular system. The latter raise concerns about the safety of cross-sex hormone treatment. Although this treatment has been used for several decades, our knowledge regarding short- and long-term effects and adverse effects of cross-sex hormone therapy is scarce, mainly due to the low prevalence of this diagnosis, lack of prospective studies and wide variations in treatment modalities between centers.

In the past years, the importance of patients'quality of life (QoL) and the changes in QoL induced by medical treatment has become increasingly important, but these data were scarce in trans persons.

The objectives of this thesis were therefore twofold: first, to investigate the short-and long-term physical changes, side effects and adverse events of cross-sex hormonal therapy in trans persons and second to study QoL of trans persons and its association with cross-sex hormone therapy.

In **Chapter 1,** a general background with a review of the available literature on effects and side effects of cross-sex hormone therapy was presented and discussed. In addition, we stated our research objectives and described our studies conducted for this purpose. We continued with material and methods used in our research.

The aim of **Chapter 2** was to investigate the effects, side effects and adverse events during the first year of cross-sex hormone therapy using a multicenter prospective study in 53 trans women

and 53 trans men. We observed that our current treatments with cyproterone acetate 50 mg daily and estrogens (< 45 years: estradiol valerate 4 mg daily and ≥ 45 years 17β estradiol patch 100 μg/24h) in trans women and 3-monthly injections with testosterone undecanoate in trans men, were all effective and safe therapies. None of our trans patients experienced severe adverse events such as cardiovascular events or death. About 5.7% of trans women experienced transient elevation of the liver enzymes whereas 15.7% had prolactin above double the upper limit of the normal range. During anti-androgen and estrogen treatment, a significant increase in breast tenderness, hot flashes, emotionality and low sexual desire was found. In trans men a small risk for erythrocytosis and transient elevation of the liver enzymes was observed. Trans men reported an increase in sexual desire, voice instability and clitoral pain (**chapter 2.1**).

In **chapter 2.2** we focused on the changes in cardiovascular risk factors during the first year of cross-sex hormonal therapy. Hormone therapy in trans women caused favourable changes in lipid profile and induced a gynoid pattern of fat distribution but less favourable changes in glucose metabolism and body composition, with an increase in insulin resistance and fat mass and decrease in lean mass. Similar to trans women, testosterone therapy induced both favourable and unfavourable changes in cardiovascular risk factors. A decrease in appendicular and trunk fat mass and an increase in lean mass and insulin sensitivity was seen. In contrast, testosterone therapy induced a poor lipid profile and an android pattern of fat distribution.

As it is well-known that androgen excess in women is associated with important dermatological effects, such as acne vulgaris, hirsutism and androgenetic alopecia with potentially important psychological disturbing effects, we also aimed to investigate dermatological changes during treatment with testosterone undecanoate (**chapter 2.3**). As expected, testosterone therapy increased facial and body hair and about 4 out of 5 trans men developed Ferriman and Gallwey scores indicative of hirsutism. In addition, we observed a rather low risk for development of androgenetic alopecia but a high risk for acne during the first 12 months of testosterone therapy. Both facial acne scores and acne scores at back or chest increased, causing the need for topical or oral acne treatment in half of partcipants. However, most men developed only mild acne and none suffered from severe or very severe acne.

In **chapter 2.3** we also described long-term effects of testosterone therapy on the skin of trans men using a study of 50 trans men, who were on average 10 years on hormonal therapy. We showed that long-term testosterone therapy further increased facial and body hair and that this further increase contributes to increased patient satisfaction because this was strongly associated with the obtained male hair pattern. In contrast, scalp hair further decreased during treatment so

that about 64% of trans men had androgenetic alopecia. Our long-term observations also show markedly lower acne scores compared to scores during the first year of treatment.

How long-term cross-sex hormone therapy affects physical health of trans persons was addressed in **chapter 2.3**, **2.4** and **2.5** using three different studies. The first two studies were performed in respectively 50 trans men and 50 trans women who were on average 10 years on cross-sex hormone therapy. We observed that 11.5% of trans women and 26.7% of trans men had supraphysiological levels, which may increase the risk for cardiovascular events. In contrast, 63% of trans women and 8.9% of trans men exhibited subphysiological sex steroid levels which put trans persons at risk for side effects known from hypogonadal states. Indeed, an important finding of these studies was that a high number of trans women (about 25%) present with osteoporosis at the lumbar spine, hip or radius, whereas in trans men no osteoporosis was diagnosed. No hormone-related cancers were observed in either trans women or men but 12% of trans women in this study experienced a cardiovascular adverse event during hormonal therapy, compared to not a single trans man.

Because the sample sizes were rather small in our clinical studies, the TransBel was initiated, a cross-sectional multicenter questionnaire study with the aim to investigate morbidity of trans persons in Belgium. As recruitment in other centers is still ongoing, we present in this thesis data from our center including 352 trans persons with an average hormone treatment duration of 7.4 years (**chapter 3.5**). We confirmed the results from our clinical studies showing a low risk for cardiovascular events and cancer in trans men, whereas our results in trans women suggested no higher risk for cancer but again an increased risk for cardiovascular disease. About five percent (5.1%) of trans women experienced venous thrombosis and 3.7% cardiovascular event during hormonal therapy. Risk factors for cardiovascular disease were older age and prevalent cardiovascular risk factors, especially smoking.

Besides physical health changes, cross-sex hormone therapy also affects sexual functioning and QoL. We already showed in our prospective study (**chapter 2.1**) that trans women experience lower levels- and trans men higher levels of sexual desire after initiation of cross-sex hormone therapy. These results were also confirmed in our TransBel study as trans women reported a decrease of sexual desire since the start of cross-sex hormone therapy whereas trans men mentioned an increase (**chapter 3.1**). Moreover, in the TransBel study, 73% of trans women never or rarely experienced spontaneous and responsive sexual desire. A third of these women also reported associated personal or relational distress, which resulted in a prevalence of hypoactive sexual desire disorder (HSDD) of 22%. In contrast, the prevalence of HSDD in trans

men was comparable to that in the general male population. Moreover, a small group of trans men (3.6%) even reported personal or relational distress due to high levels of sexual desire.

Previous research from our center showed no associations between sexual desire and serum levels of sex steroids in trans women. In **chapter 3.2** these potential associations between sexual desire and sex steroid levels were investigated in trans men. No direct associations were observed between testosterone levels and solitary or dyadic sexual desire (the desire to behave sexually by onseself or with a partner). However, an independent effect of LH levels on solitary sexual desire was found. Trans men with elevated levels of LH, indicating suboptimal testosterone therapy, reported significantly lower solitary sexual desire levels than those with low LH levels. Suppressed LH levels were also associated with having a higher need for sexual activities and a higher frequency of experiencing excessive sexual desire.

Chapter 4 discusses QoL of trans persons after long-term hormonal treatment. Firstly, we compared QoL of trans persons to an age- and gender matched control population, recruited from a population based study on sexual health in Flanders (the'Sexpert'-study). We showed that QoL of trans persons was poorer compared to our control population even after adjustment for important determinants of QoL such as age and socio-economical status. Both physical and mental functioning were significantly lower in trans persons, but the absolute difference in physical functioning was rather small, whereas it was marked for mental functioning. Secondly, we investigated the main determinants of QoL in trans persons. We showed that hormonal treatment satisfaction and employment status were independently associated with physical and mental functioning in both trans women and men. In trans women, having children and having undergone vaginoplasty were also negative determinants of physical functioning. However, the latter association was lost when we excluded trans women who underwent vaginoplasty very recently. Facial feminization surgery was also a positive predictor of mental functioning in trans women whereas having children was an additional independent positive determinant of mental functioning in trans men.

Finally, in **chapter 5**, the main findings of our different studies are summarized and discussed, together with perspectives on future research topics.

In conclusion, with this work, we have shown that current cross-sex hormone therapies are effective and safe at short-time follow-up. Alternatively, we showed that long-term cross-sex hormone therapy may be associated with important side effects such as osteoporosis and cardiovascular disease, especially in trans women. Besides these effects on physical health, we also

observed important associations between cross-sex hormonal therapy, sexual functioning and QoL of trans persons.

CHAPTER 1. INTRODUCTION

In this background chapter, we will give an overview on the prevalence, etiology, diagnosis and treatment of gender dysphoria. Further, we will summarize the regulation, synthesis and clinical effects of sex steroids as well as the clinical effects of cross-sex hormone therapy in trans persons.

1

CHAPTER 1. INTRODUCTION

1.1 GENDER DYSPHORIA

1.1.1 INTRODUCTION

Gender identity is defined as the individual's personal sense and subjective experience as being male, female or in between.

Gender role refers to the behaviors, attitudes and personality traits that a society, in a given culture and historical time, designates as masculine or feminine. Or that is seen as "more appropriate" or "typical of" the social role as men or as women.

Variations in gender identity and gender role are evident in many cultures, but with considerable differences across cultures in how such individuals are perceived[1]. In some cultures, gender non-conforming individuals, for example Acault-men of Myanmar[2] and the Hijras of India, are very well accepted in the society. Other cultures, such as our Western Society have been unreceptive to gender non-conforming persons, and enforced a clear sexual dimorphism. Nevertheless, many stories of gender variation have been described in our society[3].

In the beginning of the 20th century, Magnus Hirschfeld performed ground breaking work in this field and opposed against a clear male-female dichotomy. Together with Harry Benjamin, he initiated medical treatment of the "transsexual," a term introduced by Hirschfeld in 1923, to live a gender-appropriate life[4, 5]. Since then, the scientific, clinical and social acceptance have ameliorated and an increasing number of countries nowadays have well-organized gender teams to support trans persons in a multi-disciplinary setting.

1.1.2 DEFINITIONS AND DIAGNOSIS

Gender nonconformity refers to the extent to which a person's gender identity, gender role and/or gender expression differs from the cultural norms prescribed for people of a particular sex, within a certain society and time. This term incorporates a broad spectrum of gender variations, for example cross-dressers, androgynes, transsexuals and other individuals with a lower degree of gender dysphoria. The majority of research is based on the most extreme form of gender nonconformity, in literature described as gender dysphoria (DSM-5), gender identity disorder (DSM IV), or transsexualism (DSM III). They all refer to the distress that is caused by the discrepancy between a persons' gender identity – the sense one has of being male or female

or in between – and the sex assigned at birth. It is important to note that only some gender nonconforming people will experience gender dysphoria at some point in their lives[6].

The diagnosis of Gender Dysphoria relies on assessment by a mental health professional according to the criteria specified in the Diagnostic and Statistical Manual of Psychiatric Diseases (DSM III, DSM IV, DSM IV-TR, DSM-5)[7] or the tenth revision of the International Classification of Diseases (ICD-10)[8]. As in this thesis participants have been diagnosed mainly based DSM IV-TR criteria, both DSM IV-TR and DSM-5 criteria will be discussed.

1.1.2.1 *DSM IV-TR criteria*

A. A strong and persistent cross-gender identification (not merely a desire for any perceived cultural advantages of being the other sex).

B. Persistent discomfort with his or her sex or sense of inappropriateness in the gender role of that sex.

C. The disturbance is not concurrent with a physical intersex condition.

D. The disturbance causes clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Specify whether (for sexually mature individuals):

- Sexually attracted to males
- Sexually attracted to females
- Sexually attracted to both
- Sexually attracted to neither

1.1.2.2 *DSM-5 criteria*

A. A marked incongruence between one's experienced/expressed gender and assigned gender, of at least 6 months' duration, as manifested by at least two of the following:

A marked incongruence between one's experienced/expressed gender and primary and/or secondary sex characteristics (or in young adolescents, the anticipated secondary sex characteristics).

A strong desire to be rid of one's primary and/or secondary sex characteristics because of a marked incongruence with one's experienced/expressed gender (or in young adolescents, a desire to prevent the development of the anticipated secondary sex characteristics).

A strong desire for the primary and/or secondary sex characteristics of the other gender.

A strong desire to be of the other gender (or some alternative gender different from one's assigned gender).

A strong desire to be treated as the other gender (or some alternative gender different from one's assigned gender).

A strong conviction that one has the typical feelings and reactions of the other gender (or some alternative gender different from one's assigned gender).

B. The condition is associated with clinically significant distress or impairment in social, occupational, or other important areas of functioning.

Specify if: **With a disorder of sex development** (e.g., a congenital adrenogenital disorder such as congenital adrenal hyperplasia or an androgen resistance syndrome).

Specify if: **Post transition:** The individual has transitioned to full-time living in the desired gender (with or without legalization of gender change) and has undergone (or is preparing to have) at least one cross-sex medical procedure or treatment regimen - namely, regular cross-sex hormone treatment or gender reassignment surgery confirming the desired gender (e.g., penectomy, vaginoplasty in a natal male, mastectomy or phalloplasty in a natal female).

1.1.2.3 *ICD-10 criteria*

ICD-10 defines a range of gender identity disorders including Transsexualism[8], which has three diagnostic criteria:

1. The desire to live and be accepted as a member of the opposite sex, usually accompanied by the wish to make his or her body as congruent as possible with the preferred sex through surgery and hormone treatment.

2. The transsexual identity has been present persistently for at least two years.

3. The disorder is not a symptom of another mental disorder or chromosomal abnormality.

1.1.3 PREVALENCE OF GENDER DYSPHORIA

The prevalence of gender dysphoria is not well-known[9]. Small sample studies have been conducted in several countries. De Cuypere and colleagues[10] reviewed several studies involving eight countries and also conducted their own research. They reported that the prevalence of gender dysphoria in Belgium ranged from 1:11,900 to 1:45,000 in trans women and 1:30,400 to 1:200,000 in trans men. This wide variation between studies can be explained by use of different definitions as some prevalence numbers were based on the number of legal sex change as noted in the national register[11, 12], number of psychiatric diagnoses[13], number of persons using hormonal therapy[14-16], the number of applicants for sex reassignment surgeries (SRS)[17]or number of persons who underwent SRS[10, 18]. This implies an important selection bias as only persons who undergo treatment are included in the current calculations. Population-based research is required for a more reliable estimation of the prevalence of gender dysphoria as not all persons have access to treatment. In addition, population based research can give us an estimation on the broader spectrum of gender nonconformity. Recently, we performed a population-based study in Flanders on the prevalence of gender nonconformity in the Flemish population. We found that gender incongruence was present in 0.7% of men and 0.6% of women. More information on this study can be found in the Addendum.

1.1.4 ETIOLOGY

The development of a person's gender identity is a complex cognitive and affective learning process, influenced by biological factors such as genetics and pre- and post-natal hormonal environment and interactions with parents, peers, societal and cultural/religious norms and expectations[19].

The development of a person's gender identity and the sex development generally coincide. The actual cause of experiencing a discrepancy between a persons' gender identity and the birth sex remains unknown. Several biological, psychological, and socio-cultural correlates have been related to the existence of gender dysphoria (for review see[20]) but none of these findings can give a formal explanation. The etiology of gender dysphoria is probably based on a complex interaction between all these biological, psychological and socio-cultural factors[20].

1.1.5 TREATMENT

Once the diagnosis of gender dysphoria in adults is made, the therapeutic approach may include psychotherapy or counseling, cross-sex hormone therapy and surgery[6]. The procedure of sex reassignment therapy is presented in Figure 1.1.

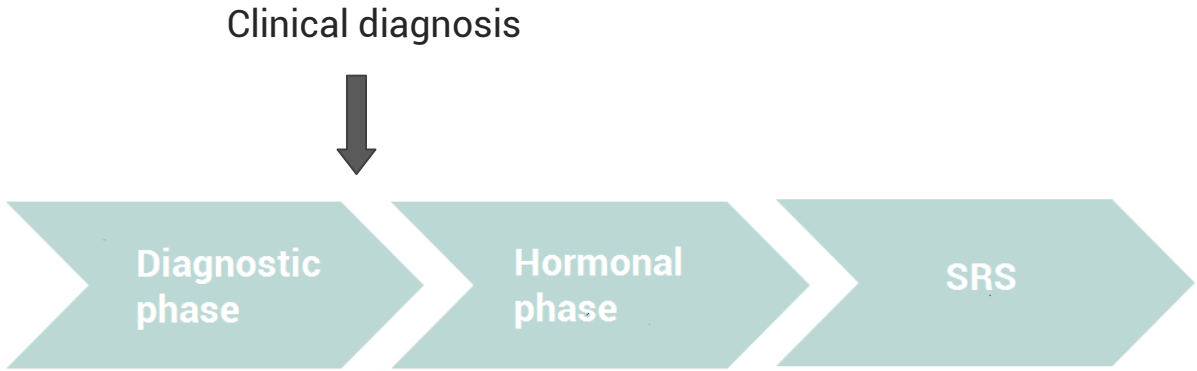

Figure 1.1. Classical procedure of sex reassignment therapy

1.1.5.1 *Psychotherapy or counseling*

Psychotherapy is often the first intervention and is highly recommended by the World Professional Association of Transgender Health (WPATH) Standards of Care[6], but is not a requirement for hormone therapy neither for surgery in the new guidelines of the Standards of Care. The overall goal of psychotherapy is to help an individual to explore gender conflicts and to find ways to alleviate gender dysphoria by setting realistic life goals in their relationships, education and work.

An important part of this exploration occurs by the real life experience. In this period, trans persons are asked to fully live in accordance with their desired gender and to inform their social environment. This real life experience is intended to confront the individual with the everyday life in the desired gender role before irreversible surgical changes are induced. Real life therapy is mostly started at the initiation of cross-sex hormone therapy.

1.1.5.2 *Hormonal therapy*

The two main goals of hormone treatment are to reduce endogenous hormone levels, and thereby the secondary sex characteristics of the individual's biological sex and assigned gender; and to replace endogenous sex hormone levels with those of the desired sex. In addition to inducing physical changes, the act of using cross-sex hormones is itself an affirmation of gender identity in many trans persons.

Trans women usually receive a combination of anti-androgen therapy together with estrogens to induce feminization. Testosteron therapy is used in trans men to induce masculinisation, often preceded by progestins to suppress menstruations. As, the focus of this thesis is to describe the clinical effects of cross-sex hormonal of trans persons, we will discuss this treatment in more detail in section 1.3.

1.1.5.3 *Surgery*

Sex reassignment surgery is often the last and the most considered step in the treatment process for gender dysphoria. Some trans persons live successfully in their desired gender role without surgery, for others surgery is needed to reduce their gender dysphoria. Surgery can involve many procedures (Table 1 and 2). SRS is the most relevant procedure and includes oophorectomy, penectomy and vaginoplasty in trans women and mastectomy, hysterectomy and ovariectomy in trans men. Trans men can proceed with a phalloplasty (creation of a full-sized phallus) or with metaidoioplasty (creation of a microphallus by surgical enhancement of the androgen dependent hypertrophy of the clitoris). Both procedures are generally combined with scrotoplasty and vaginectomy. Trans men who have a phalloplasty can proceed with the placement of an erection prosthesis.

TABLE 1. SURGICAL PROCEDURES FOR TRANS WOMEN (ADAPTED FROM (5))

BREAST SURGERY	GENITAL SURGERY	NON-GENITAL, NON-BREAST SURGERY
Augmentation mammoplasty (implants/lipofilling)	Penectomy Orchiectomy Vaginoplasty Clitoroplasty Vulvoplasty	Facial feminization surgery Liposuction and lipofilling Voice surgery and/or thyroid cartilage reduction Gluteal augmentation (implants/lipofilling) Hair reconstruction Various aesthetic procedures

TABLE 2. SURGICAL PROCEDURES FOR TRANS MEN (ADAPTED FROM (5))

BREAST SURGERY	GENITAL SURGERY	NON-GENITAL, NON-BREAST SURGERY
Subcutaneous mastectomy, creation of a male chest	Hysterectomy Ovariectomy Metadoidioplasty Phalloplasty Vaginectomy Scrotoplasty Implantation of erection and/or testicular prostheses	Voice surgery Liposuction or lipofilling Pectoral implants Various aesthetic procedures

1.2 SEX STEROIDS IN MEN AND WOMEN

Sex steroids are steroid hormones which play an essential role in sexual and reproductive functions as well as the development and maintenance of secondary sex characteristics. In addition, the sexual dimorphism of different organs and tissues also points out a major role for sex steroids in many other processes.

1.2.1 SYNTHESIS, SECRETION AND REGULATION

In both men and women, synthesis and secretion of sex steroids occurs in gonads and adrenal cortex and starts with the conversion of cholesterol into pregnenolone (Figure 1.2). After formation of pregnenolone, the specific hormones produced are dependent on the endocrine organ and cell type due to specific enzyme expression. In the adrenal cortex for example, zona glomerulosa and fasciculata lack different enzymes necessary for sex steroid production.

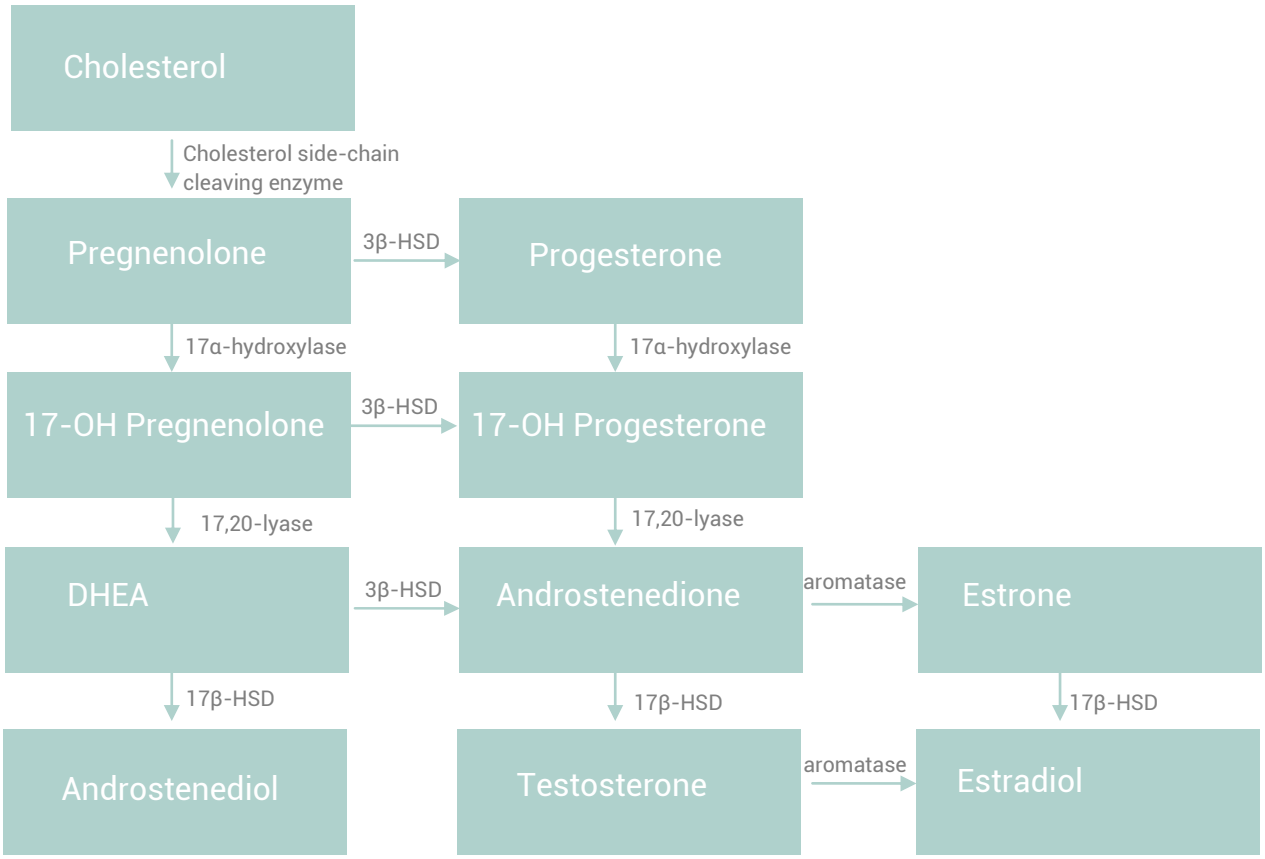

Figure 1.2. Major pathways in sex steroid synthesis (modified from[21])

While men and women both produce estrogens and androgens, the amount of sex hormone production is different and genetically predetermined. Prenatal sex steroid exposure in uterus plays a crucial role in the development of primary sex characteristics. Afterwards, production and secretion of sex steroids is very limited during childhood, apart from a transient 'infant puberty', whereas from puberty on the pulsatile frequency of gonadotropin-releasing hormone (GnRH) secretion triggers gonadotropin production, luteinizing hormone (LH) and follicle stimulating hormone (FSH). In general, the basic mechanisms of the hypothalamic-pituitary-gonadal axis are quite similar in women and men, but in women of reproductive age they are more complex due to the cyclical hormonal changes of the menstrual cycles imposed by the ovaries.

1.2.1.1 *Regulation of the hypothalamic-pituitary-gonadal axis in women*

The menstrual cycle includes a follicular phase and a luteal phase. The follicular phase is characterized by recruitment of new follicles and maturation of a dominant follicle with progressive increase of follicular estrogen secretion towards the midcycle ovulation. After ovulation, the luteal phase is initiated which is characterized by the transformation of the follicle into a corpus luteum that secretes E_1, E_2 and progesterone. If no conception takes place, the cycle ends by the demise of the corpus luteum, with decrease of progesterone and estrogen secretion, which induces menstruation.

This ovarian function is driven by coincidental pulsatile secretion of the pituitary gonadotropic hormones LH and FSH by the gonadotropes, which in turn is driven by release of the hypothalamic GnRH into the pituitary portal circulation. FSH is responsible for the early maturation of the ovarian follicles whereas both FSH and LH are needed for their final maturation. The midcycle surge of LH secretion induces ovulation and initiates the initial formation of the corpus luteum. LH stimulates conversion of cholesterol into androstenedione in the theca cell in the ovarian follicle and corpus luteum. The theca interna cell supplies androstenedione to the granulosa cell and the latter produce E_1 and E_2 through aromatization. FSH facilitates estrogen secretion by activation of FSH receptors on the granulosa cell. Mature follicles also express LH receptors and activation increases estrogen and progesterone synthesis.

GnRH is normally secreted in episodic bursts, which is essential for normal secretion of gonadotropins. Besides this episodic secretion, fluctuations in both the frequency and amplitude of the GnRH bursts also play an important role in hormonal changes during menstrual cycle. These variations are induced, at least in part, by gonadal steroid feedback (Figure 1.3). The feedback of progesterone during the luteal phase lowers the frequency of episodic GnRH secretion, which in turn favours FSH relative to LH secretion by the pituitary gonadotropes and contributes to the recruitment of new follicles in the subsequent early follicular phase of the next cycle. The early follicular phase of the cycle consists of higher frequency low amplitude gonadotropin pulses, controlled by negative feedback action of E_2 at both the pituitary and hypothalamic level. The FSH secretion is also regulated by negative feedback inhibition by inhibin B, a peptide hormone produced by the ovarian granulosa cells.

The substantial progressive increase of E_2 secretion by the dominant follicle towards midcycle results in a temporary reversal of negative feedback of E_2 on gonadotropins into a positive feedback with massive secretion of gonadotropins of only a few hours duration. This so called

'gonadotropin surge' induces ovulation. During the subsequent luteal phase, the combined negative feedback effect of E_2 and progesterone is to slow down the frequency of the hypothalamic GnRH pulse generator, with consequently a marked reduction of the frequency of gonadotropin pulses compared to the follicular phases. Because there is more time for accumulation of gonadotropins between pulses, they are of larger amplitude.

After cessation of follicular ovarian activity at menopause or after removal of the ovaries, there are no longer cyclical changes in gonadotropin secretion and in absence of any substantial negative feedback by estrogens, mean gonadotropin levels are high and characterized by frequent large amplitude pulses; because of loss of negative feedback effect by inhibin B, FSH secretion is higher than LH secretion.

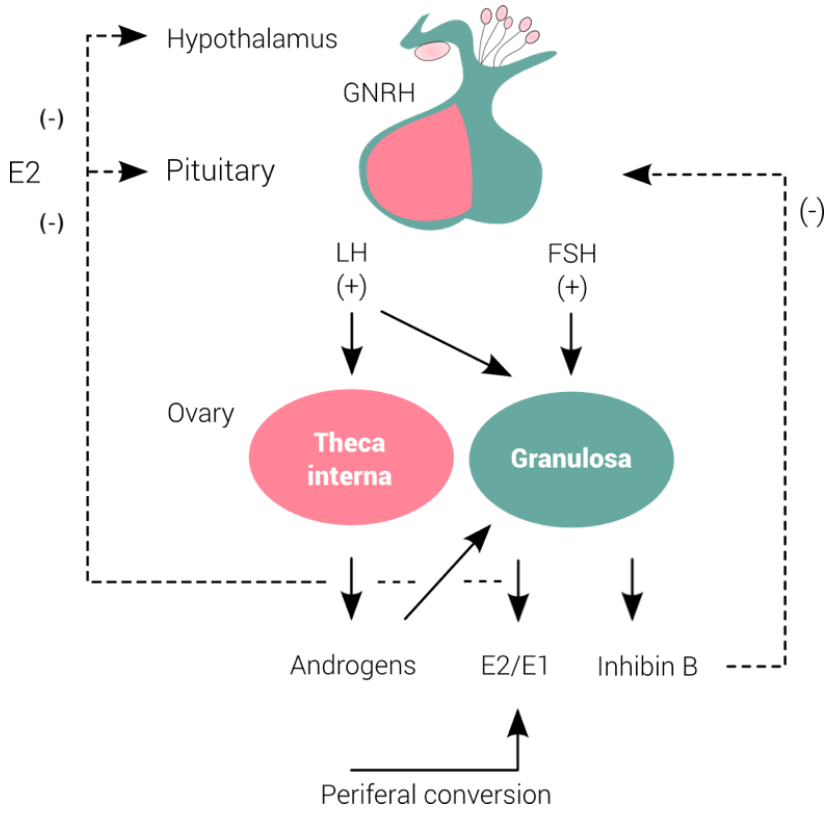

Figure 1.3 Regulation of the hypothalamic-pituitary-gonadal axis women (modified from[21])

More than 95% of E_2 in the female circulation is produced in the ovary. In contrast to E_2, approximately 50% of estrone is secreted in the ovary while the remaining is produced from peripheral conversion of mainly androstenedione, produced in the adrenal gland, and in a lesser extent E_2.

The major androgens in the serum of women (with a normal menstrual cycle) are DHEAS, DHEA, androstenedione, T and dihydrotestosterone (DHT). DHEAS is almost exclusively produced in the adrenals whereas only half of the DHEA levels in women are produced in the adrenals. The remaining 50% are produced in the the ovary (20%) or derived from peripheral conversion of DHEAS (30%). Half of serum T originates from conversion of these adrenal androgens whereas the ovaries and adrenals produce both 25% of serum T levels.

1.2.1.2 *Regulation of the hypothalamic-pituitary-gonadal axis in men*

In men, LH production and secretion, stimulates the Leydig cells to produce T where FSH stimulates spermatogenesis and inhibin B production by the Sertoli cells. The produced hormones are in turn involved in a negative feedback regulation of the gonadotropins (Figure 1.4.) Androgens exert inhibitory actions on LH production at both the hypothalamic and pituitary level, whereas inhibin B is the main regulator of FSH secretion. Besides negative feedback by T itself on LH production, major negative feedback effects on LH secretion are in fact exerted by circulating E_2 produced by aromatization of T[22].

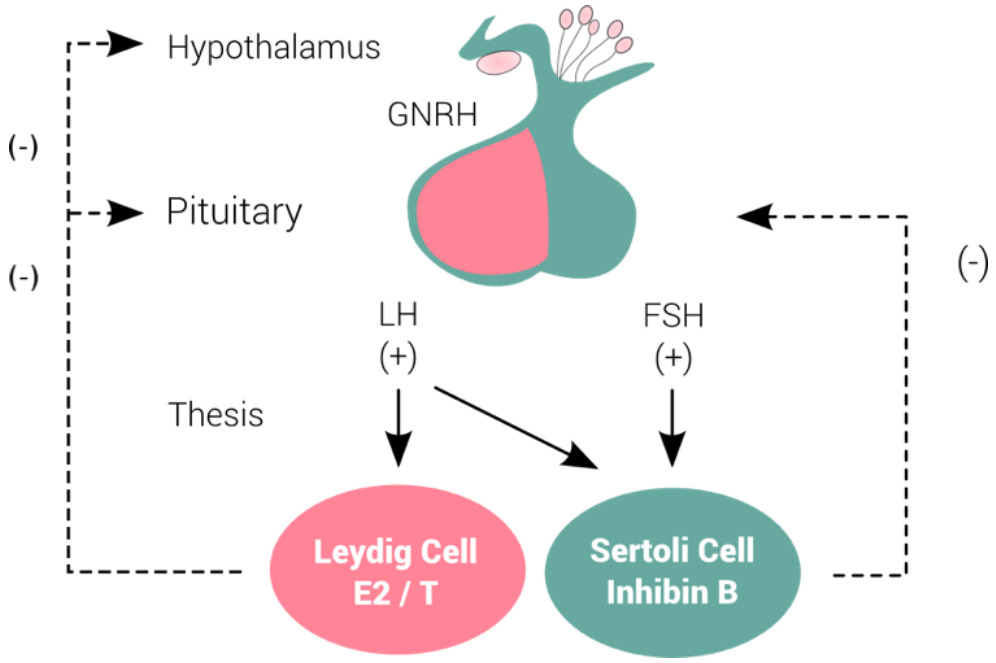

Figure 1.4. Regulation of the hypothalamic-pituitary-gonadal axis in men

Besides this production of T in testis, which accounts for 95% of serum T levels, 5% of T is secreted by the adrenals. In addition, the adrenals produce about 50% of androstenedione and almost all DHEA and DHEAS.

About 1% of the produced T in the testis is converted into E_2 through conversion by the aromatase enzyme in peripheral tissues, which accounts for 80% of the circulating E_2[23]. Aromatization mainly takes place in adipose tissue, but aromatase expression has also been observed in other tissues such as osteoblasts, muscle and brain[24]. The remaining 20% of E_2 in the male circulation is directly secreted by the testis through aromatization of androgens within the Leydig cells. In addition, a small fraction of E_2 is derived from the conversion of E_1, which itself is mainly derived from aromatization of androstenedione. Approximately 5% of T is converted to the more potent androgen, dihydrotestosterone DHT. This occurs by the 5α-reductase enzyme at the tissue level in prostate, testis, skin and adrenal glands. A smaller amount of DHT is derived from peripheral conversion of androstenedione[25] or produced in the testis[26].

1.2.2 TRANSPORT

In the circulation, T and E_2 are mostly bound to serum proteins: i.e., tightly bound to sex hormone binding globulin (SHBG) (40-60%), with much lower affinity to albumin (40-50%) and a small fraction to corticosteroid-binding globulin (CBG) (<5%)[27]. E_2 binds to SHBG with a lower affinity than T. Only a small fraction (1-4%) of sex steroids is unbound, or "free"[28]. The free fractions are directly available to enter the cell and initiate metabolic actions. In addition, some hormone fractions, weakly bound to serum albumin, may dissociate from the protein during passage in the tissues and enter the cells. Therefore, both unbound and albumin-bound sex steroids are seen as the biologically available T and E_2 fractions[29].

1.2.3 MECHANISM OF ACTION

1.2.3.1 *Nuclear binding of sex steroid hormones*

Action of sex steroids at cellular level occurs by binding to their respective nuclear receptor: estrogen receptor alpha (ERα) and beta (ERβ) for estrogens and the androgen receptor (AR) for T and DHT. The AR does not bind androstenedione or DHEA(S) and their androgenic effects are supposed by their local transformation to T. Binding of sex steroids with their respective receptor induces a conformational change in the receptors protein structure resulting in a dissociation of the AR from chaperone proteins. After dimerization, the activated receptor is translocated to the nucleus, where it binds to a specific AR- or ER-response element on the promoter regions of the target genes, stimulating transcription of a specific target gene. Additionally, specific co-regulators can enhance or reduce transcription activation[30].

1.2.3.2 *Non-genomic effects of sex steroids*

Although sex steroids are known to exert most of their action through the classical genomic regulation of gene transcription, there is also evidence for rapid activation via 'non-genomic' pathways, which do not depend on gene transcription or protein synthesis but involve steroid-induced modulation of cytoplasmic or cell membrane-bound regulatory proteins[31]. Up till now, the receptor mechanisms mediating these rapid effects are still not entirely understood.

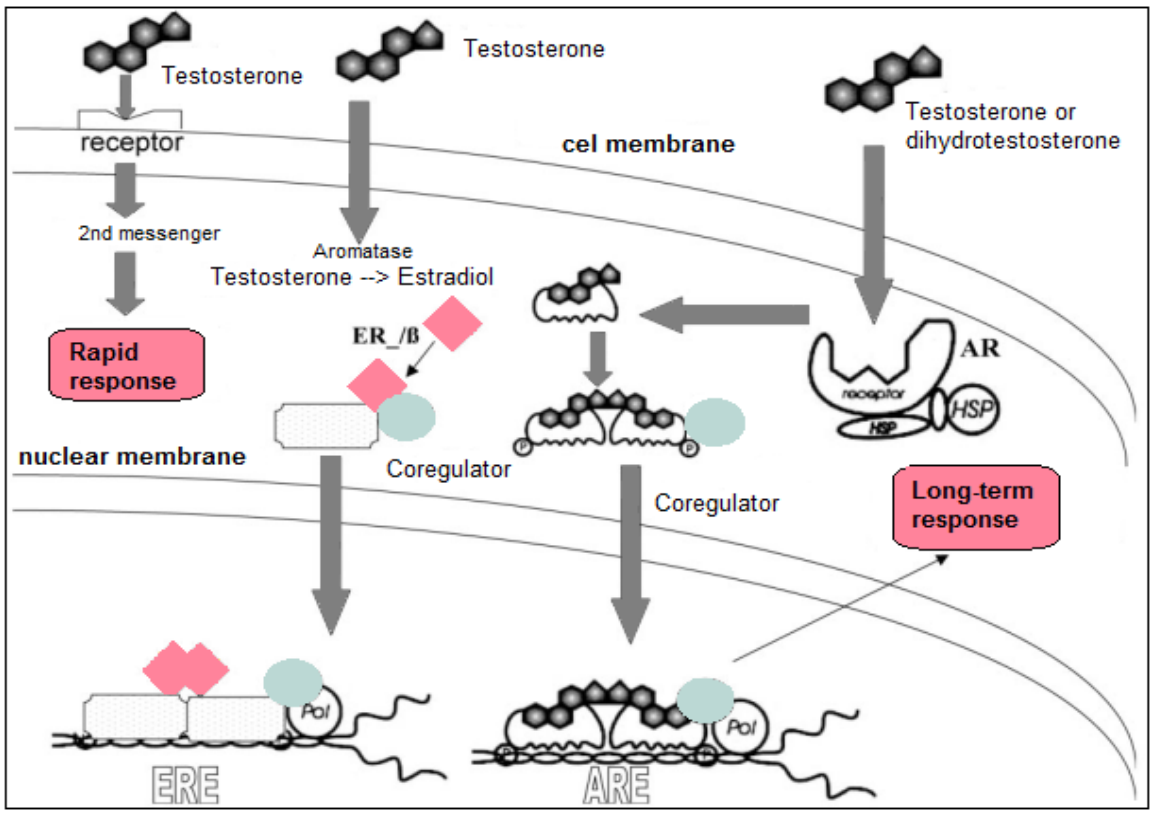

Figure 1.5. Nongenomic and genomic androgen mechanisms (modified from[32])

Left, Nongenomic effects of testosterone, which are triggered by binding to a still uncharacterized (nonclassical) membrane receptor. This activates second messengers including calcium and protein kinases, which produce typically rapid responses. Genomic effects are depicted in the *middle* and on the *right*. Testosterone (or other aromatizable synthetic androgens) crosses the cell membrane to be converted to estradiol (or the aromatic synthetic analog) by aromatase, which then binds to and activates ERα or ERβ. *Right*, DHT (or other nonaromatizable androgens) enters the cell to bind and activate the AR. Ligand-bound ER or AR dissociate their heat-shock protein (HSP), undergo conformational changes, dimerize, and translocate into the nucleus where they bind to specific sites known as estrogen response elements (ERE) or androgen response elements (ARE) located within the DNA of target nuclear genes to produce long-term genomic effects of testosterone.

1.2.4 CLINICAL EFFECTS OF SEX STEROIDS

Sex steroid receptors are widely spread throughout the body and are therefore responsible for the development and function of reproductive organs but also play a role in the development and function of many other tissues and systems, such as muscle, fat, bone, skin, the larynx, immune system, hematopoietic cells and cardiovascular system. Sex steroid receptors are also expressed in brain tissue and it is well-established that they play a major role on motivational aspects of sexual functioning. In the next section, these effects will be discussed in more detail.

1.2.4.1 *Sex steroids and the skin pilosebaceous unit*

Sex steroids are well-known to affect the skin pilosebaceous unit (PSU) and both androgen and estrogen receptors are expressed in the sebocytes and hair follicle dermal papilla[33, 34]. The biological action of T on the skin and scalp, is mostly effected by its local conversion to the more potent DHT by 5α-reductase. Two isoforms have been described; type 1 is predominant in sebaceous and sweat glands, with lesser activity in epidermal cells and hair follicles[35]. Type 2 activity dominates in beard hair follicles. T can also be converted to E_2 by the aromatase enzyme; the latter is also prominently present in the PSU[36].

Androgens play a central role in the stimulation of the sebaceous gland growth and differentiation[37, 38]. They exert also strong effects on hair growth by their effects through type 2 5α-reductase and the AR on dermal papilla cells[37]. Indeed, men with a deficiency of type 2 5α-reductase have little or no beard growth and do not develop androgenetic alopecia[39]. Also, the inhibition of type 2 5α-reductase by finasteride has been proven to slow the progression of androgenic alopecia[40]. Androgens cause an increase in the size of hair follicles and the diameter of the hair in androgen-dependent areas (beard in male adolescents, axillary and pubic hair); However in scalp follicles of susceptible men androgens paradoxically foster miniaturization and shortage of hair in the anagen phase leading to androgenic alopecia[41]. These contradictory effects may be explained by genetically determined differences in the response of papilla cells to androgens at different body areas[37].

The effects of E_2 on the PSU unit are less well-known[42]. Estrogens reduce the size and secretion of sebaceous glands indirectly by pituitary-gonadal suppression of androgen production[37].

Estrogens are also known to play an important direct role in human hair growth control[42]. They are thought to prolong the anagen phase of scalp hair growth by increasing cell proliferation rates and postponing their transition to the telogen phase[41]. Also, low dose estrogen therapy has been

found to stimulate pubic and axillary hair growth of hypogonadal girls, independently of changes in androgen levels[43]. Similar effects of E_2 are described in pregnancy: due to high E_2 levels, many scalp hair follicles remain in anagen phase[44]. However, in the postpartum, a large number of hair follicles simultaneously advance into telogen phase due to a sudden decrease in E_2 levels, causing loss of a large number of hairs, which is described as the postpartum telogen effluvium[45].

As sex steroids strongly affect the skin pilosebaceous unit, several clinical conditions of the skin such as hirsutism, acne and androgenic alopecia are often treated with anti-androgenic and/or estrogenic medication (such as cyproterone acetate[46], 5α-reductase inhibitors[47] and combined oral contraceptives (COC's)[48].

1.2.4.2 *Sex steroids, body composition, and bone*

1.2.4.2.1 Fat mass

Gender differences in fat metabolism are well-recognized. Women generally have a higher amount of body fat mass than men[49]. In addition, premenopausal women store fat mass preferentially in the subcutaneous areas, mainly at the gluteal and femoral regions, the so-called 'pear' or gynoid body shape whereas men store predominantly visceral fat mass in the abdominal area ('apple' or android body shape)[49]. It is well-known that mainly visceral fat mass is associated with detrimental effects such as dyslipidemia, insulin resistance, metabolic syndrome, systemic inflammation, diabetes, and cardiovascular disease[50-54].

As most of the differences between both genders occur from puberty on, important effects of sex steroids on body composition are suspected. Indeed, androgen receptors are expressed in both adipocytes and preadipocytes[55-56], with a higher expression in visceral compared to subcutaneous adipocytes and preadipocytes[57-58]. Androgens inhibit the differentiation of preadipocytes and interfere with lipid metabolism: T inhibits lipid uptake and lipoprotein lipase activity, and stimulates lipolysis[59-60]. Low T levels in men, for example in men treated by androgen deprivation therapy for prostate cancer, will enhance lipid uptake and decrease lipolysis mostly in the abdominal fat tissue resulting in a more centrally fat storage[59-63].

Not only androgen receptors but also estrogen receptors (ERα and ERβ) are expressed in both visceral and subcutaneous fat tissue[64]. Concerning ER-subtypes, it was found that the expression of ERα is much higher compared to ERβ. In contrast to the AR, no regional differences were found in ERα expression in subcutaneous versus visceral adipose tissue.

The important roles of estrogens are illustrated by observations in ERα KO mice and men with estrogen deficiency or resistance[65-68]: they develop an increase in total body fat mass with profound insulin resistance and impaired glucose tolerance. ERβ KO mice have a similar body weight and an equal fat distribution in comparison to wild type mice[67] indicating that ERα is more important than ERβ in preventing fat tissue deposition. Reduced ERα expression and impaired ERα function as well as some polymorphisms of the human ERα gene have been linked with an increased prevalence of several aspects of the metabolic syndrome[65, 69-71]. The molecular and cell-biological mechanisms underlying the metabolic actions of estrogen are still poorly understood[72] but evidence demonstrates that E_2 has direct effects on cultured adipocytes with the overall effect of inhibiting lipogenesis and adipogenesis[73-74]. Interestingly, although the overall effect of E_2 is to decrease lipid deposition in adipose tissue, E_2 is known to favour subcutaneous adipose tissue accumulation by central[75]and peripheral mechanisms[74, 76]. The important role of estrogens on adipose tissue can also be seen in both animal- and clinical studies after oophorectomy. These studies show that women experience an increase in abdominal fat mass after oophorectomy, putting them at a risk for glucose intolerance and cardiovascular disease. Interestingly, when supplemented with estrogens, these effects can be reversed[77-78].

The traditional view of adipose tissue as a passive reservoir for energy storage is no longer valid[79]. Adipose tissue is also an important site of steroid hormone biosynthesis and metabolism. Several steroid metabolizing enzymes such as aromatase enzyme are expressed in adipose tissue. This local sex steroid conversion may contribute to effects of T and E_2 on adipose tissue. Aromatase KO mice or patients lacking aromatase activity, with reduced endogenous production of estrogens, both show distinct metabolic phenotypes with an increased adiposity accompanied by decreased glucose tolerance and pronounced insulin resistance[65, 80-82]. Besides sex steroids metabolism, adipose tissue expresses and secretes a wide variety of bioactive peptides, known as adipokines, which can act both at local or systemic level. These adipokines appear to be involved in a wide range of physiological processes such as haemostasis (e.g., plasminogen activator inhibitor 1), lipid metabolism (e.g., apolipoprotein E), blood pressure regulation (angiotensinogen), insulin sensitivity (e.g., adiponectin), inflammation (e.g., IL-6) and angiogenesis (e.g., vascular endothelial growth factor). In addition, a variety of receptors such as cytokine-, catecholamine-, and hormonal receptors are expressed, allowing for communication with distant organs such as the central nervous system[79].

Besides a determining role of sex steroids on body composition, there is also evidence for reciprocal effects of body composition on sex steroid levels. Obesity is for example associated

with a higher aromatase activity, resulting in an increased E_2 production[83-87]. The latter will in turn decrease T levels due to suppression of the hypothalamic pituitary axis. Conversely, weight loss in obese men has been associated with increasing levels of T, and with decreasing levels of E_2, reflecting lower aromatase activity due to decreasing levels of fat mass[85]. Furthermore, adiposity is a major determinant of serum SHBG concentrations and has therefore marked effects on total levels of circulating sex steroids with obesity characterized by low SHBG and (total) sex steroid levels.

1.2.4.2.2 **Muscle mass**

Similarly as for fat mass, muscle mass differs between men and women. In general, men have more muscle mass and greater muscle strength compared to women. Indeed, the anabolic effects of T on muscle metabolism are well-established and androgens are therefore widely used among athletes and bodybuilders. Both AR as well ER (both α and β) are expressed in muscle cells[88-89]. Testosterone is found to increase myoblast protein synthesis, the cross-sectional area of muscle fibers (both type 1 and 2) and the number of myonuclei and satellite cells in a dose- and concentration-dependent manner[90-92]. Estrogens are known to exert several effects on skeletal muscle mass and functions, such as on muscle contractile properties, reducing post-exercise muscle damage and infiltration of inflammatory cells, and increasing muscle repair and regenerative processes. But the exact molecular and cell-biological pathways remain poorly understood[93-94].

Besides its role on locomotion and maintaining posture, skeletal muscle plays a key role in bone metabolism by its active loading on the bone surface and thereby stimulating bone mineralization and growth[95-96], which is also known as 'the mechanostat theory'[97]. In addition, muscle tissue contributes to glucose metabolism, as a major proportion of glucose utilization takes place in the muscles.

The importance of sex steroid effects on muscle and fat mass and function, suggests that drastic changes in sex steroids may therefore have important metabolic consequences.

1.2.4.2.3 **Bone**

Sex steroids play a pivotal role in the regulation of bone formation by both direct and indirect effects on bone growth. Prepubertal boys and girls have in general a comparable bone mass and size. However, from puberty on, bone mass and size increase in boys and girls, but in boys this increase is on average 10% greater due to a higher rate of periosteal apposition and the longer

duration of pre- and intrapubertal growth in boys. Besides these differences in size and cortical thickness, long bones in boys also acquire a relatively larger marrow cavity compared to girls.

Apart from the bone formation and achievement of peak bone mass, sex steroids also affect the maintenance of bone health through indirect effects and direct effects by AR's as well as ER's in osteoblasts, osteocytes and osteoclasts[98-101]. This maintenance of the adult skeleton is achieved by a continuous process of renewal and repair, called the 'bone remodeling'. This process occurs in basic multicellular units, which include osteocytes, osteoblasts and osteoclasts, localized within the bone remodeling cavity. Bone remodeling is strongly regulated by central, systemic and local factors and is characterized by a cycle of sequential periods of activation, bone resorption, reversal and bone formation within a basic multicellular unit. Osteocytes recruit osteoclasts to sites of microdamage and initiate bone resorption. The resulting defects are repaired by osteoblasts as they produce a new matrix which is mineralized afterwards.

The actions of E_2 on bone metabolism are multiple. In vitro research showed that E_2 increases expression of osteoprotegerin in the osteoblast[102], while decreasing that of RANKL, resulting in decreased RANK-activation, which in turn reduces osteoclast activation[103-104]. Other effects of E_2 include the reduction of osteoclastogenesis, stimulation of osteoclast apoptosis, and an increase of osteoblasts lifespan[105-107]. Alternatively, androgens are found to stimulate the proliferation and differentiation of osteoblast precursor cells[108], and to inhibit osteoclastogenesis, directly[109]or through osteoblastic activity[109-110]. Importantly, in a mice model it was found that androgens are exclusively responsible for trabecular bone development and maintenance, while both androgen and estrogen action are needed for the development and maintenance of cortical bone[111].

The clinical effects of estrogen deprivation, as seen in postmenopausal women are well-known, with a manifest increase of bone remodeling, bone resorption and thus bone loss, resulting in a high prevalence of osteoporosis[112]. A large number of studies have been conducted to evaluate the effect of estrogen administration on bone mass in postmenopausal women and generally all studies showed that estrogens (natural and synthetic, oral or transdermal) reduce bone remodeling and bone resorption and thus the loss of bone mass.

Hypogonadal men also show significantly increased bone remodeling and bone resorption, resulting in lower bone density than age- and sex-matched controls, especially at trabecular skeletal site[113], but also at cortical sites. T replacement therapy in these men results in an increase in bone mineral density[114], which is mainly substantial in hypogonadal men before closure of the epiphyseal growth plates. The effects of T during bone acquisition and in maintenance of skeletal

integrity in adult men are due to direct effects of T on bone, indirect effects of T on bone after aromatization to E2, and indirect effect of T through increase and maintenance of muscle mass[115].

1.2.4.3 *Sex steroids and cardiovascular effects*

1.2.4.3.1 Sex steroids and cardiovascular risk factors

Premenopausal women suffer less from cardiovascular disease than men, but the incidence of cardiovascular disease rises after menopause. Traditionally, the sex difference in cardiovascular morbidity and mortality has been attributed to the endocrine profiles of adult men and women, surmising that androgens are deleterious and estrogens favourable for cardiovascular health. However, this assumption is becoming increasingly questioned[116]. Nevertheless, it is well-recognized that sex steroids influence different cardiovascular risk factors such as lipids, blood pressure, hemostasis and risk of thrombosis, glucose metabolism and endothelial function. We will discuss these functions in more detail and will end with the overall relation between sex hormones and cardiovascular end points.

1.2.4.3.1.1 Glucose metabolism

As already mentioned in section 1.4.2.2.1. and 1.4.2.2.2, sex steroids exert important effects on muscle and fat mass and function, with important metabolic and cardiovascular consequences. The increase in visceral fat, as for example seen in hypogonadal men, has been related to dyslipidemia, insulin resistance, metabolic syndrome, systemic inflammation, diabetes, and cardiovascular disease[50-52]. Apart from these indirect effects of sex steroids, some studies[117-118], but not all[119], showed evidence for direct effects of sex steroids on glucose metabolism. Acute T withdrawal in men, for example, was found to decrease insulin sensitivity in the absence of any detectable changes in body composition. Also, large epidemiological studies show that the association between low T levels and insulin resistance persists after adjusting for total body fat mass[120, 121]. However, whether some of the effects of T on insulin resistance are related to peripheral conversion to E_2, is still poorly understood[118].

