MANAGEMENT OF ENDOCRINE DISEASE

Optimal feminizing hormone treatment in transgender people

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Abstract

Transgender women are assigned male at birth but identified as women. The incidence of gender dysphoria is estimated to be around 1% of the population. Gender dysphoria may be associated with depression and low quality of life, which in most cases improves during gender-affirming hormonal treatment (GAHT). Feminizing hormonal treatment for transgender women or gender non-binary people typically includes natural estrogen (estradiol). Additional testosterone-blocking treatment is often needed to ensure the suppression of the pituitary-gonadal axis and may include cyproterone acetate, a gonadotropin-releasing hormone agonist (GnRH-a), or spironolactone. The health risks of cyproterone acetate as anti-androgen treatment are debated and randomized protocols with other anti-androgen treatments are requested. Orchiectomy is performed in some transgender women after various duration of GAHT. Currently, natural progesterone is not recommended as part of GAHT due to limited knowledge on the balance between risks and benefits. In the present article, we discuss evidence regarding established and upcoming feminizing treatment for adult transgender women or gender non-binary people seeking feminization. Data on study populations with transgender women are put into a wider context of literature regarding the effects of sex steroid hormones in cisgender study populations. Relevant follow-up and monitoring during feminizing treatment is debated. The review has a special focus on the pharmacotherapy of feminizing hormonal therapy.

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Invited Author's profile

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Introduction

The term transgender is used to describe individuals, whose gender identity differs from the assigned gender at birth. Transgender women are persons assigned male at birth but identified as women. In a recent European study, 0.7–1.1% of the general population reported incongruent gender identity (1).

In transgender persons, about one out of three identifies as gender non-binary (2). Gender non-binary is an umbrella term for gender identities that are neither male nor female – identities that are outside the gender binary (2). Gender non-binary people may also seek feminization. Medical doctors are needed to prescribe hormone therapy in most of Europe, the United States, and Canada, but gender-affirming hormonal therapy (GAHT) may also be obtained from unofficial sources. In many countries, GAHT is predominantly prescribed by centers with a special interest in transgender health, also called gender teams. These multidisciplinary gender teams initiate and offer psychological support before and during the transition and prescribe GAHT. Furthermore, the gender teams help to coordinate referrals for gender-affirming surgery and the involvement of other specialists (dermatology, plastic surgery, urology, gynecology, otorhinolaryngology, etc.). Current guidelines and position statements describe the initial assessment of transgender women (3, 4).

GAHT in transgender women aims for the development and maintenance of secondary feminine characteristics and suppression of secondary masculine characteristics. Feminizing hormonal treatment usually includes natural estrogen (estradiol, E2) alone or in combination with testosterone-blocking treatment (1). E2 is administered transdermally, orally, or as injections (5). Anti-androgen treatment may include cyproterone acetate (CPA), a gonadotropin-releasing hormone agonist (GnRH-a) or spironolactone (5). Anti-androgen treatment is discontinued if the patient undergoes orchiectomy or if estrogen-only treatment has proven sufficient.

In the present review, we discuss evidence regarding established and upcoming methods for feminizing treatment in adult transgender women and gender non-binary women with a wish for feminization. We put available data on GAHT into a wider context of literature regarding the effects of sex hormones in cisgender study populations. The review has a special focus on the pharmacotherapy of feminizing hormonal therapy.

Methods

The paper was designed as a narrative review regarding GAHT in adult transgender women. We searched for available clinical trials and placebo-controlled trials in study cohorts of adult transgender women published until December 2020 in PubMed. We performed a search using the term 'transgender' in combination with one of the terms: placebo-controlled trial, clinical trial, medicine prescription, drug prescription, gender-affirming hormonal therapy. The search term 'transgender' was replaced with trans-sexualism, gender incongruence, trans female, and trans woman. We also searched for papers including each identified treatment modality: estrogen, estradiol, spironolactone, anti-androgen, androgen blocker, androgen antagonist, cyproterone acetate, cyproterone, flutamide, finasteride, gonadorelin, GnRH-a, progestogen, progesterone, progestin, thus including all hormonal feminizing treatments, in combination with one of the search terms for transgender. Further relevant studies were identified by cross-search from reference lists in identified studies. We excluded studies regarding the treatment of children/teenagers <18 years (puberty blockers) and studies focusing on surgical procedures. Dermatologic treatment of hirsutism is covered in other recent reviews (6, 7) and is not covered in this paper. Lifestyle intervention, treatment of weight problems, and gender-affirming surgical interventions are considered outside the scope of this paper. Datasets on hormone treatment in gender non-binary people are lacking and, therefore, this is not specifically addressed, although we acknowledge that some papers in this review may include gender non-binary persons seeking feminizing treatment. In case of limited literature in transgender study populations, we included and discussed studies performed in cisgender study populations. Furthermore, knowledge regarding the effects of sex hormones in cisgender study populations was discussed in the context of transgender health.