Compared to their age-matched counterparts, premenopausal women (with a normal menstrual history) have enhanced insulin sensitivity. Menopause and ovariectomy result in a marked decline in insulin sensitivity, in parallel with an increase in fat mass and rise in lower low-density lipoprotein cholesterol (LDL-C), triglycerides and fatty acids[74]; whereas restoration of E_2 in physiological doses can maintain insulin action and glucose tolerance[122]. Evidence for direct

effects of cross-sex hormones has also been described. Pregnancy and luteal phase of the menstrual cycle, both characterized by elevated E_2 and progesterone levels, are associated with reduced insulin sensitivity[123-124]. Indeed, it is argued that E_2 favours insulin sensitivity when E_2 levels stay within a tight physiological concentration[74]. On the contrary, augmentation of E_2 to supraphysiological levels were shown to induce insulin resistance by both hyperinsulinemia in the liver and reduction in GLUT 4 expression within the muscle[125].

1.2.4.3.1.2 Lipids

Women have a less proatherogenic plasma lipid profile than men. In particular, women have a higher high-density lipoprotein cholesterol (HDL-C) concentration and LDL-C, very low-density lipoprotein cholesterol (VLDL-C) and total plasma triglyceride concentrations compared to age-matched men[126-128]. In addition, the average size of circulating VLDL particles is smaller in women whereas the average size of LDL and HDL particles is larger in women than men[128-130]. It is generally thought that these sex differences in plasma lipid profile are caused by differences in sex steroid levels, but this assumption has been questioned[128]. The already mentioned important gender differences in body composition may also contribute as total body adiposity, visceral fat storage, and insulin resistance are associated with increased plasma triglyceride and LDL-C and decreased HDL-C concentrations[131]. However, many other factors such as gender differences in insulin and adipokine action, gene expression and gene imprinting may also contribute[128].

Our knowledge concerning the effects of E_2 and T on lipid profile is mostly based on studies investigating the effects of hormone therapy in men and pre- and post-menopausal women. In Table 3, a summary of these effects can be found. These studies also show that the mode of administration[132-133], i.e., oral, intramuscular or transdermal, plays an important role probably due the hepatic "first-pass" effect. In addition, different effects can be found in relation to the dosage as well as the chemical nature of the hormone preparations.

TABLE 3. EFFECTS OF SEX STEROID THERAPY ON LIPID METABOLISM (ADAPTED FROM (126))

	ESTROGENS		PROGESTOGENS	NON-AROMATISABLE ANDROGENS	
	ORAL	PARENTAL	ORAL AND PARENTAL	MEN	WOMEN
Total and VLDL Triglycerideconcentrations	↑↑	↓ or ↔	↓	↔	↔
VLDL triglyceride production	↑↑	?	↔	?	?
VLDL triglyceride removal	↔	?	↑	?	?
HDL concentrations	↑↑	↑ or ↔	↓	↓↓	↓↓
HDL apolipoprotein A-I production	↑↑	↔	↓↓	↓↓	↓↓
HDL apolipoprotein A-I removal	↔	↔	↓	↑↑	↑↑
HDL apolipoprotein A-2production	↔	?	?	?	?
HDL apolipoprotein A-2 removal	↔	?	?	?	?
LDL concentrations	↓↓	↓ or ↔	↓ or ↔	↑ or ↔	↑ or ↔
LDL apolipoprotein B-100 production	↑	↔	↑ or ↔	?	?
LDL apolipoprotein B-100 removal	↑↑	↔	↑ or ↔	?	?

↑, Increase; ↓, decrease; ↔, no effect, ? unknown . *Double arrows* indicate more pronounced effects than *single arrows*.

1.2.4.3.1.3 Endothelial function

The endothelium plays a major role in vascular physiology and contributes to maintain vascular tone and non-thrombogenic properties of the endothelial surface. Atherosclerosis, a chronic inflammatory and multifactorial process, characterized by the formation of atheromatous plaques, arterial wall thickening, and lumen narrowing, is a consequence of endothelial dysfunction[134]. Endothelial dysfunction is initiated by injury of the endothelium by shear stress and exposure to toxins (e.g., tobacco components) and atherogenic lipids. This dysfunctional endothelium is impaired to preserve vascular tone and its non-thrombogenic features. In addition, activated endothelial cells express selectins, integrins and adhesion molecules to which monocytes and T

lymphocytes bind before migration into the subendothelial space. Afterwards, monocytes differentiate into macrophages which take up modified lipoproteins, such as oxidized LDL, and further differentiate into foam cells. The latter, together with T lymphocytes, stimulate proliferation and migration of smooth muscle cells by release of inflammatory factors resulting in the so-called fatty streak, which further evolve into an atherosclerotic plaque. Endothelial function is regulated by many factors, including sex steroid hormones. ER's and AR's are present at the vascular endothelium and smooth muscle cell, but sex steroid functions are also mediated by non genomic effects[135-136]. Importantly, aromatase, 5α-reductase and other steroid-modifying enzymes are also expressed at the vascular endothelium and smooth muscle cell as well as in macrophages and platelets[137-140].

Studies in mice show mainly protective effects of E_2 on endothelial function, including vasodilatation[141], promoting re-endothelialization, inhibiting smooth muscle cell proliferation[142] and matrix deposition following vascular injury, and attenuating atherosclerotic plaque progression[143]. Importantly, different types of estrogens, estrogen dosages or route of administration may exert different effects[144].

The effects of T are less well studied. While some animal and clinical studies suggest that T indeed exerts deleterious effects on the vascular wall by enhancing vascular tone[145-147], others show that T is associated with protective effects by inducing vasorelaxation of vascular smooth muscle cells[148-150]. Besides regulation of vascular tone, smooth muscle cells also contribute in the atherosclerosis process by proliferation, migration and matrix production. In contrast to the already mentioned inhibiting effects of E_2 on smooth muscle cell proliferation and matrix deposition[142], some studies[151-152] but not all[153] showed that T increased proliferation and migration of smooth muscle cells. In addition, T may increase oxidation of LDL by macrophages. A summary of the metabolism and mode of action of sex steroids in the vascular cells can be found in Figure 1.6.

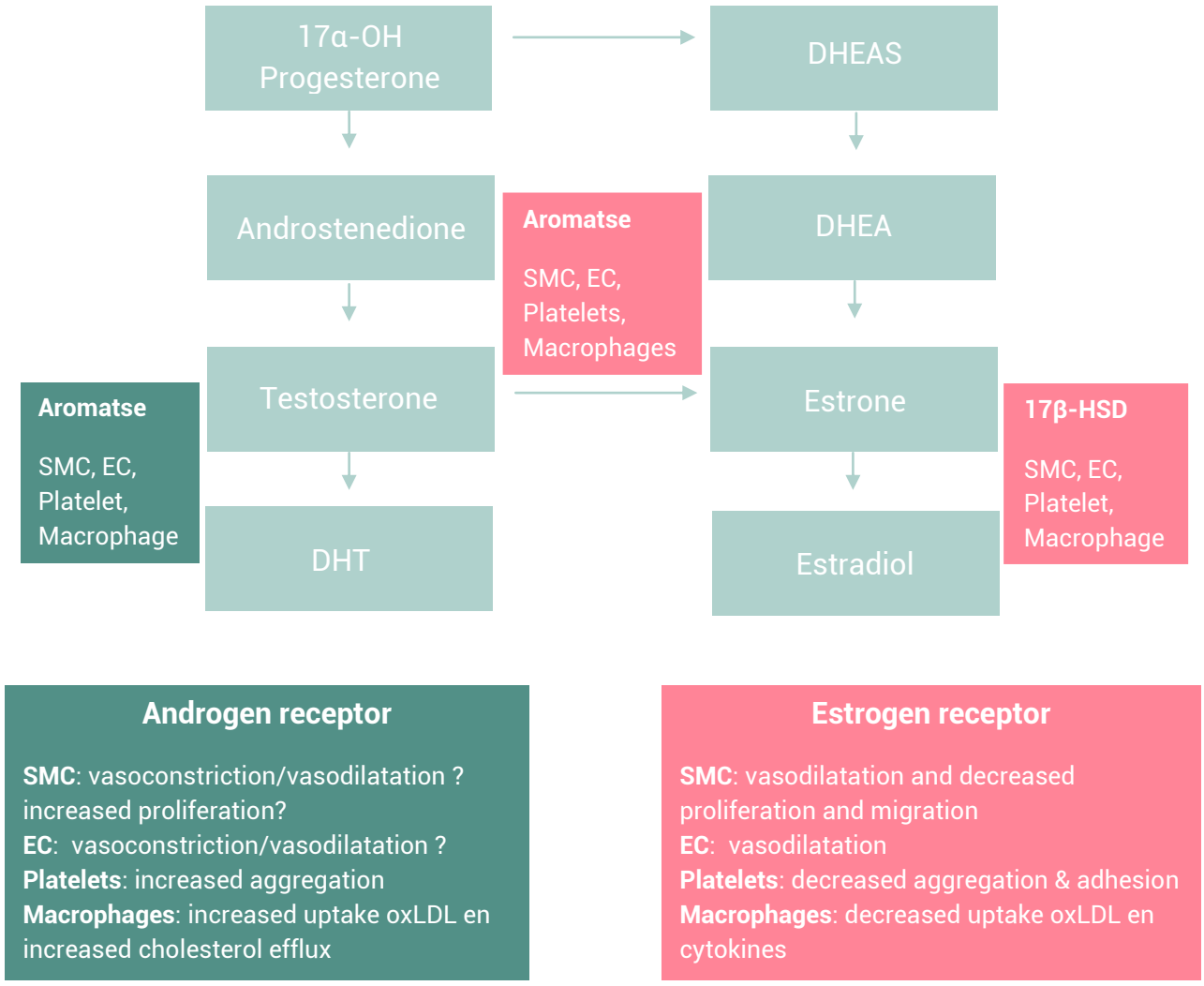

Figure 1.6. Metabolism and mode of action of sex steroids in vascular cells (modified from[154]).

SMC: smooth muscle cell, EC: endothelial cell.

1.2.4.3.1.4 Blood pressure

Men typically have higher blood pressures than premenopausal women. The sexual dimorphism in blood pressure begins at puberty and persists through adult age, until after menopause when the prevalence of hypertension is at least as prevalent in women as in age-matched men[155-157]. These findings may suggest an important role of sex steroids. Effects of sex steroids on blood pressure can be mediated by effects on the vascular tone (cfr. above), vascular growth, kidney and sympathetic nervous system. Indeed, sex steroid receptors are present at the endothelium, smooth muscle cells, the kidney and sympathetic nervous system[148, 158-159].

Support for the antihypertensive effects of E_2 can be found in the observations that during the menstrual cycle, blood pressure is lower during luteal phase than follicular phase[157, 160-161] and that pregnancy is generally associated with lower blood pressure[157,162]. In contrast, clinical studies on the effects of estrogen therapy such as combined oral contraceptives (COC) or postmenopausal hormone replacement therapy showed inconsistent results, with some studies showing a decrease of blood pressure[163-164], whereas others observed no effect[165-166] or an increase[167-169]. Once more, these findings are complicated by differences in type, dosage and route of administration of estrogens.

Few data are available on the associations between T and blood pressure in biological women. In women with or without PCOS (polycystic ovary syndrome), a positive association between serum androgen levels and blood pressure[170-171] whereas others were unable to show such an association[172]. In contrast, in men several studies[173-175] found an inverse association between serum T levels and blood pressure; and low levels of T have been reported in men with hypertension[176, 177]. Moreover, T substitution in hypogonadal men and patients with metabolic syndrome was found to decrease blood pressure levels[178-180]. Concerning supraphysiological levels of T, as seen in anabolic-androgenic steroids users, some studies observed no change in blood pressure[181-183] whereas others found a significant increase[184-186].

1.2.4.3.1.5 Hemostasis and thrombosis

Estrogens are known to affect the coagulation system. High E_2 levels, e.g., during pregnancy, are known to induce a prothrombotic state resulting in an increased risk for venous thrombosis[187]. In addition, COC use and hormone replacement therapy are both associated with a higher risk for venous thrombosis[188-189]. The haemostatic changes in women taking hormone replacement therapy and COC's are characterized by changes in procoagulant- (e.g., prothrombin), anticoagulant- (e.g., protein S and tissue factor pathway inhibitor) and fibrinolytic parameters[190].

Thrombin generation-based activated protein C (APC) sensitivity is a global test for the overall prothrombotic effect and predicts the risk of venous thrombosis[191]. Significant differences exist between different types of COC's, with third generation COC's with newer progestins showing a higher risk than second generation COC's. In addition, COC's with a higher estrogen dosage showed a higher risk than those with lower estrogen dosage[192]. Of interest, use of COC's containing cyproterone acetate may be associated with an excess risk for venous thrombosis comparable to third generation COC's or even higher[193]. As already mentioned, different effects can also be seen in relation to the route of administration. The transdermal route avoids the first pass effect of the liver and this may induce different metabolic effects. Indeed, a reduced activation of the haemostatic system in transdermal estrogens has been observed[194] and a lower venous thrombosis risk is described in women using transdermal than oral COC's and HRT[195-197].

In men, abuse of anabolic-androgenic steroids by athletes has been associated with cardiovascular morbidity[198]. T administration can induce erythrocytosis and affects the expression of platelet tromboxane A2 receptors[199], both pro-thrombotic factors. Interestingly, the risk for development of erythrocytosis during T treatment in men is greater in older men than in young men[200], and when administrating intramuscular T ester injections compared to oral or transdermal T therapy[201]. Some studies observed a dose-dependent effect of T treatment[90], whereas others were unable to confirm this[200]. Considering aromatase expression in vascular cells, some authors suggested that some pro-thrombotic effects of T therapy in men may be related to higher E_2[202].

1.2.4.3.2 Sex steroids and cardiovascular morbidity

As stated above, sex steroids strongly affect different cardiovascular risk factors. E_2 was mainly associated with beneficial effects on cardiovascular system, whereas T was mostly related to deleterious effects on cardiovascular health. Whether these associations with cardiovascular risk factors can be translated into cardiovascular morbidity will be discussed below.

Observations that cardiovascular morbidity rises after menopause, although not abruptly, are consistent with a protective cardiovascular effect of E_2. About three decades ago, data from large observational studies also suggested that hormonal therapy resulted in a reduction in coronary heart disease and mortality[203-205]. In contrast with these protective effects of estrogen treatment, data from the first large scale randomized controlled trials (The Women's Health Initiative Trials) showed no coronary protective effects of hormone replacement therapy and even harmful effects

in some women[206]. However, recent analyses show the importance of the relationship between timing of HRT initiation and underlying differences in vascular biology that exist between perimenopausal and older women. These showed that younger women at the start of menopause experienced beneficial effects from hormone replacement therapy whereas older women experienced harmful effects[207, 208]. A recent randomized controlled trial (Danish Osteoporosis Prevention Study) also showed a reduction in a combined end-point of mortality and hospitalizations for congestive heart failure or myocardial infarction in women using hormone replacement therapy[209]. Alternatively, women of reproductive age using COC's were found to have a higher risk for myocardial infarction, in a dose-dependent way[210]. In addition, the majority of evidence suggests a small but significant increase in ischemic stroke in women using COC's[211] and in postmenopausal women (even younger ones) using oral estrogens[212]. Concerning the associations between T levels and cardiovascular health in women, some studies[213, 214] but not all[215] have found a negative association with cardiovascular risk profile, but so far evidence for an association with cardiovascular events is lacking[216].

In men, most cross-sectional studies have associated lower endogenous serum T levels in men with a higher prevalence of cardiovascular disease[217]. Some prospective population-based studies showed no association[218], but the majority found a modest inverse association between T and cardiovascular disease in men[219-220, 203] (for review see[221]). Our knowledge concerning effects of T treatment on cardiovascular health is sparse with only a few, small sample size randomized controlled trials[221]. With respect to the association of endogenous E_2 and cardiovascular disease in men, a recent meta-analysis of prospective studies did not provide evidence for either protective or harmful effect of total E_2 concentrations on risk for incident cardiovascular disease in men[222].

1.2.4.4 *Sex steroids, brain and behaviour*

Testosterone and E_2 receptors are widespread throughout the entire brain[223-225] and sex steroids are thought to be responsible, at least in part, for the sexual differentiation in brain and behaviour[226]. Effects of sex steroids on the brain can be categorized as organizational or activational[226-227]. Organizational effects refer to the ability of sex steroids to form nervous system structure during development. These effects are permanent and programs activitional responses to sex steroids. In contrast, activational effects of sex steroids are transient, depend on hormone levels and include the ability to change activity of cells which then facilitates certain behaviour[226, 227].

In this section we will elaborate on some effects sex steroids on mood and behaviour, more specifically we will discuss the effects of T and E_2 on motivational aspects of sexual functioning, depressive mood and aggressive behaviour.

1.2.4.4.1 Motivational aspect of sexual functioning

T affects motivational aspects of sexual functioning such as sexual desire in men[228]. Loss of sexual desire is one of the key clinical signs of hypogonadism and numerous studies consequently showed an increase in sexual desire during T administration in hypogonadal young and aging men[229-232]. Whether supraphysiological levels of T affects sexual desire in men is not well-known, as some authors[232] described a significant increase whereas others did not[233- 234]. In contrast, the role of androgens in female sexual desire is not well-known with inconsistent and often contradictory evidence[228]. Some studies found a correlation between serum T levels and sexual desire in women[235-236], whereas other studies did not observe such an association[237-238]. In addition, several studies[239-240], but not all[241,] observed that T supplementation in women with surgical or natural menopause increased sexual desire.

Effects of estrogens on sexual desire in men and women are poorly understood[228]. Only a few studies examined the relation between sexual desire and circulating estradiol levels in men, but most studies observed no clear associations[242-243]. In contrast, administration of exogenous estrogens has been used historically as an anti-androgen treatment in prostate cancer patients and sex offenders, in whom a reduction in sexual desire was observed[228]. In women, some[244] but not all studies[245-246], observed associations between E_2 levels and sexual desire. Several studies examining the effects of estrogen therapy in postmenopausal women found an increase in sexual desire[246-247]. However, others reported that high doses of estrogens were associated with lower sexual desire[248-249].

1.2.4.4.2 Depressive mood

The lifetime prevalence of a major depressive disorder in women is almost twice that in men[250-251]. This has led to the hypothesis that sex steroids are involved in this sex difference.

Hypogonadism in young men has been associated with depressive mood and T replacement has been found to improve mood of these men[252- 253]. In older men this association between T levels and depressive symptoms is less clear with some[254] but not all[255-257] observing an association. Supraphysiological levels of T in healthy young men were found to increase ratings of manic symptoms, although most showed little change, while a few developed prominent effects[258].

Alternatively, depressive symptoms are commonly reported in men using anabolic androgenic steroids[259-260], but depression in these men can also be related due to withdrawing from steroids[259].

The role of T on depressive mood in women is not well-known and some studies found inverse relationships between depressive symptoms and total and T levels[235] whereas others failed to show such an association[261]. A recent randomized double blinded placebo controlled study observed no benefit of 150 μg T patch on mood in women with primary ovarian insufficiency[262], whereas a randomized double blinded placebo controlled study in androgen deficient women with hypopituitarism did show an improvement[263]. In postmenopausal women treated with T patch, an improvement in depressive symptoms was found[241] whereas a prospective population based study in 3302 women showed that an increase in T levels from baseline were significantly associated with higher depression scores independently of multiple covariates and confounders including menopausal status and vasomotor symptoms[264].

The role of E_2 on depressive symptoms remains unclear. Some epidemiological and clinical studies have suggested that lower levels of E_2 or a more pronounced decrease in E_2 levels were associated with depressed mood[265,-266] whereas others were unable to find such an association[267-268]. In postmenopausal women an improvement in psychological well-being was reported after treatment with HRT[269-270].

1.2.4.4.3 Aggressive behaviour

In animals studies, the association between T and aggressive behaviour is well recognized[271- 272], whereas in humans this association is more controversial. Two meta-analyses observed an overall weak positive association between T and aggressive behaviour[272-273].

Some studies[258] but not all[90, 274] found that supraphysiological levels of T, are associated with an increase in aggressive behaviour. In young men using (long-term) anabolic androgenic steroid use, aggressive behaviour is one of the most often reported side effects[258, 275]. Moreover, young males who used anabolic androgenic steroids were more likely to engage in violent acts than were those that did not use[276]. Importantly, it has also been described that these behavioural consequences are not simply determined by the presence of very high levels of androgens but through influence of the central nervous system and changes of the behavioural response to a variety of stimuli[277].

1.3 CROSS-SEX HORMONAL THERAPY IN TRANS PERSONS

As stated above, cross-sex hormone treatment often plays a key role in the treatment of trans persons. The two main aims of hormone treatment are firstly to reduce endogenous hormone levels and thereby the secondary sex characteristics of the individual's biological sex and assigned gender, and secondly to replace endogenous sex hormone levels with those of the desired sex by using cross-sex hormones. Besides inducing physical changes, the act of using cross-sex hormones is itself an affirmation of gender identity. In addition, cross-sex hormone therapy may help trans persons in changing their gender role, as many trans persons change their gender role around the time of starting hormonal therapy.

1.3.1 CROSS-SEX HORMONE THERAPY IN TRANS WOMEN

As already mentioned, a combination of estrogens and 'anti-androgens' and/or androgen lowering therapy is the most commonly used and studied treatment regimen to obtain feminization in trans women. In the literature, a wide variation of types and doses of estrogens and anti-androgens and/or androgen lowering medication have been described, all resulting in feminization (Table 4). As up till now no randomized controlled trials have been performed, little information is available on the optimal formulations and dosages.

Androgen deprivation can be achieved by reducing endogenous T levels and/or T activity by blocking androgen receptor or blocking the conversion of T to the more potent DHT. The use of anti-androgens and/or androgen lowering therapy permits lower doses of estrogen needed to suppress T, and so reduces the associated risks of high-dose exogenous estrogen exposure. Estrogens can be administered orally, transdermally or parentally using various types of estrogens.

Anti-androgen therapy and/or androgen lowering therapy can be stopped after oophorectomy and some physicians also lower estrogen dosage at that time[278]. Current endocrine guidelines also suggest adjusting the hormone dosage for comorbid health concerns, similar as in hormonal replacement therapy for hypogonadism[5].

TABLE 1. COMMON HORMONE TREATMENT REGIMENS CURRENTLY USED IN TRANS WOMEN (ADAPTED FROM (5))	
ANTI-ANDROGEN AND/OR ANDROGEN LOWERING THERAPY	**ESTROGEN THERAPY**
Spironolactone 100-200 mg daily	**Oral estradiol**
Cyproterone acetate 50-100 mg daily	Ethinyl estradiol 50-100 µg daily
GnRH agonist 3.75 mg sc monthly	Estradiol valerate 4-6 mg daily
	Conjugated equine estrogens 1.5-5 mg daily
	Transdermal estradiol
	Estradiol gel 4mg daily
	Estradiol patch 100 µg/24 h
	Parental estradiol
	Estradiol valerate 5-20 mg every 2 weeks
	Estradiol cypionate 5-10 mg weekly

The use of progestins, apart from cyproterone acetate, remains controversial[5]. Since progestins play a role in mammary development in biological women, some clinicians believe they are necessary for full breast development[279-281]. However, the available evidence does not provide support for better effects on breast size of adding progestogens to cross-sex estrogen administration in trans women[282]. However, available evidence is quantitatively and qualitatively extremely poor, which hampers any firm conclusion at this time[283]. Also, many centers use anti-androgens with progestational action, which complicates the interpretation of current data. In addition, all progestogens by definition have some progestational activity but they differ in chemical structure, metabolism, pharmacokinetics, affinity, potency and intracellular action which can translate into very different biological and clinical effects[284].

1.3.2 CROSS-SEX HORMONE THERAPY IN TRANS MEN

Cross-sex hormone therapy in trans men mainly involves T therapy. Testosterone treatment can be given orally, transdermally, or parenterally (IM). Similar to the situation for trans women, no randomized controlled trails have been performed concerning T treatment in trans men and therefore variations in treatment modalities exist between centers (Table 5.). Besides T, progestins and GnRH agonists can be used for a short period of time to assist with menstrual cessation early during hormonal therapy.

TABLE 5. COMMON HORMONE TREATMENT REGIMENS CURRENTLY USED IN TRANS WOMEN (ADAPTED FROM (5))

SUPPRESSION OF MENSES	TESTOSTERONE THERAPY
Progestagens **GnRH agonist**	**Oral Testosterone** Testosterone undecanoate 160-240 mg daily **Transdermal Testosterone** Testosterone gel 1% 2.5-10 mg daily **Parental Testosterone** Testosterone undecanoate 1000 mg every 12 weeks Testosterone enanthate or cypionate every 2 weeks

1.3.3 CLINICAL EFFECTS AND SIDE EFFECTS OF CROSS-SEX HORMONE THERAPY

1.3.3.1 *Physical changes induced by cross-sex hormone therapy*

Our knowledge concerning physical changes during cross-sex hormone therapy is largely based on clinical experience, rather than evidence based. Most physical changes occur during the first year of cross-sex hormone therapy. In trans women, a decreased facial and body hair, decreased skin oiliness, a fat redistribution and breast growth were observed[278, 285-288]. Over a longer period of time, the prostate gland and testicles underwent hypotrophy[5, 289].

Testosterone treatment in trans men is found to increase facial and body hair, skin oiliness, clitoral size and muscle mass. In addition, a redistribution of fat mass and induction of a deepened voice, breast atrophy and cessation of menses were reported[278, 285, 286, 288, 290-291].

Large prospective studies examining both the short and long-term clinical effects of cross-sex hormone therapy are needed to improve our understanding in an evidence based way.

1.3.3.2 *Cardiovascular and metabolic effects*

1.3.3.2.1 **Cardiovascular and metabolic factors**

As seen in 1.2.4.3., sex steroids can affect several cardiovascular risk factors. A summary of the literature concerning prospective changes in cardiovascular risk factors during cross-sex hormone therapy in trans women and men is shown in Table 6 and 7.

Anti-androgen and estrogen therapy in trans women and T therapy in trans men both improves and impairs some aspects of the profiles of cardiovascular risk factors. The majority of these data are based on older treatment modalities using high dose ethinyl estradiol and high dose cyproterone acetate in trans women and short-acting intramuscular T esters in trans men. Whether our newer treatment modalities in trans persons will induce similar changes in metabolic and cardiovascular parameters still needs to be examined.

In addition, it is not well known how these effects on metabolic and cardiovascular factors can be translated into hard clinical endpoints. This will be discussed in the next section.

TABLE 6. PROSPECTIVE CHANGES IN CARDIOVASCULAR RISK FACTORS IN TRANS WOMEN (ADAPTED FROM (292))

OUTCOME VARIABLE	OBSERVED CHANGE	EFFECT ON CV MORBIDITY	REFERENCES
Body composition			
Weight	Increase/no effect	↑/-	(293),(294),(295), (296) / (297), (298), (299)
Visceral fat	Increase	↑	(293), (300), (295)
Total body fat	Increase	↑	(293), (300), (295), (296), (299)
Insulin metabolism			
Fasting glucose	No effect	-	(300), (294)
Fasting insulin	Increase	↑	(300)-(295)
Insulin sensitivity	Decrease	↑	(300), (295), (301)
Lipid spectrum			
Total cholesterol	Decrease/no effect	↓/ -	(300) / (297), (298), (302)
LDL cholesterol	Decrease/no effect	↓/ -	(300) / (302)
HDL cholesterol	Increase/no effect	↑/ -	(300), (296), (299)/ (244)
VLDL cholesterol	No effect	-	(300)
Triglycerides	Increase/ no effect	↑/-	(300), (296) / (297), 248)
Fish fatty acid (DHA)	Increase	↓	(300)
Other CVD risk factors			
Heart rate	Increase	?	(294)
Diastolic blood pressure	Increase/no effect	↑/ -	(300) /(294)
Systolic blood pressure	Increase/no effect	↑ / -	(300)/(294)
Arterial stiffness	No effect	-	(294)
Hemostasis/fibrinolysis	Increase	↑	(194), (296)
Total homocysteine	Decrease	↓	(296), (302)
Inflammation marker	Increase	↑	(296), (303)

TABLE 7. PROSPECTIVE CHANGES IN CARDIOVASCULAR RISK FACTORS IN TRANS MEN (ADAPTED FROM (292))

OUTCOME VARIABLE	OBSERVED CHANGE	EFFECT ON CV MORBIDITY	REFERENCES
Body composition			
Weight	Increase/no effect	↑/-	(290), (294), (295), (302), (304)/ (305),(306)
Visceral fat	Increase	↑	(293), (300), (295)
Total body fat	Increase	↓	(290), (293), (300), (295), (307)/(305), (306)
Insulin metabolism			
Fasting glucose	No effect	↓	(300), (294), (304) / (295)
Fasting insulin	Increase	-	(300), (295), (301), (304)
Insulin sensitivity	Decrease	↓/ -	(300), (301), (304) / (300), (295)
Lipid spectrum			
Total cholesterol	Decrease/no effect	↓/ -	(258, 259) / (300), (305), (304), (306)
LDL cholesterol	Decrease/no effect	↓/ -	(300), 258, 259) / (239, (302), (306)
HDL cholesterol	Increase/no effect	↑/-	(239, (300), (294), (305), (304)/ (306), (308), (309)
VLDL cholesterol	No effect	-	(300)
Triglycerides	Increase/ no effect	↑/-	(290, 300), (305)/ (294), (295), (306), (309)
Fish fatty acid (DHA)	Increase	-	(300)
Other CVD risk factors			
Heart rate	Increase	-	(300), (294)
Diastolic blood pressure	Increase/no effect	↑/-	(290)/ (300), (294), (305)
Systolic blood pressure	Increase/no effect	↑/-	(290), (305)/ (300), (294),(243)
Arterial stiffness	No effect	↑	(194, 295)
Hemostasis/fibrinolysis	Increase	-	(296), (302)
Total homocysteine	Decrease	↑	(296), (303)
Inflammation marker	Increase	↑	

1.3.3.2.2 Cardiovascular morbidity

1.3.3.2.2.1 Cardiovascular morbidity in trans women

Current evidence suggests that cross-sex hormone therapy administration to trans women is associated with an increased risk for venous thrombosis (Table 8.)[310), (311)]. Two studies, assessing cardiovascular mortality in trans persons, observed an increased cardiovascular mortality in trans women compared to general population[312),(313)]. Interestingly, Kaplan Meier Curve diverged after about 10 years of follow-up, which could explain previous research that observed no higher cardiovascular mortality[310), (311)].

TABLE 8. STUDIES ON CARDIOVASCULAR ENDPOINTS IN TRANS WOMEN

STUDY	N	FOLLOW-UP	TREATMENT REGIMEN	OUTCOME
Asscheman 1989[310]	303	Median duration HRT of 4.4 years*	Ethinyl estradiol 100 µg/d and cyproterone acetate 100 mg/d	45-fold increase in VT and/or PE No increased cardiovascular morbidity and mortality
Van Kesteren 1997[311]	816	Mean duration HRT of 9.5 years	Ethinyl estradiol 100 µg/d or transdermal 17β-estradiol 100 µg/twice a week and cyproterone acetate 100 mg/d	20-fold increase in venous thrombosis and/or pulmonary embolism No increased cardiovascular morbidity or mortality rate
Asscheman 2011[312]	966	Median duration HRT of18.5 years*	Ethinyl estradiol 100 µg/d or transdermal 17β-estradiol 100 µg/twice a week and cyproterone acetate 100 mg/d	Higher mortality due to ischemic heart disease; SMR 1.64 (1.43-1.87) Higher mortality due to CVD; SMR 2.11 (1.32-3.21) in age group 40-64 years
Dhejne 2011[313]	191	Median time since SRS of 9.1 years*	Not specified	Higher mortality due to cardiovascular disease compared to controls
Bazarra Castro 2012[314]	58	Mean duration HRT of 6.5 ± 7.9 years	Different estrogen regimens and cyproterone acetate 50mg/d	Lower cardiovascular morbidity compared to control male and female population

HRT: hormone replacement therapy; VT: venous thrombosis; PE: pulmonary embolism; AMI: acute myocardial infarction; CVD: cerebrovascular disease; * not separately reported for trans men and trans women

Despite mainly positive changes in cardiovascular risk factors, the majority of evidence suggests an increased cardiovascular risk in trans women. The interpretation of the increased cardiovascular risk during cross-sex hormone therapy in trans women is complicated because the chemical nature of the estrogen, the route of administration, cardiovascular health status of the patient and the dosage of estrogens, may all carry significant weight as seen from studies on COC's and hormone replacement therapy.

Chemical nature

As no controlled trials or even large observational studies are available on cross-sex hormone therapy in trans persons, hard evidence concerning preference of one estrogen type above the others is lacking. However, different types of estrogens can exert different metabolic effects. Indeed, observational data suggested that use of high dose ethinyl estradiol (EE) was associated with a higher risk for venous thrombosis in trans women[311], which was also confirmed in the study from Toorians et al.[194] who found a higher APC resistance in trans women using oral EE than oral and transdermal 17-β E_2. In addition, Asscheman and colleagues[312] showed that current EE use was independently associated with a threefold increased risk for cardiovascular death.

Route of administration

As already mentioned, the transdermal route avoids the first pass effect of the liver with potential metabolic consequences. A lower venous thrombosis risk is described in women using transdermal contraceptives and HRT[195]. In trans persons, an observational study[311] observed a decrease in venous thrombosis since the use of transdermal estrogens in trans women aged above 40 years. Alternatively, although based on small sample size, Toorians and colleagues[194] observed no significant difference in APC resistance in trans women using oral than transdermal estrogens. In line with studies seen in women using hormone replacement therapy[144], Giltay and colleagues reported that oral EE increased CRP and tPA levels in trans women, whereas transdermal estrogens did not[303, 315].

Estrogen dosage

To our knowledge, not a single study investigated the effects of different estrogen dosages on cardiovascular morbidity or mortality in trans women. As already mentioned, in women using COC's it has been observed that lower estrogen dosage showed a significant reduction in venous thrombosis[192] and some also found a lower risk for myocardial infarction and stroke[210]. Whether these results can be extrapolated to trans women is not known, but as the estrogen dosage prescribed in trans women are higher than those used in contraceptives of HRT, it is undoubtedly of interest.

Cardiovascular risk factors and older age

Safety of cross-sex hormone therapy in patients with established cardiovascular disease or increased cardiovascular risk, such as older age, is not known as studies in these patient populations are lacking. It is widely believed that cardiovascular risk factors should be managed as they emerge[5], but whether hormonal therapy should be stopped or a dosage reduction should be performed at a certain cardiovascular risk profile, remains uncertain.

1.3.3.2.2.2 Cardiovascular morbidity in trans men

Similar as in trans women, only a handful of studies have examined cardiovascular morbidity in trans men (Table 9.). These showed a lower or comparable risk for cardiovascular morbidity compared to control populations[310, 311]. Concerning cardiovascular mortality, one study[263] found a higher cardiovascular mortality rate whereas others did not[310,312]. In conclusion, in contrast to the situation in trans women, the majority of evidence suggests that T treatment in trans men is relatively safe at short and medium duration of follow-up, although it should be noted that outcome studies in trans men are performed on smaller sample sizes and at significant younger ages, compared to trans women.

TABLE 9. STUDIES ON CARDIOVASCULAR ENDPOINTS IN TRANS MEN

STUDY	N	FOLLOW-UP	TREATMENT REGIMEN	OUTCOME
Asscheman 1989 [310]	122	Median duration HRT of 4.4 years*	T esters 250 mg im every 2 wks or T undecanoate 120-160 mg/d	No increased cardiovascular morbidity
Van Kesteren 1997 [261]	293	Mean duration HRT of 8.2 years	T esters 250 mg im every 2 wks or T undecanoate 160 mg/d	No increased cardiovascular morbidity or mortality rate
Asscheman 2011 [312]	365	Median duration HRT of 18.5 years*	T esters 250 mg im every 2 wks or T undecanoate 160 mg/d	No increased cardiovascular mortality rate
Dhejne 2011 [267]	133	Median time since SRS was 9.1 years*	Not specified	Higher mortality due to cardiovascular disease compared to controls
Bazarra Castro 2012 [268]	37	Mean duration HRT of 4.9 years ± 4.6	Different T preparations	No difference in cardiovascular morbidity compared to control men and women

HRT: hormone replacement therapy

* not separately reported for trans men and trans women

1.3.3.3 *Cancer*

Some tumors are hormone-dependent, therefore examining the incidence of cancer during cross-sex hormone therapy is of interest. In the next section, a review of the literature on hormone-dependent and reproductive organ carcinomas is given. A number of tumors (e.g., colon cancer) also show sex differences in their prevalence and it is reasonable to assume that sex steroids might be one of the factors to explain this sex difference, but so far no data are available concerning the incidence of these cancers during cross-sex hormone therapy[316].

1.3.3.3.1 **Cancer in trans women**

1.3.3.3.1.1 Breast cancer

One of the major concerns of long-term cross-sex hormone therapy in trans women is the induction of cancers of estrogen-sensitive tissues, such as the breast[316]. Up till now, eight individual breast cancer case reports have been reported in the literature [317-322]. Given that only a few case reports have been reported and no cases were reported in large cohorts of trans women, the risk of breast carcinoma is likely to be very low at short-term follow-up; long-term follow-up data are presently not available.

1.3.3.3.1.2 Lactotroph adenoma

Several cases of lactotroph adenoma, also known as prolactinoma, during cross-sex hormone therapy in trans women have been described[323-328]. Indeed, both estrogen and cyproterone acetate treatment were found to increase prolactin levels[329-331], although the exact onset and time course of hyperprolactinemia and development of prolactinoma during cross-sex hormone treatment in trans women is still not well-understood[5]. Similarly, as for breast cancer, only a few case reports of prolactinoma's have been reported and none were reported in large cohorts of trans women, suggesting a low risk. Current guidelines recommend to measure prolactin levels at baseline and then at least annually during the transition period and biannually thereafter[5].

1.3.3.3.1.3 Prostate cancer

The prostate is not removed during SRS, however, the risk of development of prostate cancer during anti-androgen and estrogen seems low, considering the important role of T in the development of clinical prostate cancer. Indeed, only four cases of prostate cancer in trans women have been reported[332-335], mostly hormone (T)-independent cancers with very high PSA values at diagnosis, and it is not clear whether these cancers were present prior to cross-sex hormone therapy.

1.3.3.3.2 **Cancer in trans men**

As mentioned earlier, T treatment usually reduces breast glandular tissue and the vast majority of trans men undergo mammectomy early in the course of sex reassignment treatment. In addition, ovariectomy and hysterectomy are recommended in trans men when they are eligible for SRS. Therefore, the risk for development of these cancers seems low. Indeed, only a few cases of breast and ovarian cancer have been described before[336-338] and after mammectomy[339-340].

Overall, the available evidence suggests that cancers related to cross-sex hormone treatment of trans persons are uncommon, but long-term follow-up studies addressing prevalence of cancer are scarce.

1.3.3.4 ***Bone health***

Concerning bone health in trans women, most studies[289, 297-299, 307, 341-344] but not all[287, 345] demonstrated a maintained bone mineral density or even an increase in bone mineral density after several years of cross-sex hormonal therapy.

In trans men, T treatment was found to prevent possible bone loss due to estrogen deficiency both after short[305, 307, 346] and longer-term T treatment[341-342, 347]. However, most studies were rather small and long-term follow-up data are still lacking.

1.3.3.5 ***Sexual desire and sexual desire problems***

In the past, transsexualism (especially male-to-female transsexualism) was often considered as a hyposexual condition and it was assumed that trans individuals had no sexual desire[348]. Considering the important effects of sex steroids in men and women, a significant impact of cross-sex hormone therapy in trans persons can be expected. Aside from effects of cross-sex hormone therapy, the experience of genital surgery and postsurgical outcome may also affect sexual functioning in trans persons. Indeed, some studies already showed an important impact of sex reassignment therapy on sexual functioning[349-351].

Yet, the effects of both hormonal therapy and SRS on sexual desire are not well-known, as prospective data concerning the frequency and intensity of sexual desire in trans men and women are currently lacking. In trans women, sexual desire is only investigated after SRS (Table 10), and the results can therefore be influenced by length of time since SRS and specific surgical techniques used. In addition, most studies were based on small samples and used indirect 'indicators' of sexual desire, such as frequency of masturbation or frequency of sexual activity.

Furthermore, current evidence in trans women is conflicting, as some studies showed a decrease[349, 352] or no change[353-354] whereas others showed an increase in sexual desire[350, 355-356]. When sexual desire scores are compared to control women, one study[354] observed lower sexual desire scores in trans women, whereas another study showed that trans women had an equal prevalence of HSDD[357].

Our knowledge on sexual desire in trans men is even more limited, since data from direct evaluations of sexual desire in trans men are non-existent. The available data can only be interpreted as an indication of sexual desire through measurement of the frequency of sexual activity or frequency of masturbation before and after SRS. In their sample of adult trans men, De Cuypere et al.[353] generally found an increase in frequency of masturbation after T treatment and SRS. Similarly, Lief and Hubschmann[355] reported an increase in sexual activity following SRS, whereas Smith et al.[352] and Cohen-Kettenis and van Goozen[349] reported no change or an increase in masturbation after T treatment and SRS in their adolescent trans men sample.

TABLE 10. STUDIES CONCERNING SEXUAL DESIRE IN TRANS WOMEN (ADAPTED FROM (311))

STUDY	N	FOLLOW-UP	OUCOME MEASUE	OUTCOME
Sorenson et al.,1981(358)	23	Postoperative, on average 6 years	Frequency of masturbation	Infrequent or low
Mate-Kole et al., 1990 (350)	20	Postoperative, on average 2 years	Sexual desire	Increased
Lief et al.,1993 (355)	14	Post-operative, on average 33 months	Sexual activity	Increased
Cohen-Kettenis et al.,1997 (349)	5	Postoperative, on average 2.6 years*	Frequency of masturbation	Decreased
Rehman et al., 1999 (359)	28	Postoperative, at least 3 years	Sexual desire	No change
Schroder et al., 1999 (351)	17	Postoperative	Frequency of sexual desire	Low
Smith et al., 2001(352)	7	Postoperative, on average 1.3 years*	Frequency of masturbation	Decreased
De Cuypere et al., 2005 (353)	32	Postoperative, on average 3.8 years	Frequency of masturbation	No change
Lobato et al., 2006 (356)	18	Postoperative, on average 24.9 months	Sexual activity	Increased
Elaut et al., 2008 (357)	62	Postoperative, on average 5.2 years	Prevalence of HSDD Association with T	No significant difference No significant association
Weyers et al., 2009 (354)	50	Postoperative, on average 75.5 months	Comparison of sexual desire to control population	Low sexual desire in trans women

*Not separately reported for trans men and trans women

1.3.3.6 *Other conditions*

1.3.3.6.1 Other conditions in trans women

1.3.3.6.1.1 Liver problems

Van Kesteren and colleagues[311] showed that approximately 3% of trans women experienced a transient elevation of the liver enzymes. Concerning persistent changes in liver enzymes, it was not clear wether these could be attributed to hepatitis B infection or alcohol rather than to the effects of hormonal treatment[311].

1.3.3.6.1.2 Gallbladder problems

Both observational studies as well as randomized controlled trials suggest that estrogen therapy carried an important risk for gallbladder disease[360, 361]. Indeed, an increased risk for galbladder disease during cross-sex hormone therapy has already been described in trans women[310].

1.3.3.6.1.3 Mood problems

It is well-recognized that anti-androgen and estrogen therapy induces a calming effect in trans women[281]. Besides these positive changes, Asscheman and colleagues[310] described that combined treatment with estrogen and cyproterone acetate in trans women was associated with a 15-fold increase in depressive mood changes, but with few morbidity. In contrast, others[362] report that cross-sex hormone therapy lowers anxiety and depression scores, which is possibly related to a better mental health after initiation of cross-sex reassignment therapy.

1.3.3.6.2 Other conditions in trans men

1.3.3.6.2.1 Skin problems

From clinical experience, it is known that acne is one of the most often reported side effects of T treatment in trans men, but so far only one study has investigated the effects of T treatment on the skin in trans men. Giltay and colleagues[286] examined sebum production and acne scores during the first year of cross-sex hormone therapy in 17 trans men treated with short-acting intramuscular T esters, and found indeed a significant increase in sebum production and acne scores during T treatment. The long-term dermatological effects of T treatment in trans men have not yet been addressed.

1.3.3.6.2.2 Liver problems

Similar to trans women, periodic monitoring of the liver enzymes is recommended given that up to 15% of trans men treated with T experience transient elevations in liver enzymes[311].

1.3.3.6.2.3 Mood problems

As in trans women, cross-sex hormone therapy by itself was found to induce a calming effect on most trans men[281]. However, some observed that the administration of androgens in trans men was clearly associated with an increase in anger proneness and aggression tendencies[363].

1.4 QUALITY OF LIFE IN TRANS PERSONS

The World Health Organization (WHO) defines quality of life (QoL) as "an individual's perception of their position in life in the context of the culture and value systems in which they live, and in relation to their goals, expectations, standards, and concerns". QoL is therefore an evaluation of a personal well-being, including several aspects such as emotional, social and physical functioning. In health care, QoL is often measured to determine how the individual's well-being may be affected by a certain disease or its treatment.

At this moment, only a few studies addressed QoL of trans persons (Table 11). Most studies[364-366], but not all[354], found that QoL in trans persons was poorer when compared with a general population sample or control group, but in these studies no adjustments were made for well-known determinants of QoL, such as age and socio-economic status, which can induce an important bias. Several follow-up studies have shown that cross-sex hormone therapy and SRS are related to favourable changes in many aspects of the individual's life, such as gender dysphoria feelings, psychosocial and sexual functioning[353, 367-368]. In the same way, cross-sex hormone therapy[365, 369, 370] and facial[366] and/or genital surgery[366, 371] were associated with better QoL scores. However, the main predictors of QoL in trans persons after SRS, after taking all these determinants into account, have not yet been investigated.

Only recently, other determinants of QoL in trans persons were explored. Some[371, 372] found that socio economical factors (e.g., educational level, employment status, partnership) were associated with QoL scores whereas others were unable to find such associations[369].

In summary, QoL is an underinvestigated area in transgender health care. Both hormonal therapy, facial and genital surgery have been associated with better QoL scores in trans persons in cross-sectional studies but prospective studies are still needed to confirm these observations. Little information is available on general determinants of QoL in trans persons and data concerning the main predictors of quality of life are lacking in trans persons after SRS.

TABLE 11. STUDIES CONCERNING HEALTH RELATED QUALITY OF LIFE IN ADULT TRANS PERSONS

STUDY	STUDY POPULATION	FOLLOW-UP	OUTCOME MEASURE	OUCTOME
Newfield et al. 2006 [365]	446 trans men	64.7% used HRT 37% had top surgery	Comparison with published norms Comparison depending on HRT and top surgery status	Lower QoL scores in trans men Higher QoL scores in those using HRT and those who had top surgery
Weyers et al. 2009 [354]	50 trans women	Post SRS, on average 10 years of HRT	Comparison with published norms Associations with sociodemographic correlates	No significant differences compared to controls
Kuhn et al. 2009 [364]	52 trans women 3 trans men	Post SRS (on average 15 years)	Comparison with control population	Lower QoL scores compared to controls
Ainsworth et al. 2010 [366]	247 trans women	30.4% had FFS 29% were post SRS	Comparison with published norms Association with FFS and SRS	Lower QoL in trans women Positive association with FFS
Parola N et al. 2010 [373]	15 trans men 15 trans women	Post SRS at least 2 years; all used HRT	Retrospective evaluation; Associations with gender and the subject's personality	QoL improved after SRT Better QoL scores in trans men. No association with personality traits
Wierckx et al. 2011 [372]	50 trans men	Post SRS; on average 10 years of HRT	Comparison with published norms Associations with sociodemographic correlates	Lower mental health and vitality Postive association with relationship status
Gorin-Lazard et al. 2012 [369]	31 trans women 30 trans men	72.1% used HRT Median duration HRT 20 months	Comparison depending on HRT status Comparison with control population Associations with sociodemographic correlates	Higher QoL scores in those using HRT. Comparable QoL scores except for role physical functioning (lower in trans) and general health (lower in general population)
Motmans et al. 2012 [371]	83 trans women 65 trans men	95.5% used HRT (on average 6.4 years)73.6 % were post SRS	Comparison with published norms Associations with SRT and sociodemographic correlates	Similar QoL scores in trans women. Lower mental well-being in trans men. Employment, education, income partnerschip were positively associated
Gomez-Gil et al. 2013 [370]	119 trans women 74 trans men	62.2% used HRT Different surgeries	Associations with SRT and sociodemographic correlates	HRT, family support, working/studying ara positively associated with QoL scores

1.5 RESEARCH OBJECTIVES

1.5.1 GENERAL AIMS

Gender dysphoria is a condition in which a person experiences discrepancy between the sex assigned at birth and the gender they identify with, leading to extensive personal distress. Transsexualism is considered the most extreme form of gender dysphoria and is characterized by the wish to undergo treatment to conform with the other biological sex. Cross-sex hormone therapy is an important part of the medical treatment of trans persons. The above discussed profound effects of sex steroids on several tissues, organs and systems raise questions about the safety and effects of cross-sex hormone treatment in trans persons. Although this treatment has been used for several decades, our knowledge regarding short- and long-term effects and side effects of cross-sex hormone therapy, is extremely scarce. This is mainly due to the low prevalence of this diagnosis, the small number of subjects treated in each centre, the lack of prospective studies, the lack of long-term follow-up data and the wide variations in treatment modalities between centers. In the past years, the importance of patients' QoL and its changes by medical treatment has become increasingly important, but these data are scarce in trans persons.

Therefore the general aim of this thesis is two-fold:

Firstly, we aim to investigate the short-and long-term physical changes, side effects and adverse events of cross-sex hormonal therapy in trans persons.

Secondly, to study QoL of trans persons and to investigate the main determinants of QoL in trans persons.

1.5.2 SPECIFIC AIMS

Firstly, the short-term effects of anti-androgen and/or estrogen treatment in trans women and T treatment in trans men are investigated in a multi-center prospective study (**chapter 2**). In chapter 2.1, general clinical effects including hormonal and biochemical changes, anthropometrics (waist-hip ratio, total body fat mass and muscle mass), side effects (e.g., liver dysfunction, hypercholesterolemia and hypertension) and adverse events during the first year of cross-sex hormonal therapy, are addressed. In chapter 2.2 we focus on the changes in metabolic and cardiovascular risk factors (e.g., HOMA-IR, Matsuda index, lipid differentiation, body composition by DXA and pQCT) during the first year of hormonal therapy, whereas in chapter 2.3 we investigate the dermatological changes (body hair and distribution, androgenetic alopecia and acne) in trans men. To address questions with respect to the long-term effects, side effects

and adverse events of cross-sex hormone therapy in trans women and men, two clinical studies and one large-sample questionnaire study were performed. In chapter 2.4 biochemical changes, anthropometrics, side effects and adverse events of cross-sex hormone therapy are investigated, whereas in chapter 2.5, cardiovascular and cancer morbidity of trans persons are compared to a control population recruited from a population-based study in Flanders.

In **chapter 3** (3.1 and 3.2), we focus on the effects of cross-sex hormone therapy on sexual desire, whereas in **chapter 4**, QoL of trans persons is compared to control men and women, and associations between sex reassignment therapy and QoL are investigated.

1.6 STUDIES

1.6.1 OVERVIEW OF THE STUDY POPULATIONS

A general overview on the different study populations is given in Figure 1.4.

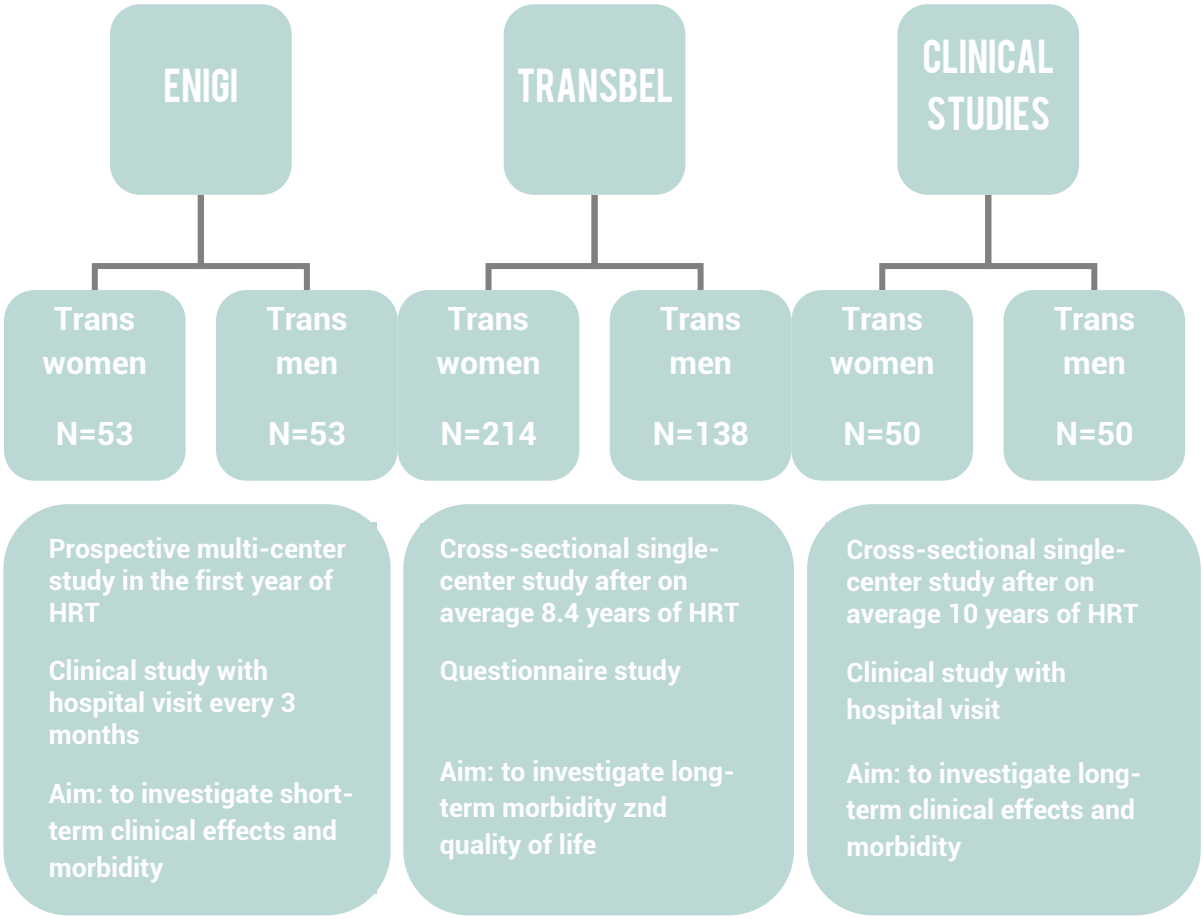

Figure 1.4. Overview of the study populations

1.6.1.1 *ENIGI: multicenter prospective study*

The ENIGI study or the European Network for the Investigation of Gender Incongruence is a multicenter prospective study involving four European institutions with established gender teams (Ghent, Oslo, Hamburg and Amsterdam), started by the psychologists and psychiatrists. The endocrinologists joined this project afterwards and developed a common standardized hormonal intervention protocol. Besides the four gender teams, Florence also joined this endocrinological protocol.

The sample used in this thesis is based on the data of Ghent and Oslo. Between February 2010 and June 2012, all patients diagnosed with gender dysphoria who were referred to our departments were invited to participate in this prospective study (N=152). Forty-five persons

were excluded resulting in a total population of 53 trans women and 53 trans men in the current analyses. Patients are followed 3-monthly during the first treatment year. After 12 to 24 months of cross-sex hormone treatment, most trans persons at our centers usually undergo sex reassignment surgery.

Trans women treated at the Ghent University Hospital were assigned to two different treatment modalities based upon the decision of the psychiatrist. Trans women who would favour a slower procedure received a dual-phase protocol. In the first phase, sex-specific features were suppressed by administration of cyproterone acetate 50 mg daily for about 3 months. In the second phase, estrogens were added to induce irreversible feminization. The other trans women received a combination of cyproterone acetate 50mg and estrogens at start. In both centers, trans women younger than 45 years received estradiol valerate 4mg daily; patients older than 45 years received transdermal estrogens (transdermal 17- β estradiol path 100μg/24h). In case of non tolerance, transdermal 17-β estradiol gel 2mg twice daily or EV 4mg was prescribed. All trans men received intramuscular T undecanoate 1000 mg (Nebido®) 3-monthly, whether or not preceded by progestin treatment to suppress menstruation. Short-acting intramuscular T esters 250 mg 2-weekly were given, in case of non tolerance of intramuscular T undecanoate. The main goal of this study was to investigate effects, side effects and adverse events of standardized cross-sex hormonal therapies at set time points during the first year of hormonal therapy. Clinical effects investigated in this study were changes in hair growth and distribution of body hair, acne, sexual desire, body composition, bone mass, cardiovascular risk factors, insulin sensitivity and biochemical parameters. This study complied with the recommendations of The Declaration of Helsinki and was approved by the Ethical Committee of the Ghent University Hospital and the University Hospital of Oslo. All participants gave written informed consent. Clinical trial number: NCT01072825.

1.6.1.2 *TransBel*

The TransBel study is a multicenter cross-sectional questionnaire study with the main aim to investigate the outcome of sex reassignment treatment. Further recruitment in other centers in Flanders and Wallonia is currently ongoing. Therefore, we included only data from our own center in this thesis. Inclusion criteria for this study were: diagnosis of gender dysphoria, use of at least 3 months cross-sex hormones and currently or formerly treated at the center for Sexology and Gender Problems at the Ghent University Hospital. Respondents received a paper version of the questionnaire or could choose to fill in the survey online. All surveys were collected between August and December 2012. We achieved a response rate of 54%, as 352 participants (214 trans

women and 138 trans men) agreed to participate in this study. This study was approved by the ethical review board of Ghent University Hospital, Belgium and all participants gave their consent for participation in the study. The main focus of this study was to investigate the morbidity and quality of life of trans persons during and after sex reassignment treatment.

1.6.1.3 *Clinical studies*

Two clinical cross-sectional studies were performed, one in 50 trans women and one in 50 trans men. Participants of both studies were Dutch-speaking, treated at the Center for Sexology and Gender Problems at the Ghent University Hospital and all underwent sex reassignment surgery. Both studies were approved by the ethical review board of Ghent University Hospital and all participants gave written informed consent. Response rate of the studies were 71.4% and 64% respectively. During a one day hospital visit, all participants received a fasting, morning blood sample, evaluation of anthropometrics, body composition, bone mineral density (dual energy x-ray absorptiometry) and questionnaires on medical history, clinical effects, quality of life, sexual functioning and surgical results. The aims of these studies were to investigate antropometrics, body composition, bone mineral density, clinical effects and biochemical parameters after long-term cross-sex hormone therapy.

1.7 METHODS

1.7.1 ANTHROPOMETRIC CHARACTERISTICS AND BODY COMPOSITION

Antropometrics and body composition were measured in the ENIGI study and in both clinical studies. Body weight (kilograms) was measured in light indoor clothing, without shoes. Standing height was measured to the nearest 0.1 cm using a wall-mounted Harpenden stadiometer (Holtain Ltd., Crymuch, UK). Body mass index (BMI) was calculated as body weight (kilograms) divided by the square of the body height (square meters). Waist-to-hip ratio is determined as the ratio of waist circumference, the abdominal circumference located midway between the lower rib margin and the iliac crest, over hip circumference, the widest circumference around the buttocks. As both WHR and BMI are only indicators of body composition, we also assessed fat and lean mass by measuring the whole body soft tissue composition using Dual-energy X-ray absoptiometry (DXA) (Hologic QDR-4500A device; software version 11.2.1; hologic Inc., Bedford, MA, USA). This technique allows us to make a distinction between fat and lean mass but also between truncal fat mass and appendicular fat mass. The coefficient of variation for both spine and whole-body calibration phantoms was less than 1%, as calculated from daily and weekly measurements, respectively.

1.7.2 EVALUATION OF CLINICAL EFFECTS

Clinical assessment of **acne** was performed in each patient with the Gradual Acne Grading Scale (GAGS) in the ENIGI study and the clinical trans men study[374]. This is a quantitative scoring system in which the total severity score is derived from the summary of six regional subscores. Each subscore is obtained by the score of the most heavily weighted lesion within each region (1 for ≥ one comedone, 2 for ≥ one papule, 3 for ≥ one pustule and 4 for ≥ one nodule) multiplied by the factor for each region (factor for forehead and each cheek is 2, chin and nose is 1 and chest and upper back is 3). A score between 6–18, 20-30 and 31-36 are considered as mild, moderate or severe acne respectively.

The effects of T therapy on **hair growth and distribution of body hair** in trans men was evaluated by the Ferriman and Gallwey classification[375]for each patient in the ENIGI study and the clinical trans men study. This scale scores 9 androgen dependent areas on a 5-point Likert-type scale (from 0= no to 4=very dense). A score for the androgen-dependent area of more than 8 is considered indicative for hirsutism.

Patients' experienced treatment-related symptoms including hot flashes, palpitations, dizziness, sleeping problems, fatigue, abdominal complaints, hair loss, acne, cognition problems, mood swings, irritability, anxiety, panic attacks, low sexual desire, high sexual desire, headache, breast tenderness, joint pain and muscle soreness were investigated in the ENIGI and TransBel study using a four-point Likert-type scale (no, mild, moderate or severe complaints).

Frequency of sexual desire was assessed in the TransBel study and clinical trans men study using a 5-point Likert-type scale as well as current sexual desire compared to after sex reassignment (5 point Likert-type scale, from 'much higher' to 'much lower'). In the clinical trans men study, sexual desire was additionally measured using the Dutch version of the Sexual Desire Inventory[376]. This self-report questionnaire contains 14 items. Subscales measure the intensity and frequency of the desire to behave sexually with a partner (dyadic sexual desire) or by oneself (solitary sexual desire). For the frequency-items, participants chose one out of seven options. For the strength items, participants scored their sexual desire on a 9-point Likert-type scale ranging from 0 (no desire) to 8 (strong desire). Participants were asked to take the previous month as a reference. Adding items resulted in a score for dyadic and solitary sexual desire. Higher scores indicate a higher level of sexual desire, with a maximum score of 62 for the dyadic subscale and 23 for the solitary subscale. The Sexual Desire Inventory has a good reliability and validity[376]. In the TransBel study, both trans men and women were additionally asked about the presence of

personal distress caused by sexual desire problems (yes or no) and to describe whether this caused distress for themselves, their partner, both of them and/or their relationship.

All studies recorded **clinical adverse events** including cardiovascular events, cerebrovascular disease, venous thrombosis and/or pulmonary embolism, cancer. In addition, in our clinical studies, reaching hormone target levels, normal for the desired gender and supra-or subnormal serum of serum parameters; e.g., development of erythrocytosis, hyperprolactinaemia, hypercholesterolemia, and hyperglycemia were also evaluated.

1.7.3 QUALITY OF LIFE

QoL was measured in the TransBel using the SF-12 questionnaire[377]. This questionnaire includes 12 questions with fixed response choices, organized in two scaled scores, based on the weighted sums of the questions in their section. These scores were converted into a summary score for each section: physical functioning and mental functioning, with higher scores indicating higher levels of functioning or well-being. The SF-12 questionnaire is the shorter version of the SF-36 questionnaire.

1.7.4 BIOCHEMICAL ANALYSIS

1.7.4.1 *ENIGI study*

Venous blood was obtained at baseline and 12 months and serum was stored at -80 degrees until batch analysis. Routine clinical blood samples were drawn at the 3 months, 6 months and 9 months time point.

Luteinizing hormone (LH), follicle stimulating hormone (FSH), SHBG, insulin, DHEAS and, prolactin were measured by electrochemiluminiscence immunoassay (ECLIA); Modular, Roche Diagnostics, Mannheim, Germany. The inter-assay CVs were as follows: LH and FSH, 2.19% and 2.55%, respectively; prolactin 4.8%, SHBG 2.8%, DHEAS 4.8% and insulin 2.3%. Estradiol (E2), estrone (E1), androstenedione, cortisol and testosterone were determined by liquid chromatography tandem mass spectrometry (AB Sciex 5500 triple-quadrupole mass spectrometer; AB Sciex, Toronto Canada). Serum limit of quantification was 0.3 pg/mL for E2 and 0.5 pg/ml for E1, and the interassay CV were 4% at 21 pg/mL for E2 and 7.6% at 25 pg/mL for E1. Serum limit of quantification was 1 ng/dL (35pmol/L) for T, and the interassay CV were 6.5% at 3 ng/dL. Dihydrotestosterone (DHT) was measured by liquid chromatography tandem mass spectrometry after paper chromatography. Serum limit of quantification was 5 ng/dL for DHT, and the interassay CV were 6% at 11 ng/dL. Hemoglobin (Hb), hematocrit

(Hct), glucose, creatinin and liver enzymes glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), cholesterol (C), LDL-C, HDL-C, and triglycerides were measured using routine clinical chemistry methods.

1.7.4.2 *Clinical studies*

Venous blood samples were obtained between 08.00 and 12.00h after overnight fasting. All blood samples were stored at -80°C until batch analysis.

Commercial kits for Radio-Immuno Assay (RIA) were used to determine the serum concentrations of total T and sex hormone binding globulin (Orion Diagnostica, Espoo, Finland); E_2 (Clinical Assay, Diasorin s.r.l., Saluggia, Italy); LH, Insulin-like growth factor (IFG-1), C-terminal telopeptides of type I collagen (CTX), 1 aminoterminal propeptide (P1NP) (electrochemiluminiscence immunoassay (ECLIA); Modular, Roche Diagnostics, Mannheim, Germany. Insulin-like growth factor-binding protein 3 (IGFBP3) was determined by an extraction method (DSL-5600; Diagnostic System Laboratories, Webster, 166 TX, USA). The intra- and interassay coefficients of variation for all assays were ≤ 10%. Hematocrit, total cholesterol, and creatinin were measured using routine clinical chemistry methods.

CHAPTER 2. CLINICAL EFFECTS OF CROSS-SEX HORMONAL THERAPY

BASED ON

Wierckx K, Van Caenegem E, Schreiner T, Haraldsen I, Fisher A, Toye K, Kaufman JM, T'Sjoen G 2014 Cross-sex hormone therapy is safe and effective at short-time follow-up: results from the European Network for the Invesitgation of Gender Incongruence. J Sex Med *(Accepted).*

Wierckx K, Van Caenegem E, Schreiner T, Lapauw B, Kaufman JM, T'Sjoen G 2014 Sex steroids and cardiometabolic risk factors: lessons from the treatment of trans women and men. (*Submitted)*

Wierckx K, Van de Peer F, Dedecker D, Verhaeghe E, Van Caenegem E, Toye K, Kaufman, JM, T'Sjoen G 2014 Short and long-term dermatological effects of cross-sex hormone therapy in trans men. J Sex Med 11(1):222-9.

Wierckx K, Mueller S, Weyers S, Van Caenegem E, Roef G, Heylens G, T'Sjoen G 2012 Long-term evaluation of cross-sex hormone treatment in transsexual persons. J Sex Med 9: 2641-51

Wierckx K, Elaut E, Declerq E, Heylens G, De Cuypere G, Kaufman JM, T'Sjoen G 2013 Prevalence of cardiovascular disease and cancer during cross-sex hormone therapy in a large cohort of trans persons: a case control study. Eur J Endocrinol 169:471-478

CHAPTER 2. CLINICAL EFFECTS OF CROSS-SEX HORMONAL THERAPY

2.1 CROSS-SEX HORMONE THERAPY IS SAFE AND EFFECTIVE AT SHORT-TIME FOLLOW-UP: RESULTS FROM THE EUROPEAN NETWORK FOR THE INVESITGATION OF GENDER INCONGRUENCE

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ABSTRACT

CONTEXT

Data on the effects of cross-sex hormone therapy (CHT) are limited due to the low prevalence of gender dysphoria, small number of subjects treated at each center, lack of prospective studies, and wide variations in treatment modalities.

OBJECTIVE

To report the short-term effects of cross-sex hormone therapy on hormonal and clinical changes, side effects, and adverse events in trans men (female-to-male transsexuals) and trans women (male-to-female transsexuals)

DESIGN

This was a multi-center 1 y prospective study.

SETTING

University Hospital setting.

PATIENTS

Fifty-three trans men and 53 trans women were included.

INTERVENTION

Trans men received injections with testosterone undecanoate every 3 months. Trans women younger than 45 y received cyproterone acetate (CA) 50mg daily and 4mg estradiol valerate (EV) daily while those older than 45 y received CA 50mg daily together with transdermal 17-β estradiol 100μg/24h.

MAIN OUTCOME MEASURES

Sex steroids, prolactin, liver enzymes, lipids, hematocrit, blood pressure, anthropometrics, Ferriman and Gallwey score, and global acne grading scale were measured. Side effects, adverse events, and desired clinical changes were examined.

RESULTS

No deaths or severe adverse events were observed one year after start of CHT. Two trans men developed erythrocytosis and two had transient elevation of the liver enzymes. Trans men reported an increase in sexual desire, voice instability, and clitoral pain (all $p \leq 0.01$). Testosterone therapy increased acne scores, facial and body hair, and prevalence of androgenetic alopecia. Waist-hip ratio, muscle mass, triglycerides, total cholesterol (C), and LDL-C increased whereas total body fat mass and HDL-C decreased. Three trans women experienced transient elevation of liver enzymes. A significant increase in breast tenderness, hot flashes, emotionality, and low sex drive (all $p \leq 0.02$). Fasting insulin, total body fat mass, and prolactin levels increased; and waist-hip ratio, lean mass, total-C and LDL-C decreased.

CONCLUSIONS

Current treatment modalities were effective and carried a low risk for side effects and adverse events at short time follow-up.

INTRODUCTION

Trans persons undergo cross-sex hormone therapy (CHT) to induce the secondary sex characteristics of the desired sex while reducing those of the natal one[1]. The act of using cross-sex hormones is also an affirmation of gender identity in many trans persons. The choice of type and dosage of hormones have not yet been established as randomized controlled trials and comparative studies are lacking[2], and so a variety of hormone preparations are currently used[3]. Female-to-male gender dysphoric persons, referred to as trans men, are treated with testosterone (T) preparations to induce virilization, sometimes preceded with progestagens to suppress menstruation[1]. To promote feminization, trans women (male-to-female gender dysphoric persons) usually receive estrogens in combination with anti-androgen and/or gonadal axis suppression medication to lower T levels and/or action[1].

Many centers in Europe use cyproterone acetate (CA), a progestational agent with androgen receptor blocking properties[4-6] whereas spironolactone, a diuretic with anti-androgen action, is mostly used in the United States[7-8]. Other centers also use gonadotropin-releasing-hormone (GnRH) analogues[9-10], non-steroidal androgen receptor blockers, or 5-alpha reductase inhibitors. In addition, type of formulation, hormone dosage and route of administration (oral, transdermal or intramuscular) may differ between centers. These wide variations in treatment modalities and the low prevalence of transsexuality, and therefore small number of subjects treated in each center, hamper our knowledge on the effects and side effects of CHT. Furthermore, hormonal therapies have changed considerably in the past years, and most current evidence is based on older treatment regimens. The use of long-acting T preparations has significantly increased in trans men, and use of ethinyl estradiol (EE) has decreased in trans women due to the increased risk for cardiovascular disease[11].

AIMS

The aim of this study was to investigate the physical and physiological effects, side effects, and adverse events of commonly used cross-sex hormone therapies. We present the first multi-center prospective study in a well-described cohort of trans persons treated according to a standardized treatment protocol.

METHODS

STUDY POPULATION AND SEX HORMONE THERAPY

This research is part of the European Network for the Investigation of Gender Incongruence (ENIGI), a collaboration of four major West European gender identity clinics (Amsterdam, Ghent, Hamburg and Oslo)[12] created to study the diagnostics and treatment of gender dysphoria. We present current data from the Department of Endocrinology at the Ghent University Hospital and the University Hospital in Oslo. All patients diagnosed with gender dysphoria and referred to our departments between February 2010 and August 2012 were invited to participate in this prospective study (N=152). We included only hormone naive trans persons. After screening, by thorough medical history and determination of serum sex steroids, 44 individuals were excluded. A total of 53 trans men and 53 trans women participated in our study (figure 1). Patients were followed every 3 months during the first treatment year.

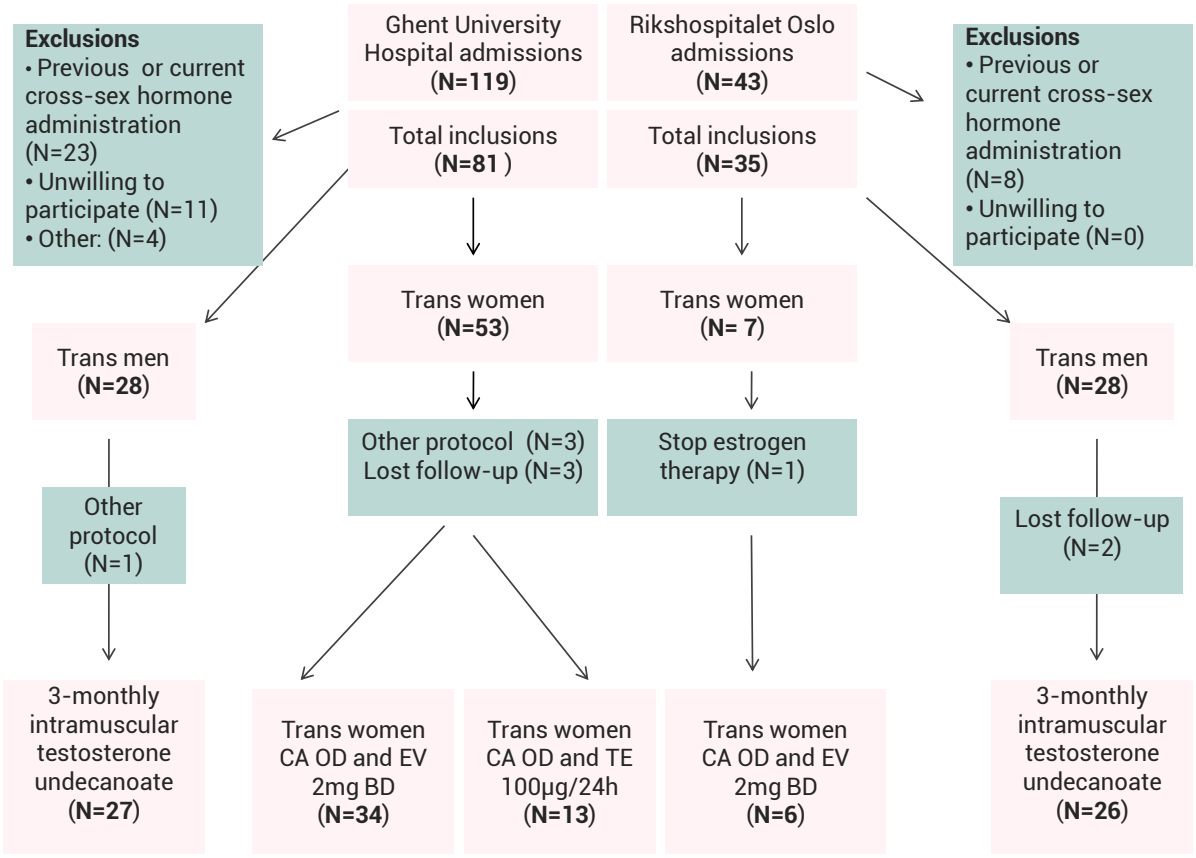

Figure 1. Subject enrollment

Trans men received injections of 1000 mg intramuscular T undecanoate (Nebido®; Bayer, Germany) at the start of the study, after 6 weeks, and every 12 weeks thereafter. Before T initiation, progestagens were sometimes taken to suppress the menstrual cycle. In case of non tolerance, injections with intramuscular T esters (T decanoate 100 mg, T isocaproate 60 mg, T fenylpropionate 60 mg, T propionate 30 mg/ml) (Sustanon 250®; MSD; Netherlands) every 2 weeks were prescribed.

All trans women younger than 45 y (N=40) received 50 mg of CA (Androcur®; Bayer, Germany) in combination with 4 mg of estradiol valerate (EV) daily (Progynova®; Bayer). Patients older than 45 y (N=13) received 50 mg of CA daily in combination with 100 μg/24 h transdermal 17-β estradiol patch (Dermestril®; Besins, Belgium). In case of non-tolerance, 2 mg of transdermal 17-β estradiol gel twice daily (Oestrogel®; Besins, Belgium) or 4 mg EV per day was given. Based on the decision of the mental health professional and patient, some trans women who favored a slower procedure and/or needed an extra diagnostic evaluation received a dual-phase protocol (N=16). In the first phase, sex-specific features were suppressed by administration of CA 50 mg daily for about 3 months, and estrogens were added to induce feminization in the second phase. This study complied with the recommendations of the Declaration of Helsinki and was approved by the ethical committee of the Ghent University Hospital and the University Hospital of Oslo. All participants gave written informed consent. Clinical trial number: NCT01072825.

MAIN OUTCOME MEASURES

MEDICAL HISTORY AND EXAMINATION

Descriptive data was collected from all individuals, including physical and psychiatric medical history, current and past medication use, familial medical history, and lifestyle factors such as smoking and alcohol consumption. Information was compared to data from medical files for accuracy and corrected if necessary.

PHYSICAL PARAMETERS

Anthropometrics

Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd, Crymuch, United Kingdom). Body weight was measured in light indoor clothing without shoes to the nearest 0.5 kg. Waist circumference, defined as the smallest abdominal circumference, and hip circumference, defined as the largest hip circumference, were determined to the nearest 0.1 cm.

Body composition

Whole body lean mass and fat mass were measured using dual-energy X-ray absorptiometry (DXA) with Hologic Discovery (Hologic Inc., Bedford, MA) in Belgium and with Lunar Prodigy Advance (GE, Madison, WI) in Norway.

Acne

Clinical assessment of acne was performed every 3 months in each patient by the same endocrinologist with the Gradual Acne Grading Scale (GAGS)[13], a semi-quantitative scoring system in which the total severity score is derived from the summary of six regional subscores. Each subscore was obtained by the score of the most heavily weighted lesion within each region (1 for one or more comedones, 2 for one or more papules, 3 for one or more pustules, and 4 for one or more nodules) multiplied by the factor for each region (the factor for forehead and each cheek is 2, chin and nose is 1 and chest and upper back is 3). Scores between 6–18, 20–30, and 31–36 were classified as mild, moderate or severe acne, respectively.

Body hair and distribution

The effects of testosterone therapy on hair growth and distribution of body hair in trans men was evaluated every 3 months using the modified Ferriman and Gallwey classification[14] in each patient by the same endocrinologist. This scale scores nine androgen-dependent areas on a 5 point likert scale (from 0= no to 4=very dense). A score for an androgen-dependent area of more than 8 indicated hirsutism. Androgenetic alopecia was assessed using the Norwood/Hamilton classification[15].

We were unable to evaluate the effects of CHT on hair growth and distribution of body hair in trans women because almost all of them underwent laser epilation during the course of the study.

SIDE EFFECTS AND ADVERSE EVENTS

Clinical adverse events, including cardiovascular events, venous thrombosis and/or pulmonary embolism, osteoporotic fractures, abnormal liver function tests, hypertension, and death (including suicide) were recorded. We also evaluated whether hormone levels reached the target values for the desired gender. Development of erythrocytosis, hyperprolactinaemia, hypercholesterolemia, and hyperglycemia was also assessed.

We evaluated symptoms possibly related to hormonal status every 3 months, including hot flashes, night sweats, sleeping problems, fatigue, memory or cognition problems, mood swings, irritability, anxiety, low or high sexual desire, migraines, nail and gum problems, breast

tenderness, joint pain, and muscle soreness using a four-point Likert scale (no, mild, moderate or severe complaints).

Biochemical determinations

Venous blood was obtained at baseline and at 12 months, and serum was stored at −80°C until hormones were analyzed in one batch. Blood samples for routine clinical parameters were drawn at the 3-, 6-, and 9-month time points.

Luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG), insulin, dehydroepiandrosterone sulfate (DHEAS), and prolactin were measured by electrochemiluminiscence immunoassay (ECLIA) (Modular, Roche Diagnostics, Mannheim, Germany). The inter-assay CVs were as follows: LH 2.19%, FSH 2.55%, SHBG 2.8%, prolactin 4.8%, DHEAS 4.8%, insulin 2.3%. Estradiol (E_2), estrone (E_1), DHEAS, androsteendione, cortisol, and testosterone were determined using liquid chromatography tandem mass spectrometry (AB Sciex 5500 triple-quadrupole mass spectrometer; AB Sciex, Toronto Canada). The serum limit of quantification was 0.3 pg/mL for E2 and 0.5 pg/ml for E1, and the inter-assay CVs were 4% at 21 pg/mL for E_2 and 7.6% at 25 pg/mL for E_1[16]. Serum limit of quantification was 1 ng/dL (35pmol/L) for T, and the interassay CV was 6.5% at 3 ng/dL. Hemoglobin (Hb), hematocrit (Hct), glucose, creatinin, and the liver enzymes glutamic-oxaloacetic transaminase (ASAT), glutamic-pyruvic transaminase (ALAT), cholesterol (C), LDL-C, HDL-C, and triglycerides were measured using routine clinical chemistry methods.

STATISTICAL ANALYSIS

Descriptive statistics were expressed as means and standard deviations, or medians [first to third quartiles] for in case of a non-normal distributions. Statistical analyses of categorical variables were carried out using $\chi 2$ and Fisher's exact tests as appropriate. Statistics of means in prospective data were carried out using the paired Student t tests or Wilcoxon signed-rank tests when variables were not normally distributed. Between two groups, statistics of means was evaluated using independent Student t tests and Mann-Whitney-U tests when variables were not normally distributed. Significance was set at $p<0.05$ (two-tailed). Data were analyzed using SPSS-software, v.21 (SPSS Inc., Chicago, IL). For all analyses, missing values were excluded.

RESULTS

GENERAL CHARACTERISTICS

General characteristics of the study population are shown in Table 1. Trans women were significant older than trans men at the time of presentation ($p<0.001$). A significantly greater proportion of trans men were at the Oslo center, and the subjects were significantly older at the Ghent center.

TABLE 1. BASELINE CHARACTERISTICS OF THE STUDY POPULATION

	TRANS WOMEN		TRANS MEN	
	GHENT (N=47)	OSLO (N=6)	GHENT (N=27)	OSLO (N=26)
Age (years)	31.7 ± 14.8	19.3 ± 2.4	27.3 ± 8.5	21.7 ± 5.1
Current smoker (%)	19.1	0	25.9	14.4
Former smoker (%)	38.3	0	33.3	30.8
Alcohol (U/week)	0 (0 - 7)	0 (0 - 0)	0 (0 – 0)	0 (0 – 0.75)
Height (cm)	178.4 ± 6.2	179.5 ± 3.8	163.4 ± 4.4	168.3 ± 6.0
Weight (kg)	76.1 ± 13.9	73.7 ± 18.5	65.7 ± 15.0	71.2 ± 15.9
BMI (kg/m²)	23.9 ± 4.1	22.9 ± 5.7	24.5 ± 5.2	25.2 ± 5.5

Data are presented as mean ±S.D or median (first to third quartiles).

HORMONAL AND BIOCHEMICAL CHANGES IN TRANS MEN

All trans men achieved T levels within the male reference range (321–1005 ng/dl) during treatment (Table 2). Three trans men (5.7%) had T levels that exceeded the upper limit of 1005 ng/dl (1 at 3-month, 1 at 9-month and 1 at 12-month time point of treatment). Hct levels gradually increased during T treatment. Two trans men developed erythrocytosis according to male reference ranges (Hct levels above 52%), one developing it after 9 months and one after 12 months of treatment. T levels were within the male reference range in these men. Erythrocytosis was present in 20.1% of trans men according to female reference ranges (Hct levels above 48%). E2, E1, prolactin, and SHBG decreased significantly, whereas DHEAS, androsteendione, and cortisol levels were not influenced by T treatment. Testosterone treatment induced a less favorable lipid profile, as total-C, LDL-C, and triglycerides increased whereas HDL-C decreased (Table 2).

HORMONAL AND BIOCHEMICAL CHANGES IN TRANS WOMEN

All trans women achieved adequate gonadotropin and T suppression during anti-androgen and estrogen administration (Table 2). Two trans women (both on oral EV) who initially had an adequate T and gonadotropin suppression showed T levels within the normal male range at 12

months, possibly due to poor adherence to treatment. Gonadotropins, T, and androstenedione decreased during oral and transdermal estrogen therapy associated with CA, whereas E2 and E1 increased.

Trans women using oral EV, unlike those using transdermal estrogen, experienced a significantly increased SHBG, decreased DHEAS, and a trend toward increased cortisol levels. Prolactin levels significantly increased during both oral and transdermal estrogen treatments (figure 2A), and during 12 weeks of CA treatment alone (figure 2B).

CHT induced a more favorable lipid profile, as total-C and LDL-C decreased during both oral and transdermal estrogen treatments. HDL decreased during both forms of estrogen treatment, and triglycerides decreased during transdermal but not oral estrogen treatment. No significant differences were observed between trans women initially treated with CA plus estrogens compared to those initially treated with CA alone (data not shown).

Figure 2. Changes in prolactin levels during CA plus oral or transdermal estrogen treatment (A)/ Changes in prolactin levels during CA plus estrogens or CA alone.

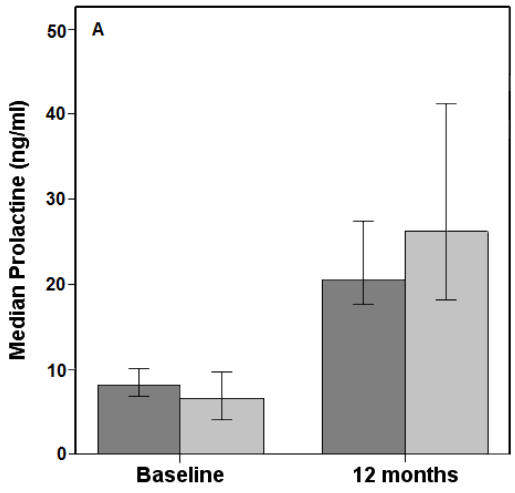

Figure 2A. Data presented as median, error bars represent 95% CI. Dark grey square: cyproterone acetate (CA) plus oral estrogens; Light grey square: CA plus transdermal estrogens. We observed a comparable increase in prolactin levels during both oral and transdermal estrogen treatment (p<0.001 and p=0.002, respectively).

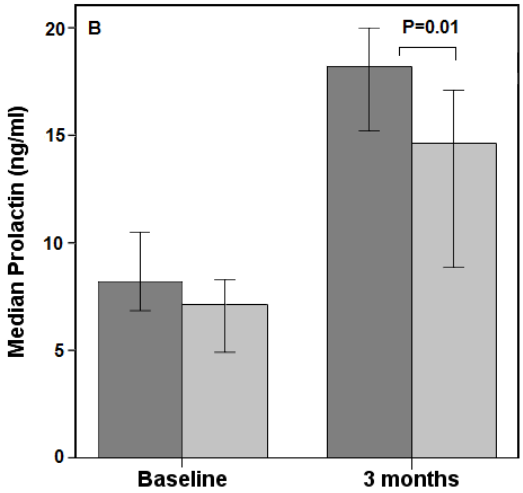

Figure 2B. Data presented as median, error bars represent 95% CI. Dark grey square: cyproterone acetate (CA) plus estrogens; Light grey square: CA alone.
We observed an increase in prolactin levels during CA + estrogen treatment (p<0.001) and during CA treatment alone (P=0.009). CA+ estrogen treatment induced higher prolactin levels compared to CA treatment alone (p=0.01).

TABLE 2. HORMONAL AND BIOCHEMICAL CHANGES IN TRANS PERSONS

	TRANS WOMEN (N=53)				TRANS MEN (N=53)	
	ORAL ESTROGENS (N=40)	P	TRANSDERMAL ESTROGENS (N=13)	P	INTRAMUSCULAR T UNDECONOATE (N=53)	P
Testosterone (ng/dl)						
Baseline	517.5 (419.2-631.9)	<0.001	567.0 (484.8-678.2)	0.001	30.2 (20.4-39.9)	<0.001
12 month	10.7 (8.3-14.3)		14.0 (12.4-17.2)		595.8 (481.1-715.7)	
Estradiol (pg/ml)						
Baseline	19.1 (15.3-24.9)	<0.001	23.6 (18.3-29.3)	0.001	50.3 (24.2-99.3)	0.001
12 month	56.5 (42.3-70.8)		95.4 (58.5-127.6)		29.4 (21.7-34.7)	
Estrone (pg/ml)						
Baseline	29.8 (24.1-40.1)	<0.001	45.8 (33.8-50.3)	0.028	51.7 (30.3-80.9)	0.01
12 month	360.9 (253.3-485.3)		57.8 (47.3-90.7)		45.5(32.9-53.8)	
SHBG (nmol/l)						
Baseline	31.2 (24.0-40.1)	<0.001	50.3 (36.0-59.7)	0.33	51.6 (23.0-81.2)	<0.001
12 month	41.7 (25.8-50.7)		48.1 (29.0-90.7)		25.1 (19.7-34.0)	
LH (U/l)						
Baseline	4.8 (3.7-6.4)	<0.001	5.5 (3.5-6.8)	0.001	6.7 (4.2-9.9)	<0.001
12 month	0.1 (0.1-0.1)		0.1 (0.1-0.1)		2.4 (0.7-5.8)	
FSH (U/l)						
Baseline	3.5 (2.8-5.3)	<0.001	5.4 (3.5-6.8)	0.001	4.8 (3.4-7.3)	0.02
12 month	0.2 (0.1-0.4)		0.2 (0.1-0.4)		4.2 (1.2-6.6)	
Prolactin (ng/ml)						
Baseline	8.1 (6.3-11.3)	<0.001	6.6 (4.1-9.3)	0.001	13.7 (9.3-19.9)	<0.001
12 month	20.6 (16.7-28.9)		26.2 (18.6-36.2)		9.6 (7.9-14.4)	
DHEAS (µg/dl)						
Baseline	289.9 (253.1-401.7)	<0.001	285.9 (220.4-404.5)	0.42	254.0 (170.1-351.4)	0.97
12 month	236.8 (192.9-348.5)		275.6 (171.9-410.3)		257.6 (174.1-355.7)	
Androstenedione (ng/dl)						
Baseline	101.2 (77.0-121.3)	<0.001	117.4 (94.6-161.4)	0.001	113.9 (90.9-150.5)	0.42
12 month	56.2 (43.4-76.4)		69.6 (55.9-106.7)		113.8 (80.2-151.9)	
Cortisol (µg/dl)						
Baseline	15.2 (12.9-18.7)	0.06	15.4 (13.4-19.9)	0.28	13.3 (8.9-16.0)	0.42
12 month	15.7 (12.8-20.8)		15.0 (11.8-18.9)		11.8 (9.4-15.2)	
Hematocrit (%)						
Baseline	45.2 ± 2.5	0.003	45.5 ± 1.7	<0.001	40.8 ± 2.9	<0.001
12 month	42.0 ± 5.7		42.0 ± 2.3		45.8 ± 3.0	
Fasting glucose (mg/dl)*						
Baseline	0.83 ± 0.1	0.78	0.92 ± 0.06	0.25	0.80 ± 0.1	0.28
12 month	0.84 ± 0.1		0.88 ± 0.1		0.77 ± 0.1	
Fasting insulin (mU/l)*						
Baseline	7.2 (4.9-9.7)	0.019	7.3 (6.6-18.7)	0.1	9.1 (5.8-16.3)	0.02
12 month	9.3 (7.2-11.3)		12.0 (9.1-17.0)		7.5 (5.2-13.2)	
Creatinin (mg/dl)						
Baseline	0.9 ± 0.1	0.001	0.93 ± 0.1	0.011	0.74 ± 0.1	<0.001
12 month	0.8 ± 0.1		0.85 ± 0.1		0.84 ± 0.1	
GOT (U/L)						
Baseline	24.1 ± 9.2	<0.001	26.8 ± 8.3	0.013	20.0 (17-23)	0.01
12 month	17.7 ± 3.6		19.6 ± 4.8		24 (18-29.5)	
GPT (U/L)						
Baseline	25.0 ± 17.4	0.01	29.9 ± 15.3	0.12	16.0 (11.5-20)	0.02
12 month	18.6 ± 9.5		21.0 ± 10.1		20 (15-25)	
Total C (mg/dl)						
Baseline	171.5 ± 32.7	0.001	227.2 ± 35.6	0.004	171.9 ± 28.1	0.04
12 month	152.3 ± 28.3		181.3 ± 20.6		178.2 ± 30.6	
LDL-C (mg/dl)						
Baseline	99.4 ± 29.0	0.03	138.3 ± 24.8	0.001	98.4 ± 26.3	0.006
12 month	92.3 ± 28.9		106.3 ± 20.4		116.1 ± 28.9	
HDL-C (mg/dl)						
Baseline	52.9 ± 13.5	<0.001	58.2 ± 15.2	0.26	56.3 ± 12.7	<0.001
12 month	45.7 ± 9.2		54.8 ± 15.7		47.8 ± 10.7	
Triglycerides (mg/dl)						
Baseline	79.5 (54.7 -108)	0.1	87.0 (68.0-176.5)	0.03	69.0 (51.7-89.5)	<0.001
12 month	70.8 (50-133.1)		85.0 (70.0-110)		81.1 (65.3-124.6)	

Data are presented as mean ±S.D or median (first to third quartiles) P value results from paired T-test or Wilcoxon signed rank test * based on subsample (N=44)

PHYSICAL CHANGES

Trans men

Total body weight significantly increased due to an increase in total lean mass, whereas total fat mass decreased. An android pattern of fat distribution was observed as the waist-hip ratio increased during treatment ($p=0.02$), mainly due to reduced hip circumference (Table 3). As expected, Ferriman and Gallwey score significantly increased ($p<0.001$), with a wide between-subject variability ranging from 2 to 28.

Trans women

In contrast to trans men, total body weight remained unchanged for trans women, although they experienced an increase in total body fat mass and a decrease in total body lean mass. A gynoid pattern of fat distribution was induced in trans women, as the waist-hip ratio decreased during treatment (Table 3).

Anti-androgen with estrogen treatment resulted in a significant increase (average 3.3 cm) in breast circumference at the nipple, with a wide inter-individual range of increase ($p<0.004$). Trans women using oral estrogens experienced similar changes in physical measures as those using transdermal estrogens (Table 3). No significant differences were observed between trans women treated with CA plus estrogens from start compared to those initially treated with CA alone (data not shown), although the former tended to have a larger breast circumference ($p=0.06$).

SIDE EFFECTS AND ADVERSE EVENTS

Trans men

We recorded no deaths, cardiovascular events, osteoporotic fractures, venous thromboses, or pulmonary embolisms in trans men. Two were switched to short-acting intramuscular T esters after 9 and 12 months, respectively, of T undecanoate therapy mainly because of muscle and joint aches. Liver enzymes increased during T therapy, but only 1.9% of trans men had liver enzymes values exceeding twice the upper limit of normal according to female reference ranges. No subject had liver enzymes values exceeding twice the upper limit of normal levels according to male reference ranges.

Blood pressure increased slightly during treatment, but none of subjects developed hypertension during our observation. Fasting insulin levels decreased ($p=0.02$), and nobody developed type 2

diabetes. GAGS acne scores increased ($p<0.001$), but the majority of trans men (94.3%) had mild acne lesions. The remaining 5.7% had moderate acne lesions after 12 months of therapy, and no individuals had severe or very severe acne lesions. Eighteen persons (34.0%) initiated topical or oral acne treatment.

Seventeen percent of trans men developed androgenetic alopecia. No indication of troublesome aggression, hostility, or sleep apnea was present. All trans men reported loss of vaginal bleeding during treatment. Spotting was reported in about one third of participants, generally limited to the first 6 months of treatment (figure 3).

Trans women

Similar to trans men, no deaths, cardiovascular events, osteoporotic fractures, venous thromboses, or pulmonary embolisms were observed in trans women. One trans woman had to stop estrogen treatment due to major depression and was excluded from our analyses. Serum prolactin levels exceeding twice the upper limit of normal were observed in 15.7% and 3.9% according to the male and female reference range, respectively. One trans woman experienced galactorea, which spontaneously stopped after several weeks. Transient elevation of the liver enzymes exceeding twice the upper limit of normal was observed in 5.7% and 1.9% according to the female and male reference ranges, respectively. One trans woman developed hypertension during the study observation (defined as a systolic blood pressure above 140 mmHg or diastolic above 90 mmHg at three different time points). Fasting insulin increased during anti-androgen and estrogen treatment ($p=0.005$), but no subject met criteria for diagnosis of type 2 diabetes. Two trans women using transdermal estrogen patches (15.4%) were switched to other therapies because of skin irritation after 3 and 9 months of treatment, respectively.

TREATMENT-RELATED SYMPTOMS

Trans men

Treatment-related symptoms were investigated every 3 months in a subsample of trans men ($N=25$). The vast majority of the trans men reported an increase in sexual desire (figure 3). Testosterone treatment resulted in variable levels of voice deepening and an increased voice instability ($p=0.024$). About 20% of trans men reported clitoral pain, with a peak incidence observed at 6 months of treatment. Symptoms of emotionality decreased ($p=0.01$).

We observed no changes in symptoms of night sweats, hot flashes, abdominal pain, anxiety, breast tenderness, irritability, palpitations, joint pain, muscle soreness, headache, mood swings, fatigue, concentration difficulties, memory, or sleep-related problems (data not shown).

Trans women

We examined treatment-related symptoms every 3 months in a subsample of trans women (N=30) treated with 50 mg of CA daily in combination with estrogens from the start. A significant increase in breast tenderness, emotionality, low sexual desire, and hot flashes was observed ($p<0.001$, $p=0.001$, $p<0.001$, and $p=0.02$, respectively)(figure 3).

We found no changes in night sweats, abdominal pain, anxiety, irritability, palpitations, skin dryness, joint pain, muscle soreness, headache, mood swings, fatigue, concentration difficulties, memory or sleep-related problems (data not shown).

No significant differences in the presence of treatment-related symptoms at the 3-month time point between trans women treated with oral or transdermal estrogen. No significant differences were observed at the 3-month time point between trans women treated with CA plus estrogens from start compared to those initially treated with CA alone (data not shown), except for a higher prevalence of breast tenderness in those women treated with CA plus estrogens ($p=0.001$).

TABLE 3. PHYSICAL CHANGES IN TRANS PERSONS

	TRANS WOMEN (N=53)				TRANS MEN (N=53)	
	ORAL ESTROGENS (N=40)	P	TRANSDERMA LESTROGENS (N=13)	P	INTRAMUSCULAR TUNDECONOATE (N=53)	P
Weight (kg)						
Baseline	73.3 ± 13.8	0.10	82.1 ± 13.1	0.99	68.4 ± 15.5	0.01
12 months	74.6 ± 14.3		82.1 ± 11.6		70.6 ± 13.2	
BMI (kg/m²)						
Baseline	23.1 ± 4.2	0.42	26.1 ± 3.5	0.91	24.8 ± 5.3	0.01
12 months	23.7 ± 4.4		26.1 ± 3.4		25.6 ± 4.4	
Total body fat mass (kg)						
Baseline	15.4 ± 7.4	<0.001	16.6 ± 5.4	0.01	22.9 ± 11.4	<0.001
12 months	20.0 ± 8.1		18.7 ± 4.6		19.9 ± 9.7	
Total body lean mass (kg)						
Baseline	5.6 ± 7.5	<0.001	62.6 ± 9.3	0.02	43.0 ± 6.6	<0.001
12 months	5.3 ± 8.0		59.7 ± 8.1		48.3 ± 5.6	
Waist circumference (cm)						
Baseline	81.2 ± 10.1	0.21	91.6 ± 11.1	0.50	80.3 ± 13.6	0.74
12 months	79.7 ± 10.5		90.9 ± 10.1		80.1 ± 11.2	
Hip circumference (cm)						
Baseline	94.2 ± 9.3	<0.001	96.9 ± 7.8	0.07	97.3 ± 10.5	0.02
12 months	98.1 ± 9.3		100.4 ± 7.1		95.4 ± 9.2	
Waist-hip ratio						
Baseline	0.9 ± 0.1	<0.001	0.94 ± 0.06	0.07	0.82 ± 0.09	0.03
12 months	0.8 ± 0.1		0.91 ± 0.07		0.84 ± 0.08	
Breast circumference (cm)						
Baseline	92.9 ± 10.0	0.03	98.2 ± 9.0	0.09	-	-
12 months	95.7 ± 11.2		101.2 ± 9.0		-	
Systolic blood pr (mmHg)						
Baseline	125.1 ± 13.8	0.005	131.6 ± 15.8	0.47	111.5 ± 12.6	0.05
12 months	118.8 ± 13.9		128.8 ± 15.5		115.6 ± 11.7	
Diastolic blood pr (mmHg)						
Baseline	76.8 ± 10.8	0.32	76.7 ± 9.0	0.43	70.2 ± 10.5	0.18
12 months	75.7 ± 10.6		79.7 ± 9.3		72.5 ± 9.2	
Ferriman and Gallwey score	-	-	-	-	0 (0-2)	<0.001
Baseline	-	-	-	-	10 (6-16)	
12 months						
Acne score	2 (0-7)	<0.001	0 (0-0)	1.0	2 (0-5)	<0.001
Baseline	0 (0-0)		0 (0-0)		7.5 (2-11.8)	
12 months						

Data are presented as mean ±S.D or median (first to third quartiles) in case of non-Gaussian distribution; P value results from paired T-test or Wilcoxon signed rank test in case of non-Gaussian distribution

*Based on sub sample (n= 20)

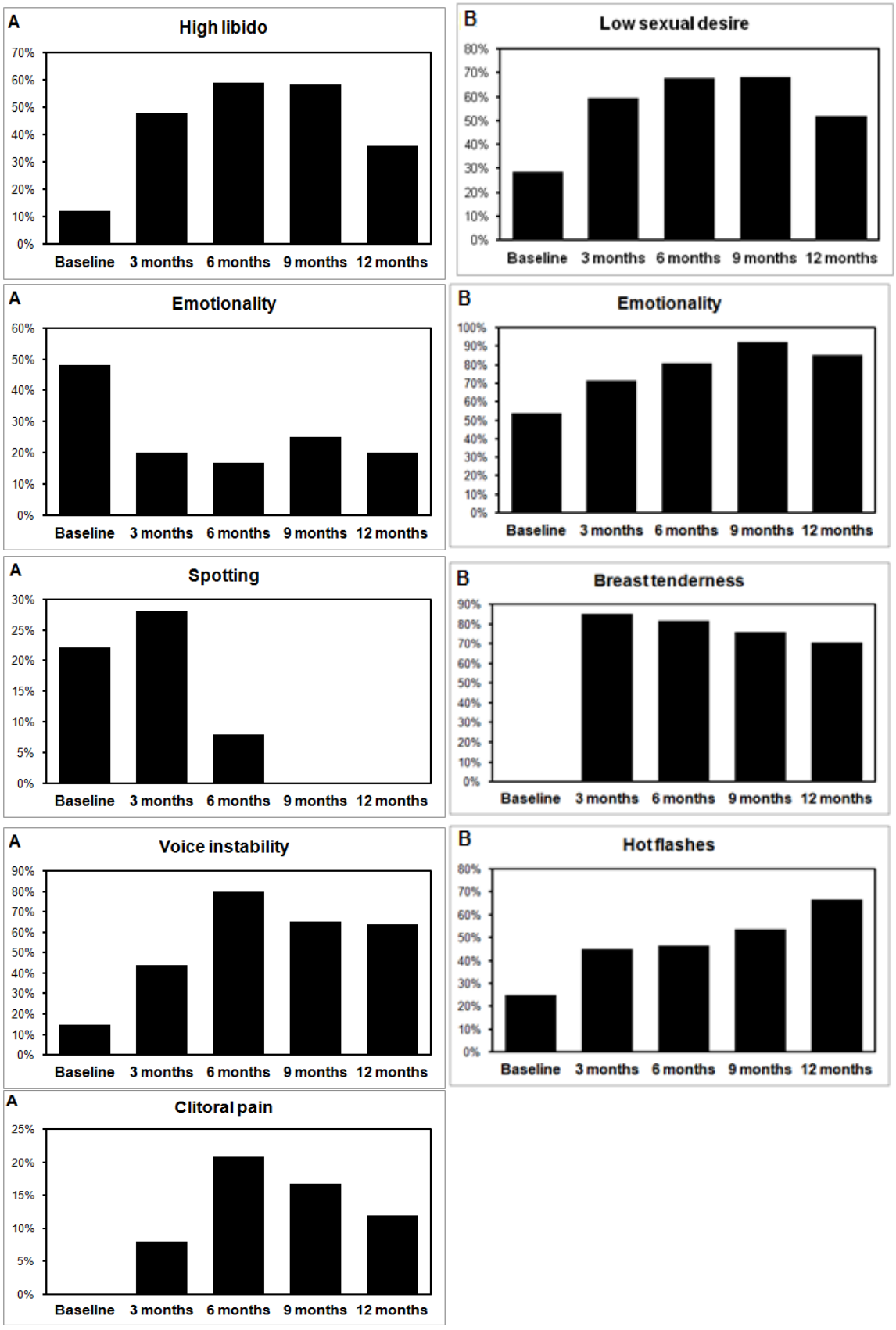

Figure 3. Prevalence of symptoms in trans men (A) Prevalence of symptoms in trans women (B)

DISCUSSION

We presented the first multi-center prospective study describing the effects of current CHT on hormonal and clinical changes, side effects, and adverse events in trans men and trans women. The main findings of our study were that our therapies (injections with T undecanoate every 3 months in trans men, and 50 mg CA plus either 4 mg oral EV or 100 μg/24 h transdermal 17-β estradiol daily in trans women) were effective and safe. The majority of trans persons had desired levels of sex steroids according to the Endocrine Society Guidelines[1] and developed the secondary sex characteristics of the desired sex. Trans men experienced cessation of menses and developed a male body habitus with an android pattern of fat distribution, a deepening of the voice, an increase in lean mass, male pattern baldness, and both facial and body hair. Trans women experienced an increase in fat mass with a gynoid pattern of fat distribution, and an increase in breast circumference.

None of the trans persons experienced severe adverse events such as cardiovascular events or death. These findings are in line with previous reports that demonstrated T treatment for trans men was effective and relatively safe in the short-term[17-19]. The results in trans women confirm a recent study[10] showing a low risk for adverse events at short-time follow-up, but contrasts earlier reports that have indicated a high incidence of venous thrombosis and/or pulmonary embolism during the first year of CHT[17, 19]. Current treatment modalities in trans women, including the avoidance of EE usage and using transdermal estrogens in older trans women, may therefore be less detrimental to the coagulation system. Indeed, Toorians and colleagues[20] described a higher activated protein C (APC) resistance with oral EE compared to oral EV and transdermal 17-β estradiol usage. In addition, Van Kesteren et al.[17] reported a decreased incidence of venous thrombosis with the use of transdermal estrogens in older trans women.

Similar to Mueller and colleagues[18], we observed a small but significant increase in systolic blood pressure during T therapy. However, considering the small increase (about 3.5%) we observed, it is likely that sample sizes in other studies[21-22] were too small to detect a statistically significant difference. Importantly, no subject developed a clinically significant blood pressure increase during our study observation. T treatment also increased liver enzymes in our study, similar to the study from Mueller and colleagues[18], although the clinical relevance of this finding remains to be determined.

Previous studies have not investigated systematically treatment-related symptoms during T therapy in trans men. However, the majority of trans men reported an increase in voice instability, acne, and sexual desire. Most acne cases were mild and none were severe or very

severe according to the GAGS. Nevertheless, about a third of our patients underwent acne treatment, indicating that even mild and moderate acne lesions were clinically relevant.

One trans woman discontinued treatment because of depression. Indeed, it has been previously reported that CHT increases depression risk [19]. However, others show CHT lowers anxiety and depression scores, possibly due to improvements in mental health after initiation of cross-sex reassignment therapy[23]. Similar to others[17, 19], we found a transient elevation of the liver enzymes during anti-androgen and estrogen treatment, although generally a decrease in liver enzymes is observed[24].