Gender-affirming hormonal treatment (GAHT)

In transgender women, the desired effect of GAHT is the development of secondary female characteristics including breast growth and female body composition. Anti-androgen treatment may be needed to ensure the full

suppression of the pituitary-gonadal axis and suppression of secondary male characteristics such as terminal body hair growth.

Estrogen treatment

Background

The most important endogenous estrogens include 17β-estradiol (E2), estrone (E1) and estriol (E3), where E2 is found at the highest concentration and is the most potent estrogen. E2 acts on the nuclear estrogen receptors α and β, which leads to different transcriptional effects. Apart from genomic effects, E2 can activate non-genomic G-coupled estrogen receptors (8). The E2 level in adult cisgender men is around 0.1 nmol/L compared to 0.4–0.5 nmol/L in premenopausal women at mid-cycle (9). In cisgender men, 75% E2 is produced by peripheral aromatization of testosterone (9). The biological roles of E2 in males include endothelial repair and regeneration, decrease of body fat, preservation of bone health, and increase of growth hormone secretion (9, 10).

Estrogen can be given synthetically as ethinyloestradiol (oral contraceptives) or naturally as E2. Pro-coagulant side effects of synthetic estrogens are well described (11), which makes treatment with ethinylestradiol obsolete for GAHT. E2 is considered as first-line drug for GAHT in transgender women. E2 can be applied orally, trans-dermally, or as i.m. injections. Table 1 gives an overview of the different treatment regimens of E2. Oral E2 is metabolized in the intestines and liver into E1 and estrogen conjugates before entering the circulation (first-pass metabolism) (12). As a result, the ratio of circulating E1/E2 is 5/1 after oral E2 compared to 1/1 after transdermal E2. Transdermal E2 gives a more constant, non-fluctuating serum E2 without circulating estrogen metabolites, which may be advantageous in terms of cardio-metabolic side effects (13). According to current treatment guidelines, transdermal E2 is considered as first-line treatment in transgender women >40 years (14, 15, 16). Intramuscular E2 is associated with a peak in E2 (Table 2) lasting for about 2 weeks and reaching values up to six times higher than the average E2 concentration in cisgender women (17). Such high serum E2 levels may potentially be associated with thromboembolic events. In most European countries, i.m. injections are not available. Buccal E2 can be used for the treatment of hot flashes in postmenopausal women (18); however, the use of buccal E2 in transgender women may be limited by lower concentrations and fluctuating E2 levels (19). Studies regarding feminizing effects of GAHT most often evaluated breast growth, whereas other outcomes are less evaluated. Studies comparing efficacy depending on

Table 1 GAHT, recommended treatment modalities in transgender women. GAHT modalities not currently recommended are not included in the table (5-alfa reductase inhibitors, flutamide, progesterone etc). Information adapted from (3, 92, 109).