Concerning the risk for hyperprolactinemia during CHT in trans women, our findings substantiate most other studies showing an increase in prolactin levels during administration of CA combined with estrogen therapy[1, 17, 25-28] and CA alone[25, 27]. However, our previous retrospective follow-up studies do not show a further increase in the long-term[5, 29]. Moreover, the clinical relevance of increased prolactin levels during CHT remains undetermined. Although a few case reports of prolactinomas have been reported following CHT, none were reported in large follow-up studies, perhaps suggesting a low risk associated with CHT.

Trans women also reported treatment-related symptoms. Although some of them such as breast tenderness and low sexual desire were well-known from our clinical practice, subjects unexpectedly reported a significant increase in hot flashes during anti-androgen and estrogen treatment. Because we did not use validated questionnaires or objective measurements to analyze these symptoms, further exploration and characterization is needed.

Investigation of cardiovascular risk factors during CHT is important, as recent studies show that trans women have an increased cardiovascular morbidity[30] and mortality[11, 31] compared to the general population. Similar to previously published studies using EE plus CA, we found a reduction in LDL-C and an increase in fat mass and fasting insulin during the first year of CHT in trans women[21, 32]. Estrogen therapy increases removal of LDL apolipoprotein B-100[33], but the pathophysiological mechanism of increased insulin resistance during anti-androgen and estrogen therapy is not fully understood. CHT induces important changes in body composition, with an increase in fat mass, which may affect glucose metabolism. Sex steroids may also exert direct effects, as acute T withdrawal in men decreases insulin sensitivity in the absence of any detectable changes in body composition[34]. Augmentation of E_2 to supraphysiological levels may also induce insulin resistance through liver hyperinsulinemia and reduced GLUT 4 expression within the muscles[35].

In line with Dittrich and colleagues[10], we observed no increase in body weight, triglycerides, or blood pressure in trans women. These findings differ from a previously published study using EE plus CA [21], and are likely to be related to differences in type of estrogen used. Different types of estrogen exert divergent metabolic effects. EE has a stronger hepatic impact due to its 17α-ethinyl group, which prevents the inactivation of the molecule and results in a slower metabolism[36-37]. Additionally, the higher estrogen dosage in the study by Elbers and colleagues[21] may also contribute to differences in these health outcomes.

HDL-C decreased in trans women (in both oral and transdermal group), which was somewhat unexpected under estrogen therapy. A potential explanation for these findings may be found in the progestagenic effects of cyproterone acetate, as progestogens decrease HDL-C concentrations[38]. Decreased HDL-C levels have been previously shown in trans women using transdermal estrogen therapy[39-40]. Because the transdermal route avoids the first pass effect of the liver, it may have different metabolic effects than oral estrogen therapy. Decreased triglycerides were also observed with transdermal, but not oral estrogen therapy. In addition, trans women using oral EV showed significantly higher E1/E2 ratio than those using transdermal therapy. A lower E2/E1 ratio has been reported in postmenopausal women receiving transdermal hormone replacement therapy[41]. No other differences were observed between these two modes of estrogen treatment, suggesting both are equally effective.

Testosterone undecanoate treatment decreased fat mass but induced a less favorable lipid profile and an android pattern of fat distribution in trans men. Although these changes were also seen in studies using 2- or 3-weekly injections of intramuscular T esters[42-43], most of the evidence suggests that T treatment is relatively safe at short- and medium-term follow-up[17-19]. However, outcome studies in trans men are generally performed in much smaller sample sizes and at younger ages compared to trans women. Large, long-term (>20 y) follow-up studies are needed to investigate the cardiovascular safety of T therapy in trans men.

As previously described[12], the age and sex ratio differed significantly between our two centers. This age difference should be kept in mind in future outcome studies, as older age may be associated with a worse cardiovascular outcome[44].

The strengths of the present study were its relatively large sample size compared to most other prospective studies; the use of widely prescribed (but scientifically not well documented) treatment modalities; our detailed description of adverse events, side effects and treatment-related symptoms; and the use of a mass spectrometry-based methodology to measure serum sex steroid levels in trans persons. Although we did not use a validated questionnaire to measure

treatment-related symptoms, we were the first to examine these symptoms systematically. Validation of such a questionnaire may be valuable for future studies, as no standardized assessment of symptoms exists presently. Secondly, our treatment protocol was to administer oral EV to trans women younger than 45 y and transdermal estrogen therapy to those over 45 y. This age discrepancy may have influenced the difference in treatment response between the two groups. In addition, some trans women initially received CA alone without concomitant estrogen use. This may have influenced our results, although no significant differences were observed between these two groups. Moreover, the lack of difference between the groups may indicate that most relevant changes occur during the first 9 months of CHT. Thirdly, we did not have a blinded clinician to determine the clinical effects of the treatment, which may possibly induce a bias. Finally, we described average differences associated with each treatment, but we observed large between-subject differences in clinical outcome measures, which may be due to differences in sex steroid metabolism or sensitivity.

CONCLUSION

We observed that our current treatment modalities in both trans men and women are effective and carry a low risk for side effects and adverse events at short-time follow-up.

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2.2 SEX STEROIDS AND CARDIOMETABOLIC RISK FACTORS: LESSONS FROM THE TREATMENT OF TRANS WOMEN AND MEN

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Submitted

ABSTRACT

INTRODUCTION

Sex steroids may explain part of the between-sex variation in cardiovascular risk. This study aimed to investigate associations between sex steroids and cardiometabolic factors and to explore the changes in cardiovascular risk profile during cross-sex hormone therapy (CSH) in trans persons.

METHODS

In this prospective intervention study, we examined cardiometabolic factors in 40 trans women (male-to-female transsexual persons) and 20 trans men (female-to-male transsexual persons) before and after 12 months of CSH. Trans men received injections with testosterone (T) undecanoate every 3 months. Trans women <45y received cyproterone acetate (CA) 50mg daily and estradiol valerate 4mg daily while those >45y received CA 50mg daily together with transdermal 17-β estradiol 100μg/24h.

MAIN OUTCOME MEASURES

Glucose metabolism (oral glucose tolerance test) and detailed lipid profile were examined.Total body fat, trunk fat mass and lean body mass were determined by dual X-ray absorptiometry. Regional fat and muscle area at the forearm and calf by peripheral quantitative computed tomography.

RESULTS

In trans women, an increase in total body fat mass, trunk fat mass and cross-sectional fat area at proximal forearm and tibia and decrease in total body lean body mass and cross-sectional muscle area at forearm was seen. Trans men experienced an increase in lean mass and a decrease in total body fat percentage. Fasting insulin levels and HOMA-IR increased during anti-androgen with estrogen treatment, but decreased during T therapy in trans men.

Fasting glucose, Matsuda index and AUC for insulin and glucose remained unchanged during CSH both in trans women and men. In trans women, all serum lipids levels lowered during CSH, apart from an increase in free fatty acids. During T therapy triglycerides, LDL cholesterol and ApoB levels increased, whereas HDL cholesterol decreased.

No associations were found between changes in sex steroid levels and changes in body composition, glucose metabolism or lipid profile. Older age was significantly associated with an increased AUC of insulin after one year in trans women. Measures of adiposity were associated

with impaired lipid profile and glucose metabolism in trans women and with an impaired lipid profile in trans men.

CONCLUSIONS

CSH increased insulin resistance but favourably affected the lipid profile in trans women, whereas it improved insulin resistance but worsened lipid profile. Adiposity was associated with a less favourable outcome in both trans women and men. In trans women, older age was related to an impaired glucose tolerance.These findings suggest that the between-sex variation in cardiovascular risk is mainly due to effects of sex steroid exposition on body composition.

INTRODUCTION

Women have a more favourable cardiovascular risk profile compared to their age- matched male counterparts, including a less proatherogenic plasma lipid profile, less visceral fat mass and a lower prevalence of impaired glucose tolerance and arterial hypertension[1-4]. Whether these sex differences in cardiovascular risk factors are simply caused by differences in sex steroid levels is a matter of debate; and the role of non-hormone mediated effects of genes located on the sex chromosomes has been put forward[5-7]. Some also suggested that sex differences in the relationship between cardiovascular risk factors (e.g., impact of insulin resistance on cardiovascular disease) may explain the advantage of young women compared to men[8]. The effects of cross-sex hormone therapy in trans persons may offer new perspectives on these sex differences and the role of sex steroids herein.

Current knowledge in the field of transsexualism is hampered by a lack of prospective studies, small study samples and wide variations in treatment modalities between centers. In addition, most previous studies investigating changes in lipid profile and insulin sensitivity[9-12], used former treatment modalities in male-to-female transsexual persons (trans women) including high dose cyproterone acetate (CA) and high dose oral ethinyl estradiol (EE). However, since the observations that this estrogen type was associated with a higher risk for venous thrombosis and increased cardiovascular mortality[13-15], its use has been abandoned. Most studies in female-to-male transsexual persons (trans men) used short acting T esters[9-12], instead of long-acting intramuscular T undecanoate, that was more recently marketed. The latter is increasingly used, as the long intervals between intramuscular injection make this formulation attractive for T supplementation, particularly in these healthy, young persons who will need life-long treatment.

The aims of this study were twofold: (1) to investigate possible associations between sex steroids, body composition, insulin sensitivity and lipid profile in trans persons, and (2) to evaluate changes in cardiovascular risk profile during treatment with current cross-sex hormone therapies.

METHODS

STUDY POPULATION AND SEX HORMONE THERAPY

This study is conducted at the department of Endocrinology at the Ghent University Hospital. Between February 2010 and September 2012, all adult patients diagnosed with gender dysphoria referred to our department were invited to participate in this prospective study (N=119). After screening by thorough medical history and determination of serum sex steroids, participants were

excluded because of current or previous use of cross-sex hormone therapy (n=23), unwillingness to participate (n=11), pre-existent impaired glucose tolerance (n=7), anorexia (n=2) and other reasons (n=3). In addition, four participants were excluded due to protocol violation and another four trans persons were lost to follow-up. Baseline results were not statistically different when participants who were lost to follow-up were excluded (data not shown). A total number of 40 trans women and 20 trans men were included in the current study.

All trans women younger than 45y (n=29) received cyproterone acetate (CA) 50mg daily (Androcur®; Bayer, Germany) in combination with estradiol valerate (EV) 4mg daily (Progynova®; Bayer, Germany). Patients older than 45y (n=11) received CA 50mg daily in combination with transdermal 17-β estradiol patch 100μg/24h (Dermestril®; Besins, Belgium). In case of non-tolerance due to allergic skin reaction, transdermal 17-β estradiol gel 2mg twice daily (Oestrogel®; Besins, Belgium) (n =1) or EV 4mg was prescribed (n=1). Generally, CA and estrogens were given from start (treatment protocol 1; n=24). Based upon the decision of the mental health professional and patient, some trans women who favoured a slower procedure and/or need an extra diagnostic evaluation received a dual-phase protocol (treatment protocol 2; n=16). In the first phase, sex-specific features were suppressed by administration of CA 50 mg daily for a median duration of 16 weeks whereas in the second phase, estrogens were added to induce feminization.

All trans men received injections with intramuscular T undecanoate 1000mg every 3 months (Nebido®; Bayer, Germany), sometimes preceeded by progestagens to suppress the menstrual cycle. In case of non-tolerance, 2-weekly injections with intramuscular T esters (T decanoate 100 mg, T isocaproate 60 mg, T fenylpropionate 60 mg, T propionate 30 mg/ml) (Sustanon 250®; MSD; Netherlands) was prescribed (n=1).This study complied with the recommendations of The Declaration of Helsinki and was approved by the Ethical Committee of the Ghent University Hospital and the University Hospital of Oslo. All participants gave written informed consent. Clinical trial number: NCT01072825.

MAIN OUTCOME MEASURES

MEDICAL HISTORY AND EXAMINATION

Descriptive data were collected from all individuals including: physical and psychiatric medical history, current and past medication use, familial medical history, and lifestyle factors including smoking and alcohol consumption. Information was compared to data from medical files for accuracy and corrected if necessary.

ANTROPOMETRICS

Standing height was measured to the nearest 0.1 cm using a Harpenden stadiometer (Holtain Ltd, Crymuch, United Kingdom). Body weight was measured in light indoor clothing without shoes to the nearest 0.1 kg. Waist circumference, defined as the smallest abdominal circumference and hip circumference, defined as the largest hip circumference, were determined to the nearest 0.1 cm.

BODY COMPOSITION

Body composition (whole body lean mass, fat mass, trunk fat mass and appendicular fat mass) was determined using dual-energy X-ray absorptiometry (QDR-4500 A, software version 11.2.1; Hologic, Inc., Bedford, MA, USA). We used quantitative computed tomography (XCT-2000, Stratec Medizintechnik, Pforzheim, Germany) to measure cross-sectional muscle area (muscle CSA) and fat area (fat CSA) at dominant forearm and lower leg (66% from distal end). This fat CSA at the forearm and tibia is a measure of subcutaneous fat.

GLUCOSE METABOLISM

After an overnight fast, a two-hour oral glucose (75 g) tolerance test (OGTT) was carried out at baseline and after 12 months of hormonal therapy, with glucose, insulin and triglycerides measured at 0, 30, 60, and 120 minutes. Postprandial hypertriglyceridemia was also found to be an independent cardipovascular risk factor.

To evaluate the degree of glucose tolerance and β-cell function, several indexes derived from either fasting or OGTT-stimulated measurements were used: homeostatic model assessment of insulin resistance (HOMA-IR)[16], Matsuda index[17] and incremental glucose and insulin areas under the curve (AUC glucose/insulin).

BIOCHEMICAL DETERMINATIONS

Fasting venous blood samples were obtained at baseline and 12 months between 08.00 and 10.00h and stored at -80 degrees until batch analysis.

Luteinizing hormone (LH), follicle stimulating hormone (FSH), sex hormone binding globulin (SHBG) and insulin were measured by electrochemiluminiscence immunoassay (ECLIA); Modular, Roche Diagnostics, Mannheim, Germany. The inter-assay CVs were as follows: LH and FSH, 2.19% and 2.55%, respectively; SHBG 2.8%, insulin 2.3%. Estradiol (E2), and testosterone (T) were determined by liquid chromatography tandem mass spectrometry (AB

Sciex 5500 triple-quadrupole mass spectrometer; AB Sciex, Toronto Canada). Serum limit of quantification was 0.3 pg/mL for E2 and 35pmol/L for T , and the interassay CV were 4% at 21 pg/mL for E2 and 6.5% at 3 ng/dL. Radioimmunoassays were used for leptin (Bio-connect diagnostics, Huissen, the Netherlands) and insulin-growth factor 1 (IGF1) (Cisbio diagnostics, Codelet France).

Hemoglobin (Hb), hematocrit (Hct), glucose, creatinin, cholesterol (C), LDL cholesterol (LDL-CH), HDL cholesterol (HDL-C), triglycerides (TG), Apolipoprotein (apo)A1, ApoA2, ApoB, free fatty acids, C-reactive protein (CRP) and uric acid were measured using routine clinical chemistry methods.

STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation or median [1st–3rd quartile] when criteria for normality were not fulfilled. Statistical analyses of categorical variables were carried out using χ^2 and Fisher's exact tests as appropriate. Statistics of paired data were carried out using the paired Student t test and Wilcoxon rank sum tests when variables were not normally distributed. We calculated the differences between year one and baseline, denoted as Δ, for lipids, glucose metabolism parameters, body composition parameters (total body fat, subcutaneous and trunk fat mass). We created a multiple regression model using Δlipids and Δglucose and Δinsulin and Δbody composition as dependent variables and age, Δbody composition and treatment protocol (this only for trans women) as independent variables in multiple linear regression analysis (stepwise). Significance was set at $p<0.05$ (two-tailed). Data were analyzed using SPSS-software, version 21 (SPSS Inc., Chicago, IL). For all analyses, missing values were excluded.

RESULTS

GENERAL CHARACTERISTICS

Table 1 summarizes the general characteristics of the study population and hormone levels at baseline and after 12 months of cross-sex hormone therapy. In trans women, gonadotropins, T, hematocrit and creatinine decreased, whereas E2, leptin and IGF1 increased during anti-androgen with estrogen therapy (all $p<0.01$). Changes were comparable in both treatment protocols (data not shown).

Trans men achieved T levels within the male reference range (321-1005mg/dl) during treatment. Hematocrit and creatinin levels increased whereas E2, prolactin, gonadotropins, SHBG and leptin decreased. IGF1 levels were not influenced by T treatment.

Systolic blood pressure decreased during treatment in trans women, whereas no change was observed in trans men.

TABLE 1. DEMOGRAPHICS AND HORMONE MEASUREMENTS AT BASELINE AND AFTER 12 MONTHS OF HRT

	TRANS WOMEN (N=40)	P	TRANS MEN (N=20)	P
Age at baseline (years)	31.5 ± 12.9	-	25.6 ± 8.0	-
Current smoker at baseline (%)	17.5	-	20.02	-
Alcohol at baseline (U/week)	0 (0-6)	-	0 (0-0)	-
Systolic blood pressure (mmHg)				
Baseline	128.2 ± 14.3	0.020	114.1 ± 12.3	ns
12 months	122.7 ± 12.6		119.5 ± 14.4	
Diastolic blood pressure (mmHg)				
Baseline	77.8 ± 9.7	ns	71.2 ± 11.6	ns
12 months	76.8 ± 9.0		74.2 ± 10.6	
Mean arterial blood pressure				
Baseline	94.6 ± 9.5	ns	85.4 ± 11.5	ns
12 months	91.2 ± 10.5		89.3 ± 10.0	
Hematocrit %				
Baseline	45.5 ± 2.4	<0.001	41.2 ± 3.0	<0.001
12 months	41.6 ± 2.4		45.0 ± 2.8	
Creatinin (mg/dl)				
Baseline	0.94 ± 0.1	<0.001	0.79 ± 0.1	0.03
12 months	0.84 ± 0.1		0.86 ± 0.1	
Testosterone (mg/dl)				
Baseline	543.5 (433.7-676.7)	<0.001	30.0 (19.6-35.8)	<0.001
12 months	12.0 (10.2 -15.5)		647.2 (492.6-	
Estradiol (pg/ml)			795.9)	
Baseline	20.5 (16.7 - 28.6)	<0.001		0.006
12 months	57.5 (40.7 -78.6)		69.3 (24.6-113.2)	
SHBG (nmol/l)			31.2 (23.8- 36.1)	
Baseline	35.2 (25.3-41.8)	0.009		<0.001
12 months	42.4 (27.1-51.4)		74.5 (46.9-86.2)	
LH (U/l)			37.2 (20.2-45.8)	
Baseline	4.5 (3.7- 6.4)	<0.001		0.006
12 months	0.1 (0.1 -0.1)		4.8 (3.7-7.2)	
FSH (U/l)			1.9 (0.1-4.3)	
Baseline	3.6 (2.8-5.8)	<0.001		ns
12 months	0.2 (0.1 -0.4)		4.3 (2.9-6.2)	
Leptin (ng/ml)			4.6 (1.0-6.7)	
Baseline	4.7 (2.8-7.8)	<0.001		<0.001
12 months	11.0 (7.0- 16.0)		9.5 (6.6-14.1)	
IGF1 (ng/ml)			5.3 (2.8-6.5)	
Baseline	224.1 (182.5-307.2)	<0.001		ns
12 months	287.2 (210.1-320.0)		287.4 ± 109.7	
			285.4 ± 83.6	

Data are presented as mean ±S.D or median (first to third quartiles); *P* value: from paired T-test[a] or Wilcoxon signed rank test[b]

ANTHROPOMETRICS AND BODY COMPOSITION (TABLE 2).

In trans women, there was an increase in total body fat mass, trunk fat mass and fat CSA at proximal forearm and tibia. Total body lean mass and muscle CSA at proximal forearm decreased, hip circumference increased and waist-hip ratio decreased during treatment. During T therapy, trans men experienced an increase in whole body lean mass and a decrease in fat percentage, but no changes in total body fat mass, trunk fat or appendicular fat mass were seen. Fat CSA at the tibia decreased whereas muscle CSA at forearm and tibia increased.

TABLE 2. ANTROPOMETRICS AND BODY COMPOSITION AT BASELINE AND AFTER 12 MONTHS OF HRT

	TRANS WOMEN (N=40)	P	TRANS MEN (N=20)	P
BMI (kg/m²)				
Baseline	23.8 ± 4.3	ns	22.6 ± 3.2	0.02
12 months	24.2 ± 4.4		24.6 ± 3.2	
Whole body fat mass (kg)				
Baseline	15.1 ± 6.6	<0.001	16.6 ± 5.6	ns
12 months	18.9 ± 7.0		16.2 ± 5.2	
Whole body percentage fat (%)				
Baseline	19.3 ± 5.5	<0.001	27.1 ± 6.0	0.04
12 months	24.1 ± 5.1		24.1 ± 5.3	
Whole body trunk fat mass (kg)				
Baseline	7.1 ± 3.7	0.001	6.6 ± 2.9	ns
12 months	8.2 ± 3.8		6.4 ± 2.8	
Whole body lean mass (kg)				
Baseline	58.1 ± 9.2	<0.001	41.6 ± 5.2	<0.001
12 months	55.4 ± 8.9		47.5 ± 5.4	
Waist (cm)				
Baseline	83.8 ± 11.3	ns	73.0 ± 9.4	ns
12 months	82.9 ± 11.4		75.0 ± 9.8	
Hip (cm)				
Baseline	95.5 ± 9.1	<0.001	95.9 ± 4.1	ns
12 months	99.6 ± 8.6		95.9 ± 5.8	
Waist-hip ratio				
Baseline	0.88 ± 0.1	<0.001	0.76 ± 0.1	ns
12 months	0.83 ± 0.1		0.78 ± 0.1	
Proximal forearm fat CSA (cm²)				
Baseline	8.6 ± 5.0	<0.001	10.7 ± 3.4	ns
12 months	12.8 ± 5.2		9.7 ± 3.4	
Proximal forearm muscle CSA (cm²)				
Baseline	41.5 ± 8.3	<0.001	28.0 ± 4.1	<0.001
12 months	37.0 ± 8.3		33.0 ± 4.1	
Proximal tibia fat CSA (cm²)				
Baseline	17.3 ± 9.2	<0.001	27.4 ± 9.2	0.02
12 months	22.8 ± 7.7		24.5 ± 9.7	
Proximal tibia muscle CSA (cm²)				
Baseline	78.8 ± 16.4	ns	64.3 ± 12.0	<0.001
12 months	76.7 ± 15.0		72.6 ± 11.1	

Data are presented as mean ±S.D or median (first to third quartiles); P value: from paired T-testa or Wilcoxon signed rank testb; CSA: cross-sectional area

GLUCOSE METABOLISM AND LIPID METABOLISM (TABLE 3)

Serum fasting glucose levels remained unchanged both in trans men and trans women (Table 3). Fasting insulin levels and HOMA-IR increased during anti-androgen with estrogen treatment, and decreased during T therapy in trans men.

TABLE 3. SERUM LIPIDS AT BASELINE AND AFTER 12 MONTHS OF CROSS-SEX HORMONE THERAPY

	TRANS WOMEN (N=40)	P	TRANS MEN (N=20)	P
Total-CH (mg/dl)				
Baseline	183.5 ± 39.1	<0.001	162.5 ± 19.6	0.1
12 months	159.9 ± 30.2		173.3 ± 27.4	
LDL-CH (mg/dl)				
Baseline	115.7 ± 34.4	<0.001	98.8 ± 19.8	0.008
12 months	100.3 ± 27.4		112.5 ± 23.6	
ApoB (mg/dl)				
Baseline	70.6 ± 21.5	0.001	54.8 ± 13.2	0.005
12 months	61.8 ± 16.5		72.0 ± 20.3	
ApoA1 (mg/dl)				
Baseline	137.0 ± 27.7	0.01	129.4 ± 29.1	ns
12 months	125.6 ± 25.4		135.0 ± 24.7	
ApoA2 (mg/dl)				
Baseline	0.30 ± 0.05	0.07	0.3 ± 0.06	ns
12 months	0.28 ± 0.06		0.3 ± 0.06	
HDL-CH (mg/dl)				
Baseline	44.9 ± 10.8	0.01	49.5 ± 11.5	0.01
12 months	41.4 ± 9.8		42.5 ± 6.8	
VLDL-CH (mg/dl)				
Baseline	23.2 ± 16.7	0.02	14.3 ± 3.6	0.06
12 months	19.5 ± 13.4		18.2 ± 9.6	
TG (mg/dl)				
Baseline	87 (58 -121.8)	0.02	65.5 (48.5-77.5)	0.06
12 months	74.5 (54.3 – 90.5)		66.5 (53.3-102.0)	
FFA (µEq/L)				
Baseline	481 (359.8-570.5)	0.05	504 (367.8-605.3)	ns
12 months	627.5 (437.5-729)		430 (333.5-504.3)	
Uric acid (mg/dl)				
Baseline	5.5 ± 1.0	<0.001	3.9 ± 0.6	<0.001
12 months	4.3 ± 0.8		5.1 ± 0.8	
HOMA-IR				
Baseline	0.7 (1.2-2.0)	0.02	1.7 (1.0-3.1)	0.05
12 months	2.0 (1.4-2.9)		1.4 (1.0-2.3)	
Matsuda index				
Baseline	4.4 (3.7-6.6)	ns	4.4 (3.3-6.2)	ns
12 months	3.9 (2.7-6.0)		4.4 (3.2-6.3)	

Data are presented as mean ±S.D or median (first to third quartiles); P value: from paired T-test or Wilcoxon signed rank test; CH: cholesterol; APO: apolipoprotein; LDL: low density lipoprotein; HDL: high density lipoprotein; VLDL: very low density lipoprotein;TG: triglycerides; FFA: free fatty acids; HOMA: homeostatic model assessment

Matsuda index, reflecting whole-body insulin sensitivity, and AUC for insulin and glucose remained unchanged during cross-sex hormone therapy both in trans women and trans men.
In trans women, apart from increasing free fatty acids, all levels of lipids and uric acid lowered during treatment.
In trans men, TG at all time points of the OGTT, LDL-CH, ApoB and uric acid increased, whereas HDL-CH decreased after 1 year of testosterone therapy. CRP remained unchanged in both groups.

ASSOCIATIONS BETWEEN SEX STEROIDS, BODY COMPOSITION, LIPID PROFILE, AGE AND GLUCOSE METABOLISM IN TRANS WOMEN

Age

Older age was significantly associated with a greater decrease in LDL-CH and total-CH and increased AUC of insulin after 12 months (respectively p=0.003, βsd=-0.461 and p=0.001, βsd=-0.514 and p=0.003, βsd=0.410, p=0.003, βsd=0.486) and a less increased fat mass and percentage fat, and less decreased lean body mass percentage during treatment (respectively p=0.028, βsd=-0.348, p=0.004, βsd=-0.442 and p=0.003, βsd=0.458).

Type of hormonal treatment

No major differences were found between Δlipids, Δglucose metabolism parameters and Δbody composition parameters and oral or transdermal estrogen treatment (data not shown).

Changes were comparable in both treatment protocols but participants in treatment protocol 1 showed a tendency towards greater increase in total body fat mass (p=0.05), trunk fat mass (p=0.09), appendicular fat mass (p=0.03), hip circumference (p=0.08) and proximal forearm fat (p=0.06).

Changes in total-CH, LDL-CH, VLDL-CH, HDL-CH were comparable in trans women receiving treatment protocol 1 or 2 (data not shown), but ApoA1, A2 and ApoB were more markedly decreased in treatment protocol 2 vs. 1, which remained after adjustment for total fat mass and age.

No differences were observed in glucose metabolism changes in trans women receiving treatment protocol 1 or 2 (data not shown).

Fat mass

A greater increase in total body fat mass and trunk fat mass (but not subcutaneous fat mass) was associated with increased AUC of insulin (both $p=0.001$, $\beta sd=0.561$ and $p=0.004$, $\beta sd=0.458$), decreased Matsuda index (both $p=0.030$, $\beta sd=-0.574$ and $p=0.016$, $\beta sd=-0.399$) and increased leptin (both $p<0.001$; $\beta sd=0.492$ and $p<0.001$, $\beta sd=0.477$), increased ApoA2 (both $p=0.001$, $\beta sd=0.499$ and $p<0.001$, $\beta sd=0.528$) and ApoB (both $p=0.001$, $\beta sd=0.524$ and $p<0.001$, $\beta sd=0.537$).

The ratio of trunk fat over appendicular fat (as measured by DXA) was positively associated with changes in fasting TG, VLDL-CH, ApoA2, ApoB and uric acid and negatively with HDL-CH (data not shown).

Sex steroids

After adjustment for age, no associations were found between sex steroid changes (E2, T and SHBG) and changes in lipids, glucose metabolism and body composition.

ASSOCIATIONS BETWEEN SEX STEROIDS, BODY COMPOSITION, LIPID PROFILE, AGE AND GLUCOSE METABOLISM IN TRANS MEN

Age

No associations were found between age and changes in lipids, glucose metabolism and body composition in trans men (data not shown).

Fat mass

No associations were found between measures of body composition and changes in glucose metabolism in trans men (data not shown). A smaller decrease in all measures of fat mass (total body fat, trunk fat as well as subcutaneous fat mass) was associated with a greater increase in ApoB changes during testosterone therapy (after adjustment for age: all $p<0.05$ and $\beta sd>0.473$). Changes in trunk fat mass were negatively associated with ΔHDL-CH ($p=0.035$, $\beta sd=-0.486$) and positively with Δleptin ($p=0.036$, $\beta sd=-0.483$), which remained after correction for age.

Sex steroids

No associations were found between sex steroid changes (E2, T and LH) and changes in lipids, glucose metabolism and body composition.

DISCUSSION

The present study investigated possible associations between sex steroid levels and cardiometabolic factors and explored changes in cardiovascular risk profile during cross-sex hormone therapy in trans persons. Our results demonstrate that CSH has important effects on body composition, glucose metabolism and lipid profile. In trans women, anti-androgen and estrogen therapy increased in insulin resistance and generally improved lipid profile, whereas T therapy showed opposite effects in trans men.

Our observation that insulin resistance increased during treatment in trans women corroborates with previous studies using high dose CA and EE[10, 12]. Cross-sex hormone therapy in trans women induces a decrease in lean mass and an increase in fat mass, which is hypothesized to deteriorate glucose metabolism. Some[18, 19] but not all[20] studies also demonstrated that sex steroids may exert direct effects in the absence of any detectable changes in body composition. Our results generally support the first hypothesis, as we found that changes in total body fat mass and trunk fat mass (but not subcutaneous fat mass) were associated with these less favourable changes in insulin sensitivity, whereas no associations were found with serum sex steroid levels. However, the variable timing of sampling in relation to the last dosing may have obscured possible associations.

Similar as for glucose metabolism, less favourable changes in lipid profile such as increased ApoB and TG and decreased HDL-CH concentrations were not related to individual sex steroid levels but were associated with changes in trunk fat or the ratio of trunk fat over appendicular fat in both trans men and women. This again underscores the importance of body composition changes during cross-sex hormone therapy.

In line with Dittrich and colleagues[21], significant differences were observed compared to previously published studies using EE plus CA[10] as we observed no increase in body weight and TG in trans women. These discrepancies may be related to differences in type of estrogen as it has been shown that EE has a stronger hepatic impact due to its 17α-ethinyl group that prevents the inactivation of the molecule and results in a slower metabolism[22, 23]. In addition, the higher estrogen dosage used in the study by Elbers and colleagues[10] may also contribute to these previous findings.

In trans men, our observations that T treatment decreased insulin resistance in trans men are in contrast with previous reports showing no change[10] or increase[12] in insulin resistance. The beneficial changes in body composition in this study with an increase in percentage lean mass, a

decrease in percentage body fat and no change in trunk fat mass and waist circumference, may be an explanation for our findings, even though we were unable to show such a significant association. In contrast, others showed an increase in visceral fat mass during the first 12 months of T therapy[10] and a larger waist circumference compared to natal females after long-term T therapy (mean 9.9 years)[24]. The different type and dosage of T administered may give an explanation. Short-acting intramuscular T esters are known to induce more sub- and supraphysiological T levels compared to long-acting intramuscular T undecanoate[25]. In young healthy men, changes in T concentrations were found to be associated with dose-dependent and region-specific changes in total body lean and fat mass[26]. Future randomized controlled trials are needed to investigate this hypothesis.

Trans men developed a less favourable lipid profile with higher fasting TG, LDL-CH and ApoB and lower HDL-CH levels. These results largely confirm those from Mueller and colleagues[27] who also observed an increase in TG's and decrease in HDL-CH levels. The observed increase in LDL-CH levels in our study are in contrast with other studies in trans men showing no change[9, 28] or decrease[10, 29]. However, studies in women receiving T therapy mainly showed an increase or no change in LDL-CH[7, 30- 31]. Similar as in trans women, changes in body composition were also associated with the lipid profile alterations. Although T therapy induced a lower fat percentage overall, trans men that had less decreases in fat mass, had higher levels of ApoB and lower levels of HDL-CH after 12 months of T therapy. ApoB is present as a single molecule in LDL, IDL and VLDL lipoproteins and a growing body of evidence has now demonstrated the superiority of apoB measurement over that of LDL cholesterol for assessment of CVD risk[32].

From our study we can derive several clinical implications. Firstly, in comparison with older treatment modalities in trans women and short-acting intramuscular T esters in trans men, it seems that the current treatment modalities displayed a more favourable cardiovascular risk profile: we observed no increase in TG and total body weight in trans women and an reduction of insulin resistance in trans men. The question remains whether these more favourable effects on cardiovascular risk profile will also be translated into a lower risk for cardiovascular disease during long-term follow-up.

Secondly, we found that a higher body fat mass was associated with less favourable cardiometabolic factors in both trans women and men. Therefore, avoiding obesity is also important to improve the cardiometabolic risk profile of trans persons, and weight management should be a part of the entire endocrine treatment plan.

Thirdly, older age at treatment initiation was associated with more detrimental changes in glucose metabolism in trans women despite more modest changes in body composition, and there should be a low threshold to screen for impaired glucose tolerance in older trans persons.

The strengths of the present study are our detailed descriptions of lipid profile, the use of pQCT to measure subcutaneous fat mass, the use of increasingly used state-of-the-art treatment modalities and the use of mass spectrometry to measure serum sex steroid levels. Our study is hampered by a relatively small sample size, although we achieved a larger sample size in comparison with most other prospective studies. Recruitment is continuously ongoing. Finally, our comparisons between both treatment modalities in trans women are hampered by the age difference between the two groups, which is inherent to the treatment protocol.

In conclusion, we showed that cross-sex hormone therapy in trans women increased insulin resistance and generally improved lipid profile, whereas trans men experienced less insulin resistance but a worsening of lipid profile. Adiposity was associated with a less favourable outcome in both trans women and men. These findings suggest that the between-sex variation in cardiovascular risk is mainly due to effects of sex steroid exposition on body composition.

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2.3 SHORT AND LONG-TERM DERMATOLOGICAL EFFECTS OF T TREATMENT IN TRANS MEN.

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ABSTRACT

INTRODUCTION

Our knowledge concerning the effects of testosterone (T) therapy on the skin of trans men (female-to-male transsexuals) is scarce.

AIM

To evaluate the short and long-term clinical effects of T treatment on the skin of trans men.

METHODS

We conducted a prospective intervention study in 20 hormone naïve trans men and a cross-sectional study in 50 trans men with an average of 10 years on T therapy.

MAIN OUTCOME MEASURES

Acne lesions were assessed using the Gradual Acne Grading Scale, hair patterns using the Ferriman and Gallwey classification (F&G), and androgenetic alopecia using the Norwood Hamilton Scale.

RESULTS

T treatment increased facial and body hair growth. The F&G score increased progressively from a median value of 0.5 at baseline to a value of 12 after 12 months of T administration. After long-term T treatment, all but one transman achieved an F&G score indicative of hirsutism in women, with a median value of 24. Only one transman acquired mild fronto-temporal hair loss during the first year of T treatment, whereas 32.7% of trans men had mild frontotemporal hair loss and 31% had moderate to severe androgenetic alopecia after long-term T therapy. The presence and severity of acne increased during the first year of T therapy, and peaked at 6 months. After long-term T treatment, most participants had no or mild acne lesions (93.9%). Dermatological outcome was not demonstrably related to individual serum T or dihydrotestosterone levels.

CONCLUSIONS

T treatment increased facial and body hair in a time-dependent manner. The prevalence and severity of acne in the majority of trans men peaked 6 months after beginning T therapy. Severe skin problems were absent after short- and long-term T treatment.

INTRODUCTION

Androgens and estrogens are known to affect the skin pilosebaceous unit (PSU), and both androgen and estrogen receptors are expressed in the sebocytes and hair follicle dermal papilla[1-2]. The biological action of testosterone (T) on peripheral tissues, such as the scalp, is in part affected by its local conversion to dihydrotestosterone (DHT) by 5α-reductase (type 1 and 2). T can also be converted to estradiol (E2) by the aromatase enzyme, which is also prominently present in the PSU[3]. Androgens are required for sexual hair and sebaceous gland development and play a central role in stimulation of sebaceous gland growth and differentiation[4, 5]. In addition, androgens have been shown to increase the size of the hair follicle, the diameter of the hair, and the proportion of time that terminal hairs spend in the anagen phase[4-5, 6]. The effects of E2 on the PSU unit are less understood but estrogens are known to play an important role in human hair growth control[7]. Furthermore, given that T is aromatized into E2 in many tissues, it may be possible that some effects of T on the PSU unit are mediated by E2. Alternatively, the local balance between E2 and androgens may determine local E2 and androgen action[7-8]. Androgen excess in women is associated with important dermatological effects, such as acne vulgaris, hirsutism, and androgenetic alopecia[9-11], with potentially important psychological disturbing effects[12]. However, androgens are not the sole contributors in to the pathogenesis of these disorders[4, 13], and wide inter-individual variability in androgen effects has been described. Susceptible persons may experience these pathologies with normal female androgen levels whereas others experience no skin problems with markedly elevated androgen levels[4, 13-14]. Female-to-male transsexual persons (herein referred to as trans men) receive T treatment to induce virilization and suppress menstruation[15]. Given the important effects of sex steroids on sebum production and distribution as well as on the growth of body and scalp hair, dermatological changes during cross-sex hormone treatment are important to address. However, to our knowledge, only one study has previously investigated the effects of T treatment on the skin in trans men. Giltay and colleagues[16] examined the changes of hair growth and sebum production during the first year of cross-sex hormone therapy in 17 trans men, all treated with intramuscular T esters every 14 days. However, the short-term dermatological effects of T undecanoate, a long-acting depot preparation administered every 3 months, has not been previously addressed in trans men. In addition, long-term dermatological outcomes of T treatment have not yet been described in this patient population.

The aim of the current study is to investigate the short- and long-term dermatological effects of T treatment in a relatively large group of trans men.

METHODS

Study population and study procedures

All trans participants were diagnosed with Gender Identity Disorder (Diagnostic and Statistical Manual of Mental Disorders- III-R and DSM-IV, 302.85) and were treated at the center for Sexology and Gender Problems at the Ghent University Hospital (Ghent, Belgium). Two different studies were performed.

Prospective intervention study

Twenty Caucasian trans men before start of cross-sex hormone therapy and sex reassignment surgery were included in this study. All men received intramuscular T undecanoate (1000 mg) (Nebido®) every 3 months. Patients were followed and monitored every 3 months during the first treatment year (Clinical trial number: NCT01072825).

Cross-sectional study

This study included 50 trans men who underwent sex reassignment surgery (SRS), including hystero-oophorectomy and mastectomy. On average, participants were 8.7 years after SRS (range, 9 months to 22 years). All started hormonal therapy at least 2 years before SRS. The majority of participants were Belgians (n=48), with one Dutch subject and one Iranian subject. Detailed descriptions of this study population can be found elsewhere[17-18].

Trans men had been using T treatment for an average of 9.9 years, (range, 3.2 to 27.5 years). Current cross-sex hormonal therapy consisted of intramuscular T treatment with either a mixture of T esters (T decanoate 100 mg, T isocaproate 60 mg, T fenylpropionate 60 mg, and T propionate 30 mg/ml) every 2 or 3 weeks (n=35), T undecanoate (1000 mg) every 12 weeks (n=7), or transdermal T (50 mg) daily (n=8). One participant used both oral T undecanoate (40 mg, once daily) and T gel (50 mg per 5 g, 50 mg daily). All trans men had physiological male T levels. Exclusion criteria for both studies included treatments or disorders affecting sex hormone status: untreated hypo- or hyperthyroidism, Cushing syndrome, alcohol abuse, mucoviscidosis, malabsorption, cirrhosis, chronic kidney failure, or current (<2 years) or prolonged use of corticosteroids, anabolic steroids, and anti-androgens. Both studies complied with the recommendations of the Declaration of Helsinki and were approved by the Ethics Committee of the Ghent University Hospital. All participants gave their written informed consent.

MAIN OUTCOME MEASURES

MEDICAL HISTORY

In both studies, a self-constructed questionnaire was completed with questions pertaining to medical history, current and past hormonal treatment, medication use, and dermatological history.

DEGREE OF HAIR GROWTH

The degree of hair growth was subjectively assessed according to the modified Ferriman and Gallwey method (F&G)[19] in which nine sites (lip, chin, chest, upper back, sacroiliac region, upper abdomen, lower abdomen, arm, and medial thigh) are graded according to the following: 0=none, 1=slight, 2=moderate, 3=dense, and 4=very dense. The F&G score has a minimum value of 0 and a maximum of 36. Only terminal hair growth is considered in the scoring. A score of greater than 8 in an androgen-dependent area was considered indicative of hirsutism. Androgenetic alopecia was assessed using the Norwood/Hamilton classification[20].

SATISFACTION MALE HAIR PATTERN

Satisfaction with the male hair pattern was assessed using a five point Likert scale from very unsatisfied to very satisfied.

ACNE EVALUATION

Clinical assessments of current acne lesions were performed on the face and back of subjects according to the Global Acne Grading Scale (GAGS)[21]. The GAGS considers six locations on the face and chest/upper back, with a factor for each location based roughly on surface area, distribution, and density of pilosebaceous units. The borders on the face are delineated by the hairline, jaw line, and ears. No magnifying glass or skin stretching is allowed and good lighting is taken into account. The chest and upper back have been included because they are critical in order to assess the severity of the acne. Each of the six locations is graded separately on a 0-4 scale, with the most severe lesion within a location determining the local score. These grading scores are then multiplied by the factor of each location (forehead ×2, right cheek ×2, left cheek ×2, nose ×1, chin ×1, chest and upper back ×3). The global score is the summation of all the local scores (=grades × factors). The global scores are subdivided into categories: no active acne lesions (score=0), mild active acne lesions (score=1-18), moderate active acne lesions (score=19-30), severe active acne lesions (score=31-38), and very severe acne lesions (score>39).

SEBUM PRODUCTION AND ACNE SCAR EVALUATION

In the cross-sectional study, we additionally evaluated sebum production using the Dual Tape Sebum Test Kit, Cortex Technology (Cuderm Corporation, www.cortex.dk) 1 hour after thorough alcohol cleansing. The Sebutape technique provides information about sebaceous gland function expressed in a score ranging from 1 to 10: low production of sebum (values 1, 2, or 3), normal production of sebum (values 4, 5, 6 or 7) and high production of sebum (values 8, 9, or 10). The area in the midline of the forehead was tested because this is an area where sebaceous glands are the largest and most numerous. In this study, we also assessed acne scarring on the face of subjects according to the Échelle d'evaluation Clinique des cicatrices d'acné (ECCA) grading scale (min score=0; max score=540)[22].

BIOCHEMICAL DETERMINATIONS

In all subjects, venous blood samples were obtained between 0800 and 1000 h. Using these blood samples, hematocrit (Sysmex-XE-2100 Hematology Analyzer, Goffin Meyvis, Etten-Leur, Netherlands), creatinine, and total cholesterol (Cobas C501 + Modular, Roche Diagnostics, Mannheim, Germany) were determined in the serum. Commercial immunoassay kits were used to determine serum concentrations of sex hormone-binding globulin (SHBG) (Orion Diagnostica, Espoo, Finland), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (Modular; Roche Diagnostics, Mannheim, Germany). Baseline T levels in hormone naïve trans men were determined by using liquid chromatography-mass spectrometry (LC-MS; AB Sciex 5500 triple-quadrupole mass spectrometer, AB Sciex, Toronto, Canada). T levels within the male range were determined using immunoassay kits (Modular; Roche Diagnostics, Mannheim, Germany). DHT was measured by LC-MS after paper chromatography. The intra- and interassay coefficients of variation for all assays were ≤ 10%.

STATISTICAL ANALYSES

The normal distribution of all variables was tested using the Kolmogorov-Smirnov one-sample test. Variables with a normal distribution were described in terms of the mean and standard deviation or in the case of non-normal distribution, the median [first to third quartiles]. Correlations between normally distributed parameters were calculated using Pearson's correlation coefficient and Spearman's correlation coefficient. Analysis of variance (ANOVA) tests for repeated measurements were used to explore dermatological changes during the first year of T treatment. We used multiple regression analyses with absolute changes in dermatological variables as the dependent variable and absolute changes in endocrine measurements as the independent

variable to investigate associations of changes in endocrine measurements with dermatological changes. PASW 21.0 software package (SPSS Inc., Chicago) was used for all analyses. A *P* value <0.05 was considered statistically significant all *P* values were two-tailed.

RESULTS

The general characteristics of the study populations are summarized in Table 1.

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY POPULATION

	PROSPECTIVE STUDY BASELINE VISIT (N=20)	CROSS-SECTIONAL STUDY (N=50)
Age (years)	26.6 ± 8.3	37 ± 8.2
Age at SRS (years)	–	30 ± 8.2
Height (cm)	163.7 ± 4.7	165 ± 6.7
Weight (kg)	63.9 ± 14.0	67.5 ± 11.5
BMI (kg/m²)	23.7 ± 4.5	24.8 ± 3.8
Use of hormone therapy (%)	100	100
Current smoking (%)	25.0	28
T (ng/dl)	30.3 (20.6 – 46.5)	631.1 (466.0 – 1019.2)
DHT (ng/dl)	13.1 (9.1–18.7)	64.5 (46.3 – 117.3)
Estradiol (pg/ml)	88.3 (44.6 – 140)	34.4 (24.7 – 49.7)
SHBG (nmol/l)	67.7 (17.8 – 84.5)	30.1 (22.5 – 38.5)
LH (U/l)	5 (3.7 – 8.1)	3.7 (0.2 – 28.5)
Creatinin (mg/dl)	0.8 (0.7 – 0.8)	0.9 (0.9 – 1.0)
Hematocrit (%)	41.6 ± 3.0	48.8 ± 2.8
Total cholesterol (mg/dl)	172.2 ± 29.1	207 ± 35

Data are presented as mean ±S.D or median (first to third quartiles) in case of non-Gaussian distribution.

HAIR GROWTH AND HAIR DISTRIBUTION

Prospective study

T treatment increased facial and body hair growth in all subjects. The F&G score increased progressively from a median of 0.5 at baseline to 12.0 after 12 months of T administration (Table 2.) After 6 months of T treatment, more than half of the participants (53.8%) had scores >8, which indicates hirsutism in women. After 12 months, the majority of trans men (80.0%) had scores >8 (Figure 1). Wide inter-individual variability was observed as the absolute increase in F&G scores ranged from 2 to 25.

Cross-sectional study

All but one participant (n=49) acquired an F&G score >8, with a median score of 24 (range: 6-34) (Figure 1). The subject with an F&G score of 6 had been on T therapy for 43 months. Satisfaction with the obtained male hair pattern was positively associated with the F&G score (r=0.49, *P*<0.001). No associations were observed between duration of T therapy, and type of T therapy, and F&G score (data not shown).

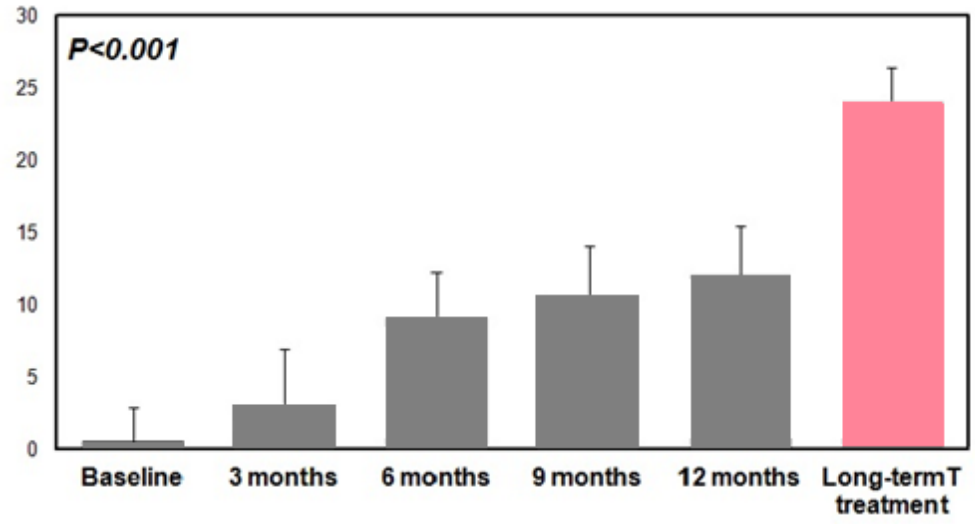

Figure 1. Ferriman and Gallwey scores during T treatment

Data are presented as the median F&G score; error bars represent 95% confidence intervals (CI). Long-term T treatment represents median F&G scores from the cross-sectional study. *P* value results from ANOVA repeated measures analyses

EVALUATION OF ANDROGENETIC ALOPECIA

Prospective study

One subject developed a mild frontotemporal hair loss during the first year of T treatment (Norwood/Hamilton score II).

Cross-sectional study

Eighteen participants (36.7%) experienced no scalp hair loss (Norwood/Hamilton score I), whereas 32.7% (n=16) had mild frontotemporal hair loss (Norwood/Hamilton score II). Fifteen participants (31%) had moderate to severe alopecia (Norwood/Hamilton score ≥ III). A significant correlation between alopecia and age was observed (r=0.31, P=0.03). No associations were observed between duration of T therapy, and type of T therapy, and alopecia (data not shown).

ACNE EVALUATION

Prospective study

At baseline, 35% of the subjects had facial acne and 15% had acne on the back and/or chest. After 6 months of androgen administration, facial acne was present in 82.4% of subjects and in 88.2% of subjects on the back and/or chest. After 12 months of androgen therapy, facial acne was still present in 55% of participants and 50% of participants on the back and/or chest. The presence of acne, as well as the severity increased during T administration (Figure 2). However, during our observations, the majority of trans men had mild acne lesions, 20% had moderate acne lesions, and none had severe or very severe acne lesions according to the GAGS.

During the first year of androgen treatment, 50% of trans men used one or more topical product [benzoyl peroxide (5% gel) (n=6), adapalene (0.1%) and benzoyl peroxide (2.5%) gel (n=6), or over the counter topical products (n=4)]. Three subjects used oral antibiotics during the course of the study to treat acne.

Cross-sectional study

Participants had a median total GAGS of 3.0 (range 0-24) (Figure 2C). According to this scale, the majority (63.3%) had mild acne lesions. Three participants (6%) had moderate acne scores, and 15 participants (30.6%) had no active acne lesions. Younger participants had more acne lesions (P=0.05). The majority of participants (65%) had no acne scars, according to the ECCA grading scale. The mean score was 12.55 (SD: 25.4; range 0-115).

SEBUM PRODUCTION EVALUATION IN THE CROSS-SECTIONAL STUDY

Sebum production was performed using the Sebutape technique on the forehead in 40 trans men. A median value of 2.0 was observed with a range from 1 to 9. Twenty-five participants (62.5%) had a low sebum production one hour after alcohol cleansing; however, up to 95% of all participants (n=38) had low to normal sebum production (value 1 to 6). Only two subjects showed elevated levels of sebum production. No significant associations were found between sebum production and acne lesions, duration of hormonal therapy, and type of hormonal therapy (data not shown).

ASSOCIATIONS BETWEEN DERMATOLOGICAL OUTCOME AND SEX STEROIDS

Prospective study

No associations were observed between absolute changes in F&G scores, male pattern baldness, acne lesions, and absolute changes in LH, T, DHT, E2, or SHBG (data not shown).

Cross-sectional study

No significant associations were found between F&G scores, male pattern baldness, sebum production, acne lesions, and LH, T, DHT, E2, or SHBG apart from a positive between F&G scores and E2 (linear regression; P=0.03).

CONCLUSIONS

This study investigated the short- and long-term dermatological effects of T treatment in trans men. We observed that the majority of trans men acquired an F&G score indicative of hirsutism in biological women during the first year of T therapy. These findings corroborate those from Giltay and colleagues[16]. As expected, long-term T administration further increased F&G scores. All but one participant achieved an F&G score >8, with wide inter-individual variability. Our results therefore suggest that substantial changes in hair growth and hair distribution occur during the first year of T treatment. However, slower but important increases in hair growth can be expected after. Moreover, the further increase in F&G scores contributes to increased patient satisfaction because it was strongly associated with the obtained male hair pattern.

With respect to the dermatological side effects of androgen therapy, we observed that the presence and severity of acne lesions increased during T treatment. However, most men developed only mild acne and none suffered from severe or very severe acne according to the Global Acne Grading Scale. However, the fact that half our participants began using topical agents or oral antibiotics during our study period shows that, although mild and moderate, these acne lesions are clinically significant.

Similar to the observation of Giltay and colleagues[16], we found that most acne lesions occurred within the first 6 to 9 months of treatment, with a decrease at 12 months. These results may be related to the acne treatment used by several of our patients. Alternatively, it may indicate that the initial increase in sebum production and the associated acne lesions resulting from male T levels in biological females attenuate over time. Indeed, our long-term observations also show markedly lower median acne scores compared to scores during the first year of treatment. Furthermore,

after long-term T therapy, the vast majority of trans men had no or mild acne scars, suggesting that the long-term dermatological side effects related to acne are relatively rare in this specific patient population.

It was also found that the risk of developing androgenetic alopecia was low during the first year of T administration. However, as expected, with longer T exposure, the prevalence of androgenetic alopecia increased. The prevalence of moderate or severe androgenetic alopecia after a mean T treatment period of 10 years in trans men between the ages of 18 and 50 (25%) was significantly lower compared to the general population, in which approximately 42% of males aged between 18 and 50 years suffer from this condition[23]. It is likely that shorter exposure times of T may explain these observations and that further increases can be expected with longer durations of T administration. Another possible explanation might be that the age at which T exposure is commenced may play a significant role in the pathogenesis of androgenetic alopecia. Alternatively, because aromatase levels in the frontal hair follicles from women were found to be approximately six times higher than those in males, it is possible that natal women are less prone to develop androgenetic alopecia due to differences in local T and E2 ratios[24]. However, whether this is the case in trans men after long-term T treatment remains unknown.

As expected, we observed no associations between serum T or DHT and dermatological outcome considering the single-point measurements of T in these men, the differences in types of T administration, and the variable timing of sampling in relation to the last T dosing in our cross-sectional study. Furthermore, most studies have described a wide inter-individual variability and observed only weak or no association between T and DHT levels and acne or hirsutism scores[14, 16]. The variability in PSU responsiveness to androgens may be related in part to variations in androgen metabolism; for example, variations in 5α reductase activity, 3β-hydroxysteroid dehydrogenase activity, aromatase activity, or differences in androgen receptor sensitivity[14-25].

Interestingly, a positive relationship was observed between E2 and F&G score in our cross-sectional study. One explanation could be that given the variability of T levels due to single-point measurements of T, the differences in the types of T administration and the variable timing of sampling in relation to the last T dosing, E2 may be a more stable marker of T exposure in trans men.

Some limitations of our study need to be addressed. First, we used the modified F&G method to evaluate hair growth and hair distribution. This method has some limitations, such as its

subjective nature, the failure to account for a focally high score, and the lack of consideration of other androgen sensitive areas (for example, sideburns and buttocks)[26]. Objective tools are available to examine hair growth and distribution, such as determining the density of terminal hairs by direct counting[27] or photography[28]. However, these techniques are predominantly useful for assessing hair growth rates and the extent of terminal hair density in a specific body area. They are considered much less useful to assess the extent of total body hair or face terminal hair density[29], which was the main aim of our study.

Secondly, the cross-sectional design of our long-term follow-up study implies no causal relationships can be drawn. A long-term prospective study is preferable. Nevertheless, because this is the first study addressing long-term dermatological outcomes in trans men, our findings add valuable information.

In conclusion, T treatment time-dependently increased facial and body hair. The prevalence and severity of acne in the majority of trans men peaked at 6 months after beginning T therapy. However, severe skin problems were absent after short and long-term T treatment. Dermatological outcome was not demonstrably related to individual serum T or DHT. The observed significant association with E2 remains to be established.

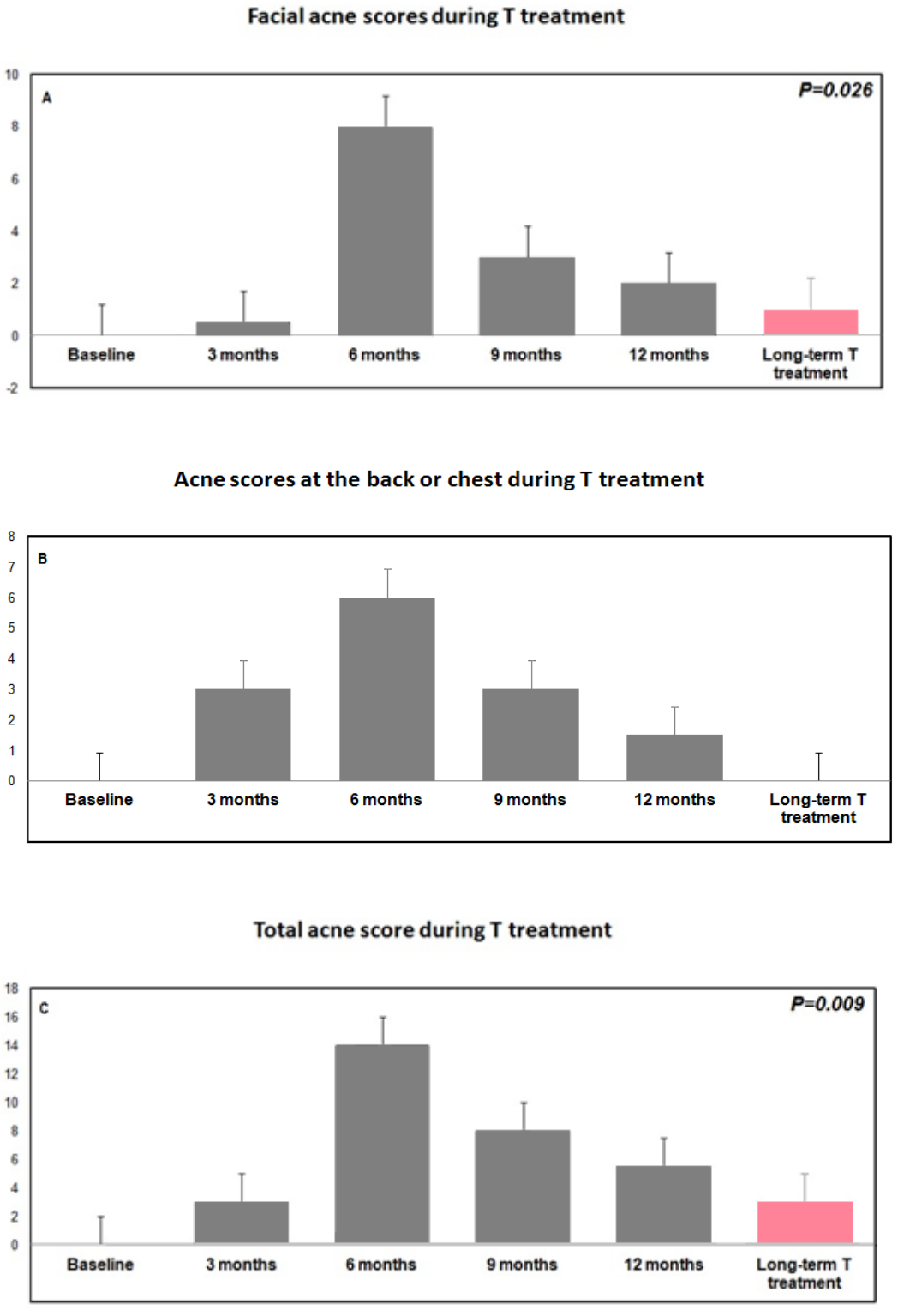

Figure 2. Acne scores during T treatment

Data are presented as the median score; error bars represent standard error. Long-term T treatment represents the median score from the cross-sectional study. *P* value results from ANOVA repeated measures analyses.

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2.4 A LONG-TERM EVALUATION OF CROSS-SEX HORMONE TREATMENT IN TRANSSEXUAL PERSONS.

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ABSTRACT

INTRODUCTION

Long-term effects and side effects of cross-sex hormone treatment in transsexual persons are not well-known.

AIM

The aim of this study is to describe the effects and side effects of cross-sex hormone therapy in both transsexual men and women.

MAIN OUTCOME MEASURES

Hormone levels were measured by immunoassays. Physical health was assessed by physical examination and questionnaires on general health and specific side effects, areal bone parameters by dual energy X-ray absorptiometry.

METHODS

Single center cross-sectional study in 100 transsexual persons post-sex reassignment surgery and on average 10 years on cross-sex hormone therapy.

RESULTS

Transsexual men did not experience important side effects such as cardiovascular events, hormone-related cancers, or osteoporosis. In contrast, a quarter of the transsexual women had osteoporosis at the lumbar spine and radius. Moreover, 6% of transsexual women experienced a thromboembolic event and another 6% experienced other cardiovascular problems after on average 11.3 hormone treatment years. None of the transsexual women experienced a hormone-related cancer during treatment.

CONCLUSION

Cross-sex hormone treatment appears to be safe in transsexual men. On the other hand, a substantial number of transsexual women suffered from osteoporosis at the lumbar spine and distal arm. Twelve percent of transsexual women experienced thromboembolic and/or other cardiovascular events during hormone treatment, possibly related to older age, estrogen treatment, and lifestyle factors. In order to decrease cardiovascular morbidity, more attention should be paid to decrease cardiovascular risk factors during hormone therapy management.

INTRODUCTION

The current treatment regimens for transsexual persons usually involve hormonal therapy as well as sex reassignment surgery (SRS). In 1986, at the start of our multidisciplinary team, a dual-phase hormonal schedule was used. First, during the reversible part, sex specific features were suppressed using cyproterone acetate 50–100 mg, together with starting the real-life test. Cyproterone acetate is a synthetic derivative of 17-hydroxyprogesterone and acts primarily as an androgen receptor antagonist. It has also a progestational and weak glucocorticoid activity that inhibits luteinizing hormone (LH) releasing and in turn reduces testosterone levels. After 6 months up to 1 year treatment with cyproterone acetate, cross-sex hormones were added[1]. Recently, we changed our hormonal protocol, and we now prescribe antiandrogens (mostly cyproterone acetate 50 mg) and estrogens simultaneously to the majority of transsexual women (male-to-female transsexual persons). However, some transsexual women favour a slower procedure, and they receive the dual-phase hormonal protocol. Also, the type and doses of estrogens have changed during the past years. Currently, at the start of cross-sex hormone treatment, ethinyl estradiol and conjugated estrogens are rarely prescribed, and below the age of 40, estradiol valerate 4 mg daily is now recommended. After the age of 40, transdermal estrogens (17β estradiol gel 2 mg daily or 17β estradiol patch 100mg twice a week) is usually recommended. In transsexual men (female-to-male transsexual persons), testosterone administration has been and is currently started after suppression of the menstruation by a progestin.The goals of hormonal treatment are to induce the development of the secondary characteristics of the new sex and to diminish those of the natal sex[2]. Consequently, transsexual men are treated with testosterone to induce virilization, which includes the development of a male hair growth pattern and body composition, cessation of menses, a deepening of the voice, and clitoral enlargement. To obtain feminization, transsexual women at our center receive a combination of antiandrogen therapy and estrogen therapy. Feminization consists of breast formation, reduction of masculine hair growth, and a more female fat distribution.

A number of studies demonstrated the efficacy of several hormonal preparations to induce masculinization and feminization in transsexual persons[3–6]. After SRS, which usually involves gonadectomy, hormone treatment is continued life-long to maintain virilization and feminization in transsexual men and women, respectively, and to avoid signs or symptoms of hormone deficiency. Follow-up data on the long-term effects and side effects of hormone treatment on physical health are still scarce in this specific population[7,8] and, as no randomized controlled trials are available, the optimal formulations and dosages of cross-sex hormone treatment are unknown at present. Current treatment modalities for hormonal replacement therapy are similar

to those of hypogonadal persons and aim at hormone values in the normal physiological range[2]. Sustained supraphysiological levels of both testosterone and estrogen increase the risk for serious adverse reactions such as thrombosis, whereas subphysiological levels may induce the effects known from hypogonadal states. Although based on limited evidence, a serum concentration of LH within the normal range may be a reliable marker of adequate dosing[8,9]. In this study, we will describe the long-term effects and side effects of hormonal therapy in a relatively large number of transsexual men and women.

METHODS

STUDY POPULATION AND STUDY PROCEDURES

We performed two independent studies. First, in 2007, all Dutch-speaking transsexual women who underwent SRS at least 6 months before recruitment and who consulted a member of the gender team for treatment or follow-up during 2006 were invited by mail (N = 70).We had no further inclusion criteria, and we included 50 transsexual women in our study. The others did not respond (N = 17) or declined to participate because they wished not to be reminded of their past (N = 3)[9,10]. Second, in 2010, all Dutch-speaking transsexual men who underwent SRS between 1987 and 2009 at our hospital (N = 79) received a written invitation in which they were asked to confirm their participation by telephone or electronic mail. Fifty individuals agreed to participate[11–13]. Two participants could not be reached because of change of address. The others were not willing to participate.

All transsexual women underwent SRS (orchidectomy and phallectomy in combination with vaginoplasty) at least 6 months before recruitment and after at least 2 years of hormonal treatment. All but two women received breast augmentation. Before SRS, hormonal therapy had been initiated using antiandrogen therapy (cyproterone acetate 50–100 mg/day) up to a maximum of 1 year, followed by the addition of exogenous estrogen administration (different formulations). Post-SRS, all but three participants received estrogen treatment. On average, transsexual women were 6.3 years after SRS, with a minimum of 6 months and a maximum of 32.2 years. All transsexual men except for one underwent ablative SRS (hystero-oophorectomy and mastectomy) at least 2 years before inclusion in this study. Almost all (N = 46) participants underwent phalloplasty, eight of whom had a previous metaidoioplasty, one person had metaidoioplasty alone, and three participants expressed no wish for further genital surgery. All started testosterone therapy at least 2 years before SRS. On average, participants were 8.7 years after SRS, with a minimum of 9 months and a maximum of 22 years.

STUDY PROCEDURES

Transsexual women received questionnaires on medical history, dermatological features, changes in voice, quality of life, sexual functioning, surgical results, and psychological functioning during their hospital visit. They completed their study protocol between March and June 2007.

Transsexual men who agreed to participate in the study received questionnaires on medical history, dermatological changes, voice, quality of life, sexual functioning, fertility wish, surgical results, and psychological functioning by regular mail. Subsequently, they visited Ghent University Hospital between November 2009 and April 2010 for further evaluation. Both studies were approved by the ethical review board of Ghent University Hospital, Belgium. All participants gave written informed consent for participation in the study.

MAIN OUTCOME MEASURES

MEDICAL HISTORY AND EXAMINATION

As sex steroid treatment is known to be associated with specific side effects, data relevant to the long-term use of estrogen and testosterone therapy were collected from both samples. A self-constructed questionnaire was completed concerning medical history, experience of adverse effects (such as hormone-related cancers and thromboembolic and other cardiovascular events), current and past hormonal treatment, medication use, and smoking habits. Information was compared with data from medical files for accuracy and corrected if necessary.

ANTHROPOMETRY, AREAL BONE MINERAL DENSITY, AND BODY COMPOSITION

Body weight and anthropometrics were measured in light indoor clothing without shoes. Standing height was measured using a wall-mounted Harpenden stadiometer (Holtain, Ltd, Crymuch, UK). Areal BMD at the lumbar spine, at the proximal femur (total hip region), and at both distal forearms were measured using dual energy X-ray absorptiometry (DXA) with a Hologic QDR-4500A device (software version 11.2.1; Hologic, Bedford, MA, USA). T- and Z-scores for areal BMD were calculated using controls provided by the National Health and Nutrition examination Survey-0 (NHANES-0) study group for the hip[14] and by the manufacturer for the lumbar spine, distal forearm, and total body BMD[15]. Male references were used in transsexual women and female references for transsexual men as all participants underwent normal pubertal development, with well-known effects on bone mass and size. The coefficient of variation (CV %) was <1% as calculated from daily spine phantom measurements.

BIOCHEMICAL DETERMINATIONS

Venous blood samples were obtained between 08.00 AMand 12.00 AMafter overnight fasting. All blood samples were stored at -80°C until batch analysis. Commercial kits for radioimmuno assay were used to determine the serum concentrations of total testosterone (T) and sex hormone binding globulin (SHBG) (Orion Diagnostica, Espoo, Finland); estradiol (E2) (Clinical Assay, Diasorin s.r.l., Saluggia, Italy), according to amodified protocol that doubles the serum amount[16]; LH, insulinlike growth factor, C-terminal telopeptides of type

I collagen (CTX) as a marker of bone resorption, protocollagen 1 aminoterminal propeptide (P1NP), which reflects bone formation (electrochemiluminiscence immunoassay [ECLIA]; Modular, Roche Diagnostics, Mannheim, Germany). Insulin-like growth factor-binding protein 3 (IGFBP3) was determined by an extraction method (DSL-5600; Diagnostic System Laboratories, Webster, TX, USA).The intra- and interassay coefficients of variation for all assays were ≤10%. For all measurements, samples from transsexual men and transsexual women were assayed in a same assay run. Serum free T was calculated from the total serum hormone concentration, serum SHBG, and serum albumin, using a validated equation derived from the mass action law[15]. We defined supra- and subphysiological levels of T, estradiol, and LH as hormone levels exceeding the upper or lower limit of the reference ranges according to values from our local laboratory. Hematocrit, total cholesterol, and creatinin were measured using routine clinical chemistry methods. Prolactin and prostate-specific antigen (PSA) were additionally investigated in transsexual women.

STATISTICAL ANALYSIS

Descriptive statistics are expressed as means and standard deviations, or, in case of a non-normal distribution. Between-group differences of categorical variables were calculated with x2 tests, or a Fisher exact test was used. Differences between both groups of linear variables were calculated using the student *T*-test or linear regression analysis while covarying for age, weight, and/or height. Significance was set at $P < 0.5$ (two-tailed). Data were analyzed using PASW-software, v.18 (SPSS Inc., Chicago, IL, USA). For all analyses, missing values were excluded.

TABLE 1. GENERAL CHARACTERISTICS AND BIOCHEMICAL LEVELS OF THE STUDY POPULATION

	TRANSSEXUAL MEN (N=50)	TRANSSEXUAL WOMEN (N=50)	P
Age at time of the interview (ys)	37 ± 8.2	43.0 ± 10.4	0.003
Age at SRS (ys)	30 ± 8.2	36.7 ± 9.8	0.001
Height (cm)	165 ± 6.7	175.1 ± 8.3	<0.001
Weight (kg)	67.5 ± 11.5	77.8 ± 18.3	0.01
Use of hormone therapy (%)	100	94.0	0.24
Testosterone (ng/dl)[a]	631.1 [466.0-1019.2]	29.6 [21.8-38.2]	<0.001[a]
Free testosterone (ng/dl)[a]	15.5 [9.2-25.0]	0.3 [0.18-0.49]	<0.001[a]
Estradiol (pg/ml)[a]	34.4 [24.7-49.7]	50.9 [27.7-73.7]	0.012[a]
SHBG (nmol/l)[a]	30.1 [22.5-38.5]	66.1 [48.0-110.3]	<0.001[a]
LH (U/l)[a]	3.7 [0.2-28.5]	27.0 [17.5-39.4]	0.001[a]
Creatinin (mg/dl)[a]	0.9 [0.9-1.0]	0.8 [0.7-0.9]	<0.001
Hematocrit %	48.8 ± 2.8	41.0 ± 2.4	<0.001
IGF1 (ng/ml)	225.5 ± 65	229.1± 103	0.713
PSA (ng/ml)[a]	-	0.003 [0.03-0.09]	-
Prolactin (ng/ml)[a]	-	9.1 [6.0-12.2]	-

Data are presented as mean ±S.D or median (first to third quartiles) in case of non-Gaussian distribution. Categorical variables using chi-square-test; Linear variables using Linear regression analysis [a] adjusted for age, height, and weight.

RESULTS

GENERAL CHARACTERISTICS

General characteristics of the study population (N = 100) are summarized in Table 1. All transsexual men were on testosterone replacement therapy for about 10 years (Figure 1); in total 496 hormone treatment years.Three transsexual women were not on estrogen therapy because of previous thromboembolic events and were therefore excluded from further hormonal, biochemical, and bone measurements. The remaining transsexual women were on average 9.2 years on hormone therapy (in total 473 hormone treatment years). Most transsexual men used intramuscular testosterone treatment (parental testosterone esters 250 mg/2 or 3 weeks;N = 35 or testosterone undecanoate 1000 mg/12 weeks; N = 7), while seven men applied transdermal testosterone gel (50 mg daily). One participant used both oral testosterone undecanoate 40 mg (daily) and transdermal testosterone gel 50 mg daily. Transsexual women used transdermal estradiol (17β estradiol gel 1.5 mg/24u:N = 22; estradiol patch 50 mg/24u: N = 3) or oral estrogens (estradiol valerate 2 mg: N = 19; estriol 2 mg: N = 1; ethinyl estradiol 50 mg: N = 1; ethinyl estradiol 120 mg: N = 1). Participants using ethinyl estradiol (N = 2) were excluded from estradiol analysis as this compound is not measured in the assay.

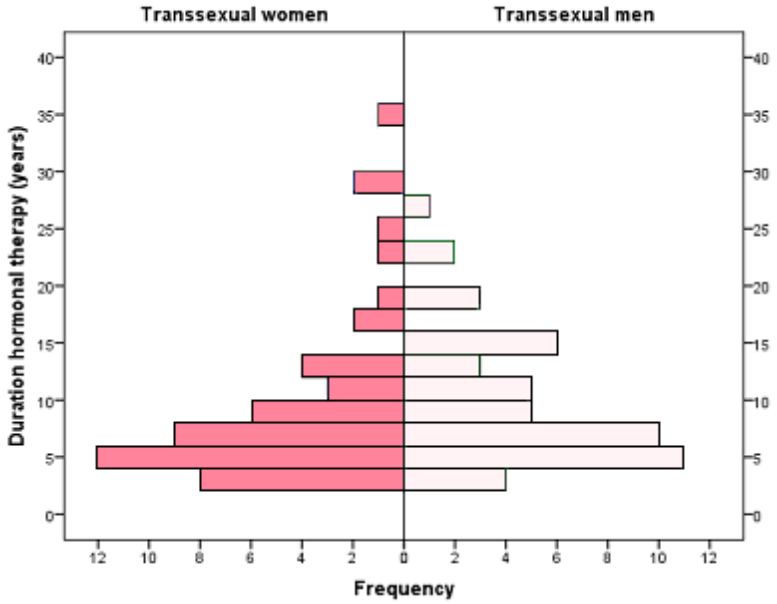

Figure 1. Duration of cross-sex hormone therapy

HORMONAL AND BIOCHEMICAL PARAMETERS

Given that the goal of hormone treatment in transsexual men and women is to obtain hormonal concentrations in the normal physiological range for natal men and women, respectively, hormonal levels differed significantly between both groups (Table 1). At the time of measurement, 8.9% of transsexual men exhibited levels below the reference values of our laboratory (testosterone<321 ng/dL), whereas 26.7% exceeded the upper limit of 1005 ng/dL. Sixty-three percent of transsexual women had estrogen levels below the lower limit (estradiol <55 pg/mL) and 11.5% above the upper limit (200 ng/L). When compared directly, transsexual women had higher LH levels compared with transsexual men (85% vs. 42%, respectively). In 14 transsexual men (28.5%), we observed hematocrit levels above 50%, eight of whom (16.3%) had erythrocytosis (hematocrit levels above 52%) with one participant having a hematocrit level of 55%. Hematocrit levels in transsexual men were negatively associated with LH levels (linear regression: $P = 0.021$; $\beta = -0.35$), but not with other hormonal parameters (testosterone, SHBG) or age (data not shown). Hematocrit levels were significantly higher in participants who used conventional intramuscular testosterone esters compared with those who used intramuscular testosterone undecanoate or transdermal testosterone (linear regression: $P = 0.042$; $\beta = 0.29$). Four transsexual women (8.2%) had slightly elevated prolactin levels (range: 21.4–30.5 ng/mL) compared with the normal male range at our laboratory (4–17 ng/mL). Only one transsexual woman had a prolactin level above the normal female range (6–30 ng/mL). None had elevated PSA levels.

HORMONE-RELATED CANCERS

Transsexual men did not experience hormonerelated cancers during hormone treatment, whereas one transsexual woman had a macroprolactinoma. As this patient's macroprolactinoma was diagnosed before the start of cross-sex hormone treatment, she was excluded from prolactin analyses.

CARDIOVASCULAR RISK FACTORS

Cardiovascular risk factors were comparable in both groups, although transsexual women evidenced lower mean arterial blood pressure and lower serum triglycerides levels (Table 2). A similar number of transsexual men and women were overweight (24% vs. 22%) or obese (14% vs.14%). Three transsexual women had a body mass index above 35 kg/m2 as compared with none of the transsexual men. Hypercholesterolemia (cholesterol >190 mg/dL) was observed in 60% of transsexual women and 64% of men. A comparable number of transsexual women and men had an elevated blood pressure at the time of investigation and/or used antihypertensive medication (26% vs. 28%).

TABLE 2. CARDIOVASCULAR RISK FACTORS

	TRANSSEXUAL MEN (N=50)	TRANSSEXUAL WOMEN (N=50)	P
Current smoking (%)	34.7	36.0	0.39
Smoking years	11.6 ± 14.0	15.7 ± 14.3	0.15
Sports activity (%)	50.0	40.0	0.32
BMI (kg/m^2)	24.8 ± 3.8	25.3 ± 5.4	0.80
Total Cholesterol (mg/dl)	199.1 [185.0 -220.0]	198.0 [168.0 -227.3]	0.44[a]
Triglycerides (mg/dl)	114.5 [85.5-182.0]	83.0 [64.3-127.8]	***0.001*** [a]
Systolic Blood pressure (mmHg)	124.7 ± 14.4	124.8 ± 16.6	0.208
Diastolic Blood pressure (mmHg)	81.3 ± 10.7	77.1 ± 10.1	***0.002*** [a]
Mean arterial blood pressure (mmHg)	95.8 ± 10.1	93.0 ± 11.2	***0.008*** [a]

Data are presented as mean ± S.D. Non-Gaussian distribution: data presented as median (first to third quartiles) Categorical variables using chi-square test; Linear variables using Linear Regression analysis [a] Adjusted for age and weight.

THROMBOEMBOLIC AND CARDIOVASCULAR EVENTS

No transsexual men reported cardiovascular events such as myocardial infarction (MI), cerebrovascular disease, or deep venous thrombosis. Three transsexual women experienced thromboembolism (two cerebral and one deep venous thrombosis) during hormone treatment (Table 3). One participant experienced deep venous thrombosis and pulmonary embolism before starting of cross-sex hormone treatment. Estrogen treatment was given in combination with anticoagulants. Additionally, four transsexual women experienced other cardiovascular diseases: transient ischemic attack (TIA) (N = 1), venous ulcer (N = 1), and MI (N = 2). One participant experienced MI prior to hormone therapy. Another transsexual woman underwent surgery for peripheral arterial disease during the course of hormone treatment, but the presence of diabetes mellitus was a likely cause in this woman. All participants except one, who experienced thromboembolic or other cardiovascular events, were smokers at the time of event (on average 24 smoking years).

AREAL BONE MINERAL DENSITY USING DXA

Transsexual women experienced significantly more osteoporosis and osteopenia as comparedwith transsexual men. In transsexual men, no osteoporosis (T-scores< -2.5) was diagnosed, while in transsexual women, osteoporosis was observed in 23.4% of patients at the lumbar spine, 8.7% at femoral neck, 2.1% at the total hip, and 25.5% at the left radius. In addition, the mean Z-scores in transsexual women were negative at all sites (at the lumbar spine: -1.0 ± 1.4, total hip: -0.4 ± 1.0, femoral neck: -0.7 ± 1.0, distal radius: -1.3 ± 1.3, and total body: -1.0 ±1.0). No differences in bone density were found between patients using transdermal or oral estrogen administration (data not shown). Finally, no associations were found in bone mineral density at lumbar spine, femoral neck, total hip and serum testosterone, LH, or estradiol levels in both transsexual women and men (data not shown). Compared with the normal male range at our laboratory (<0.58 ng/dL), all but four transsexual women had normal CTX levels (range: 0.62– 1.24 ng/dL), while two transsexual women had higher P1NP levels above the normal male range (102 ng/mL) (range: 106–125 ng/mL).

TABLE 3. TROMBOEMBOLIC AND OTHER CARDIOVASCULAR EVENTS IN TRANSSEXUAL WOMEN (N=6)

EVENT	AGE AT EVENT (YEAR)	DURATION OF HRT AT EVENT (YEARS)	TYPE OF ESTROGEN THERAPY AT EVENT (ALL ORAL ADMINISTRATION, DAILY INTAKE)	SMOKING YEARS AT EVENT	CURRENT SMOKER
Thromboembolic events					
DVT*	52	21	Conjugated estrogens 0.625 (Premarin®)	18	No
Cerebral thrombosis**	58	20	Ethinyl estradiol 50 µg	45	Yes
Cerebral thrombosis	46	1	Cyproterone acetate 50 mg	-	Yes
TIA at SRS*	33	2	Conjugated estrogens 0.625 + cyproterone acetate 50 mg	18	No
Other cardiovascular events					
Peripheral arterial disease	46	8	Estradiol valerate 2 mg[b]	18	Yes
Venous ulcer	45	7	Ethinyl estradiol 20 µg	13	Yes
MI	43	21	Estrogen therapy[a]	31	Yes

DVT: deep venous thrombosis, MI: myocardial infarction, TIA: transient ischemic attack. [a] Not further specified [b] Diagnosis of type 2 diabetes 9 years before event
* same participant ** homozygeous carrier mutant methylhydrofolatereductase (MTHFR) allele

DISCUSSION

This study presents follow-up data on physical health after hormone therapy and SRS (on average after 10 treatment years) in transsexual men and women.We demonstrate that at our center, transsexual men are less at risk for severe side effects than transsexual women. In addition, we found that none of the transsexual men experienced cardiovascular events or hormone-related cancers. These findings are in line with previous studies, which demonstrated that cross-sex hormone treatment was acceptably safe in the short- and medium-term for transsexual men[17,18]. However, risks may become more apparent as subjects grow older and the duration of hormone exposure increases[6,18]. Moreover, the presence of several cardiovascular risk factors such as obesity, poorer lipid profile, or elevated serum hematocrit raises the concern for possible future cardiovascular events[4]. Several risks factors were present in a substantial part of our group of transsexual men at the time of investigation: being overweight (24%), obesity (14%), hypercholesterolemia (64%), smoking (30%), erythrocytosis (14.3%), and elevated blood pressure (22.5%). Long-term studies are needed to provide specific data on the effects of hormonal treatment in transsexual men on cardiovascular health[6]. A healthier lifestyle may reduce the impact of these risk factors, while some risk factors such as erythrocytosis are related to the specific pharmacokinetic effects of the type of testosterone therapy[19,20] and can be avoided. In contrast with previous findings in natal men[20], older age was not associated with a higher risk of erythrocytosis during testosterone administration. However, in comparison with the study by Coviello et al. [20], none of our transsexual men were aged between 60 and 75 years.

Transsexual women showed a similar number of cardiovascular risk factors compared with transsexual men in the current sample. Except for smoking, which occurred more in the transgender participants, similar or even less cardiovascular risk factors were present in comparison with the general Belgian population[21–25]. Nevertheless, 12% of transsexual women experienced thromboembolic and/or other cardiovascular complications during hormone treatment after a mean duration of 11.4 hormone treatment years. For a comparable duration of hormone treatment years, cross-sex hormone treatment seems to have more harmful effects in transsexual women than in men. This is in line with Asscheman et al.[26], who observed higher cardiovascular mortality after an average of 20 years of cross-sex hormone therapy in transsexual women, but not transsexual men, as compared with the general population. Whether these thromboembolic and other cardiovascular events are caused by cross-sex hormone therapy, older age of transsexual women, or represent the preexisting sex differences in cardiovascular events, remains to be determined. Long-term prospective studies in a larger study sample are needed in this regard.

The incidence of venous thrombosis in the present study (2% or estimated 21/10,000 user years) was lower than the one reported by Van Kesteren et al.[17]. Their study[17] showed that 6.4% (58/10,000 user years) in their group of transsexual women experienced a deep venous thrombosis or pulmonary embolism thrombosis during hormonal therapy, which was a 20-fold increased incidence compared with the general Dutch male population. However, a more appropriate control group might be women using estrogens and/or progestagens such as hormone replacement therapy (HRT) or combined oral contraceptives (COCs). COC therapy and HRT are both associated with an increased risk of venous thromboembolism[27-28]. A recent Danish population study[29] found that the incidence of venous thromboembolism in women using COC was 3.01/10,000 user years, which is still significantly lower than the risk observed in transsexual women. The estrogen levels of many of our transsexual women were below the normal female range. This may be a reason why relatively few transsexual women experienced venous thrombosis and pulmonary embolism compared with other centers. However, inadequate estrogen levels can cause side effects known from hypogonadal states. As we felt the need to increase the estrogen dosage in our treatment protocol in the past years, we chose 17β estradiol as this formulation is thought to induce less changes in hemostatic factors[30]. Currently, more attention is also paid to monitor LH levels especially when estradiol levels cannot be measured by the immunoassay. Also, in the presence of low bone density, clearly supraphysiological LH levels will influence our decision on estrogen dosage.

We observed a higher incidence of cerebrovascular disease in our transsexual women compared with the general male and female population[31].

Both COC and HRT are known to increase the risk for cerebrovascular disease[32,33]. However, other factors such as smoking, hypercholesterolemia, or hypertension are even more detrimental[34]. Indeed, the majority of transsexual women who experienced cerebrovascular complications had other important risk factors besides estrogen therapy including genetic predisposition, smoking, and hypercholesterolemia. Although we always strongly recommend to all transsexual women to quit smoking, it appeared that many individuals continued despite prior experience of a thromboembolic or cardiovascular event. In our clinical experience, it remains difficult to convince transsexual women to discontinue smoking but also to adopt a healthier lifestyle. Importantly, during and after transition, more attention should be paid to treat cardiovascular risk factors such as hypercholesterolemia and hypertension.

One patient developed a TIA at SRS, and perioperative thromboses in transsexual women have been described in other centers as well[17,35]. As surgery and immobilization are well-known risk

factors for development of thrombosis, we currently advise to discontinue hormonal therapy at least 2 weeks before SRS or other elective surgery and to restart at mobilization. However, the existing evidence regarding the necessity to discontinue hormonal therapy before surgery in transsexual women remains limited. Also, the time period hormonal therapy should be discontinued is presently unknown. In our current treatment regimens especially ethinyl estradiol is no longer used given possible association with higher thromboembolic risks[30,35].

With regard to hormone-related cancers, it should be noted that all transsexual men treated at our center receive bilateral mastectomy, hysterectomy, and ovariectomy, within 1 year of testosterone treatment, so that risk of development of related cancers in these areas is very low. However, breast cancer has been reported in one transsexual man after bilateral mastectomy under long-term hormone treatment as well as development of an ovarian cancer in another transsexual man during hormone treatment and before ovariectomy[36,37]. None of the transsexual women developed hormone-related cancers during hormone therapy. So far, only a few case reports of prolactinomas, breast cancers, and prostate carcinomas in transsexual women have been reported[38]. Nevertheless, it deserves mention that the incidence of hormone-related cancers can increase as the duration of hormone exposure increases[38]. Moreover, it is possible that transsexual persons feel uncomfortable with medical exams concerning their native sex such as prostate examinations in transsexual women or gynecological examinations in transsexual men leading to an underinvestigation of cancers in these subjects. However, with appropriate care, these medical checkups are possible[39]. All transsexual women had normal PSA levels and only one participant developed a slightly elevated prolactin level compared with normal female range. This is in line with Dietrich et al.[40], who did not find an increase in prolactin levels in 60 transsexual women treated with monthly injections of gonadotropin-releasing hormone analogue (GnRH) analogs and oral estradiol valerate 6 mg daily, but it is in contrast with Asscheman et al.[35], who observed that more than 50% of trans women experienced elevated prolactin levels under cross-sex hormone treatment (mostly cyproterone acetate 100 mg and 100 mg ethinylestradiol daily). The time course and exact mechanism of increased prolactin levels during cross-sex hormone treatment in transsexual women has not been fully elucidated. Type and dose of HRT are likely to be at least partly responsible for the observed differences, but head-to-head comparisons of different treatment regimens are to be performed.

Finally, long-term bone health is also a matter of concern in the treatment of transsexual persons. In line with most[41–43] but not all studies[44], we found that cross-sex hormone therapy maintained areal bone mineral density in all transsexual men, possibly due to a direct effect of

testosterone on bone and/or an indirect effect of testosterone after aromatization to estradiol. In contrast to most other studies[45–48], but consistent with previous results from our center[49], we observed a high prevalence of osteoporosis and osteopenia in our group of transsexual women. However, the prevalence of osteoporosis and osteopenia is also dependent based on which gender (natal or desired) is used as a reference. As all our transsexual men and women underwent normal puberty, with well-known effects on bone mass and size, we generally recommend the use of the natal gender as a reference. Although this can be debated especially in adolescent transsexual persons. Data on bone health in transsexual women compared with control men are extensively described elsewhere[10]. Multiple causes might explain the high number of osteoporosis in our transsexual women compared with other centers. First, it is possible that our treatment regimen in the past using cyproterone acetate alone up to a maximum 1 year without concomitant use of exogenous estrogen therapy has led to a decrease in bone mineral density as cyproterone acetate decreases testosterone levels. Previous studies in men treated for prostate cancer[50,51] support these findings and show a decrease in BMD during androgen deprivation therapy. Moreover, men with prostate cancer treated with androgen deprivation therapy exhibited a higher fracture risk compared with those not receiving this therapy[52,53]. In sex offenders, treatment with cyproterone acetate was also associated with significant bone loss[54]. Second, given the use of a cross-sectional design in the present investigation, it cannot be ruled out that our study group differed at baseline because of cultural differences in physical activity, height, or calcium intake. Lower levels of physical activity in Belgium as compared with other European as well as non-European countries have been described in both female and male adults and adolescents[55–57]. Finally, low estrogen levels and high gonadotrophines in our participants might indicate inadequate estrogenization. However, the rather high SHBG levels and the absence of clinical symptoms of insufficient treatment such as hot flushes do not support this hypothesis. The normal values for markers of bone turnover in this study population are also not suggestive for a state of estrogen deficiency with active bone loss, characterized by an increased bone turnover. Future prospective studies examining bone health in transsexual women may clarify the current findings. Studies comparing treatment regimens of cyproterone acetate with or without concomitant estrogen administration, as well as multicenter studies with adjustment for confounding effects (e.g., physical activity, height and calcium intake), may solve these questions.

Our study has several limitations. The relatively small sample size disallows us to provide accurate prevalence and incidence rates of morbidity. Comparisons with the general population therefore need to be interpreted with caution. Also, some adverse events based on biochemical

variations and osteoporosis may be explained to some extent by increased screening. Third, as in all follow-up studies, selection bias of our participants cannot be excluded. Participants who agreed to this study may have a more favourable outcome than those who refused to participate. Fourth, as older age is associated with a higher morbidity, it should be noted that transsexual women in our sample were on average 6 years older than transsexual men. However, given that transsexual men mostly seek treatment at younger ages, transsexual women will be older if a comparable duration of hormone therapy is evaluated[58]. Finally, the nature of the cross-sectional design implies that we cannot draw any causative conclusions. In addition, given the absence of a baseline measurement, we cannot rule out that some of the findings were already present before cross-sex hormone therapy. Yet, despite these limitations, we feel that the present data contribute to the investigation of the effects and side effects of hormone therapy in transsexual persons, especially as follow-up data on hormone administration in this specific group remain scarce.

CONCLUSIONS

In conclusion, we have shown that after an average of 10 years of cross-sex hormone treatment, transsexual men did not experience important side effects such as hormone-related cancers or cardiovascular events. Osteoporosis was also absent in transsexual men. On the other hand, a substantial number of transsexual women suffered from osteoporosis at the lumbar spine and distal arm. Twelve percent of transsexual women experienced thromboembolic and/or other cardiovascular events during hormone treatment, possibly related to older age, estrogen treatment, and lifestyle factors. In order to decrease cardiovascular morbidity, more attention should be paid to decrease cardiovascular risk factors during hormone therapy management.

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2.5 PREVALENCE OF CARDIOVASCULAR DISEASE AND CANCER DURING CROSS-SEX HORMONE THERAPY

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ABSTRACT

OBJECTIVE

This study evaluates the short- and long-term cardiovascular- and cancer-related morbidities during cross-sex hormone therapy in a large sample of trans persons.

METHODS

A specialist centre cross-sectional study compared 214 trans women (male-to-female transsexual persons) and 138 trans men (female-to-male trans persons) to an age- and gender-matched control population (1 to 3 matching). Participants were on cross-sex hormone therapy for an average of 7.4 years. We assessed physical health and possible treatment-related adverse events using questionnaires.

RESULTS

Five percent of trans women experienced venous thrombosis and/or pulmonary embolism during hormonal therapy. Five of these adverse events occurred during the first treatment year, while another 3 occurred during sex reassignment surgery.

Trans women experienced more myocardial infarctions compared to control women (P=0.001) but a similar proportion compared to control men. Cerebrovascular disease prevalence was higher in trans women compared to control men (P=0.03). Trans men had similar rates of myocardial infarction and cerebrovascular disease compared to control male and female subjects. Type 2 diabetes prevalence was higher in both trans men and women compared to their respective controls, whereas cancer rates were similar to control men and women.

CONCLUSION

Morbidity rate during cross-sex hormone therapy was relatively low, especially in trans men. We observed a higher prevalence of venous thrombosis, myocardial infarction, cerebrovascular disease and type 2 diabetes in trans women compared to control population. Trans men had similar morbidity rates compared to controls aside from increased type 2 diabetes prevalence.

INTRODUCTION

Hormonal therapy is an established part of gender identity disorder treatment and induces secondary sex characteristics development of the desired gender while reducing those of the natal sex[1]. Female-to-male transsexual persons, denoted as trans men, generally receive progestins to suppress menstruation and intramuscular testosterone preparations to induce virilisation. Male-to-female transsexual persons (trans women) generally receive anti-androgen therapy together with oral or transdermal estrogens to induce feminization. Optimal formulations and dosages of cross-sex hormone treatment are unknown at present[2], but current guidelines recommend aiming for hormonal levels within the normal physiological range[3]. Sustained supraphysiological levels of sex steroids increase the risk of adverse events such as thrombosis[3], whereas subphysiological levels may induce the effects of a hypogonadal state.

Although cross-sex hormone therapy can induce several side effects; it is surprising that only a handful of studies[4-9] have examined morbidity and mortality in trans persons. In general, current evidence suggests that testosterone administration in trans men is not very harmful[10]. However, anti-androgen and estrogen administration may negatively impact cardiovascular health in trans women[2]. Both anti-androgens and estrogens increase the risk of venous thrombosis and/or pulmonary embolism[7], and two recent mortality studies showed a higher cardiovascular mortality rate in trans women compared to the general population[4-5]. However, the relationship between cross-sex hormone treatment and cardiovascular risk profile in trans women is complex, as the chemical nature of the estrogens[5], the route of administration[11], the dosage of estrogens[12-13], and the patient's cardiovascular health status may all have an effect.

Cancer has not been reported frequently in trans individuals[2, 14]. However, because some tumours are hormone-dependent, examining the incidence of cancer during cross-sex hormone therapy may be important[15].

The aim of this study was to examine the prevalence of cardiovascular- and cancer-related morbidity during cross-sex hormonal therapy in the patients treated at our centre since 1986.

SUBJECT AND METHODS

STUDY POPULATION AND STUDY PROCEDURES

All persons diagnosed with Gender Identity Disorder (GID) (Diagnostic and Statistical Manual of Mental Disorders- III-R and DSM-IV, 302.85; International Classification of Diseases, 10th revision, F64.0) at the Center for Sexology and Gender Problems at the Ghent University Hospital (Ghent, Belgium) between 1986 and June 2012 and underwent at least 3 months of cross-sex hormonal therapy were invited by letter to participate in this study. Respondents received a paper version of the questionnaire by post or filled out the survey online. A reminder message was sent to non-responders. All surveys were collected between August and December 2012. We achieved a response rate of 54% as 352 participants, including 214 trans women and 138 trans men, agreed to participate in this study (Figure 1). Ten of the invited persons died during the follow-up period.

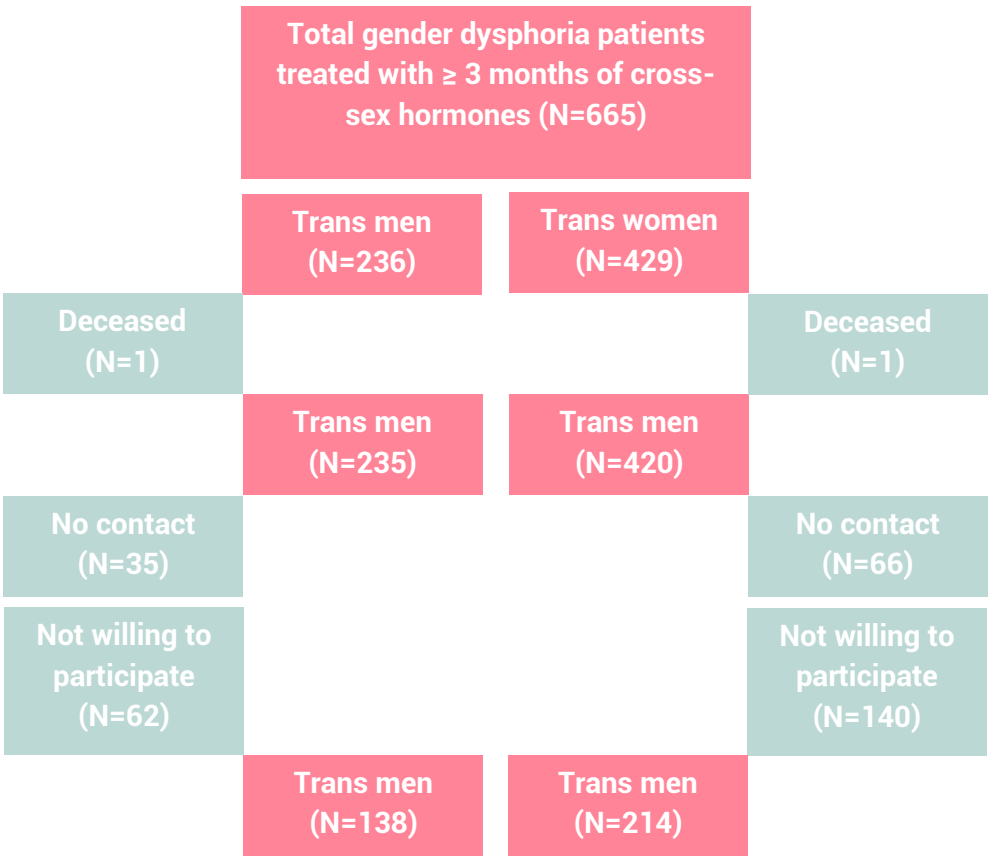

Figure 1. Subject enrollment

Ten trans women were no longer on estrogen therapy due to previous thromboembolic events (n=5), dissatisfaction (n=2) or other reasons (n=3). The remaining trans women were on hormone therapy for an average of 7.7 years (range: 3 months-35 years) (Figure 2). In trans women, current cross-sex hormonal therapy consisted of transdermal estradiol [17-β estradiol gel 1.5 mg/24u (n= 76; 35.5%); estradiol patch 50 µg/24u (n=29;13.6%); or daily intake of oral estrogens; estradiol valerate 2 mg (n=91; 42.5%), estriol 2 mg (n=1; 0.4%), ethinyl estradiol 50 µg (n=2; 0.9%), and oral contraceptive ethinyl estradiol 30-50µg (n=5; 2.3%)]. A majority of the trans women (n=129; 65%) in our study underwent orchiectomy.

Trans men were on testosterone replacement therapy for an average of 9.4 years (range: 3 months to 49 years). Cross-sex hormonal therapy in trans men consisted of intramuscular testosterone treatment with either a mixture of testosterone esters (testosterone decanoate 100 mg, testosterone isocaproate 60 mg, testosterone fenylpropionate 60 mg, testosterone propionate 30 mg/ml) every 2 or 3 weeks (n=64; 46.4%); testosterone undecanoate 1000mg per 12 weeks (n=62; 44.9%); transdermal testosterone 50 mg daily (n=9; 6.5%); or oral testosterone undecanoate (n=2; 1.4%). Eighty-six percent of trans men (n=118) underwent hysterectomy/ovariectomy.

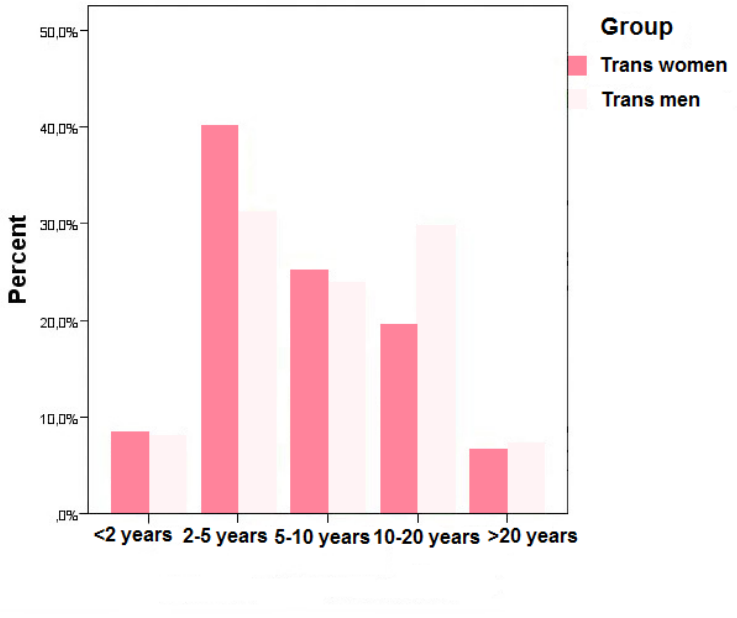

Figure 2. Duration of hormonal therapy

Participants were questioned about their physical health, incidence of possible treatment-related adverse events, socio-demographic status, health-related quality of life (QOL), treatment-related symptoms, surgical results, and satisfaction with hormonal and surgical treatment. Data concerning QOL, treatment satisfaction, and sexual desire are addressed elsewhere[16-17]. For both trans men and women, age-matched female and male control groups were used to compare data concerning cardiovascular and cancer morbidities. We focused on relatively common morbidities in the general population to provide accurate estimates of their prevalence. We also studied morbidities that could be assessed reliably by a questionnaire. The control group for trans women consisted of 619 women and 640 men, and the control group for trans men consisted of 414 women and 414 men. This control group was randomly selected (3 control men and 3 control women for each subject) and was recruited from a population-based study in Flanders. Methods for this study are described elsewhere[18]. Briefly, the control population was recruited from a survey of sexual health characteristics and bio-medical, psychological, demographic and socio-cultural correlates in persons between 14 and 80 years. Data were collected on 1832 respondents (response rate: 40.0% of the eligible respondents) between February 2011 and January 2012. Respondents were randomly drawn from the Belgian National Register. All data were gathered via face-to-face interviews using a combination of computer-assisted personal interviewing (CAPI) and computer-assisted self-interviewing (CASI). Both studies were approved by the ethical review board of Ghent University Hospital, Belgium. All participants gave their consent to participate in the study.

MAIN OUTCOME MEASURES

PHYSICAL HEALTH AND INCIDENCE OF POSSIBLE TREATMENT RELATED ADVERSE EVENTS

Medical history, medication use, smoking habits, weight, height, current and past hormonal treatment, and clinical adverse events (e.g. acute myocardial infarction, venous thrombosis and/or pulmonary embolism, type 2 diabetes, transient ischemic attack, stroke, and cancer) were addressed using a questionnaire that we developed. Morbidity data before the start of hormonal therapy were retrieved from medical files. Medical information was corrected from medical files.

STATISTICAL ANALYSIS

Descriptives were expressed as mean and standard deviation (SD) or median (first to third quartile) when criteria for normal distribution were not fulfilled. Linear regression analyses were used to compare linear variables between groups with group being the independent variable. Dichotomous and categorical variables were analyzed using logistic regression and chi-square

tests, respectively. Morbidity, QOL, and physical symptoms comparisons between groups were adjusted for age. Data were analyzed using PASW-software, v.19 (SPSS Inc., Chicago, IL). Statistical significance was set at $P < 0.05$, and all tests were two-tailed.

RESULTS

GENERAL CHARACTERISTICS

General characteristics of the study population are described in Tables 1 and 2.

Almost 30% (n=179) of the women in the trans women control group used sex steroid medication, including oral contraceptives (n=93; 15%), hormonal coil (n=33; 5.9%), hormonal replacement therapy (n=21; 34%), vaginal ring (n=14; 2.3%) or others (n=17; 2.8%). One man in the trans women control group used oral testosterone undecanoate and another used intramuscular testosterone esters.

In the control group of the trans men, 36.2% (n=150) of women used sex steroid medication, including oral contraceptives (n=89; 21.5%), hormonal coil (n=34; 8.2%), hormonal replacement therapy (n=8; 1.9%), vaginal ring (n=9; 2.2%), or others (n=10; 2.4%). One man in this control group used intramuscular testosterone esters.