Content/form	Content	Dosage	Time to peak	Pharmacokinetics
E2				
Patch	E2 in polymeric acrylate or vinylacetate	One patch every 3 days 0.05–0.15 mg E2/24 h	8–12 h	E1/E2 ratio: 1; Constant delivery of E2 for 7 days; No accumulation of E2; Half-life: 24 h
Gel/spray	E2 in alcoholic gel	Gel daily 0.8–3 mg/day	4–6 h	E1/E2 ratio: 1; No accumulation of E2; Steady state within 3 days; Half-life: 36 h
Tablet	E2	2–6 mg/day	2–4 h	High E1/E2 ratio (due to first-pass metabolism in gut and liver); Gradual accumulation of E2 in blood; Steady state reached within days; Half-life: 12 h
Intramuscular injection	E2 in oily solution	2–10 mg/injection every 1–2 weeks (depending on solution)	2–4 days (depending on solution)	E1/E2 ratio: 0.5; Half-life: 1–2 weeks
Cyproterone acetate				
Tablet	Cyproterone acetate	≤10 mg/day	2–3 h	Metabolized in liver; Half-life: 48–72 h
Spironolactone				
Tablet	Spironolactone	100–400 mg/day	1–4 h	Metabolized in liver; Half-life: 16–22 h
GnRH-a				
Subcutaneous or i.m. injection	Leuprolide/triptoreline	3.75–45 mg/1–6 months	1–4 h	Metabolized in liver; Half-life: 3 h

E1, estrone; E2; estradiol.

Table 2 GAHT, directions for choice of treatment.

	First choice	Second choice	Not recommended	Debated
Estrogen	E2: Dermal; Oral (< 40 years old)	E2: Oral	Oral contraceptives	Buccal E2
Anti-androgen	Spironolactone; CPA; GnRH-a		5-alfa reductase inhibitors; Flutamide	CPA vs spironolactone vs GnRH-a; Timing of orchiectomy; Reduction of CPA dosage
Adjunctive treatment				Progesterone: Natural vs progestins

CPA, cyproterone acetate; E2, estradiol.

the route of E2 application in transgender women are sparse (20) and are discussed further below. Most studies applied combined treatment regimens, which makes the individual effects of different estrogen preparations and antiandrogen drugs difficult to evaluate.

Anti-androgen treatment

The most commonly used antiandrogen treatments are CPA, GnRH-a, and spironolactone. Anti-androgen treatment is, for many, a cornerstone of feminizing treatment. E2 treatment in transgender women ensures serum E2 within reference interval for cisgender women, but the pituitary-gonadal axis will often not be sufficiently suppressed by E2 treatment alone. Testosterone is an important masculinizing and anabolic hormone. The masculinizing effects of testosterone include the stimulation of the male reproductive tract and development of secondary sex characteristics (e.g. terminal hair growth), whereas the anabolic effects of testosterone include the stimulation of somatic tissue, such as muscle. Testosterone acts as a prohormone and is converted to the biologically active derivate dihydrotestosterone (DHT) by the enzyme 5α-reductase or to E2 by aromatase. Aromatization of androgens to estrogens occurs in adipose tissue muscle, bone and brain (9). Many of the masculinizing effects of endogenous testosterone until initiation of feminizing treatment during puberty and adult life will not be reverted despite partial or complete suppression of androgen levels. Therefore, in most cases, additional treatment is needed, which may include voice training, surgical intervention on the vocal cords, laser treatment of terminal hair growth and maybe facial surgery (1). Importantly, anti-androgen treatment will result in testicular atrophy and azoospermia within few months. After a longer treatment duration (2–3 years), the decrease in fertility is considered, at least partially, irreversible, therefore, sperm cryopreservation should be carefully discussed before the prescription of anti-androgen treatment (21, 22). In the case of orchiectomy, anti-androgen treatment can be stopped and spare the patient from the side effects of anti-androgen treatment. The timing of orchiectomy will very much depend on local traditions including the possibility for genital reconstruction surgery.

The choice of anti-androgen treatment will very much depend on national availability, pricing and financing of drugs (1). In Europe, the most commonly prescribed androgen-lowering medication for feminization is CPA. Spironolactone is commonly prescribed in the US where CPA is not available (1). GnRH-a is provided free of charge to transgender women by the National Health Service in the United Kingdom, whereas, for example, in Belgium, Denmark, and Australia, GnRH-a is provided by the hospital for puberty suppression only (23).

The mechanism for testosterone suppression differs between different anti-androgen treatments. In overview, anti-androgen treatments include androgen receptor antagonists (spironolactone and flutamide), whereas GnRH-a and progestogens suppress the hypothalamic-pituitary-gonadal axis. CPA has a dual action on the androgen receptor and the hypothalamic-pituitary-gonadal axis. 5-alfa reductase inhibitors block the conversion of testosterone to dihydrotestosterone.