TABLE 1. GENERAL CHARACTERISTICS OF TRANS WOMEN AND THEIR CONTROL GROUP

	TRANS WOMEN (N=214)	CONTROL MEN (N=640)	CONTROL WOMEN (N=619)	P MEN	P WOMEN
Age at time of study (years)	43.7 ± 12.6	43.4 ± 13.7	43.1 ± 13.1	NS	NS
Current in a relationship (%)	47.2	82.7	83.9	<0.001	<0.001
Work status (%)					
Unemployed	14.2	1.8	7.2	<0.001	<0.001
Employed	55.9	77.2	70.8		
Retired	9.5	10.3	6.2		
Student	5.2	9.0	8.4		
Unable to work	13.7	1.7	4.3		
Household	1.4	0	7.4		
Income (%)					
≤999 euro	10.3	0.2	2.9	<0.001	<0.001
1.000-1.999 euro	42.2	16.6	20.9		
2.000-2.999 euro	27.5	29.0	28.5		
3.000-5.999 euro	16.7	49.2	44.8		
≥ 6.000 euro	3.4	5.1	2.9		
Educational level (%)					
School going	5.2	3.8	4.1	NS	NS
None / Primary School	6.1	13.0	16.7		
Primary High School	17.3	24.8	19.6		
Secondary High School	29.4	27.0	23.0		
Bachelor/master	41.1	31.5	36.7		
BMI (kg/m²)	24.4 [21.7-27.9]	24.8 [22.7-27.4]	23.5 [21-26.5]	0.07	NS
SRS (%)	64.8	-	-	-	-
Time since SRS (years)	6.0 [2-11]	-	-	-	-
Duration HRT (years)	6.0 [3-11]	-	-	-	-
Sex steroid therapy (%)	95.3%	0.3	29.1	<0.001	<0.001

Data are presented as mean (S.D) or median (first to third quartiles) in case of non-Gaussian distribution. Categorical variables using Chi Square; Linear variables using ANOVA analysis. NS: not significant; SRS: sex reassignment surgery

TABLE 2. GENERAL CHARACTERISTICS OF TRANS MEN AND THEIR CONTROL GROUP

	TRANS MEN (N=138)	CONTROL MEN (N=414)	CONTROL WOMEN (N=414)	P MEN	P WOMEN
Age at time of study (years)	37.5 ± 11.0	37.1± 11.9	37.4 ± 10.1	NS	NS
Current in a relationship (%)	62.3	77.3	82.6	<0.001	<0.001
Children (%)	23.9	44.1	58.1	<0.001	<0.001
Work status (%)					
Unemployed	9.4	1.3	4.2	0.001	0.02
Employed	64.5	77.2	69.6		
Retired	1.5	2.3	2.0		
Student	13.0	18.2	16.5		
Unable to work	9.4	1.0	3.7		
Household	2.2	0	4.0		
Income (%)					
≤999 euro	14.2	0	1.5	<0.001	<0.001
1.000-1.999 euro	35.8	14.0	18.1		
2.000-2.999 euro	24.6	28.3	25.1		
3.000-5.999 euro	23.9	52.6	52.6		
≥ 6.000 euro	1.5	5.1	2.6		
Educational level (%)					
School going	13.1	8.6	9.3	NS	NS
None / Primary School	8.7	9.6	11.2		
Primary High School	10.2	22.6	17.6		
Secondary High School	28.3	26.7	24.7		
Bachelor/master	39.3	32.5	37.2		
BMI	24.3 [22.2-27.5]	23.1 [20.9-26.2]	24.2 [22.2-26.6]	NS	0.02
SRS (%)	85.5	-	-	-	-
Time since SRS (years)	7.0 [4-13]	-	-	-	-
Duration HRT (years)	7.0 [4-13]	-	-	-	-
Sex steroid therapy (%)	100%	0.2	36.2	<0.001	<0.001

Data are presented as mean (S.D) or median (first to third quartiles) in case of non-Gaussian distribution. Categorical variables using Chi Square Test; Linear variables using ANOVA analysis. NS: not significant; SRS: sex reassignment surgery

MORBIDITY IN TRANS PERSONS COMPARED TO CONTROL POPULATION

Cardiovascular disease and cancer-related morbidities in trans women and men and their respective control groups are shown in Table 3 and 4, respectively.

TABLE 3. PREVALENCE OF MORBIDITY IN TRANS WOMEN COMPARED TO AN AGE-MATCHED CONTROL POPULATION

	TRANS WOMEN BEFORE HRT*	TRANS WOMEN	AGE-MATCHED CONTROL MEN	AGE-MATCHED CONTROL WOMEN	P MEN	P WOMEN
VT and/or PE	9.2	60.7	-	-	-	-
Myocardial infarction	4.7	18.7	12.5	0	NS	*0.001*
TIA/CVD	4.7	23.4	9.4	14.9	*0.03*	NS
Obesity	56.0	116.8	92.0	107.6	NS	NS
Diabetes Mellitus type 2	37.3	42.0	6.2	14.9	*0.04*	*0.021*
Cancer	18.7	28.0	21.9	24.9	NS	NS

Data are presented cases/1000 persons; logistic regression adjusted for age
NS: not significant; VT: venous thrombosis; PE: pulmonary embolism; TIA: transient ischemic attack; CVD: cerebrovascular disease
* retrieved from patient files

Eleven trans women (5.1%) experienced venous thrombosis and/or pulmonary embolism during hormonal therapy. Almost half of these incidents occurred during the first treatment year (n=5), another three at the time of sex reassignment surgery (SRS), one after 3 years of hormonal therapy, another after 11 years, and one after 22 years of hormonal therapy. In all but one incident, one or more of the following risk factors was present: smoking (n=7), immobilization (n=3), and/or clotting disorder (n=1). Hormonal treatment at the time of the incident consisted of 50 mg cyproterone acetate (n=2), 4 mg estradiol valerate in combination with 50mg cyproterone acetate (n=2), 4mg estradiol valerate (n=1), 2mg transdermal 17β estradiol (n=3), 50 µg ethinyl estradiol (n=1), 0.625mg conjugated equine estrogens (n=1), or unknown (n=1).

Three cases of acute myocardial infarction were diagnosed within the first 2 years of combined anti-androgen and estrogen treatment. Patients were 48 years old on average at the time of the event (Table 5).

Transient ischemic attack (TIA) or cerebrovascular disease (CVD) was diagnosed in 5 trans women after an average of 7.2 years of hormonal therapy (range 2-20). Patients were 51 years old on average at the time of event (Table 5).

Trans women had a similar BMI compared to control men and women (data not shown). Trans men had a similar BMI as control men but a higher BMI compared to control women (*P*=0.02). Prevalence of obesity in trans women and men was similar to the control population. Trans

persons had a similar prevalence of cancer compared to control men and women. Three cases of colon carcinoma, 2 cases of melanoma, and 1 case of lymphoma were diagnosed in trans women. None of our trans men experienced cancer during follow-up.

TABLE 4. PREVALENCE OF MORBIDITY IN TRANS MEN COMPARED TO AN AGE-MATCHED CONTROL POPULATION

	TRANS MEN BEFORE HRT*	TRANS MEN	AGE-MATCHED CONTROL MEN	AGE-MATCHED CONTROL WOMEN	P MEN	P WOMEN
VT and/or PE	14.5	14.5	-	-	-	-
Myocardial infarction	0	0	7.3	0	NS	NS
TIA/CVD	0	0	7.3	7.3	NS	NS
Obesity	79.7	137.7	92.0	118.6	NS	NS
Diabetes Mellitus type 2	14.5	36.2	7.3	0	*0.06*	*<0.001*
Cancer	0	0	19.2	21.4	*0.05*	*0.04*

Data are presented cases/1000 persons; logistic regression adjusted for age
NS: not significant; VT: venous thrombosis; PE: pulmonary embolism; TIA: transient ischemic attack; CVD:cerebrovascular disease
* retrieved from patient files

MORTALITY IN TRANS PERSONS

Ten trans persons (one transman and nine trans women) died during our follow-up. Causes of death were suicide (n=6), cardiovascular disease (n=2), cancer (n=1), and unknown (n=1). Three patients committed suicide during hormonal therapy and before SRS, while three committed suicide after SRS.

DISCUSSION

This study presents follow-up data related to cardiovascular disease and cancer-related morbidities during short- and long-term cross-sex hormone therapy administration in a large group of trans persons. Similar to others[7, 9], we observed a relatively high risk of venous thrombosis and/or pulmonary embolism during cross-sex hormone therapy in trans women. The incidence of venous thrombosis and/or pulmonary embolism in our study (5.1%) was lower than Van Kesteren and colleagues[7], who showed that 6.4% of their sample experienced this during hormonal treatment. The use of high-dose oral ethinyl estradiol (100μg OD) may explain the higher incidence in the latter study, as this estrogen type and route of administration is known to affect the coagulation system negatively[5, 19-20]. However, Ott and colleagues[21] did not observe any venous thrombosis and/or pulmonary embolism incidents during their follow-up study of 162 trans women (mean age 36 years) who received transdermal 17β estradiol for an average of 4.4 years. This may be a safer type of estrogen and route of administration although 37% of the trans women who experienced venous thromboses in our sample received transdermal 17β estradiol. The older age of our participants and longer follow-up period in our study may also contribute to the higher incidence of these events.

The increased risk of cerebrovascular diseases in trans women compared to control men corroborates with our previous follow-up study[6] and with Asscheman and colleagues[5]. The latter found a higher mortality rate due to cerebrovascular disease in trans women aged between 40 and 64 years. Use of oral contraceptives and hormonal replacement therapy are associated with an increased risk of cerebrovascular disease[22-23], and our results suggest this may also be true for cross-sex hormone therapy in trans women.

We also showed that trans women had a higher prevalence of myocardial infarction compared to control women, but similar compared to control men. Asscheman and colleagues[5] also observed that long-term hormonal replacement therapy does not decrease the risk of coronary events in trans women compared to community dwelling men. It may be that these results represent the pre-existing gender differences in coronary events[24] as a considerable part of this discrepancy in cardiovascular health cannot be explained by lifestyle or other cardiovascular risk factors. Moreover, we observed in our previous follow-up study that aside from a higher smoking rate, trans persons had a similar number or fewer cardiovascular risk factors compared to the general population[6]. Another explanation may be that similar to the timing hypothesis in postmenopausal women[25-26], estrogen therapy effects in trans women may depend on the individual's cardiovascular health at the start of the therapy. Estrogen therapy can aggravate

preexisting cardiovascular disease in older trans women, whereas it may exert beneficial cardiovascular effects in young and healthy trans women. Our results, may support this hypothesis as the majority of trans women who experienced myocardial infarction or cerebrovascular disease were aged over 50 years, had one or more cardiovascular risk factors (mainly smoking), and had undergone cross-sex hormone therapy for a short duration. Although cardiovascular risk factors should be managed in trans women[3], current guidelines do not specify at what age and how patients with cardiovascular risk factors should be treated. Based on these preliminary results, we may recommend that older trans women with well-known cardiovascular risk factors, especially smoking, should be closely monitored, in particular at the start of cross-sex hormone treatment. Furthermore, cardiovascular risk factors including hypertension, hypercholesterolemia, and smoking should be managed before initiating cross-sex hormone therapy in trans women at risk for cardiovascular disease. Further research is needed to investigate possible mechanisms contributing to increased cardiovascular disease risk during cross-sex hormone treatment, as well as the safety of different cross-sex hormone therapies in older trans women.

Similar to others[7-9], we found that testosterone treatment in trans men was relatively safe in relation to short- and medium-term cardiovascular health. However, risks may become more apparent as subjects grow older and the duration of hormone exposure increases[27-28].

To our knowledge, previous morbidity studies[7, 9] have not investigated the prevalence of type 2 diabetes in trans persons compared to control individuals. We discovered both trans men and women had a higher prevalence of type 2 diabetes compared to control men and women, with almost all diagnoses made before starting hormonal therapy in trans women. Because trans persons are carefully screened by endocrinologists before hormonal therapy, the presence of type 2 diabetes may be diagnosed more frequently. Incidence of type 2 diabetes during hormonal therapy was also higher in trans men compared to control men and women, although the overall number of cases we observed was relatively low. Hyperandrogenism in biological women has been associated with an increased insulin resistance due to direct effects on skeletal muscle and adipose tissue insulin action[29-30], altered adipokine secretion, and increased visceral adiposity[31]. Furthermore, one study investigating the effects of androgen administration on insulin sensitivity, evaluated by euglycemic clamp[32], observed that testosterone treatment significantly decreased insulin-mediated glucose disposal in trans men, although another study from the same group[33] was unable to reproduce these findings. Trans men also had a higher BMI compared to control women. This suggests lifestyle differences between the groups may contribute to this

discrepancy, although obesity prevalence was not different between trans men and control women.

We saw no difference in cancer incidence in trans persons compared to our control population, which are in line with the results from Asscheman and colleagues[5]. However, Dhejne and colleagues[4] found the cause-specific risk of death from neoplasms in trans persons was double that of control individuals, although this was unlikely to be related to cross-sex hormonal treatment. Although the incidence of hormone-related cancers was not higher in the trans groups at either short- or medium-term follow-up, the incidence of hormone-related cancers may increase with longer duration of hormone treatment[10].

Trans women had a higher mortality rate compared to trans men, although the number of mortalities was relatively small. Similar to others[5, 7], we found that prevalence of suicide in trans persons (902/100 000 persons), was higher compared to the general Belgian female (12.8/ 100 000 persons) and male population (34.9/100 000 persons) within the same age category, especially in trans women[34]. Even though cross-sex reassignment therapy has been associated with better functioning in terms of gender dysphoria relief, QOL improvement, psychological functioning, and decrease in suicide attempts[35-39], the relatively high suicide rate during and after transition shows the fragile psychological position of some trans persons.

The main limitation of this study is the possible selection bias as we had a modest response rate of 54 %. Furthermore, the relatively small sample size prevents us from determining accurate prevalence of morbidity and mortality. Joining databases from other specialized centres may help solve these problems and improve the safety and clinical care related to cross-sex hormone therapy. Additionally, because many of the women in the control groups used hormonal therapy, we may be underestimating the observed differences in trans person morbidities compared with the control population. However, because the control population was randomly drawn from the national register, we assume a representative population sample was used in our analysis.

In conclusion, the data of the current study indicates relatively low morbidity rates during cross-sex hormone therapy, especially in trans men. We observed a higher cardiovascular morbidity in trans women compared to female and male controls.

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CHAPTER 3. SEXUAL DESIRE IN TRANS PERSONS

BASED ON

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CHAPTER 3. SEXUAL DESIRE IN TRANS PERSONS

3.1 SEXUAL DESIRE IN TRANSSEXUAL PERSONS: PREVALENCE AND ASSOCIATIONS WITH SEX REASSIGNMENT TREATMENT

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ABSTRACT

INTRODUCTION

Sex steroids and genital surgery are known to affect sexual desire, but little research has focused on the effects of cross-sex hormone therapy and sex reassignment surgery on sexual desire in trans persons. We aim to explore associations between sex reassignment therapy (SRT) and sexual desire in a large cohort of trans persons.

METHODS

A cross-sectional single specialized center study including 214 trans women (male-to-female trans persons) and 138 trans men (female-to-male trans persons).

MAIN OUTCOME MEASURES

Questionnaires assessing demographics, medical history, frequency of sexual desire, hypoactive sexual desire disorder (HSDD) and treatment satisfaction.

RESULTS

In retrospect, 62.4% of trans women reported a decrease in sexual desire after SRT. Seventy-three percent of trans women never or rarely experienced spontaneous and responsive sexual desire. A third reported associated personal or relational distress resulting in a prevalence of HSDD of 22%. Respondents who had undergone vaginoplasty experienced more spontaneous sexual desire compared to those who planned this surgery but had not yet undergone it (P=0.03). In retrospect, the majority of trans men (71.0%) reported an increase in sexual desire after SRT. Thirty percent of trans men never or rarely felt sexual desire; 39.7% from time to time and 30.6% often or always. Five percent of trans men met the criteria for HSDD. Trans men who were less satisfied with the phalloplasty had a higher prevalence of HSDD (P=0.02). Trans persons who were more satisfied with the hormonal therapy had a lower prevalence of HSDD (P=0.02).

CONCLUSION

HSDD was more prevalent in trans women compared to trans men. The majority of trans women reported a decrease in sexual desire after SRT, whereas the opposite was observed in trans men. Our results show a significant sexual impact of surgical interventions and both hormonal and surgical treatment satisfaction on the sexual desire in trans persons.

INTRODUCTION

Hormonal therapy is an established part of Gender Dysphoria treatment and induces secondary sex characteristics development of the desired sex while reducing those of the natal sex[1]. Trans women (male-to-female transsexual persons) at our center generally receive cyproterone acetate together with oral or transdermal estrogens to induce feminization[1]. Female-to-male transsexual persons, denoted as trans men, generally receive progestins to suppress menstruation and intramuscular testosterone preparations to induce virilisation[1-2].

It is well-known that sex steroids play a role in motivational aspects of sexual functioning such as sexual desire, particularly in cisgender men[3]. Numerous studies have observed an improvement in sexual desire during T administration in hypogonadal young and aging men[4-6] but not in eugonadal men[7]. Whether T treatment resulting in supraphysiological levels of testosterone increases sexual desire is less clear as one study[8] observed a significant increase whereas another did not[9].

In contrast, our current knowledge about the role of androgens in female sexual desire is still scarce with inconsistent and often contradictory evidence[3]. In epidemiological studies, serum T levels were not correlated with sexual desire in cisgender women[10-11] whereas other studies did observe an association[12-13]. Also, evidence concerning the effects of oral contraceptives on sexual desire, which are known to decrease free T levels[14], are conflicting[15]. Furthermore, several studies[16-17], but not all[18], observed that T supplementation in surgical or natural menopausal women increases sexual desire.

Effects of estrogens on sexual desire in both cisgender men and women are poorly understood[3]. Given that T is aromatized into estradiol (E_2) in many tissues, it may be possible that effects of T on sexual desire are mediated by E_2. Only a few studies examined the relation between sexual desire and circulating E_2 levels in cisgender men but most studies observed no clear associations[19-20]. However, as E_2 is known to exert an important role in the negative feedback of T in men at both the hypothalamic and pituitary level, administration of exogenous estrogens has been used historically as an anti-androgen treatment in prostate cancer patients and sex offenders, where a reduction in sexual desire was observed[3]. In cisgender women, some[21] but not all[22-23] studies, observed associations between E_2 levels and sexual desire. Several studies examining the effects of estrogen therapy in postmenopausal women found an increase in sexual desire[23-24]. However, others reported that high doses of estrogens were associated with lower sexual desire[25-26].

In view of the effects of sex steroids on sexual desire in cisgender men and women, several effects of cross-sex hormone therapy on sexual desire in trans persons can be hypothesized. In trans women, a decline in serum T levels or T action together with increased SHBG and high E_2 levels may lower sexual desire; while in trans men, increasing serum T levels may facilitate sexual desire. However, these theoretical effects may be influenced in trans persons due to prior brain masculinization or feminization. Aside from effects of cross-sex hormone therapy, the experience of breast augmentation and removal, genital surgery and postsurgical outcome clearly affects sexual functioning in trans persons[27-32]. Sex reassignment surgery (SRS) in trans women consists of orchidectomy, penectomy and vaginoplasty. SRS in trans men includes mastectomy, hysterectomy and oophorectomy. Due to availability and extensive experience at our center, most trans men proceed immediately with phalloplasty (creation of a full-sized phallus)[33].

Considering the important potential effects of both hormonal treatment and genital surgery, the current knowledge of the effect of sex reassignment therapy on sexual desire is limited and based on small sample studies. Evidence concerning sexual desire in trans women is conflicting as some studies using direct or indirect measures of sexual desire observed no change[34-35] or decrease[27-28; 36] whereas others observed an increase after sex reassignment treatment[29; 37-38]. In trans men a single study investigated sexual desire directly[39] whereas others used indirect measures such as frequency of masturbation[27, 35-36] and sexual activity[37].

The present study aimed to investigate the effects of sex reassignment therapy on sexual desire in a well-described, large cohort of both trans men and women almost all treated by the same endocrine and surgical team.

METHODS

STUDY PROCEDURES AND POPULATION

All persons who were diagnosed with Gender Dysphoria/ Transsexualism (Diagnostic and Statistical Manual of Mental Disorders- DSM-5/International Classification of Diseases, 10th revision, F64.0) at the Center for Sexology and Gender Problems at the Ghent University Hospital (Ghent, Belgium) between1986 and June 2012 and underwent at least 3 months of cross-sex hormonal therapy were invited by letter to participate in this study. Respondents received a paper version of the questionnaire by post or filled out the survey on-line. A reminder message was sent to non-responders. All surveys were collected between August and December 2012. Three hundred fifty two participants including 214 trans women and 138 trans men agreed to participate in this study resulting in a total response rate of 54% (51% in trans women and 58.7% in trans men). Ten of the invited persons had died by the time of the follow-up (Figure 1).

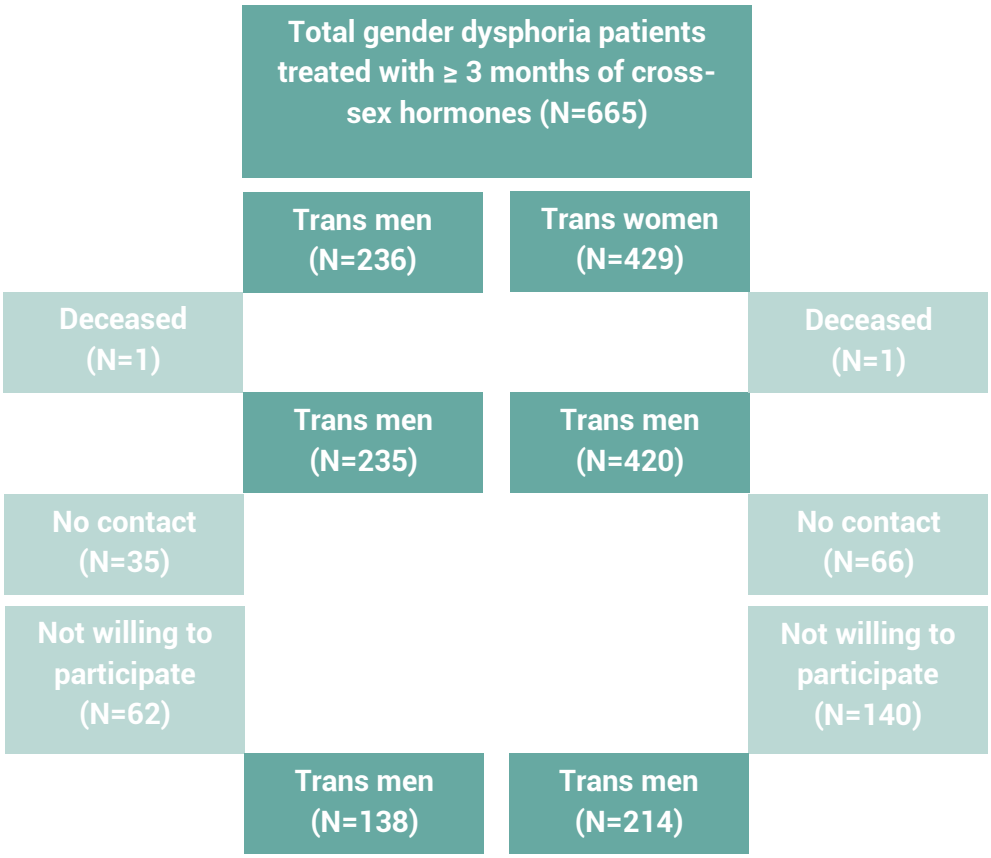

Figure 1. Subject enrollment

This study was part of a multi-disciplinary study in which participants were questioned about their physical health and incidence of possible treatment related adverse events, socio-demographic status, health-related quality of life (QoL), and treatment related symptoms such as changes in sexual desire, surgical results, and satisfaction with hormonal and surgical treatment. Data concerning morbidity and QoL are addressed in other articles[40-41].

The trans women were on hormonal therapy for a median duration of 7 years (range: 3 months - 35 years). One hundred thirty-nine trans women (65%) underwent SRS (orchidectomy, penectomy and vaginoplasty), 60 trans women (28%) had plans for this surgery in the future, 4 (1.8%) were still in doubt. The others (n= 11; 5%) did not wish this surgery or did not undergo it for medical reasons. About half of trans women (53.5%) underwent breast augmentation, 17.8% underwent vocal cord surgery or cricoid reduction and 21.5% had facial feminizing surgery.

Trans men were on testosterone treatment for a median duration of 6 years (range 3 months - 49 years). Eighty-six percent of the trans men underwent hysterectomy/oophorectomy. Seventy-six men (59.4%) had undergone phalloplasty. Nine men were treated with metoidioplasty, 8 of whom subsequently underwent a phalloplasty. Sixty-three percent of those who had undergone a phalloplasty, had an erection prosthesis implanted (n=48).

This study was approved by the ethical review board of Ghent University Hospital, Belgium. All participants gave their consent to participate in the study.

MAIN OUTCOME MEASURES

GENERAL CHARACTERISTICS

Civil status, current working situation, current job and household income were addressed. All participants were asked whether they had children (yes/no) and if so, to describe the method of conception for each child.

SEX REASSIGNMENT TREATMENT

Current and past hormonal treatments were addressed through a self-developed questionnaire. Patients were questioned about the medical procedures they underwent during transition. Satisfaction with different surgical procedures was evaluated by the participants on a 5-point Likert scale from very unsatisfied to very satisfied. Moreover, trans men were asked whether they had experienced surgical complications of metoidioplasty, phalloplasty or implantation of

erection prosthesis. Trans women were asked whether they had experienced surgical complications of the vaginoplasty procedure.

DEFINITION OF HSDD

The definition of Hypoactive Sexual Desire Disorder (HSDD) in this particular patient population is difficult. The renewed definitions of HSDD in women and men that will be used in DSM-5 may not be applicable for trans persons given the important interference of sex reassignment treatment with the potential to experience genital arousal in these patients. Indeed, previous research from our center observed that the female sexual functioning index (FSFI) was not unequivocally suitable for trans women, especially concerning sexual arousal[42].

We evaluated HSDD in trans women as defined by Elaut and colleagues[43] who used the Sexual Function Health Council's consensus definition[44]. They defined HSDD as 'the persistent or recurrent deficiency (or absence) of sexual fantasies, thoughts, desire for sexual activity alone or with a partner and the inability to respond to sexual cues that would be expected to trigger responsive sexual desire. These symptoms must be causing personal distress'.

Sexual desire in trans women was therefore assessed by the evaluation of spontaneous and responsive sexual desire in the past month using a 5-point scale from never to almost always. In addition, respondents were asked about the presence of distress caused by low sexual desire (yes or no) and to describe whether this caused distress for themselves, their partner, and/or their relationship. Respondents met the distress level when they experienced personal or relational distress. HSDD in trans women was scored when a participant indicated that (1) she never/rarely experienced either spontaneous (2) or responsive sexual desire, and when this was (3) causing her personal or relational distress.

HSDD in trans men was defined according to DSM-IV-TR. HSDD was scored when a participant indicated that (1) he never/rarely experienced sexual desire in the past month, and when this was (2) causing him personal or relational distress.

RELATIONAL AND SEXUAL FUNCTIONING

Participants also reported on the following items: marital status, duration of relationship, current sexual orientation (5 point scale from only attracted to men to only attracted to women or other), comparison of sexual desire before and after SRS (5-point scale from much higher to much lower), frequency of experiencing too low sexual desire in the past month (5-point scale

from never to almost always, experience of symptoms of high sexual desire, experience of symptoms of low sexual desire (4-point scale from no to severe).

PHYSICAL AND MENTAL FUNCTIONING

Quality of life (QOL) was measured using the Dutch version of the Short Form-36 Health Survey (SF-12). This questionnaire includes 12 questions with fixed response choices, organized in 2 scaled scores, based on the weighted sums of the questions in their section. These scores were converted into a summary score for each section: physical functioning and mental functioning, with higher scores indicating higher levels of functioning or well-being [45]. Internal consistency with the SF-12 was high (total group: Cronbach's $\alpha = 0.8$).

STATISTICAL ANALYSIS

The normal distribution of all variables was tested by the Kolmogorov-Smirnov one-sample test. Normally distributed variables were described in terms of mean and standard deviation (SD) and skewed variables in terms of median, first and third quartiles. Comparisons of continuous variables between trans women and men were made by linear regression analyses with group as independent variable. Dichotomous and categorical variables were analyzed using respectively logistic regression and Chi Square test. Data were analyzed using PASW-software, v.19 (SPSS Inc., Chicago, IL). Statistical significance was set at $P < 0.05$ and all tests were two tailed. Given the well-known association between age and sexual desire, linear and logistic regression analyses were adjusted for age.

RESULTS

PATIENT CHARACTERISTICS

Patient characteristics are summarized in Table 1.

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY POPULATION

	TRANS WOMEN (N=214)	TRANS MEN (N=138)	P
Age at time of study (years)	45 [32.8-52]	37.5 ± 11.0	<0.001
Nationality (%)			
Belgian	86.0	87.0	NS
Other	14.0	12.5	
Civil status (%)			<0.001
Married/living together	36.0	44.5	
Not married or living together	36.4	47.4	
Divorced	25.2	8.0	
Widow	2.3	0	
Children (%)	41.1	23.9	<0.001
Birth before HRT (%)	81.8	36	<0.001
Work status (%)			0.001
Unemployed	14.2	9.4	
Employed	55.9	64.5	
Retired	9.5	1.5	
Student	5.2	13.0	
Unable to work	13.7	9.4	
Household	1.4	2.2	
Monthly income (%)			NS
≤999 euro	10.3	14.2	
1.000-1.999 euro	42.2	35.8	
2.000-2.999 euro	27.5	24.6	
3.000-5.999 euro	16.7	23.9	
≥ 6.000 euro	3.4	1.5	
SRS (%)	64.8	85.5	<0.001
Time since SRS (years)	6.0 [2-11]	7.0 [4-13]	NS
Duration of hormonal therapy (years)	6.0 [3-11]	7.0 [4-13]	0.041
Active smoking (%)	29.9	29.4	NS
BMI	24.4 [21.7-27.9]	24.3 [22-27.5]	NS

Data are presented as % or median (first to third quartiles). Categorical variables using Chi square test; Continuous variables using linear regression analysis. NS: not significant; SRS: sex reassignment surgery (defined as orchidectomy/penectomy/ vaginoplasty in trans women and hysterectomy/ovariectomy in trans men)

Ten trans women were no longer on estrogen therapy due to previous thromboembolic events (N=5), dissatisfaction (N=2) or another cause (N=3). Current hormonal treatment in trans women mostly consisted of transdermal estradiol [17-β estradiol gel 1.5 mg/24u (n= 76; 35.5%); estradiol patch 50 μg/24u (n=29;13.6%); or daily intake of oral estrogens; estradiol valerate 2 mg (n=91; 42.5%), estriol 2 mg (n=1; 0.4%)), ethinyl estradiol 50 μg (n=2; 0.9%), and oral contraceptive ethinyl estradiol 30-50μg (n=5; 2.3%)]. Cross-sex hormonal therapy in trans men consisted of intramuscular testosterone treatment with either a mixture of testosterone esters (testosterone decanoate 100 mg, testosterone isocaproate 60 mg, testosterone fenylpropionate 60 mg, testosterone propionate 30 mg/ml) every 2 or 3 weeks (n=64; 46.4%); testosterone undecanoate 1000mg per 12 weeks (n=62; 44.9%); transdermal testosterone 50 mg daily (n=9; 6.5%); or oral testosterone undecanoate (n=2; 1.4%).

SEXUAL DESIRE IN TRANS WOMEN

Sexual desire and HSDD in trans women

The majority of trans women (83.4%) never or rarely experienced spontaneous sexual desire; whereas 6.5% often or always experienced spontaneous sexual desire. Almost seventy-six percent (75.8%) never or rarely experienced responsive sexual desire. Fourteen percent experienced responsive sexual desire from time to time, 10.1% often or always. Seventy-three percent never or rarely experienced either spontaneous or responsive sexual desire. About one in three trans women, who never or rarely experienced either spontaneous or responsive sexual desire, indicated to be distressed by this, personally or within the relationship, resulting in a prevalence of HSDD of 22.1%. (Figure 2). However, the vast majority of trans women did not experience this lack or absence of sexual desire as distressing.

Of the trans women who reported to experience spontaneous sexual desire often or always (6.5%), only one reported associated personal or relational distress.

One fourth of trans women (24.2%) often or always experienced too low sexual desire (Table 2). In retrospect, the majority of trans women (69.7%) described their current sexual desire lower or much lower as compared to before sex reassignment therapy. In contrast, 17.4% reported no change in sexual desire and 13% of participants described an increase in sexual desire (Figure 3).

TABLE 2. SEXUAL DESIRE IN TRANS PERSONS

		TRANS WOMEN (N=214)	TRANS MEN (N=138)	P
Current in relationship (%)	yes	47.2	62.3	<0.001a
	no	52.8	37.7	
Duration of relationship (%)	< 6 months	7.1	4.7	NS b
	6-12 months	4.0	5.8	
	1-2 years	14.1	14.0	
	2-5 years	16.2	19.8	
	> 5 years	58.6	55.8	
Sexual orientation (%)	(mainly) attracted to males	36.0	10.2	<0.001b
	bisexual	11.2	6.5	
	(mainly) attracted to females	47.7	81.8	
	Other	5.1	1.4	
Current sexual desire (%) compared to sexual desire before sex reassignment treatment	Much higher	5.5	31.3	<0.001b
	Higher	7.5	39.7	
	Equally	17.4	16.8	
	Lower	26.4	9.2	
	Much lower	43.3	3.1	
Frequency of experience of too low sexual desire (%)	Almost never or never	46.0	74.0	<0.001b
	Rarely	19.7	15.3	
	From time to time	10.1	5.3	
	Often	10.1	4.6	
	Almost always or always	14.1	0.8	
Experience of symptoms of low sexual desire (%)	No	40.8	81.6	<0.001b
	Mild	16.8	13.2	
	Moderate	15.2	4.4	
	Severe	27.2	0.9	<0.001b
Experience of symptoms of high sexual desire (%)	No	82.3	31.6	
	Mild	9.2	35.1	
	Moderate	4.9	22.8	
	Severe	3.8	10.5	
Hypoactive sexual desire disorder (%)	Yes	22.2	5.0	<0.001a
	No	77.8	95.0	

Data are expressed as %; logistic regression analyses are adjusted for age; NS: not significant
[a] Logistic regression analysis; [b] Chi Square Test

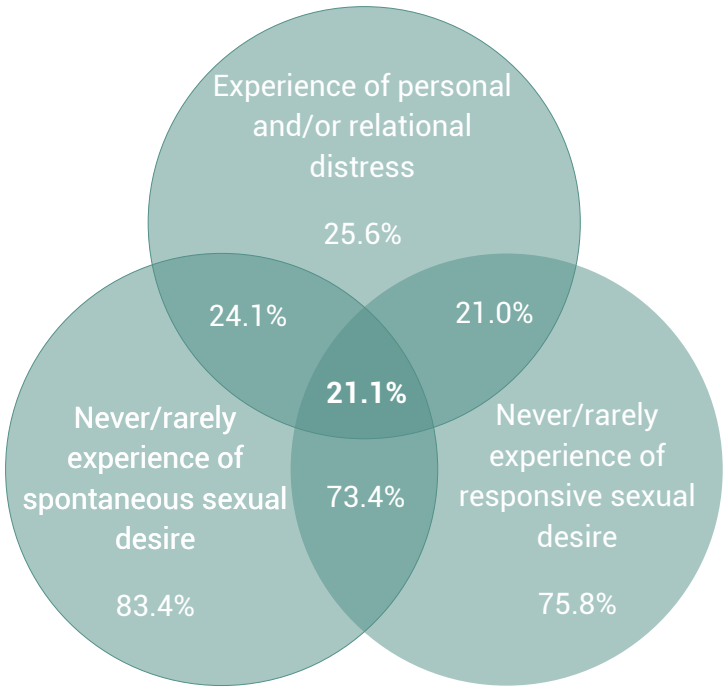

Figure 3. Prevalence of HSDD in trans women

Associations between sexual desire and sex reassignment treatment in trans women

Cross-sex hormonal therapy

Type of hormonal therapy, duration of hormonal therapy and satisfaction with hormonal therapy were not associated with frequency of spontaneous or responsive sexual desire or prevalence of HSDD (data not shown).

Sex reassignment surgery

Respondents who already had undergone a vaginoplasty, experienced more spontaneous sexual desire (P=0.002) compared to those who were still scheduled for this surgery. Satisfaction with genital reassignment surgery and experience of complications of vaginoplasty were not associated with sexual desire scores (data not shown).

Sexual orientation and relationship

Involvement in a romantic relationship or relationship duration were not related to frequency of spontaneous or responsive sexual desire (data not shown) but respondents involved in a relationship had a higher prevalence of HSDD ($P<0.001$). Trans women (mostly) attracted to men had higher levels of spontaneous and responsive sexual desire compared to trans women (mostly) attracted to women ($P=0.008$ and $P=0.009$, respectively) (Figure 4A and 4B) but sexual orientation was not associated with prevalence of HSDD (data not shown).

General characteristics

Age was negatively associated with frequency of spontaneous and responsive sexual desire ($P=0.008$ and $P<0.001$, respectively). Employment status, having children, physical and mental well-being were not associated with frequency of sexual desire or prevalence of HSDD (data not shown).

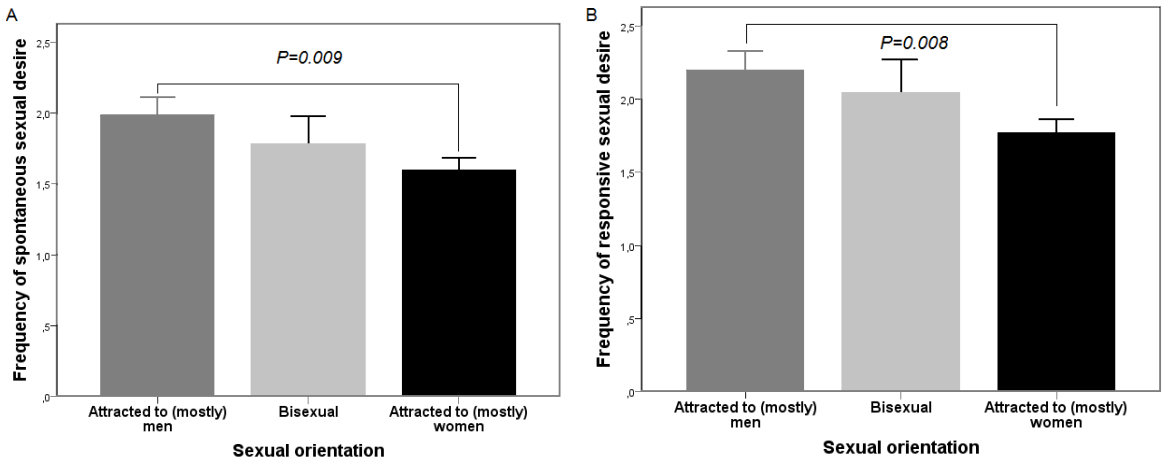

Figure 4. Frequency of sexual desire according to sexual orientation in trans women/ Bars represent mean and whiskers 2 standard error of mean. *P* value result from post-hoc ANOVA.

SEXUAL DESIRE IN TRANS MEN

Sexual desire and HSDD in trans men

Forty percent of trans men reported experiencing sexual desire from time to time whereas 30.6% often or always experienced this. Thirty percent of trans men never or rarely experienced sexual desire and about one in six trans men in our sample indicated to be distressed by this, personally or within the relationship, resulting in a prevalence of 5.0% of HSDD in our sample of trans men.

Of the trans men who reported experiencing sexual desire often or always (30.6%), a majority reported no associated distress (76.9%), 12.8% reported personal or relational distress and 10.3% expressed that this caused no personal distress but exclusively stress for their partners. Of the total sample, 3.6% of trans men often or always experienced sexual desire and reported this caused personal or relational distress.

In retrospect, the majority of trans men (71.0%) reported an increase in sexual desire after sex reassignment treatment, whereas 12.3% reported a decrease. Almost 17% experienced no change in sexual desire.

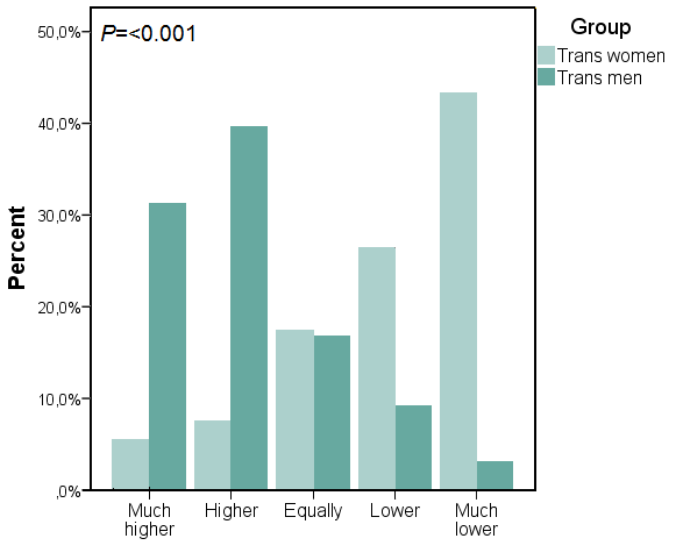

Figure 3. Current sexual desire in trans men and women compared to sexual desire before sex reassignment treatment

Associations between sexual desire and sex reassignment treatment in trans men

Cross-sex hormonal therapy

Shorter duration of testosterone treatment was significantly associated with more symptoms of high sexual desire (P=0.005). We observed no associations between type of hormonal therapy and satisfaction with HRT and sexual desire scores (data not shown).

Sex reassignment surgery

Whether or not trans men had undergone phalloplasty surgery and/or the implantation of an erection prosthesis did not affect sexual desire scores. In addition, time since genital surgery, satisfaction with genital reassignment surgery and experiences of complications of phalloplasty and/or erection prosthesis were not associated with frequency of experiencing sexual desire (data

not shown). However, HSDD was more prevalent in trans men who were less satisfied with their phalloplasty (Fisher exact; P=0.02).

Sexual orientation and relationship

Shorter relationship duration was associated with a higher frequency of experiencing sexual desire (P=0.03). No associations were found between sexual orientation, involvement in a relationship and frequency of sexual desire and HSDD in trans men (data not shown).

General characteristics

No association was observed between age and frequency of sexual desire (data not shown) in trans men. Having children, physical and mental well-being were not associated with frequency of sexual desire or prevalence of HSDD (data not shown). Unemployed trans men had a lower frequency of sexual desire (P=0.015).

SEXUAL DESIRE IN TOTAL TRANSSEXUAL SAMPLE

Comparison between trans women and trans men

A comparison between trans women and trans men on relational and sexual desire parameters are shown in Table 2. Trans men were more frequently involved in a romantic relationship compared to trans women. More symptoms of high sexual desire were observed in trans men, whereas trans women reported more symptoms of low sexual desire. HSDD was more prevalent in trans women compared to trans men (P<0.001). We observed no difference in satisfaction with surgical reassignment of genitalia between trans men and women (data not shown).

Associations between sexual desire and sex reassignment treatment in total transsexual sample

Cross-sex hormonal therapy

Dissatisfaction with hormonal therapy was associated with a higher prevalence of HSDD (P=0.02) (Figure 5). No association was observed between sexual desire scores and duration of hormonal therapy in the total trans sample (data not shown).

Sex reassignment surgery

We observed no association between sexual desire scores and satisfaction with surgical reassignment of genitalia in the total transsexual sample.

Relationship

Trans persons involved in a romantic relationship had higher levels of sexual desire (P=0.02) and also had a higher prevalence of HSDD (P=0.001).

General characteristics

Age was negatively associated with frequency of sexual desire (respectively: P<0.001). Employment status, having children, physical and mental well-being were not associated with frequency of sexual desire or prevalence of HSDD (data not shown).

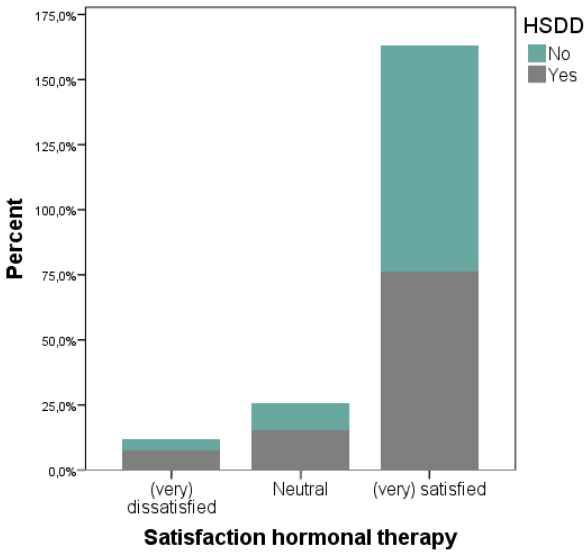

Figure 5. Hormonal satisfaction in trans men and women and prevalence of HSDD / Bars represent percentage. We observed a higher ratio of trans persons experiencing HSDD in trans persons who are dissatisfied with the hormonal therapy.

DISCUSSION

The present study described the sexual desire frequency (and prevalence of HSDD) and associations between sex reassignment treatment and sexual desire in a large, well-described group of both trans women and men. We observed that almost three quarters of trans women rarely or never experienced spontaneous and responsive sexual desire. About a third of these women with low sexual desire reported associated distress and met criteria for HSDD, a ratio also reported by studies in cisgender women[46-47]. We observed a lower prevalence of HSDD in trans women (22%) compared to a previous multi-center study[43] in 62 trans women (33%), possibly related to methodological differences. Trans women in our study had a higher prevalence of HSDD compared to male and female population studies using a similar definition, taking distress levels into account, as they found that between 0.5 and 6% of men and 3 to 14.2% of women reported HSDD[48-52]. In trans men, a similar prevalence of HSDD was found (5.0%) compared to the general male population (range: 0.5-6%)[48-50, 52].

Trans women had a higher prevalence of HSDD and described more often a decrease in sexual desire after sex reassignment therapy compared to trans men. Sexual desire is a multifaceted process, resulting from the triggering of a sexual response system with a specific arousability based on genetics, the presence of sex steroids and neurotransmitters as well as by lifelong psychosocial learning experiences. Those sexual stimuli can be both internal (such as sexual thoughts) or external cues (sensory stimuli experienced as erotic)[53]. Since trans women have mostly grown up with a highly testosterone dependent sexual response system and have often had sexual experiences in the male gender role, it requires a certain 'reconditioning' of the sexual response system within a less androgenic hormonal milieu. The fact that two in three trans women in our group did *not* experience this lack or absence of sexual desire as distressing, points to the presence of factors that might be compensating for the loss of the 'androgen-driven sexuality'. This might consist of factors the current study could not assess, such as an increasingly positive self-image due to the transition and a boosted self-esteem from being recognized as female.

Regarding determinants of sexual desire, it was found that trans women attracted to women experienced lower levels of sexual desire. These findings support previous results from Weyers and colleagues[42]. Interestingly, no differences in distress levels were observed between trans women attracted to men or attracted to women suggesting that trans women attracted to women may be less interested in sexual activity or are less open to sexually adequate stimuli compared to those attracted to men. Indeed, it has already been observed[42] that trans women attracted to

women attributed the lowest importance to sex in comparison with the others. These results may also corroborate with observations in lesbian women, as the latter had lower levels of sexual desire compared to heterosexual women[54]. Similar to studies in the general population, it was found that respondents involved in a partner relationship had higher distress levels[46] resulting in a higher prevalence of HSDD.

Concerning the associations between cross-sex therapy and sexual desire, it was observed that trans women who had undergone vaginoplasty experienced higher levels of sexual desire compared to those who were scheduled to undergo this surgery. It is likely that the relief of gender dysphoria due to a body image more congruent with the gender identity has positive effects on sexual functioning or the other way around that the presence of male genitalia has negative effects on sexual functioning. As a result, trans women may experience more satisfying sexual relationships after genital surgery. In contrast, no associations were found between type of surgery and sexual desire scores in trans men, but satisfaction with phalloplasty was negatively associated with prevalence of HSDD. Interestingly, no associations were observed between surgical satisfaction and sexual desire scores in trans women.

These different associations between trans women and trans men are not well-understood. One explanation could be that trans men attribute a higher degree of importance to functionality of their newly-formed genitalia compared to trans women. Many underlying factors may contribute to these findings such as differences between functional and esthetical surgical satisfaction, body image, self-esteem etc. This is a point for further exploration and study.

Hormonal therapy satisfaction was related to a lower prevalence of HSDD in the total transsexual sample. Overall the results underline the importance of different surgical interventions and treatment satisfaction on sexual desire in trans persons. In earlier research in the same cohort, we observed that both hormonal and surgical satisfaction were strongly related to mental and physical well-being[40]. The current results underline the importance of high quality sex reassignment treatment on the sexual well-being of trans persons.

As to the limitations of this study, we had a modest response rate (54%) and selection bias cannot be excluded. As in most follow-up studies, respondents who participated in this study may have experienced a more favourable outcome than those who refused. Secondly, our observations on changes in sexual desire after sex reassignment treatment in both trans men and women are based on retrospective data, and should be interpreted with caution; prospective studies are needed to confirm our results. Thirdly, the aim of this study was to investigate the

associations between sex reassignment treatment and sexual desire. However, we are aware that the subjective experience of sexual desire for an individual results from a complex interaction between the individuals' sexual response system and adequate sexual stimuli and consequently can be affected by many biological, psychological, social, sexual and relational factors[53], which were not all addressed in the current study. Also, gender differences in intrinsic and extrinsic factors affecting sexual desire have led to another conceptualization of sexual desire in men and women[55]. Given these gender differences in sexual desire, distinct definitions of HSDD in men and women have been proposed[56-57]. We defined HSDD in trans women and trans men according to the definition of the desired gender, due to changes in both hormonal environment and/or genital anatomy and function in combination with transitioning to the desired gender role. However, this approach has certain limitations considering the presence of male or female genetics, gender differences in imprinting of brain systems, and lifelong psychosocial learning experiences in the natal gender role. In addition, because of surgical availability and expertise, and a well-organized health insurance system, allowing coverage for sex reassignment surgery, the majority of trans persons at our center who receive cross-sex hormone therapy undergo sex reassignment surgery. Also, postoperative sexual functioning is highly dependent on the quality of, and satisfaction with surgery[34] and this may differ from center to center. These factors may hamper the generalizabililty of our results.

CONCLUSION

Trans women had a higher prevalence of HSDD compared to what has been reported in previous research for cisgender women, while trans men had similar or lower prevalence rates of HSDD in comparison with cisgender men. Most trans women reported a decrease in sexual desire after sex reassignment treatment whereas the opposite was observed in trans men. Our results point out a significant sexual impact of surgical procedures and both hormonal and surgical treatment satisfaction on the sexual desire in trans persons.

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CATEGORY 3

(a) Final Approval of the Completed article

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3.2 SEXUAL DESIRE IN FEMALE-TO-MALE TRANSSEXUAL PERSONS: AN EXPLORATION OF THE ROLE OF T REPLACEMENT.

Wierckx K, Elaut E, Van Caenegem E, Van de peer F, Dedecker D, Vanhoudenhove E, T'Sjoen G

European Journal of Endocrinology 2011; 165:331–337

ABSTRACT

OBJECTIVE

To describe sexual desire in female-to-male transsexual persons post sex reassignment surgery (SRS). The associations between serum androgen levels and sexual desire are examined.

DESIGN

Single center cross-sectional study.

METHODS

Forty-five female-to-male transsexual persons post SRS completed a standardized questionnaire assessing sexual desire (Sexual Desire Inventory). In addition, participants were asked questions on sexual desire before starting hormone treatment and having SRS. Serum levels of testosterone, LH and sex hormone-binding globulin were measured on fasting morning serum samples.

RESULTS

In retrospect, 73.9% of the participants reported an increase in sexual desire after hormone treatment and SRS. Solitary sexual desire scores were significantly correlated with frequency of masturbation ($r=0.835$; $P<0.001$), whereas frequency of sexual intercourse with a partner was not. No direct associations were found between testosterone and solitary or dyadic sexual desire. However, ANOVA showed an independent effect of LH on solitary sexual desire ($P<0.001$). Post hoc analysis revealed that female-to-male transsexual persons with elevated levels of LH, indicating suboptimal testosterone therapy, reported significantly lower solitary sexual desire levels (than those with low LH levels; $P=0.007$). Suppressed LH levels were also associated with having a higher need for sexual activities ($P=0.009$) and a higher frequency of excessive sexual desire ($P=0.007$).

CONCLUSION

Most female-to-male transsexual persons report on a marked increase in sexual desire after testosterone treatment and SRS. No direct associations between levels of testosterone and solitary or dyadic sexual desire were found. However, measures of sexual desire were inversely associated with LH levels.

INTRODUCTION

It is now widely believed that testosterone (T) plays an important role in motivational aspects of sexual functioning as sexual desire[1]. Testosterone therapy seems to enhance the frequency of sexual thoughts and the intensity of sexual feelings, especially in young hypogonadal men[2-3]. During the process of sex reassignment, transsexual persons receive cross-sex hormone replacement which transforms their hormonal milieu. After sex reassignment surgery (SRS), which often includes gonadectomy, cross-sex hormone treatment is continued as in other hypogonadal subjects. In view of a possible impact of T on sexual desire, it has been suggested that the effect of continuous T administration in female-to-male transsexual persons might lead to an increase in sexual desire[4]. Also, care givers working in treatment centers for gender dysphoria sometimes encounter complaints of low sexual desire in male-to-female transsexual persons while this is hardly the case in female-to-male transsexual persons. A previous study from our group found that hypoactive sexual desire was reported in one-third of transsexual women. While levels of free T in the group of male-to-female transsexual persons were markedly lower, the intensity and frequency of sexual desire was very similar to control women not using hormonal contraception. These findings argue against a major role of T in the sexual desire in male-to-female transsexuals[5]. In a pilot study, Kronawitter et al.[6] treated hypoactive sexual desire in male-to-female transsexual persons with a T patch. A significant improvement in sexual desire (as measured by the Brief Profile of Female Sexual Function (B-PFSF) was observed.

Our knowledge on sexual desire in female-to-male transsexual persons is even more limited since data from validated questionnaires on sexual desire in female-to-male transsexual persons are non-existent[4]. The available data can only be interpreted as an indication of sexual desire through measurement of the frequency of sexual activity or frequency of masturbation before and after SRS. In their sample of adult female-to-male transsexual persons, De Cuypere et al.[7] generally found an increase in frequency of masturbation after T treatment and SRS. Similarly, Lief and Hubschmann[8] mentioned an increase in sexual activity following SRS. In their adolescent female-to-male sample, Smith et al.[9] and Cohen-Kettenis and van Goozen[10] reported no change or an increase in masturbation after T treatment and SRS. The present study aimed at providing a validated measure of sexual desire in this population[11]. Additionally, we hypothesized a marked association between serum androgen levels and levels of sexual desire in female-to-male transsexual persons.

MATERIAL AND METHODS

STUDY POPULATION

All Dutch speaking female-to-male transsexual persons who received SRS between 1987 and 2009 at our hospital were invited by letter (n=79), in which they were asked to confirm their participation by telephone or electronic mail. Two participants could not be reached due to change of address. Those who had not replied after one month were contacted by telephone or were left a voice message as a reminder. If necessary, potential participants were contacted a second time. A total number of 47 persons agreed to participate in the study, consisting of one day hospital visit, which resulted in a response rate of 64%. Three participants, were informed by others and offered to participate in the study themselves, resulting in a study sample of 50.

Exclusion criteria included treatments or disorders affecting sex hormone status, body composition and bone metabolism: untreated hypo- or hyperthyroidism, Cushing syndrome, alcohol abuse, mucovisidosis, malabsorption or eating disorders, cirrhosis, chronic kidney failure, auto-immune rheumatoid arthritis, the current (<2 years) or prolonged use of corticosteroids, estrogens, anti-androgens, bisphosphonates, calcitonine and fluorides. The first year after SRS is often called the honeymoon period; a period which does not represent a realistic picture of long-term sexual and psychological status. Participants who underwent SRS during the last year, were therefore excluded. One participant had to be excluded based on these criteria. Further, four participants were not willing to complete the Sexual Desire Inventory, resulting in a final study population of 45 for the current paper.

In our center, female-to-male transsexual persons are treated through a multidisciplinary approach consisting of hormone therapy and SRS for most. SRS in female-to-male transsexual persons includes mastectomy, hysterectomy and bilateral ovariectomy. Due to the extensive experience in phalloplasty at our center[12-13], most female-to-male transsexual persons in this study (n=38) immediately preceeded with a phalloplasty and less frequently, with metaidoioplasty. The majority of participants who initially chose for metaidoioplasty (n=9) preceeded towards phalloplasty afterwards (n=8). Two female-to-male transsexual persons had not yet made up their minds about having further genital surgery. All participants started hormonal therapy at least two years before SRS. On average, participants were 8 years after SRS, with a minimum of 2 and a maximum of 22 years. All participants were on long-term T therapy. While current cross-sex hormone therapy was not standardized, almost all participants were treated by the same endocrinologist. Cross-sex hormone therapy consisted of: intramuscular T

treatment (parental T esters 250 mg/2 or 3 weeks; n=32); T undecanoate 1000 mg/12 weeks (n=7) and transdermal T gel (50mg daily; n=5). One participant used both oral T undecanoate 40 mg (daily) and transdermal T gel 50 mg daily.

We chose not to include community dwelling men, women or male-to-female transsexual persons as a control group since hormonal status, psychosocial factors and sexual functioning are very specific in this study population.

To contextualize the levels of sexual desire in the present sample, we will use previous data of our research group on sexual desire in community dwelling men[14] and male-to-female transsexual persons[5] using the same validated questionnaire. The data on sexual desire in community dwelling men consist of baseline measures collected during a study in 55 heterosexual couples in which the effect of three different forms of hormonal contraception on female sexual desire was assessed[14]; data from the male-to-female transsexual persons were collected during a previous study of our group on sexual desire and the association with T[5].

STUDY PROCEDURES

Female-to-male transsexual persons, who agreed to participate in the study, received all questionnaires by regular mail. Subsequently, they visited the Ghent University Hospital between November 2009 and April 2010 for further evaluation. The visit included a fasting morning blood sample, dermatologic, urological, speech, bone and body composition evaluations; data that will be reported in other publications[15].

This study complied with the recommendations of The declaration of Helsinki and was approved by the Ethical committee of the Ghent University Hospital. Informed consent was obtained from all participants.

MEASURES

Sexual desire was measured using the Dutch version of the Sexual Desire Inventory[11]. This self-report questionnaire contains 14 items. Subscales measure the intensity and frequency of the desire to behave sexually with a partner (dyadic sexual desire) or by oneself (solitary sexual desire). For the frequency-items, participants chose one out of seven options. For the strength items, participants scored their sexual desire on a 9-point Likert scale ranging from 0 (no desire) to 8 (strong desire). The participants were asked to take the previous month as a reference. Adding items resulted in a score for dyadic and solitary sexual desire. Higher scores indicate a higher level of sexual desire with a maximum score of 62 for the dyadic subscale and 23 for the

solitary subscale. The Sexual Desire Inventory has a good reliability and validity[11]. Internal consistency in the present study population was high (Cronbach's α=0.89).

Other items concerning sexual desire and sexual functioning in the past month were added: frequency of experiencing sexual desire, frequency of experiencing sexual desire towards their partner, frequency of sexual activities, frequency of masturbation (5 point Likert-scale from not at all to daily), current sexual desire compared to after sex reassignment (5 point Likert-scale from much higher to much lower), frequency of experiencing excessive sexual desire (5 point Likert-scale from almost never to almost always), time one can live without any sexual activities (9 point Likert-scale from always to less than a day), sexual satisfaction with the current partner (5-point Likert scale from very unsatisfied to very satisfied, or not applicable).

BIOCHEMICAL DETERMINATIONS

Venous blood samples were obtained between 0800 and 1200 h after overnight fasting, due to practical reasons regardless of the timing of T administration. All blood samples were stored at -80 °C until batch analysis. Commercial kits for Radio-Immuno Assay (RIA) were used to determine the serum concentrations of total T and sex hormone binding globuline (SHBG) (Orion Diagnostica, Espoo, Finland); Luteinizing hormone (LH) (electrochemiluminiscence immunoassay (ECLIA); Modular, Roche Diagnostics, Mannheim, Germany. Intra- and interassay coefficients of variations were less than 10 en 15% for all measurements respectively. For all measurements, samples from female-to-male transsexual persons were assayed in a same assay run. Serum free T was calculated from the total serum hormone concentration, serum SHBG and serum albumin, using a validated equation derived from the mass action law[15]. We defined supra- and subphysiological levels of T and LH as hormone levels exceeding the upper or lower limit of the reference ranges according to values of our local laboratory.

STATISTICAL ANALYSIS

The normal distribution of all variables was tested by the Kolmogorov-Smirnov one-sample test. Normally distributed variables were described in terms of mean and standard deviation and skewed variables in terms of median, first and third quartiles. Kruskall Wallis tests were used to determine differences in the hormone levels according to type of T treatment and to determine differences in sexual desire between female-to-male transsexual persons, male-to-female transsexual persons and community dwelling men. ANOVA was used to explore associations between hormonal levels (T, free T and LH) and measures of sexual desire. Post hoc analyses were performed by LSD tests. Mann Whitney U tests were used to test differences in sexual

desire between participants with T levels above the P75 and below P25, participants with supra or sub physiological levels of T and the rest of the group.

All hypothesis tests were two-sided. The level indicating statistical significance was 0.05. Internal consistency within a set of items was assessed through Cronbach's alpha metric. Statistical analyses were performed using PASW (version 18, 0; SPSS Inc., Chicago, IL).

RESULTS

PATIENT CHARACTERISTICS

Patient characteristics are summarized in Table 1.

Twelve participants reported one or more chronic disease(s): autoimmune hypothyroidism (n=3), hypercholesterolemia (n=2), obesity (n=2), hypertension (n=3), liver function problems (n=2), epilepsy, migraine, colitis ulcerosa, psoriasis, Graves' disease, chronic fatigue syndrome, unspecified disease (n=1).

TABLE 1. PATIENT CHARACTERISTICS

	MEAN (S.D.)	RANGE
Age (years)	37 (8.17)	22-54
Age at time of SRS (years)	30 (7.68)	16-44
Height (cm)	165.11 (6.73)	147.4-183.9
Weight (kg)	69.17 (12.04)	45.0-98.5
BMI (kg/m²)	25.33 (3.88)	18.3-34.0
Active smoking (%)	37	28
Stopped smoking (%)	34.8	
Use of testosterone therapy (%)	100	
Duration of Ttherapy (years)	9.42 (5.82)	3.0-27.0

SEXUAL DESIRE AND SEXUAL FUNCTIONING

All but four participants completed the Sexual Desire Inventory. In two questionnaires one item was missing, we inserted there the personal mean of the other items of this subscale. The majority of the participants mentioned an increase in sexual desire after SRS: 38.6% (n=17) described the current sexual desire as much higher; 34.1% (n=15) as higher. In contrast, 25.0% (n=11) described no change in sexual desire after SRS and one participant mentioned a decrease in sexual desire. One participant was not willing to answer this question. Most participants masturbated after SRS. Sixty percent expressed to masturbate weekly or daily. A quarter expressed to masturbate once or twice a month. A minority masturbated less than monthly (4.4%) or not at all (11.1%). Frequency of masturbation was strongly correlated with solitary

(r=0.803; *P*<0.001) and dyadic sexual desire (r=0.466; *P*=0.001). Up to one third (32.1%) of the participants currently in a relationship reported to have sexual intercourse several times a week. Almost half of the participants (48.1%) reported to have sexual intercourse once or twice a month. Twenty-two percent of the participants were not sexually active with their partner in the past month. Frequency of sexual intercourse was not correlated with either dyadic (r=0.201; *P*=0.315) nor solitary sexual desire (r=0.204; *P*=0.307). Sixty-four percent of the participants currently in a relationship expressed their satisfaction with their sexual life, 18% remained neutral, whereas 18% expressed to be (very) unsatisfied. Comparison of female-to-male, male-to-female transsexual persons and community dwelling men shows a difference in dyadic sexual desire (Kruskal-Wallis test; *P*<0.001) but not in solitary sexual desire (Kruskal-Wallis test; *P*=0.074). Further analysis shows that female-to-male transsexual persons score significantly higher on solitary and dyadic sexual desire compared to male-to-female transsexual persons but score similarly as community dwelling men.

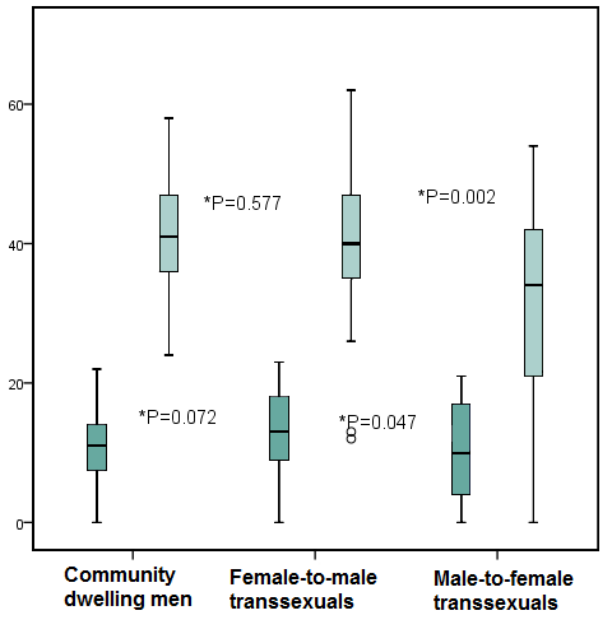

Figure 1. Sexual desire scores. Dark green square: dyadic sexual desire; Light green square: solitarysexual desire. * Mann-Whitney U test between female-to-male transsexuals and community dwelling men or male-to-female transsexuals

HORMONAL DATA

As expected, the time between the last T administration and the date of visit was significantly different according to the type of T replacement therapy ($P < 0.001$). Consequently further ANOVA analyses were corrected for this parameter. We could not detect a statistical difference in the levels of total T, calculated free T, LH and hematocrit between groups based on the type of T replacement therapy (Table 2).

Testosterone treatment in female-to-male transsexual persons aims at T concentrations in the normal physiological T range for men. At the time of measurement, almost nine percent (8.9%) had levels below the reference values of our laboratory (T < 321 ng/dl), whereas 26.7% exceeded the upper limit of 1005 ng/dl. All participants with T values below 321 ng/dl were treated with classical T esters. No difference was observed in type of T therapy in participants with T above the upper limit.

TABLE 2. SERUM HORMONE LEVELS ACCORDING TO THE TYPE OF TESTOSTERONE REPLACEMENT THERAPY

	T undecanoate 1000 mg N=7	Parental Testers 250 mg N=32	Transdermal T gel N=6	P*	Reference values **
T (ng/dl)					
Mdn	878.9	549.75	807.7	0.11	321-1005
IQR	527.1-1161.0	393.9-1033.2	574.4-1405.5		
Free T (ng/dl)					
Mdn	20.1	13.5	20.7	0.11	6-25
IQR	13.8-30.2	8.1-25.5	15.7-38.2		
SHBG (nmol/l)					
M	31.0	31.0	30.8	0.91	16-61
SD	8.9	11.9	11.8		
LH (U/L)					
Mdn	6.8	3.5	20.5	0.50	1-9
IQR	0.1-17.5	0.44-28.4	0.4-49.1		
Hematocrit (%)					
M	48.5	49.0	46.1	0.078	39.8-52.2
SD	2.2	2.8	2.4		

Note. IQR: interquartile range; M: mean; Mdn: median; SD: standard deviation
To convert T and FT to nanomoles per liter, multiply by 0.0347.
*Kruskall-Wallis test ** Reference values based on males values at local laboratory

ASSOCIATION BETWEEN SEXUAL DESIRE AND HORMONAL LEVELS.

No associations were found between levels of free T and solitary or dyadic sexual desire using ANOVA after correction for the type of T replacement therapy and time since last T administration (Testosterone undecanoate 1000 mg: P=0.885; Parental testosterone esters 250mg: P=0.316; Transdermal testosterone gel: P= 0.709).

Also, no significant associations were observed between total T and solitary or dyadic sexual desire (data not shown). We found no differences in measures of solitary or dyadic sexual desire between participants with total or free T levels below P25 and higher than P75 (Mann Whitney U test; TT: *P*=0.30; *P*=0.60 FT: *P*=0.24; *P*=0.40). Also, no differences were observed in measures of sexual desire between participants with supra or sub physiological T levels in comparison with T levels within the normal range (data not shown).

Supraphysiological LH levels are assumed to reflect inadequate hormone therapy while suppressed levels indicate an excess of sex hormone substitution. Hence, we explored the associations between sexual desire scores and LH by ANOVA: this shows an independent association of LH on solitary and dyadic sexual desire after adjustment for age and weight (*P*< 0.001; *P*=0.024 respectively).

Post hoc analysis showed that female-to-male transsexual persons with low LH levels have significantly higher levels of solitary sexual desire than participants with higher LH levels (Figure 2). Participants with LH levels above P75 reported a significantly lower solitary sexual desire than those with LH levels below P25, but a similar level to those with LH levels within the P25-P75 range. Post hoc analysis for dyadic sexual desire demonstrated only a significant difference between those with LH levels above P75 and P50-75 (*P*=0.046*)* and below P25 and P50-75 (*P*=0.011).

LH levels were also inversely associated with a higher frequency of experiencing excessive sexual desire (ANOVA; *P*= 0.007). Post hoc analysis showed that participants with LH levels below P25 reported more frequent excessive sexual desire than participants with LH levels between P25-50 (LSD; *P*= 0.001) or LH levels above P75 (LSD; *P*= 0.001). LH levels were also inversely associated with a shorter time of being able to live without any sexual activities (ANOVA; *P*=0.009). Participants with LH levels below P25 reported having a higher need of sexual activities than those with LH levels between P50-75 (LSD; *P*= 0.004) or LH levels above P75 (LSD; *P*= 0.005).

DISCUSSION

Almost three quarters of our participants mentioned an increased sexual desire after SRS. This could be attributed to many changes associated with a sex reassignment procedure: improvement in general well being due to relief of gender dysphoria, T therapy, or more satisfactory sexual relationships after SRS. Furthermore, the majority of the participants currently in a relationship reported a satisfactory sexual life. These findings are in line with previous studies who generally found an improvement in sexual functioning after SRS[7; 9-10; 14].

To our knowledge, this is the first study investigating sexual desire in female-to-male transsexual persons using a validated questionnaire. We found a correlation between the desire to behave sexually by oneself (solitary sexual desire) and frequency of masturbation, but no relation between the desire to behave sexually with a partner (dyadic sexual desire) and frequency of sexual activities with a partner. Female-to-male transsexual persons experience significantly higher frequency and intensity of sexual desire than male-to-female transsexual persons, a finding also reported in literature on sexual desire differences in biological men and women[16-17]. The higher level of androgens after sex reassignment in comparison with male-to-female transsexual persons might be one explanation for this finding. However, many biological, psychological as well as socio-cultural factors have been proposed to explain gender differences in sexual desire and motivation[18-19].

With regard to the hormonal levels, we observed no statistical differences between the levels of total T, calculated free T, LH and hematocrit according to the type of T replacement therapy. However, borderline significance is observed for (free) testosterone and hematocrit. The differences in sample size according to the type of T therapy as well as the limited power of this study should be considered in this regard. Almost 9% of our participants reached the cut off levels of hypogonadism. This finding can be explained as blood sampling was performed regardless of the moment of last administration of T (Testosterone undecanoate 1000mg median: 37 days, range: 1-97 days; Parental testosterone esters 250 mg median: 9 days, range: 1-30 days). However, the higher LH levels measured in these participants indicate the need to optimally adjust T dosage. In contrast, more than a quarter of our participants exceeded the upper limit of 1008ng/dl.

In this study, we could not establish a direct association between sexual desire and T levels in female-to-male transsexual persons. However, we found an inverse association between solitary sexual desire and LH levels. Similarly, Van kesteren et al.[20] observed in their study on the effect of sex steroid treatment on bone health of transsexual persons that LH was a more adequate

indicator than the levels of sex steroids themselves. Inadequate T therapy, as indicated by high LH levels were associated with lower levels of solitary sexual desire compared to participants with low LH levels, but not than those with LH levels within the normal range. Participants with LH levels below P25 (all below the lower limit of our laboratory indicating an excess of T replacement therapy) scored significantly higher on solitary sexual desire than those with LH levels within the normal range. Furthermore, participants with LH levels below P25 reported more often an excessive sexual desire and a having greater need of sexual activities than the others.

The reasons for not establishing a direct association between sexual desire and T in female-to-male transsexual persons could be multiple. First, the differences in sample size between the types of T therapy in our participants could have hampered finding a relation, especially considering the relatively small sample size. Second, the response to T supplementation may differ individually[21-22], further complicating finding an association. Third, several studies have indicated that T levels above a certain threshold value of T do not have a significant impact on sexual functioning[23-26]. Fourth, serum androgen levels are only one aspect in the androgen cascade. Other factors, such as genetic variation of the transcriptional activity induced by the androgen receptor may modify T action[27-28].

Hormonal therapy in female-to-male transsexual persons aims at T levels within the normal male physiological range[29]. Adequate hormone therapy in female-to-male transsexual persons is needed to maintain for instance bone mass[20,30]. However, supraphysiological doses of T increases the risk of adverse effects as erythrocytosis and liver dysfunction[31]. Notably, the findings of our study reveal the importance of adequate T therapy for a satisfactory sexual life since suppressed LH levels are associated with increased frequency of excess of sexual desire and higher LH levels with lower measures of sexual desire. Further investigation of these observations in larger prospective samples are needed to confirm our findings as well as investigating the clinical relevance of experiencing high or low levels of sexual desire, as could be indicated by the levels of distress.

Certain limitations of this study should be noted. First, the power of this study was limited, despite the fact that we were able to study a substantial number of female-to-male transsexual persons. Secondly, the cross sectional design implies that no causal relations could be drawn. Third, we could only establish a difference in sexual desire between participants with LH below P25 and the others. We found no difference between participants with LH levels above P75 and LH levels between P25-50 and P50-75. However, the high variance in sexual desire scores in the

group with LH levels above P75 as well as the limited power of this study should be considered. Also, selection bias of our study is another possible limitation. As in all follow-up studies, participants who agreed to this study may have a more favourable outcome than those who refused to participate. Nevertheless, the response rate is relatively high with 64% of those contacted willing to participate in a study that required a full day hospital visit. Finally, sexual functioning after SRS is partly dependent on the quality of and satisfaction with surgery which hampers generalization to all centers.

In conclusion, the data of the current study indicate the majority of the participants reported an increase in sexual desire after cross sex hormone treatment and SRS. No direct associations between levels of T and measures of sexual desire were found. However, measures of sexual desire were inversely associated with LH levels.

DECLARATION OF INTEREST

There is no conflict of interest to be declared.

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CHAPTER 4. QUALITY OF LIFE

BASED ON

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4

CHAPTER 4. QUALITY OF LIFE

4.1 QUALITY OF LIFE IN TRANS PERSONS: A CASE CONTROL STUDY.

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ABSTRACT

In this cross-sectional study, we (1) compared the quality of life (QoL) of trans individuals during and after sex reassignment with an age- and sex-matched control population and (2) investigated the predictors of QoL in trans persons. Participants included 352 adults diagnosed with gender dysphoria (214 natal males, 138 natal females) who were treated at the Ghent University Hospital in Belgium. QoL was assessed with the SF-12 questionnaire. Possible predictors of QoL included age, sex, sexual orientation, partnership and parenthood status, education, employment, income, satisfaction with hormonal and surgical treatments, SRS, phalloplasty, erection prosthesis, facial feminization surgery, breast augmentation surgery, and occurrence of surgical complications. We found that trans women had lower physical and mental functioning scores compared with age-matched male and female counterparts, even after correcting for socio-economic status. Similarly, trans men had lower mental functioning scores, but not physical functioning scores, compared with control individuals after correcting for socio-economic status. Multivariate regression analysis revealed that hormonal treatment satisfaction and employment status were independent positive predictors of physical and mental functioning in trans women and men. In trans women, having children and undergoing vaginoplasty were negative predictors of physical functioning, whereas facial feminization surgery was a positive predictor of mental functioning. In trans men, having children was another independent positive predictor of mental functioning. Therefore, these predictors should be considered within efforts to enhance QoL among trans individuals.

INTRODUCTION

Gender dysphoria refers to the incongruence individuals experience between their assigned sex and their gender identity—the sense one has of being male, female, or in between—which causes extensive personal distress. Characteristic symptoms include strong and persistent cross-gender identification and a sense of inappropriateness toward one's gender. Therapeutic approaches to gender dysphoria may include psychotherapy or counseling, cross-sex hormone therapy, and surgery[1].

Quality of life (QoL) is an individual's perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards, and concerns. In health care, QoL is often measured to determine how an individual's well-being is affected by a disease or condition and its treatment. Little information is currently available on how QoL differs between trans persons and the general population. In general, QoL in trans persons has been found to be poorer than that compared with control groups of the general population[2-5]. However, none of these studies corrected for age, gender, or socio-economic status, which are well-known determinants of QoL in the general population [6-7], thereby possibly introducing a bias. Yet, a good comparison between trans persons and the general population is required to inform health care providers, policy makers, and the general public about the needs of the trans population.

Most research on self-reported QoL shows that trans persons who receive cross-sex hormone therapy [2, 8-9] facial feminization and genital surgery[5,10] had better QoL scores compared to those who did not receive these therapies. QoL refers to the general well-being of individuals and includes many aspects such as emotional, social and physical functioning. Apart from these clinical factors, QoL in trans individuals may also be related to other determinants such as social and economic factors. Some of these determinants have only recently been explored in the group of trans persons. Overall, most studies show that better employment status, social status, and social support are associated with higher QoL scores [8-10]. A better understanding of the strongest predictors of QoL in trans persons can help health care providers and policy makers utilize resources more effectively. However, this information is scarce and only available from trans individuals before sex reassignment surgery [8-9].

Thus, the aims of this study were two-fold: (1) to compare QoL between trans individuals and a control population after correcting for age, gender, and socio-economic status and (2) to

investigate the contribution of clinical and general determinants of QoL in trans persons after cross-sex hormone therapy and/or sex reassignment surgery.

METHODS

STUDY POPULATION AND STUDY PROCEDURES

All persons who were diagnosed with gender dysphoria/transsexualism (Diagnostic and Statistical Manual of Mental Disorders-5, 302.85/ International Classification of Diseases, 10th revision, F64.0), treated at the Center for Sexology and Gender problems at the Ghent University Hospital (Ghent, Belgium) between January 1986 and June 2012, and who used cross-sex hormones for at least 3 months were invited to participate in this study. Respondents received a paper version of the questionnaire by post or could choose to complete the survey on-line. A reminder message was sent to non-responders. All survey responses were collected between August and December 2012. Our response rate was 54% (51% for trans women, 58.7% for trans men), with a final total of 352 participants (214 trans women, 138 trans men) (Figure 1).

Participants were 41 years old on average (range: 18-86 years). Some trans women were not on estrogen therapy due to previous thromboembolic events (N=5), dissatisfaction (N=2), or other reasons (N=3), but the majority of trans women had been on hormonal therapy for a median duration of 7 years (range: 3 months–35 years). Cross-sex hormonal therapies were transdermal estradiol (17-β estradiol gel, 1.5 mg/24 h; (N=76, 35.5%), estradiol patch (50 μg/24 h; N=29, 13.6%), or daily intake of oral estrogens (estradiol valerate, 2 mg; N=91, 42.5%), estriol (2 mg; N =1, 0.4%), ethinyl estradiol (50 μg; N=2, 0.9%), or oral contraceptives (N = 5, 2.3%). Among trans women, 139 (65%) underwent sex reassignment surgery (SRS; orchidectomy, penectomy, and vaginoplasty), 60 (28%) were planning for this surgery, 4 (1.8%) were in doubt, and 11 (5%) did not want surgery or did not undergo surgery for medical reasons. About half of the trans women (53.5%) underwent breast augmentation, 17.8% underwent vocal cord surgery or cricoid reduction, and 21.5% underwent facial feminizing surgery

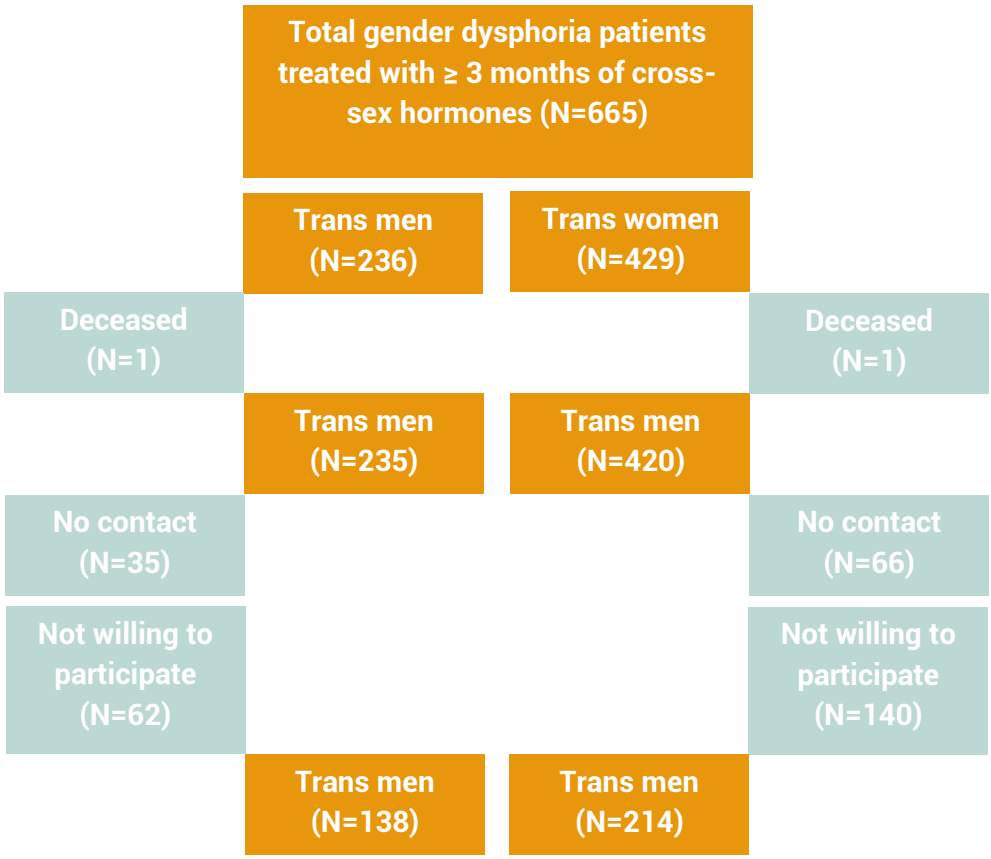

Figure 1. Subject enrollment

Trans men had been on testosterone replacement therapy for an average of 9.4 years (range: 3 months–49 years). Cross-sex hormonal therapies were intramuscular testosterone treatment with a mixture of testosterone esters (testosterone decanoate, 1000 mg; testosterone isocaproate, 60 mg; testosterone fenylpropionate, 60 mg; and testosterone propionate, 30 mg/ml; treatment every 2 or 3 weeks; N = 64, 46.4%), testosterone undecanoate (1000 mg for12 weeks; N = 62, 44.9%), transdermal testosterone (50 mg/24 h; N = 9, 6.5%), or oral testosterone undecanoate (N = 2, 1.4%). Among trans men, 119 (86%) underwent hysterectomy/oophorectomy, 76 (59.4%) underwent phalloplasty, and 9 (0.06%) underwent metaidioplasty, 8 of whom subsequently underwent phalloplasty. Forty-eight (63%) trans men who underwent phalloplasty had an erection prosthesis implanted.

For both trans men and women, we used age-matched (± 3 years) female and male control subjects to compare QoL scores. These subjects were randomly selected (3 control men and 3 control women for each patient) and were recruited from Sexpert, a population based study on sexual health in Flanders. Methods of this study are extensively described elsewhere[11]. Briefly, control individuals were recruited from a population (range: 14–80 years of age) who completed a survey containing extensive information on sexual health characteristics and biomedical,

psychological, demographic, and socio-cultural correlates. Control data were collected between February 2011 and January 2012. The final database consists of 1832 respondents (response rate: 40% of eligible respondents). Respondents were randomly drawn from the Belgian National Register. All data for this study were collected from face-to-face interviews and a combination of computer-assisted personal interviewing and computer-assisted self-interviewing. Both studies were approved by the ethical review board of Ghent University Hospital in Belgium, and all participants gave informed consent for participation in this study.

OUTCOME MEASURES

Physical health and incidence of possible treatment related adverse events

Medical history, medication use, smoking habits, weight, height, current and past hormonal treatment and clinical adverse events were addressed by a specifically designed questionnaire.

Sociodemographic variables

Information on age, sexual orientation (five-point scale from only attracted to men to only attracted to women or other), marital status, educational level (ISCED codes), employment status, and household income was collected. All participants were asked whether they were currently involved with a partner (yes/no) and the length of the relationship. Participants were also asked whether they had children (yes/no) and to describe the method of conception for each child.

Health-related quality of life

QOL was measured using the SF-12, a brief 12-item version of the Short Form-36 Health Survey (SF-36). All items had fixed response choices and were organized into two sections (physical functioning and mental functioning), with scores based on the weighted sums of responses (1–100) in each section. Participants were asked to answer the questions based on their experiences within the previous month. Higher scores indicate higher levels of functioning or well-being[12]. Internal consistency for the SF-12 was high (total group: Cronbach's $\alpha = .8$).

Surgical results

Participants evaluated their satisfaction with the outcome of transition-related surgical procedures using a five-point scale from very unsatisfied to very satisfied. Trans men were asked whether they experienced complications related to metaidioplasty, phalloplasty, or implantation of an erection prosthesis (yes/no). Trans women were asked whether they experienced complications related to vaginoplasty (yes/no).

Statistical analysis

Continuous variables were expressed as mean and standard deviation (SD) or median (first to third quartile) when criteria for normal distribution were not fulfilled. Qualitative variables were expressed as percentages. Comparisons of linear variables between groups were performed using independent Student's t-tests (parametric) or Mann-Whitney U tests (non-parametric). Dichotomous and categorical variables were analyzed using chi-square tests. Internal consistency within questionnaire scales used was assessed using Cronbach's α. Univariate associations with QoL scores were determined by one-way analysis of variance (ANOVA). Multiple linear regression analysis was performed to determine which variables were independently associated with mental and physical functioning scores. Variables were included in the regression model based on their clinical interest and association with QoL in the univariate analysis (age, genital surgery, surgical satisfaction, hormonal treatment satisfaction, facial feminization surgery, partnership status, having children, educational level, and income). In Figure 3, bars represent means, and whiskers indicate 95% confidence intervals. Statistical significance was set at $p < .05$, and all tests were two-tailed. Data were analyzed using SPSS software, v.21 (SPSS Inc., Chicago, IL).

RESULTS

GENERAL CHARACTERISTICS

General characteristics of the study population are described in Table 1. About half of trans women (47.7%) were mainly attracted to females, 36% were mainly attracted to males and 11.2% were equally attracted to females and males. Majority of trans men (81.8%) were mainly attracted to females, 10.2% were mainly attracted to males and 6.5% were equally attracted to females and males.

TREATMENT SATISFACTION

Most trans women (79.8%) were very satisfied or satisfied with vaginoplasty, whereas some (8.3%) were unsatisfied or very unsatisfied. Some (16%) also experienced one or more complications after vaginoplasty. Satisfaction with vaginoplasty was negatively associated with the occurrence of complications ($p < .001$). Almost all trans men were very satisfied with hysterectomy and ovariectomy. The vast majority (84.9%) who underwent phalloplasty were very satisfied or satisfied, although many (54.7%) reported having one or more postoperative complications. Over half of trans men who received an erection prosthesis (57.4%) felt very satisfied or satisfied, whereas nearly a quarter (24.1%) were very dissatisfied or dissatisfied. A third (33%) experienced one or more postoperative complications. Occurrence of phalloplasty- or erection prosthesis-related complications was negatively associated with surgical satisfaction (p

< .001 and p = .001, respectively). In both trans women and men, satisfaction with hormonal therapy was high (78% vs. 87.7%), but trans women were more often dissatisfied with hormonal therapy than trans men (9.8% vs. 2.1%, p = .002). We observed no association between type of hormonal therapy and treatment satisfaction (data not shown).

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY POPULATION

	TRANS WOMEN (N=214)	TRANS MEN (N=138)	P
Age at time of study (years)	43.7 ± 12.6	37.5 ± 11.0	<0.001
Nationality(%)			
Belgian	86.0	87.0	NS
Other	14.0	12.5	
Civil status (%)			
Married/living together	36.0	44.5	
Not married or living together	36.4	47.4	<0.001
Divorced	25.2	8.0	
Widow	2.3	0	
Children (%)	41.1	23.9	<0.001
Children born before HRT (%)	81.8	36.0	<0.001
Work status (%)			
Unemployed	14.2	9.4	
Employed	55.9	64.5	0.001
Retired	9.5	1.5	
Student	5.2	13.0	
Unable to work	13.7	9.;4	
Household	1.4	2.2	
Monthly income (%)			
≤999 euro	10.3	14.2	
1000-1999 euro	42.2	35.8	
2000-2999 euro	27.5	24.6	NS
3000-5999 euro	16.7	23.9	
≥ 6000 euro	3.4	1.5	
SRS (%)	64.8	85.5	<0.001
Time since SRS (years)	6.0 [2-11]	7.0 [4-13]	NS
Duration of HRT (years)	6.0 [3-11]	7.0 [4-13]	0.02
Mental functioning	58.4 ± 14.8	60.8 ± 14.3	0.11
Physical functioning	71.5 ± 23.6	78.3 ± 21.5	0.003

Data are presented as %, medium ± s.d. or median (first to third quartiles). Categorical variables using Chi square test; Continuous variables using independent student T test or Mann-Whitney-U test. NS: not significant; SRS: sex reassignment surgery (defined as orchidectomy/penectomy/ vaginoplasty in trans women and hysterectomy/ovariectomy in trans men)

Quality of life compared to age-matched control population

Trans men and women were less likely to be involved in a relationship, less likely to be employed, and had lower incomes than age-matched male and female control counterparts (p<.001). No difference in educational level was observed between trans and control individuals (data not shown). Compared to control men and women, trans women had lower physical and mental functioning scores ($p < .001$) (Figure 2AB), even after adjusting for age, partnership status, having children, employment status, educational level, and income (physical functioning: $p < .001$, $\beta st = .21$ (control men) and $p = .003$, $\beta st = .13$ (control women); mental functioning: $p < .001$, $\beta st = .54$ (control men) and $p < .001$, $\beta st = .30$ (control women)).

Physical functioning scores for trans men were similar to those for control women but lower than those for control men ($p = .003$) (Figure 2C). Trans men had lower mental functioning scores compared with those for control women and men (ps < .001) (Figure 2D). After adjusting for age, partnership status, having children, employment status, educational level, and income, trans men had lower mental functioning scores but not physical functioning scores compared to control men and women (physical functioning: $p = .2$, $\beta st = .06$ (control men) and $p = .6$, $\beta st = -.04$ (control women); mental functioning: $p < .001$, $\beta st = .50$ (control men) and $p < .001$, $\beta st = .24$ (control women)).

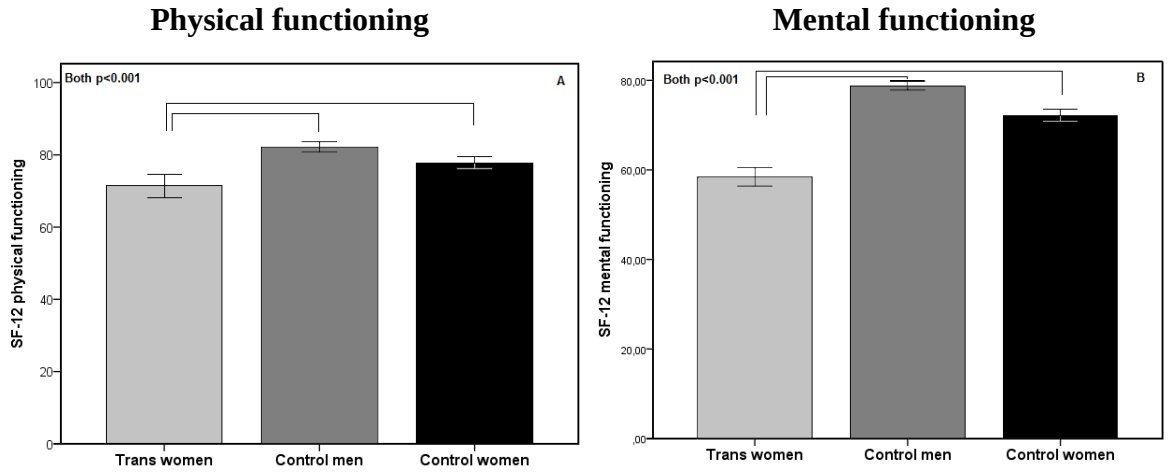

Figure 3A and 3B. Physical and mental functioning of trans women compared to control population. Bars represent mean and 95% confidence interval. p value result from post-hoc ANOVA.

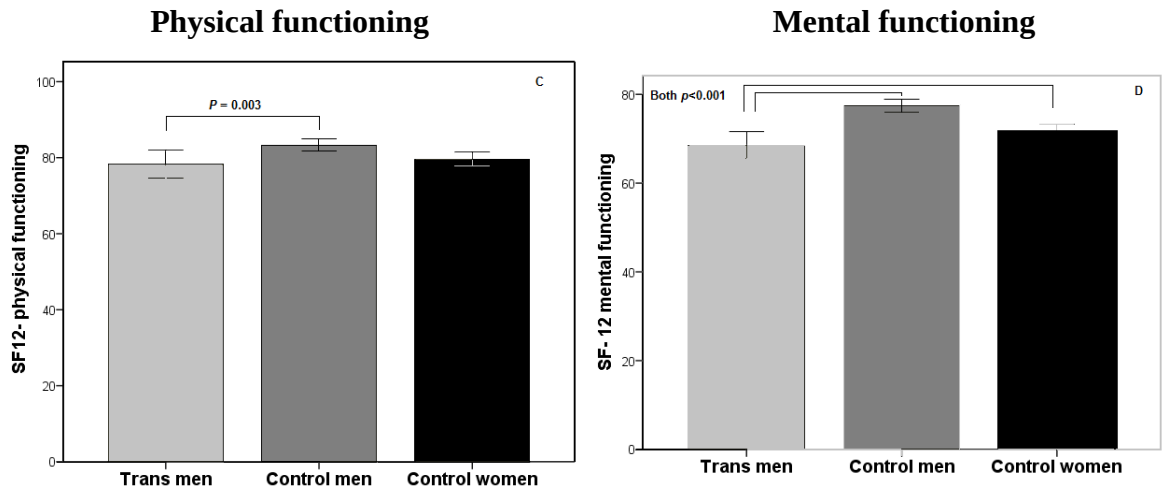

Figure 3C and 3D. Physical and mental functioning of trans men compared to control population. Bars represent mean and whiskers 95% confidence interval. *P* value result from post-hoc ANOVA.

Univariate associations with general and clinical determinants of QoL in trans persons

The results of univariate analysis are presented in Table 2.

Among all trans individuals, physical functioning scores were negatively associated with age and positively associated with educational level. Employment status and higher household incomes were positively associated with physical and mental functioning scores. Participants who lived with a partner had higher mental functioning scores than those who did not live with a partner. For trans men, having children was positively associated with mental functioning scores, whereas no such association was found for trans women. Having undergone SRS was associated with lower physical functioning scores. When only trans persons who had undergone SRS more than 1 year ago were included, this association was no longer statistically significant (F = 3.3, p = .07).

Trans women who underwent breast augmentation or vocal cord surgery had similar

QoL scores compared with those who did not undergo surgery (data not shown). Facial feminization surgery was associated with higher mental functioning scores (F = 4.4; p = .04). Trans men who underwent phalloplasty had higher physical and mental functioning scores (F = 5.8, p = .02 and F = 4.6, p = .03, respectively). Phalloplasty-related complications were negatively associated with mental functioning scores (F = 6.5, p = .001). Also, trans men with an erection prosthesis had a tendency toward higher mental functioning scores (F = 3.3, p= .07). Erection prosthesis-related complications were not associated with QoL scores (data not shown).

TABLE 2. UNIVARIATE ASSOCIATION BETWEEN QOL SCORES AND GENERAL AND CLINICAL DETERMINANTS

	TRANS WOMEN		TRANS MEN		TOTAL TRANS SAMPLE	
	PHYSICAL FUNCTIONING	MENTAL FUNCTIONING	PHYSICAL FUNCTIONING	MENTAL FUNCTIONING	PHYSICAL FUNCTIONING	MENTAL FUNCTIONING
Living with partner						
No	71.2 ± 24.1	57.8 ± 15.1	75.9 ± 23.2	57.8 ± 16.1	72.9 ± 23.8	57.8 ± 15.5
Yes	72.1 ± 22.7	59.4 ± 14.1	81.3 ± 18.9	64.5 ± 10.6	76.3 ± 21.4	61.8 ± 12.8
F	0.0	0.5	2.0	7.8 **	1.7	5.9 *
Children						
No	76.1 ± 20.9	59.2 ± 15.3	78.8 ± 21.3	59.5 ± 14.8	77.3 ± 21.1	59.3 ± 15.1
Yes	65.1 ± 25.6	57.3 ± 14.0	76.9 ± 22.1	64.8 ± 11.7	68.4 ± 25.2	59.4 ± 13.8
F	10.0 **	2.7	0.2	3.5 ¥	10.8 **	0.5
Employment						
No	54.3 ± 26.9	56.4 ± 16.3	69.4 ± 25.0	55.1 ± 15.5	66.1 ± 26.3	55.9 ± 16.0
Yes	77.1 ± 18.9	59.9 ± 13.4	83.0 ± 17.7	63.9 ± 12.6	79.6 ± 18.6	61.6 ± 13.2
F	9.5***	3.4*	15.3***	7.1***	23.3***	9.9 ***
Household Income						
< mean	66.8 ± 25.2	56.7 ± 15.5	73.6 ± 23.9	58.1 ± 15.7	69.4 ± 24.9	57.3 ± 15.2
≥ mean	76.3 ± 20.4	60.1 ± 14.1	82.6 ± 17.9	63.2 ± 12.3	78.9 ± 19.6	61.4 ± 13.4
F	8.4 **	2.5	0.9	4.4 *	14.8 ***	6.6 **
Sexual orientation						
Same birth sex	72.1 ± 23.7	56.5 ± 14.7	78.8 ± 20.6	60.9 ± 14.6	76.2 ± 22.1	59.2 ± 14.5
Other birth sex	67.1 ± 27.5	57.7 ± 16.7	69.4 ± 33.2	54.2 ± 15.5	67.8 ± 28.6	56.7 ± 16.2
Other	73.3 ± 22.3	60.1 ± 13.9	78.9 ± 19.5	64.0 ± 9.9	73.9 ± 22.0	60.5 ± 13.5
F	0.6	1.3	0.8	1.3	1.9	0.9
Satisfaction HRT						
(very) dissatisfied	46.5 ± 27.4	45.1 ± 14.1	58.3 ± 26.0	47.5 ± 15.6	48.2 ± 27.0	45.5 ± 13.9
Neutral	66.1 ± 22.4	51.7 ± 13.6	59.9 ± 33.7	51.0 ± 17.2	63.8 ± 26.8	51.4 ± 14.7
(very) satisfied	75.0 ± 21.5	60.8 ± 14.0	80.7 ± 18.7	612.0 ± 13.4	77.4 ± 21.0	61.3 ± 13.8
F	14.1 ***	12.5 ***	7.0 ***	4.8 **	21.9 ***	18.1 ***
SRS						
No	77.2 ± 17.7	59.2 ± 13.7	80.6 ± 18.4	62.1 ± 12.1	77.9 ± 17.6	59.8 ± 13.2
Yes	68.4 ± 25.8	58.0 ± 15.4	77.9 ± 22.0	60.6 ± 14.6	72.8 ± 24.5	59.2 ± 15.1
F	6.6 **	0.3	0.3	0.2	3.3 ¥	0.1
Complication SRS						
No	70.1 ± 24.9	59.0 ± 15.4	85.2 ± 13.3	66.5 ± 12.1	74.3 ± 23.2	61.2 ± 14.9
Yes	66.3 ± 27.0	56.5 ± 15.4	79.0 ± 19.8	60.3 ± 13.4	72.6 ± 24.4	58.4 ± 14.5
F	0.7	0.8	2.4	4.6 *	0.3	2.0
Satisfaction SRS						
(very) dissatisfied	62.5 ± 32.8	52.8 ± 15.6	75.0 ± 26.5	52.5 ± 17.7	73.9 ± 23.1	61.1 ± 14.2
Neutral	71.7 ± 26.3	52.1 ± 16.4	77.1 ± 25.2	59.2 ± 15.3	73.6 ± 25.6	54.5 ± 16.1
(very) satisfied	68.4 ± 25.2	59.3 ± 15.0	82.0 ± 16.6	63.5 ± 12.6	66.3 ± 30.5	52.7 ± 15.5
F	0.4	2.2	0.5	1.7	0.6	3.8 *
Duration HRT						
< 6 years	75.7 ± 20.6	55.7 ± 14.2	75.0 ± 24.1	58.3 ± 16.1	76.8 ± 21.0	59.5 ± 14.5
≥ 6 years	67.4 ± 25.6	58.1 ± 15.3	79.9 ± 20.4	62.1 ± 13.5	72.1 ± 24.4	59.2 ± 14.8
F	6.4 *	0.0	0.1	0.0	3.5 ¥	0.0
Time since surgery						
< 6 years	71.3 ± 24.8	60.0 ± 14.4	78.8 ± 21.8	61.1± 14.8	72.8 ± 24.5	59.3 ± 15.1
≥ 6 years	65.5 ± 26.6	55.9 ± 16.2	77.9 ± 21.4	60.6 ± 14.0	72.8 ± 24.6	59.1 ± 15.1
F	1.7	2.4	1.4	1.9	0.0	0.0

One-way anova; *significant at the level $p \leq .05$; ** significant at the level $p \leq .01$; ** significant at the level $p < .001$;
¥ significant at the trend level

TABLE 3. PREDICTIVE FACTORS FOR QOL IN TRANS PERSONS (MULTIVARIATE REGRESSION ANALYSIS)

	TOTAL TRANS SAMPLE		TRANS WOMEN		TRANS MEN	
	PHYSICAL FUNCTIONING	MENTAL FUNCTIONING	PHYSICAL FUNCTIONING	MENTAL FUNCTIONING	PHYSICAL FUNCTIONING	MENTAL FUNCTIONING
	R^2 = .27 F = 9.7*** βsd	R^2 = .17 F = 5.1*** βsd	R^2 = .27 F = 8.7*** βsd	R^2 = .14 F = 3.6*** βsd	R^2 = .31 F = 6.2*** βsd	R^2 = .25 F = 4.6*** βsd
General determinants						
Age	-0.16*	-0.07	-0.07	-0.02	-0.29***	-0.26**
Living with partner (yes)	-0.04	-0.06	-0.05	-0.04	-0.02	-0.14
Children (yes)	-0.12*	0.04	-0.20*	-0.04	-0.05	0.15
Employed (yes)	0.26***	0.15*	0.25***	0.08	-0.30***	-0.26**
Above mean income (yes)	0.13¥	0.04	0.12	0.00	-0.10	-0.07
Clinical determinants						
Hormonal satisfaction	0.32***	0.28***	0.29***	0.30***	0.32***	0.26**
SRS (yes)	-0.10*	-0.02	-0.16*	-0.05	-0.13	-0.14
FFS (yes)	-	-	-0.03	0.14¥	-	-
Phalloplasty (yes)	-	-	-	-	0.11	0.16
Erection prosthesis (yes)	-	-	-	-	-0.10	-0.03

β= Standardized beta coefficient; F: F test

*significant at the level $p \leq .05$; ** significant at the level $p \leq .01$; ** significant at the level $p < .001$; ¥ significant at the trend level

SRS= sex reassignment surgery; FFS= facial feminization surgery

Multivariate regression analysis in trans persons

Results of multivariate regression analysis in total trans sample, trans women and trans men can be found in Table 3.

Among all trans individuals, the regression model accounted for 27% of the variance in physical functioning scores and 17% of the variance in mental functioning scores. Employment status and hormonal treatment satisfaction contributed to both QoL sub-scores. Age, having children, and undergoing SRS were also independent predictors of physical functioning scores. In trans men, having children was an independent positive predictor of mental functioning scores. In trans women, having children and vaginoplasty were negative predictors of physical functioning scores, whereas facial feminization surgery was a positive predictor of mental functioning scores. Surgery satisfaction and complication rate were excluded from the model due to an interaction with the occurrence of genital surgery. A model including surgery satisfaction or complication rate instead of genital surgery showed similar results (data not shown). Surgery satisfaction and complication rate were not independent predictors of QoL scores (data not shown).

DISCUSSION

This study was designed to improve our understanding of health-related QoL in trans persons during and after sex reassignment by (1) comparing QoL between trans persons and an age-matched population and (2) investigating the main predictors of QoL in trans persons. Our findings are consistent with previous studies[2-5] showing lower QoL scores in trans persons during and after sex reassignment compared to the general population. We found that trans persons had markedly poorer socio-economic status, were less likely to be involved in a relationship, were more likely to be unemployed, and had lower income. To evaluate whether differences in QoL scores among trans and control populations could be attributed to differences in socio-economic status, we corrected our analyses for these variables and found that trans men and women still had lower QoL scores.

Of note, both physical and mental functioning scores were significantly lower in trans persons compared with control individuals, but the absolute difference between groups was smaller for physical functioning scores than for mental functioning scores. First, although cross-sex hormone therapy and SRS induce important physical changes and are associated with side effects and/or complications, these drastic outcomes do not seem to considerably affect physical functioning in the majority of trans persons. Second, although that sex reassignment therapy generally improves mental well-being of trans persons[8; 13-14] trans persons still have lower mental

functioning scores after this treatment. Minority stress couls also explain the lower mental functioning scores due to social stigma, prejudice, and discrimination in response to gender nonconformity. Minority stress includes actual experiences of rejection and discrimination, perceived rejection and expectations of being stereotyped or discriminated, and hiding minority status and identity out of fear of harm[15-16]. It is indeed reported that the majority of trans individuals experienced discrimination and the majority of them even experienced violence (verbal (78.9%), physical (26.8%) or sexual (31.6%) because of their gender identity or presentation[17-19].

Alternatively, trans persons may experience co-existent psychiatric morbidity prior to sex reassignment and therefore may be more prone to psychiatric problems after treatment[20-23]. However, a recent multi-center European study has shown that mainly affective and anxiety disorders were more prevalent in trans persons[23]. The incongruence trans persons experience between their gender identity and social life and/or bodily characteristics can cause much "dysphoria" that may lead to anxiety and effective problems or even disorders. In addition, the phenomenon of minority stress can also give explanation for the increased prevalence of affective disorders[23-24].

Providing a better understanding of the strongest predictors of QoL in trans persons may also help health care providers and policy makers utilize resources more effectively. Therefore, we investigated the main determinants of QoL in trans persons using a regression model including both clinical and socio-demographic determinants. We found that hormonal treatment satisfaction and employment status were independent predictors of QoL in both trans women and men. To the best of our knowledge, previous studies have not investigated the main predictors of QoL in trans persons after SRS. However, two previous studies that assessed predictors of QoL in trans persons before SRS[8-9] report that hormonal therapy was an independent predictor of QoL. As almost all our respondents were on hormonal therapy, we were unable to investigate this hypothesis, but our findings seem to confirm the importance of hormonal therapy on the QoL of trans persons, even after SRS. Indeed, a recent prospective study also showed that cross-sex hormonal treatment is associated with better mental health in both trans men and women as lower levels of anxiety, depression, psychological symptoms and functional impairment were reported after 12 months of hormonal therapy[24]. Cross-sex hormone therapy is an essential part of the medical treatment of trans persons and induces body features and shape of the desired gender, which results in a reduction of self-reported distress[25]. Besides inducing physical changes, the act of using cross-sex hormones is itself an affirmation of

gender identity. In addition, many trans persons change gender role around the time of starting hormonal therapy, which could also improve social and mental functioning[24]. Our results show that not only the initiation of cross-sex hormone therapy but also satisfaction with the outcome of hormonal treatment is important for QoL. Trans persons who were more dissatisfied with their hormonal therapy might be less satisfied with their body changes or more likely to have experienced bothersome side effects, which could reduce mental and physical functioning. Therefore, further investigations of the predictors of hormonal treatment satisfaction, such as body image, expectations of hormonal effects and the experience and severity of side effects, is of clinical interest.

Depression[8] as well as family support and working status[9] were previously found to be independent predictors of QoL in trans persons. Diagnosis of depression and strength of social support were not considered in our study, but we did observe an impact of family structure on QoL. Specifically, parenthood negatively affected QoL in trans women but positively affected QoL in trans men. A possible explanation for this finding may be that the majority of children of trans women (82%) were conceived before sex reassignment therapy, whereas the opposite was true for trans men (36%). As it is well-known that trans men present themselves at a younger age than trans women[27-28], our finding may thus reflect higher numbers of familial difficulties encountered by trans women due to sex reassignment treatment.

In line with previous studies, we observed that employment status was an independent predictor of both physical and mental functioning. For instance, Motmans et al. (2012)[10] also found that trans persons who were employed had better general health, less bodily pain, greater physical functioning, and fewer role-physical and role-emotional problems. From clinical practice, it is known that trans persons experience more problems finding employment than the general population, especially during sex reassignment. Therefore, the enhancement of employment programs for trans persons may improve their QoL.

Somewhat unexpectedly, surgical satisfaction and surgical complication rate were not found to be independent predictors of QoL scores. However, it should be noted that complication rate was only assessed as a binary variable. That is, minor adverse events, such as genital tract infections, may have been reported as complications[4] and thus may have influenced our results. Thus, other conclusions could have been reached had the severity of complications been taken into account. Future studies should take this into account. Also, we found a significant negative association between SRS and physical functioning, but this association disappeared after excluding trans individuals who had recently undergone SRS. Furthermore, our results

concerning mental functioning are in line with our previous survey[10] which showed no association between QoL and having undergone SRS, phalloplasty and erection prosthesis. These findings may be explained by the fact that many participants, who did not have these surgeries, were already planned for these surgeries in the near future. As previously suggested[10], another explanation may also lie in the state funded care (genital surgery and breast augmentation are for a large part reimbursed in Belgium) and in the quality and experience of caregivers. At our center, trans persons receive at least 6 contacts with mental health practitioners during the diagnostic phase, contacts that are continued throughout hormone therapy and before and after surgery. Surgery is generally performed after two years of cross-sex hormone therapy but this is no longer required according to the Standards Of Care[1] and has become more of a personal choice.

In line with Ainsworth and Spiegel (2010)[5], it was found that trans women who underwent facial feminization surgery had a better QoL compared to those who did not. We also observed that facial feminization surgery was an independent positive predictor of mental functioning. This may be due to the better passability of trans women who underwent facial feminization surgery and/or improvements in body image and self-esteem. Moreover, better passability was recently found to be associated with less experience of violence[19].

Strengths of the present study are its relatively large sample size and the comparison of QoL between trans individuals and a representative control population after correcting for age, sex, and socio-economic status. Also, we were able to investigate predictors of QoL in trans persons after SRS. However, our study is hampered by our limited response rate, which may have resulted in selection bias, as trans persons who participated in this study may have had a better outcome than those who refused. However, compared to other long-term follow-up studies, we achieved similar[13] or even better response rate[29]. In addition, the generalizability of our results to other centers may be limited considering the availability of surgery and expertise in our center and the well-organized health insurance system in Belgium, which covers most of the costs of SRS. Another limitation of this study is that we measured only family status but did not examine perceived social support, which should be taken into account in future studies. Finally, considering our relatively large sample size, we may have found statistically significant results that may not be clinically relevant. However, to our knowledge, no information is available indicating what changes in QoL can be considered clinically relevant.

In conclusion, our findings are consistent with the results of most other retrospective follow-up studies in that they indicate poorer QOL in trans persons compared with the general population,

even after adjusting for key determinants such as age, gender, and socio-economic status. Hormonal treatment satisfaction and employment status were the strongest predictors of physical and mental functioning of trans persons during and after sex reassignment.

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CHAPTER 5. SUMMARY OF CONTRIBUTIONS AND GENERAL DISCUSSION

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Gender dysphoria is a condition in which a person experiences discrepancy between the sex assigned at birth and the gender they identify with, leading to extensive personal distress. Transsexualism is considered the most extreme form of gender dysphoria and is characterized by the wish to undergo treatment to conform to the other sex. Cross-sex hormone therapy is an important part of this medical treatment, when desired, and aims to acquire the secondary sex characteristics of the desired sex while reducing those of the natal one.

Besides these effects on secondary sex characteristics, sex steroids are also known to play an important role in many other tissues and systems such as muscle, fat, bone, skin, the larynx, the immune system, hematopoietic cells, brain cells and the cardiovascular system. This implies that cross-sex hormone therapy can induce important side effects. Despite the widespread use of this therapy, data concerning its effects and side effects are scarce due to the low prevalence of this diagnosis, the small number of subjects treated in each centre, the lack of prospective studies and the lack of long-term follow-up studies. In this thesis, we investigated both short- and long-term safety of cross-sex hormone therapy in trans women and men. In addition, as treatment related QoL is becoming increasingly important, we also examined QoL of trans persons and its associations with cross-sex reassignment therapy.

5.1 MAIN FINDINGS AND GENERAL DISCUSSION

5.1.1 SHORT-TERM EFFECTS AND SAFETY OF CROSS-SEX HORMONE THERAPY

The physical and physiological effects, side effects and adverse events of currently used cross-sex hormone therapies in both trans women and men were investigated in the ENIGI study (**chapter 2.1**). The main findings of this study were that cyproterone acetate 50 mg daily with estrogen treatment (< 45y estradiol valerate 4mg daily and ≥ 45y 17β estradiol patch 100 μg/24h) in trans women and injections with T undecanoate 1000 mg every 3 months in trans men, were effective and safe therapies. All trans persons acquired adequate sex steroid levels and secondary sex characteristics of the desired sex. Importantly, none of the trans participants experienced severe adverse events such as cardiovascular events or death. These findings were in line with previous reports in trans men, which showed that T treatment was effective and acceptably safe in the short-term[290,311]. Our results in trans women confirmed those from Dittrich and colleague[297],

indicating a low risk for adverse events at short-time follow-up. However, these findings were in contrast with earlier reports showing a high incidence of venous thrombosis and/or pulmonary embolism during the first year of cross-sex hormone therapy[310-311]. This may suggest that current treatment modalities in trans women, which avoid the use of high dose EE and use transdermal estrogens in older trans women, may induce less detrimental effects on the coagulation system. Indeed, it has been previously found that EE induced a more pronounced APC resistance compared to other estrogen types[194]. In addition, our colleagues in Amsterdam also observed a lower incidence of venous thrombosis since the introduction of transdermal estrogens in older trans women[311].

Beside this increased risk for thromboembolic disease, anti-androgen and estrogen therapy may induce liver dysfunction and hyperprolactinemia[5]. Indeed, our findings corroborate with previous studies using other treatment modalities[310-311] showing a risk for transient elevation of the liver enzymes and hyperprolactinemia. Importantly, the clinical relevance of these elevations during the first year of hormonal therapy seemed limited. Similar as in trans women, a small risk for transient elevation of the liver enzymes was found in trans men. In addition, a small increase in blood pressure was observed during T therapy as also observed by Mueller and colleagues[290].

It is known that androgen excess in women is associated with important dermatological effects, such as acne vulgaris, hirsutism, and androgenetic alopecia with potentially important psychological disturbing effects. As only one study[286], using short-acting T esters, previously addressed this topic, we also aimed to investigate dermatological changes during treatment with long-acting intramuscular T undecanoate (**chapter 2.3**). We observed a rather low risk for development of androgenetic alopecia but a high risk for acne during the first 12 months of T therapy. Both incidence and severity of acne lesions increased during T treatment but most men developed only mild acne and none suffered from severe or very severe acne according to the Global Acne Grading Scale. Importantly, half our patients initiated acne treatment during this study period and showed that, although mild and moderate, these acne lesions are of clinical significance. Similar to the observations of Giltay and colleagues[286], we observed that most acne lesions occurred within the first 6 to 9 months of treatment, with a decrease at 12 months. These results may indicate that the initial increase in sebum production and the associated acne lesions resulting from male T levels in biological females, attenuate over time. A wide between-subject variability was found which suggests an important variability in PSU responsiveness to androgens due to variations in androgen metabolism; for example, variations in 5α reductase activity, 3β-

hydroxysteroid dehydrogenase activity, aromatase activity, or differences in androgen receptor sensitivity.

Clinicians working with trans persons will acknowledge that many trans persons attribute complaints to their hormonal therapy. As this was not addressed in a structured manner in previous research, we examined prospectively both the incidence and severity of possible hormone-related symptoms in the ENIGI study. The majority of trans men experienced voice instability with a peak incidence observed at 6 months, whereas a quarter of them experienced clitoral pain, which also peaked at 6 months. Trans men also reported a decrease of emotionality and an increase in sexual desire whereas trans women reported the opposite. In addition, many trans women experienced breast tenderness and hot flashes. The observed increase in hot flashes was somewhat unexpected under estrogen therapy. However, as no validated questionnaires or objective measurements were used in this study, further exploration and characterization is needed. The exact mechanism of hot flashes is still not fully understood, but it seems that complex neuroendocrine pathways involving norepinephrine, E_2, T and endorphins are involved in regulating the thermoregulatory nucleus at the hypothalamus. Therefore, it may be possible that the profound changes in sex steroids levels in trans women also affect these neuroendocrine pathways. Whether these hot flashes will diminish over time also needs to be examined. Future research may also evaluate possible treatments.

Finally, in view of recent studies showing an increased cardiovascular mortality in trans women[312-313], one of the main aims of this thesis was to investigate possible changes in cardiovascular risk factors during cross-sex hormone therapy (**chapter 2.1 and chapter 2.2**). Changes in cardiovascular risk factors during T therapy in men have been previously investigated, but most studies had a rather small sample size and used short-acting T esters instead of long-acting intramuscular T undecanoate, which are more recently marketed. The latter are increasingly used as the long intervals make this formulation attractive for T supplementation, particularly in these healthy, young persons who will need lifelong treatment. We showed, in line with studies using 2 or 3-weekly injections of intramuscular T esters, that this treatment induced favourable changes in cardiovascular risk factors including a decrease in total body fat mass and an increase in lean body mass. Alternatively, unfavourable changes such as worsening of the lipid profile and an android pattern of fat distribution were also induced. Our findings concerning glucose metabolism, showing an improvement in insulin sensitivity, contrasts with studies in trans men using 2 or 3-weekly injections of intramuscular T esters, showing no changes[300] or a worsening in insulin sensivity[301]. The beneficial changes in body composition with an increase in

muscle mass and a decrease in total body and trunk fat mass in the ENIGI study may be an explanation for our findings, even though we were unable to show such a statistical significant association in our sample, possibly due to insufficient power. In contrast, others[300] observed an increase in visceral fat mass during T therapy. Whether these differences may be related to differences in type of T or other factors, remains unknown and needs to be explored in future studies.

In trans women, most evidence concerning changes in cardiovascular risk factors are also based on older regimens using high dose cyproterone acetate and high dose EE[295, 300]. Given that different types and dosages of estrogens can exert different metabolic effects, we aimed to investigate changes in cardiovascular risk factors induced by our current treatment modalities. On the one hand, we confirmed findings from studies using EE, showing a reduction in LDL cholesterol and an increase of total body and trunk fat mass and insulin resistance during the first year of cross-sex hormone therapy in trans women[295, 300]. On the other hand, in contrast to most previous research, we observed no increase in body weight, triglycerides or blood pressure during the first year of therapy, which may suggest more favourable changes with these newer treatments. However, it is clear that randomized controlled trials are needed in this field to compare the benefit and harm of different types of cross-sex hormone therapies, both on intermediate and hard clinical end points.

Of note, we observed that a higher adiposity was associated with poorer cardiometabolic factors in both trans women and men. Therefore, avoiding obesity is also in trans persons an important way to improve the cardiovascular and metabolic risk profile, and weight management should be considered as an important part of the entire endocrine treatment plan. In addition, older age was also associated with more detrimental changes in glucose metabolism in trans women, which implies that screening for impaired glucose tolerance in the older trans population should not be forgotten.

5.1.2 LONG-TERM EFFECTS AND SAFETY OF CROSS-SEX HORMONE THERAPY

Cross-sex hormone therapy is generally continued after SRS and it has been assumed that the principles of this treatment are similar to those of other hypogonadal persons aiming at sex steroids levels within the physiological range of the desired sex. Whether in the long-term all functions of sex steroids in trans persons are effectively covered by cross-sex hormones and whether the administration of these therapies is appropriately safe, remains unknown[292]. Therefore, we also aimed to investigate the longer term clinical effects, side effects and safety of

cross-sex hormone therapy in both trans women and men (**chapter 2.3, chapter 2.4 and 2.5**). In **chapter 2.4,** we examined 100 trans persons post SRS and on average 10 years on cross-sex hormone therapy. We observed that 11.5% of trans women and 26.7% of trans men had supraphysiological levels according to reference values of the desired sex, which may increase the risk for cardiovascular events. In contrast, 63% of trans women and 8.9% of trans men exhibited subphysiological sex steroid levels which put trans persons at risk for side effects known from hypogonadal states. Indeed, an important finding of these studies was that a high number of trans women (about 25%) experienced osteoporosis at the lumbar spine, hip, or radius whereas in trans men no osteoporosis was diagnosed. Our results in trans men substantiated others whereas our findings in trans women are in contrast to most other studies[289, 297-299, 307, 341-344]. This may be related to past treatment regimens where cyproterone acetate was generally prescribed alone for 12 months without concomitant use of estrogens as it is well-established that androgen deprivation induces bone loss. Another explanation could be that trans women had an inadequate estrogenization, given the low estrogen and high gonadotropin levels observed in these women. However, the absence of clinical symptoms and high SHBG levels did not suggest this. Biochemical markers of bone turnover were also not increased in these women and were therefore not suggestive for an increased bone turnover. Finally, given our cross-sectional study it may be that trans women at our center had low bone mass before the start of cross-sex hormone therapy. Indeed, our research group recently found that trans women, before any treatment, had a higher prevalence of osteoporosis compared to age-matched control men, possibly related to the lower physical activity and lower vitamin D levels found in trans women compared to controls[378]. Why trans women in our center experience more osteoporosis compared to other centers, is not well-known. One could speculate that trans women in Belgium might have a lower physical activity rate compared to those in other centers as lower levels of physical activity were observed in the Belgian population compared to most other European countries[379, 380]. But future multicenter studies such as the ENIGI study are needed to investigate the underlying mechanisms of the higher prevalence of osteoporosis at our center.

In chapter 2.1 and chapter 2.2, we described that anti-androgen and estrogen therapy in trans women and T therapy in trans men both improved and impaired some aspects of the profiles of cardiovascular risk factors during the first year of treatment. How long-term cross-sex hormone therapy affects cardiovascular risk factors, was addressed in our clinical studies (**chapter 2.3**.). We observed that trans women had a similar prevalence of cardiovascular risk factors including hypercholesterolemia, hypertension, smoking and obesity compared to trans men. Additionally, we compared these prevalence rates to published ones of the general population and found that

similar or even less cardiovascular risk factors were present apart from smoking, which occurred more in trans persons. In spite of these relative comparable cardiovascular risk factors and comparable duration of cross-sex hormone therapy, 12% of trans women in this study experienced a cardiovascular adverse event during hormonal therapy compared to not a single trans man. The younger age of trans men may be a possible explanation as for a comparable duration of hormonal therapy, trans women will be significant older in comparison with trans men. Indeed, it is well-established that trans men present themselves at younger ages[381], which was also confirmed in our ENIGI study. Nevertheless, others also found that cross-sex hormone therapy in trans men was acceptably safe whereas trans women exhibited a higher rate of cardiovascular disease[241, 261]. With regard to hormone related cancers, we observed none in our clinical studies in both trans women and men.

Because the sample sizes were rather small in our clinical studies and the lack of an age-matched control group to compare morbidity rates of trans persons to the general population, we initiated the TransBel study. This is a multicenter cross-sectional study in Belgium, with the aim to investigate morbidity of trans persons in Belgium. As recruitment in other centers is still ongoing, we presented in this thesis data from our center and compared it to an age- and gender-matched control population recruited from a population based study on sexual health in Flanders (Sexpert study). We presented one of the largest samples published in literature including 352 trans persons who were on average 7.4 years on hormonal therapy (**chapter 3.4**). We confirmed the results from our clinical studies, showing a low risk for cardiovascular events and cancer in trans men whereas our results in trans women suggest no higher risk for cancer but an increased risk for cardiovascular disease.

Firstly, a relatively high incidence of venous thrombosis (5%) was observed in our TransBel study, with half of these events occurring during the first year of therapy. This is in contrast with our prospective ENIGI study, where not a single case was observed during our 12 month observation. As already mentioned, these results may suggest that our newer treatment regimens, which avoid the use of EE and use transdermal estrogens in older trans women, have less detrimental effects on the coagulation system. In line with others[260-261], we observed an increased risk for venous thrombosis and/or pulmonary embolism at the time of SRS, which confirms the well-known risk for major abdominal and pelvic surgery and immobilization on thrombosis development. Alternatively, it is well-known that oral contraceptives and hormone replacement therapy increase the risk of thrombosis during and after surgery. However, the existing evidence regarding the necessity to discontinue hormonal therapy before surgery in trans women remains

limited, as there are no general guidelines available at this moment. Nevertheless, we currently advise to discontinue hormonal therapy at least two weeks before SRS or other elective surgeries and to restart at mobilization. However, it may be that this time period is too short, as it is not well-known at which time point changes in clotting factors are restored after stopping cross-sex hormone therapy. Future studies are needed to resolve this research question.

Secondly, the majority of trans women in our study, who experienced myocardial infarction or cerebrovascular disease were aged over 50 years, had one or more cardiovascular risk factors (mainly smoking), and had undergone cross-sex hormone therapy for a short duration. These findings may possibly suggest that estrogen therapy can aggravate pre-existing cardiovascular disease, which has also been described in postmenopausal hormone replacement therapy in older women as suggested by the "timing hypothesis". One potential explanation for these findings may be that besides beneficial changes such as in lipid status, estrogen therapy also increases proinflammatory factors such as matrix metalloproteinase 9, which destroy the matrix of the atheromatous plaque causing instability and rupture[382-383]. In younger women, estrogen therapy also increases these proinflammatory factors, but in the absence of a significant plaque there is no substrate on which these proinflammatory factors would act[384].

This brings us to the safety of cross-sex hormone therapy in women with established cardiovascular disease and older trans women. Unfortunately, studies in these populations are currently lacking, but it is widely believed that cardiovascular risk factors should be managed before the start of cross-sex hormone therapy and when they emerge[5]. However, whether hormonal therapy should be stopped or a dosage reduction needs to be performed depending on certain cardiovascular risk profiles, is uncertain. In trans persons, the decision to stop or lower estrogen dosage is also complicated by the explicit wish of many trans women to continue high dose estrogen therapy. Based on our data we may recommend that older trans women or women with known cardiovascular risk factors, especially smoking, should receive low dose estrogens, as higher estrogen dosages were found to be associated with a higher risk of cardiovascular disease in women using COC's and hormone replacement therapy. It may also be that these women may benefit from the transdermal route of administration of estrogen therapy as the latter induces fewer changes in proinflammatory markers. However, data concerning the effects of transdermal estrogens on cardiovascular end points in women using COC's and hormonal replacement are conflicting. A nested case control study on hormonal replacement therapy in postmenopausal women observed that users of high dose transdermal estrogen therapy had a higher stroke risk compared to those on high dose oral estrogens, but that low dose transdermal estrogen therapy

had a lower risk, than low dose oral estrogen treatment[385], whereas another national observational study in almost 700 000 Danish women aged between 51 and 69 found a lower risk for myocardial infarction in women using transdermal HRT compared to those on oral HRT[386]. In women of reproductive age, use of transdermal contraceptives was associated with a similar risk for development of MI[210, 387]. Concerning the risk for thrombotic stroke, a recent large population study observed a higher risk in women who used contraceptive patches or a vaginal ring[210] in comparison with those on oral treatment. Nevertheless, there is a need for studies determining the safety of different cross-sex hormone therapies in older patients and patients with established cardiovascular risk factors and disease. In addition, guidelines are needed to assist clinicians in follow-up and management of cardiovascular risk factors during cross-sex hormonal therapy.

Our findings concerning cancer in trans persons after long-term cross-sex hormone therapy are in line with others, indicating a low risk risk for development of hormone related cancers. Moreover, the prevalence of cancer was even lower in trans men compared to the control population in our TransBel study. Because of the bilateral mammectomy, oophorectomy and hysterectomy, trans men have a lower risk for development of these cancers, which are commonly diagnosed in cisgender women. Nevertheless, some cases of breast and ovarian cancer have already been described before surgery[336-338] and after mammectomy[339-340]. Several important notes should be made concerning cancers in trans persons. Firstly, the current evidence is mainly based on trans persons who almost all underwent SRS. Therefore, these results cannot be extrapolated to trans persons who do not undergo SRS due to personal convictions or financial restraints. As T can also be aromatized to E_2, the endometrium of trans men without hysterectomy for example is exposed to unopposed action of estrogens and the latter is known to increase the risk for endometrial carcinoma. Caregivers and trans persons themselves should be aware of this, and trans persons should be screened conform the biological sex if reproductive organs have not been surgically removed. Given the discomfort that trans persons experience with medical exams concerning their native sex, such as gynecological examinations in trans men, it is unlikely that many trans persons themselves will take initiative for screening.

In addition, it is likely that the current follow-ups were too short as it takes years to induce cancers. Therefore, the incidence of hormone related cancers may possibly increase with longer hormone exposure time. This hormone exposure time will also drastically increase in the future, as presently, adolescent trans persons can be eligible for hormonal treatment and trans persons often receive hormonal therapy beyond menopausal age. This leads us to the point how long

hormonal therapy should be continued in trans persons. No guidelines for this issue are presently available, but this matter will become increasingly relevant as the trans population ages. An important consideration in this discussion is whether the guidelines for hormone treatment of postmenopausal women can be applied to trans women or likewise those for T treatment in elderly males, to trans men.

Nevertheless, as stated above, it may be that longer sex steroid exposure increases the risk for hormone dependent cancers. Indeed, in postmenopausal hormone replacement therapy it was found that breast cancer incidence increased in women receiving about 7 years of hormone replacement therapy, compared to those receiving placebo. This would argue in favour of estrogen treatment cessation at a certain age or duration of hormonal therapy in trans women. As already mentioned, how long-term cross-sex hormone therapy affects cardiovascular health, remains to be determined. Alternatively, one can speculate that stopping hormonal therapy in trans women, who underwent gonadectomy, will increase the prevalence of hot flashes. In addition, stopping hormonal therapy will drastically decrease bone mineral density as observed for example in women receiving treatment for cancer, who experience a more profound estrogen depletion compared to postmenopausal women. Keeping in mind the already high prevalence of osteoporosis before the start of treatment, this would mean that many trans women might experience severe osteoporosis. However, besides hormonal therapy, other treatment options are currently available for osteoporosis prevention and treatment.

Although cross-sex reassignment therapy has been associated with better functioning in terms of gender dysphoria relief, psychological functioning, and decrease in suicide attempts[367, 388] we observed a high number of suicide in trans persons, especially in trans women. Our results hereby confirm others[310, 312] showing an increase of suicide in this specific patient population. Moreover, the strikingly high prevalence of suicide attempts of 41% reported in a recent nation-wide U.S.-based survey of 6456 self-identified transgender/gender non-conforming individuals[389] shows the fragile position of many trans persons. In general, this phenomenon has been related, at least in part, to minority stress caused by a number of factors such as discrimination, stigma and rejection. This also implies that clinicians working in trans persons such as endocrinologists should be thoughtful about this, especially as many trans persons quit psychological follow-up after transition. Hopefully, research such as ours, will add to respect in the scientific community for this patient group. This may reflect in political and juridic actions and eventually society's view on gender dysphoria, and lead to the amelioration of QOL of trans persons.

5.1.3 SEXUAL DESIRE

Sexual health is an important element of general health, also for trans persons. However, despite major effects of both hormonal and surgical therapy on sexual functioning of trans persons, this is still an underexposed topic by health care professionals dealing with trans individuals. From a previous study of our center[353], it was found that transsexuals' expectations were less met at a sexual level in comparison with a social or emotional level, indicating the need to counsel trans persons about possible sexual changes. Therefore, we also aimed to investigate possible effects of cross-sex hormone therapy on sexual desire of trans women and men, as it is known from cisgender men and women that sex steroids strongly affect sexual desire.

Previous cross-sectional studies concerning the effects of cross-sex reassignment therapy on sexual desire in trans women showed conflicting results with some observing an increase, whereas others described a decrease or no effect. In trans men, not a single study investigated the effects of T therapy on sexual desire specifically. We are the first to present a prospective evaluation of sexual desire during the first year of cross-sex hormone therapy and described that trans women experienced significant lower levels and trans men significant higher levels of sexual desire (**chapter 2.1**). These results were also confirmed in our TransBel study as trans women reported a decrease of sexual desire since the start of cross-sex hormone therapy whereas trans men mentioned an increase (**chapter 3.1**). Moreover, in the TransBel study, 73% of trans women never or rarely experienced spontaneous and responsive sexual desire. A third of these women also reported associated personal or relational distress which results in a prevalence of HSDD of 22%. This prevalence is higher compared to the general male and female population. In contrast, the prevalence of HSDD in trans men was comparable to the general male population. Moreover, a small group of trans men (3.6%) even reported personal or relational distress due to high levels of sexual desire. Whether sexual desire levels in trans men are related to sex steroid levels, was not addressed previously. Therefore ,we investigated these potential associations in the clinical trans men study (**chapter 3.2**). No direct associations were observed between T and solitary or dyadic sexual desire, but an independent association of LH on solitary sexual desire was found. Trans men with elevated levels of LH, indicating suboptimal testosterone therapy, reported significantly lower solitary sexual desire levels than those with low LH levels. Suppressed LH levels were also associated with having a higher need for sexual activities and a higher frequency of experiencing excessive sexual desire. In general, our results suggest an important impact of cross-sex hormones on sexual desire in both trans women and men, with the majority of trans women experiencing a decrease of sexual desire whereas the opposite was found

in trans men. This implies that trans persons and their partners should also be counseled about the changes in sexual desire when choosing cross-sex hormone therapy. The old paradigma of no sexual desire in trans persons can be rejected. Although a previous study from our center was unable to show associations between serum T levels and sexual desire in trans women[357], larger scale studies using LC-MS MS and standardization of blood sampling relative to timing of treatment administration are needed to fully confirm this observation. In addition, randomized studies are needed to determine whether low dose T therapy in trans women suffering from HSDD after starting cross-sex hormonal therapy may benefit these patients.

5.1.4 QUALITY OF LIFE AFTER SEX REASSIGNMENT TREATMENT

QoL is an evaluation of personal well-being and includes several aspects such as emotional, social and physical functioning. In health care, QoL is often measured to determine how the individual's well-being may be affected by a certain disease or its treatment. Most previous studies[364-366] showed that QoL in trans persons was poorer when compared with a general population sample or control group but previous studies made no adjustments for well-known determinants of QoL such as age, gender and socio-economic status. In **Chapter 4**, we compared QoL of trans persons to an age- and sex matched control population and found, in line with previous studies, that QoL of trans persons was indeed poorer compared to our control population, even after correction for socio-economical status. Both physical and mental functioning were significantly lower in trans persons, but the absolute difference in physical functioning was rather small, whereas it was marked for mental functioning. This may suggest two things. First, cross-sex hormone therapy and SRS induce important physical changes and both therapies are associated with side effects and adverse events, but these drastic effects do not seem to affect physical functioning considerably in the majority of trans persons. Secondly, although it is generally acknowledged that sex reassignment therapy improves mental well-being of trans persons, trans persons still had a clinically significant poorer mental health also after these treatments.

As mentioned earlier, one explanation can be found in the minority stress transgender people face due to social stigma, prejudice and discrimination because of their gender nonconformity. It is indeed known that the majority of trans individuals have reported discrimination. This social discrimination is also suggested by our findings that trans persons had a marked lower socio-economical position despite a similar or even higher educational level. In addition, many trans persons even experienced violence (both verbal as physical) because of their gender identity or presentation. Motmans and colleagues[390] recently reported that 78.9% of trans individuals (living

in Belgium, the Netherlands or France) experienced verbal violence whereas more than a quarter experienced physical (26.8%) and sexual violence (31.6%). Considering the minority stress model, increasing public policy initiatives are needed to change the social norms, attitudes and structures, in order to reduce psychological distress and improve QoL of trans individuals. In addition, efforts to prevent discrimination and violence towards trans individuals are urgently needed.

Providing a better understanding of the strongest predictors of QoL in trans persons may also help health care providers and policy makers to make the best use of resources. Therefore, we also investigated the main determinants of QoL in trans persons using a regression model including both clinical and socio-demographic correlates, as this was not addressed previously in trans persons after sex reassignment therapy. It was found that hormonal treatment satisfaction and employment status were independent predictors of QoL in trans persons. These findings corroborate with those from Gómez-Gil et al.[370] and Gorin-Lazard et al.[369], who have assessed the predictors of QoL in trans persons before SRS as they both observed that hormonal therapy was an independent predictor of QoL. We were unable to investigate this hypothesis, as almost all our respondents were on hormonal therapy, but our findings seem to confirm the importance of hormonal therapy on the QoL of trans persons, even after SRS. Cross-sex hormone therapy is an essential part of the medical treatment of trans persons and induces body features and shape of the desired gender, which results in a reduction of self-reported distress. Besides inducing physical changes, the act of using cross-sex hormones is itself an affirmation of gender identity. In addition, many trans persons change gender role around the time of starting hormonal therapy, which could also improve social and mental functioning. Our results show that not only the initiation of cross-sex hormone therapy is important, but also the satisfaction with hormonal treatment. Trans persons who were more dissatisfied with their hormonal therapy might have been less satisfied with their body changes or experienced bothersome side effects, which could all affect mental and physical functioning. Future research investigating the main predictors of hormonal therapy satisfaction is of interest; this may include body image satisfaction, experience of side effects, severity of side effects, duration of hormonal therapy etc.

In addition, it was previously found that family support and working status were independent predictors of QoL in trans persons. Strength of social support was not investigated in our study, but we also found the importance of family structure: parenthood was negatively associated with QoL scores in trans women, whereas the opposite was seen in trans men. A possible explanation may be that the majority of children in trans women (82%) were conceived before sex reassignment therapy, whereas in trans men the opposite is the case (36%). It is well-known that

trans men present themselves at a younger age compared to trans women and our findings may thus reflect the higher numbers of familial difficulties trans women encounter due to sex reassignment treatment. In line with others, we also observed that working status was an independent predictor of both physical and mental functioning scores. From clinical practice it is well-known that many trans persons experience problems finding a job, especially during sex reassignment. A potential way to improve QoL is the stimulation of employment programs for this population.

In line with others, we observed that trans women who underwent facial feminization surgery had a better QoL compared to those who did not. Moreover, we observed that facial feminization surgery was an independent positive predictor of mental functioning in trans women. The better passability of trans women who underwent facial feminization surgery may improve body image satisfaction and self-esteem, but recent research also showed that a better passability was associated with less experience of discrimination and violence[390]. For that reason, reimbursement of this surgery might also be considered to improve QoL of trans women.

5.2 LIMITATIONS AND PERSPECTIVES

We provided in this thesis several findings concerning the short- and long-term clinical effects, side effects and adverse events of cross-sex hormone therapy in trans men and women and its associations with QoL. From these findings, we are already able to inform our future clients, their families, and other caregivers more accurately on the desired effects, side effects, and adverse events of these therapies in our daily practice. Nevertheless, the overall knowledge concerning this topic remains scarce when compared to other patient groups. Firstly, we presented one of the largest prospective and cross-sectional samples published in literature so far, but our sample sizes were still too limited to provide accurate estimates of morbidity rates. To this end, we plan to continue and expand our ENIGI study and several other gender clinics such as Amsterdam and Florence recently joined this study. In addition other centers such as Tel Aviv and Copenhagen showed interest. Also in the TransBel study, recruitment in other Belgian centers is ongoing with the aim to increase our statistical power. Upon completion, TransBel will be the first nationwide study available.

In light of the aging trans population, safety of both short- and long term hormone therapy use needs to be examined in older trans persons, especially considering our observations that older trans women experienced a higher risk for cardiovascular disease. In this regard, we will further include trans persons in our ENIGI study, with additional attention to the aging group, and we will also continue to invite them for follow-up measurements in the next years.

Further, during the ENIGI study we aimed to describe effects of currently used treatment modalities and we compared our results with previously published findings. From this it seems that, especially in trans women, newer treatment modalities may be associated with fewer risks. However, to prove the benefit of certain types and dosages of anti-androgens, estrogens and T therapies above others, randomized controlled trials are needed. A detailed study protocol for a randomized trial conducted in several European gender clinics is already available, but implementation is hampered at this moment due to financial restraints.

In addition, we observed that breast development was not satisfactory to many trans women. It has been recently suggested[391] that excessive estrogenic action may negatively affect breast development. This brings up the question whether a step up dose of estrogens would be preferable to enhance breast development in trans women. No data are currently available regarding the effects of this treatment on breast development of trans women, but step up dosages of unopposed estrogens are usually prescribed for puberty induction, for example in

Turner girls. This therapy is mainly prescribed to avoid acceleration of bone maturity by high dose estrogen treatment resulting in a reduced final height. But, beneficial effects on breast development have been suggested, although rather clinical than experienced based. Considering the importance of breast development for many trans women, future studies are needed to examine this research question. In this regard, studies in adolescent trans women may also be of interest.

Finally, we have identified many clinical effects, side effects but also adverse effects of cross-sex hormone therapy. Nevertheless, we certainly did not study all possible effects in this work. Sex hormones may, for example, also influence brain and behavior as distinct sex differences have been described on both structural and functional MRI data. To date, the exact psychological and cognitive effects and side-effects of cross-sex hormonal treatment are not clear. To further examine these potential effects, we have very recently performed a cross-sectional fMRI study in 20 trans men and 20 trans women after long-term cross-sex hormone therapy compared to an age-matched control population. Data are currently being analysed.

ABOUT THE AUTHOR

Katrien Wierckx was born on the 8th of July, 1985 in Ghent, Belgium. After her high-school education at the Don Bosco College in Ghent, she started her Medicine studies in 2003 at the Ghent University and obtained her master degree in Medicine in 2010 (magna cum laude). Afterwards, she started as a PhD student at the Department of Endocrinology at the Ghent University Hospital under supervision of Prof. Dr. Guy T'Sjoen, with a research focus on clinical effects of cross-sex hormone therapy in trans persons. From April 2014, she will continue her training in Internal Medicine.

Katrien Wierckx is currently (co)-author of 18 international publications. She won the Young investigator's Award of the Belgian Endocrine Society in October 2013.

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ADDENDUM

PREVALENCE OF GENDER INCONGRUENCE IN FLANDERS

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ABSTRACT

Gender nonconformity refers to the extent to which a person's gender identity, gender role and/or gender expression differs from the cultural norms prescribed for people of a particular sex, within a certain society and time. Most data on the prevalence of gender nonconformity focus on the prevalence of gender dysphoria (which also includes a distress factor) or on the number of legal sex changes. However, not every gender nonconforming individual experiences distress or applies for treatment. Population-based research on the broad spectrum of gender nonconformity is scarce and more information on the variance outside the gender binary is needed. This study aimed to examine the prevalence of gender incongruence (stronger identification with the other sex than sex assigned at birth) and gender ambivalence (equal identification with the other sex as the sex assigned at birth) in a population-based survey in 1,832 Flemish persons and in 2,472 lesbian, gay and bisexual (LGB) individuals in Flanders. In the general population, gender ambivalence was present in 2.2% of male and 1.9% of female participants whereas gender incongruence was found in 0.7% of men and 0.6% of women. In LBG individuals, the prevalence of gender ambivalence and gender incongruence were 1.8% and 0.9% in males and 4.1% and 2.1% in females, respectively.

This study was one of the first population-based surveys to assess gender nonconformity in a probability sample. Considering a current Flemish population of about 6 million, our results indicate the presence of gender incongruent men between 17,150 and 17,665 and between 14,473 and 15,221 gender incongruent women in the Flemish population. Gender incongruence and gender ambivalence were generally more present in LGB women but not in LGB men.

INTRODUCTION

Gender nonconformity refers to the extent to which a person's gender identity, role or expression differs from the cultural norms prescribed for people of a particular sex, within a certain society and time. Gender dysphoria refers to the discomfort or distress that is caused by the incongruence between a person's gender identity and that person's sex assigned at birth (and the associated gender role and/or primary and secondary sex characteristics). The World Professional Association for Transgender Health stresses that only some gender nonconforming people experience gender dysphoria at some point in their lives[1]. Gender dysphoria however, is the most known, but also the most extreme form of a broad spectrum of gender nonconformity (DSM-5, 302.85; ICD-10, F64.0).

Most data on the prevalence of gender nonconformity only focused on the prevalence of gender dypshoria. The prevalence in Belgium was 1: 12,900 for male-to-female and 1: 33,800 for female-to-male transsexual persons, based on the number of sex reassignment operations done in Belgium[2]. The definition of gender dysphoria in current research has not been uniform: prevalence numbers were based on the number of legal sex change as noted in a national register[3], the start of cross-sex hormonal therapy[4-6], the number of applicants for sex reassignment surgeries (SRS)[7], attending a gender clinic[8], or the number of sex reassignment surgeries (SRS) performed by different surgeons[1]. This implies an important selection bias as only those who seek and/or have access to medical and/or surgical treatment will be counted. Indeed, a primary care study revealed that 40% of people defined as having a gender identity problem who consulted a general practitioner, had not received hormonal therapy or SRS[9]. This suggests that many gender incongruent persons are probably not attending a secondary care service or gender clinic. Some persons may be reluctant to seek health care and treatments due to financial restraints, shame, or fear for potential for social exclusion, e.g., losing one's family, job, friends or partner[1, 11-12]; whereas others may not desire hormonal treatment, SRS, or change of official documents. Our knowledge concerning this broader spectrum of gender nonconformity is currently scarce[11-12]. However, this information is needed to inform society on gender diversity and to address to potential needs of this group.

Population-based research is required to address an estimation of this broader spectrum of gender nonconformity. The aims of this study were to investigate the prevalence of gender identity nonconformity in the general population in Flanders (Belgium). In particular, we measured gender incongruence (stronger identification with the other sex than sex assigned at

birth) and gender ambivalence (equal identification with the other sex as the sex assigned at birth).

Although it is not evidence based, it is presumed, also by some health care professionals, that gender dysphoria is more frequent in LGB individuals. Therefore, the same questions on gender identity were included in a large study in LGB individuals as well.

METHODS

PARTICIPANTS

This study used data from the survey "Sexual Health in Flanders"[13], a large-scale representative survey on sexuality, sexual health and relations in Flanders (the northern, Dutch speaking part of Belgium that has about 6 million inhabitants). The survey contained extensive information on sexual health characteristics and demographic, biomedical, psychological, and sociocultural correlates and aimed to investigate sexual health in Flanders and sought for possible explanations using bio-medical, psychological, demographic and socio-cultural correlates.

Participants aged between 14 and 80 years were randomly drawn from the Belgian National Register. Data were collected between February 2011 and January 2012. The final database consisted of 1,832 participants (response rate: 40.0% of the eligible participants).

In order to enhance statistical power in each of the three pre-defined age categories, we used a stratified sample implying that one-third of the sample consisted of adolescents (aged 14 to 25), one-third in the middle age group (aged 26 to 49), and one-third in the oldest group (50 to 80 years old). After data collection, the data were weighted by gender, age, and schooling level in order to make them representative of the population of Flanders aged 14–80.

All data were gathered via face-to-face interviews, with a combination of computer-assisted personal interviewing (CAPI) and computer-assisted self-interviewing (CASI). In particular, all sensitive information, i.e., a wide range of sexual health characteristics, was gathered in a CASI set-up, so that participants never had to share private information about their sexual health with the interviewer. Detailed study design have been described elsewhere[13].

The second study used data from the internet based survey "Click Out of the Bed Room", a large-scale survey on sexuality, sexual health, and relations in LGB individuals in Flanders. The questionnaire was identical to the first study but significantly shorter to avoid respondent drop out. Therefore, we only aimed to investigate the current sexual health in LGB individuals. Bio-medical, psychological, extensive demographical and socio-cultural correlates were limited.

Participants between 13 and 86 years of age were included. Data were collected between September 2011 and March 2012. The final database consists of 3,702 participants. It was also important to recruit LBG individuals who do not identify themselves as gay, lesbian or bisexual. Therefore, we set up a neutral as well as a LGB-oriented campaign. The neutral campaign refers to 10,000 posters that were spread all over Flanders containing an image that did not refer to being lesbian, gay or bisexual in particular. The message on the poster presented the survey as related to sexual health in general. Banners, adds on the Internet, and press releases also contained this neutral image and message. In addition, we also set up a specific recruitment strategy to target LGB individuals including the following channels to broadcast a request for participants: specific locations such as LGB discotheques, LGB parties, and LGB events; advertisements in the written press; LGB-specific and non-LGB-specific associations and organizations were invited to spread the invitation; electronic mailings were sent and the Internet was used (posting banners on LGB-specific websites). More details on the recruitment have been described elsewhere[14].

Out of the total number of respondents, 35.4% found our site through a social network site (mainly Facebook), 18.5% through electronic mailing, 15.3% through television, radio, newspaper or magazine, 10.6% through clicking on a banner on a website, 6.7% through their school or work, 3.7% through a gadget or flyer, 2.2% through an association or activity, 1.7% through a poster and 5.8% through other means.

Both studies were approved by a Medical Ethical Committee and all participants gave consent for participation to the study. The general characteristics both sampled are displayed in Table 1.

TABLE 1. GENERAL CHARACTERISTICS OF THE STUDY POPULATIONS

	POPULATION SURVEY (N=1832)	SEXUAL MINORITY INDIVIDUAL SURVEY (N=2472)
Gender		
Women	49.8	37.4
Men	50.2	62.6
Age (years)		
≤29	27.0	49.0
30- 49	34.1	37.5
50-80	39.1	13.5
Education		
School going	11.6	27.8
None / Primary School	18.2	1.4
Primary high School	20.4	4.6
Secondary high School	22.5	17.8
Bachelor/master	27.0	48.3
Partner		
Yes	77.4	36.7
No	22.6	63.3

Data are presented as (%)

MEASURES

Gender identity and role was assessed in both studies as described by Schoonacker, Dumon and Louckx[15] using 6 questions, each scored on a 5-point Likert-scale (1 = totally disagree, 5 = totally agree). Participants were asked to answer following items: "I feel like a woman" (Item 1), "I feel like a man" (Item 2), "I look feminine" (Item 3), "I look masculine" (Item 4), "I wish to be more feminine" (Item 5), "I wish to be more masculine" (Item 6). Based on the first two items, the presence of gender ambivalence (yes/no) and gender incongruence (yes/no) was assessed similarly to previous research[11,16]. Gender ambivalence was considered if the same answer was given on both Item 1 and 2 (scores 1–1; 2–2; 3–3; 4–4; and 5–5). Incongruent gender identity reflects a higher score on the scale measuring feeling like the opposite birth sex than on those of the sex assigned at birth (scores 1–2; 1–3; 1–4; 1–5; 2–3; 2–4; 2–5; 3–4; 3–5; 4–5).

In the LGB survey, sexual orientation was conceptualized as a three dimensional construct measuring self-identification, sexual behavior, and sexual desire[17]. These dimensions were used to obtain a broader definition of LGB, as it is important to include persons who display homosexual behavior but do not identify as LGB[18-19]. The definition should not be too broad either, as we did not want to include people who scored on one or two dimensions completely heterosexual. Sexual self-identification was assessed with the question, "How would you identify yourself?". Participants could answer on a 5-point Likert scale (i.e., straight, more straight then

gay/lesbian, bisexual, more gay/lesbian then straight, gay/lesbian). An open-end response category was added for participants who did not identify with any of these labels (referred to as "other"). To measure sexual behavior, we first asked participants "Throughout your life, with how many people did you have sex?" (open-ended numeric answer category). Then we asked "Were these people men, women or both?" Participants could answer on a 5-point Likert scale (from 1 = only women to 5 = only men). To measure sexual desire we asked participants "Do you sexually fantasize about men, women or both?" and "Do you feel sexually attracted to men, women or both?" Participants could answer on a 5-point Likert scale (from 1= only about or to women to 5= only about or to men). Participants could also answer these questions with "only about or to none" With the information from these four items, we created a dichotomous variable categorizing participants as non-heterosexual (i.e.,"0") or heterosexual (i.e.,"1"). They were identified as non-heterosexual when they reported to identify as gay/lesbian, bisexual or more gay/lesbian than straight or when they reported to have at least as many same-sex sexual fantasies as opposite sex fantasies, or when they reported to feel at least as often attracted to the same-sex as to the opposite sex, or when they reported to have had at least as many same-sex sexual contacts as opposite sex sexual contacts[14]. This resulted in a final number of 2,472 non-heterosexual persons included in our analyses; heterosexual defined persons were excluded.

STATISTICAL ANALYSIS

Chi square analysis was used to evaluate differences in gender incongruence and gender ambivalence between men and women and to compare the prevalence of gender incongruence and ambivalence with previous research[11]. All data were analyzed using SPSS-software version 21 (SPSS Inc., Chicago, IL).

RESULTS

POPULATION BASED STUDY

In Flemish men, 1.4% reported feeling to be a woman and 2.3% did not feel being a man. Of all men, 2.4% desired to be more feminine and 47.6% did not want to be more masculine. When asked about their appearance, 1% of men reported to look feminine and 2.2% noted not to look masculine.

In Flemish women, 1.7% felt being a man and 1.5% did not feel being a women. 1.7% reported to look masculine and 1.9% noted not to look feminine. 1.1% wished to be more masculine and 58.7% disagreed on wishing to be more feminine.

Gender ambivalence was found in 2.2% of males and 1.9% of females. The prevalence of gender incongruence was 0.7% in men and 0.6% in women (Table 2). Prevalence rates of gender incongruence and ambivalence were not significantly different between men and women, χ^2= 1.28, p= .55. Not all gender incongruent or gender ambivalent persons wished to be more like the opposite sex and a variation in appearance was observed (Table 3).

TABLE 2. PREVALENCE OF GENDER AMBIVALENT AND INCONGRUENT IDENTITY IN THE POPULATION-SURVEY

	MEN (N=894)	WOMEN (N=905)	TOTAL POPULATION (N=1799)
Gendercongruent	97.1 (96.0-98.2)	97.5 (96.5-98.5)	97.4 (96.7-98.1)
Genderambivalent	2.2 (1.5-3.4)	1.9 (1.0-2.8)	2.0 (1.4-2.7)
Genderincongruent	0.7 (0.2-1.3)	0.6 (0.2-1.3)	0.6 (0.2-1.0)

Data are presented as % (95% confidence interval)

TABLE 3. SELF-PERCEIVED APPEARANCE

	GENDER AMBIVALENT	GENDER INCONGRUENT	GENDER CONGRUENT
MEN	100 (N=20)	100 (N=6)	100 (N=868)
I look masculine (disagree/totally disagree)	36.9	59.7	1.3
I look feminine (agree/totally agree)	5.8	0	0.9
I wish to be more masculine (disagree/totally disagree)	79.2	75.5	48.6
I wish to be more feminine (agree/totally agree)	10.6	54.0	1.8
WOMEN	100 (N=17)	100 (N=5)	100 (N=883)
I look masculine (agree/totally agree)	11.9	57.3	1.3
I look feminine (disagree/totally disagree)	15.8	86.4	0.9
I wish to be more masculine (agree/totally agree)	11.9	0	0.8
I wish to be more feminine (disagree/totally disagree)	60.1	60.4	60.3

Data are presented as % within gender ambibalent, gender incongruent or gender congruent sample

LGB SURVEY

In the LGB survey, the prevalence of gender incongruence was 0.9% in men and 2.1% in women (Table 4). The prevalence of gender ambivalence was 1.8% in males and 4.1% in females. Prevalence rates of gender ambivalence and incongruence were significantly different between men and women (GA: χ^2= 10.95, p<0.001; GI: χ^2=5.39, P=0.02). A higher prevalence of gender incongruence and gender ambivalence was found in LGB women compared to women in the population based survey (GA: χ^2= 6.84, p= .009; GI: χ^2= 6.60, p= .01). No differences were observed in prevalence rates of gender incongruence and gender ambivalence in LGB men compared to men in the population based survey (GA: χ^2= .29, p= .59; GI: χ^2= .09, p= .76).

TABLE 4. PREVALENCE OF GENDER AMBIVALENT AND INCONGRUENT IDENTITY IN THE LGB SURVEY

	MEN (N=1549)	WOMEN (N=923)	TOTAL POPULATION (N=2472)
Gendercongruent	97.3 (95.5-98.1)	93.8 (92.3-95.4)	96.0 (95.2-96.8)
Genderambivalent	1.8 (1.1-2.5)	4.1 (2.8-5.4)	2.7 (2.1-3.3)
Genderincongruent	0.9 (0.4-1.4)	2.1 (1.2-3.0)	1.3 (0.9-1.8)

Data are presented as % (95% confidence interval)

COMPARISON WITH PREVIOUS RESEARCH

A comparison of the current results to Kuyper & Wijsen (2014)[11] is shown in Table 5. In this study in a neighbouring country, the Netherlands, Kuyper et al. asked participants if they felt psychologically as male and/or as female in an internet based population survey on two items, scored on a 5-point Likert-scale (1 = *totally disagree*, 5 = *totally agree*). The Dutch participants were recruited, by use of a random sample, from a large Internet panel for online surveys and members received so-called "clix" participating in online surveys, which they could use to buy products on the Internet. During data gathering, representativeness for the Dutch population was checked and recruitment was adapted to complete shortages or limit overrepresented population groups.

The observed percentages of gender incongruence in Flanders were comparable to those of the Dutch survey. The prevalence of gender ambivalence in men was higher in the Dutch survey (Table 5).

TABLE 5. COMPARISON WITH KUYPER ET AL.

	SEXPERT (N=1799)	KUYPER (2014) (N=8064)	χ^2	P*
Genderambivalent men	2.2 (1.5-3.4)	4.6 (4.0-5.2)	9.97	0.002
Genderambivalent women	1.9 (1.0-2.8)	3.2 (2.7-3.7)	3.90	0.05
Genderincongruent men	0.7 (0.2-1.3)	1.1 (0.8-1.4)	0.79	0.37
Genderincongruent women	0.6 (0.2-1.3)	0.8 (0.5-1.1)	0.17	0.68

Data are presented as % (95% confidence interval); *Chi square test

DISCUSSION

This is the first study investigating the prevalence of gender nonconformity in the Flemish population and one of the few studies assessing gender nonconformity in a population-based survey. We found a prevalence of gender incongruence of 0.7% and 0.6% and gender ambivalence of 2.2% and 1.9% in men and women, respectively. Extrapolated to the current number of Flemish inhabitants, gender ambivalence would concern between 54,256 and 55,162 Flemish men (95% confidence interval) and between 47,020 and 47,865 women and gender incongruence between 17,150 and 17,665 men and between 14,743 and 15,221 women. These numbers are much higher than the prevalence of gender dysphoria in clinical settings. As mentioned before, a prevalence rate of transsexualism in Belgium was estimated at 1: 12,900 for male-to-female and 1: 33,800 for female-to-male transsexual persons[2]. Our findings may therefore suggest that prevalence rates based on the number of individuals seeking medical help might underestimate the prevalence of gender dysphoria in the general adult population. Indeed, two recent population surveys reported similar findings[11-12]. In the latter[12], 28,662 residents of Massachusetts were explicitly asked if they would consider themselves to be transgender and 0.5% of the participants identified themselves as transgender in a telephone health survey[12]. In the Netherlands, Kuyper et al.[11] asked participants if they felt psychologically male and/or female in an internet based population survey. Ambivalent gender identity was reported in 4.6% of men and 3.2% of women and incongruent gender identity in 1.1% of males and 0.8% of females. This Dutch survey had a similar prevalence rate of gender incongruence but a higher prevalence of gender ambivalence compared with our results. Differences in methodology should be considered (a representative stratified sample randomly drawn from the National Register versus internet based stratified sample and differences in response rate (20.9% versus 40.0%) as well as different phrasing of the questions.

In our LGB survey, we found a prevalence of gender incongruence of 0.9% and 2.1% and gender ambivalence of 1.8% and 4.1% in men and women, respectively. These findings were slightly lower compared to those from a United States LGB study, though the phrasing of the question was different. In the latter, 2.1% of the gay and bisexual men and 1.9% of the lesbian and bisexual women identified as transgender[21]. Of note, the observed prevalence of gender incongruence and gender ambivalence were generally more present in LGB women but not in LGB men.

We also found that gender incongruent or gender ambivalent persons varied in their reported appearance and desire to be more masculine or feminine. Our results may thus support the fact

that only some gender-nonconforming people experience gender dysphoria at some point in their lives[1]. Therefore, the prevalence rates of gender non conformity was expected to be much higher than earlier figures based upon those who underwent SRS in Belgium[2]. Indeed, Kuyper and Wijsen, (2014)[11] observed that only a minority of gender incongruent or gender ambivalent persons were dissatisfied with their body and wished to undergo treatment. However, as we have not investigated the prevalence of dysphoria or desire for sex reassignment treatment in our current studies, we cannot confirm these findings.

The strengths of our study are (1) the use of a large representative population survey and (2) the use of identical questionnaires in a large sample of LGB individuals. Our study is hampered by the differences in methodology between these two studies, which also resulted in a different socio-demographic population. However, LGB individuals are considered as a hard-to-reach population. In addition, in national surveys, the numbers of men and women identified as being non-heterosexual are often too small to allow for thorough statistical analysis. In order to acquire a sufficient number of LGB individuals, very large population-based samples are required. The latter, together with the costs and efforts that are typically associated with population-based representative surveys, especially in small communities like Flanders, makes it very hard to acquire adequate data[14]. Therefore we believe that we added valuable information on prevalence of gender ambivalence and gender incongruence in LGB individuals, despite these methodological shortcomings. Future studies should preferentially investigate these prevalence rates in very large population-based representative surveys to ensure sufficient power.

Another limitation of this study can be found in the nature of our questions measuring gender ambivalence and gender incongruence. However, to our knowledge, no validated questionnaires are presently available. Finally, information on experience of gender dysphoric feelings and wish for treatment in gender ambivalent and gender incongruent persons were not included in our study. Future studies should add this.

In conclusion, we found, in a large representative population survey on sexuality, 2.0% of gender ambivalent and 0.6% gender incongruent persons, together 2.6% of gender nonconformity. Evidence is growing that gender nonconformity is more prevalent, but it is of note that not all gender-nonconforming persons desire sex changes. To determine an appropriate approach, support, and potential need for intervention in these individuals, more detailed population-based research should be performed.

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