Feminization and androgen suppression are the primary goals of anti-androgen treatment as part of GAHT. One Cochrane review in transgender women found no study regarding efficacy and safety of hormone therapy with antiandrogens or E2 alone, or in combination (20). One recent systematic review found four studies regarding the effects of different anti-androgen treatment modalities on testosterone levels in transgender women (23). The authors concluded that the addition of CPA, GnRH-a and progestin may be more effective than spironolactone or estradiol alone at suppressing the serum total testosterone concentration (23). The authors found no eligible studies regarding the effects of antiandrogens on breast development or facial and body hair reduction (23). Serum levels of testosterone may not be meaningful endpoints for feminization especially for androgen receptor antagonists (23). The recent review by Angus *et al.* (23) did not include

a discussion regarding the safety and dosage of individual anti-androgen treatment modalities, and these issues will be further discussed in the present review.

Cyproterone acetate (CPA)

CPA inhibits the testosterone receptor and acts as synthetic progesterone (progestin), which results in the suppression of the hypothalamic-pituitary-gonadal axis. CPA is a part of oral contraceptives with an anti-androgen effect (G03HB01, containing 2 mg CPA per tablet), which can be prescribed as second-line oral contraceptives to treat hirsutism in cisgender women. For transgender women, CPA has the benefit of being relatively cheap, and it is administered orally in contrast to GnRH-a (1). After oral ingestion, the maximal plasma concentration is reached after around 3 h and the turnover time is slow with plasma half time of 2–3 days. Therefore, CPA can also be administered every 2nd or even every 3rd day.

CPA is a strong inhibitor of serum testosterone. A recent paper found significantly lower total testosterone concentrations (0.8 nmol/L (0.6–1.20 nmol/L)) during CPA in transgender women compared to testosterone levels during spironolactone (2.0 nmol/L (0.9–9.4 nmol/L) and E2 alone (10.5 nmol/L (4.9–17.2 nmol/L) (24)). The median daily CPA dosage in the study was 50 mg (24). The progestin effect of CPA may improve breast development (1). However, as mentioned previously, no data are available regarding the feminizing effect of CPA alone in transgender women (23).

Several reservations may apply to the use of CPA. Observational studies in transgender women reported adverse lipid status (low HDL) during CPA (25, 26), and insulin resistance tended to increase during feminizing treatment including CPA (27), but findings could in part be explained by changed body composition with higher body weight and higher waist-hip ratio (27). Prolactin levels increased during CPA (28, 29), but serum prolactin levels normalized after stopping CPA (28). Prolactin is also an adipokine, and high prolactin levels outside reference intervals could infer higher metabolic risk (30). A possible association between high prolactin and breast cancer is undetermined (31). The risk of breast cancer increases during feminizing treatment, but most tumors are estrogen and progesterone receptor-positive (32). The known association between CPA therapy and the development of meningiomas (33) depends on cumulated dose (33). Thus international health authorities recommend that CPA should be used with caution to avoid the long-term risk of meningioma (34). At present, nine cases of meningioma have been described in transgender women, which is an elevated relative risk, but still very low absolute risk (35). In many centers, the dosage of CPA has been 25–50 mg/day by the initiation of feminizing treatment, but in the future, the starting dosage of CPA could be reduced to 12.5 mg/day and further down-titration of CPA can be considered after 6–12 months. CPA 10 mg/day should be administered for no more than 2 years to keep below the maximal cumulative dose. Alternative anti-androgen use and earlier orchiectomy should be considered in order to avoid meningiomas during CPA. Furthermore, a healthy lifestyle should be supported to avoid weight gain and adverse metabolic profile during CPA treatment.

Spironolactone

Spironolactone is a non-selective mineralocorticoid and is an androgen and progesterone receptor antagonist. Spironolactone blocks the binding of dihydrotestosterone to its androgen receptor, thereby inhibiting androgen effects (24). The binding capacity of spironolactone to the androgen receptor is weaker than CPA (24). Spironolactone also acts as an inhibitor of 17α-hydroxylase and 17,20-lyase (enzymes in the testosterone biosynthetic pathway), which lowers testosterone to a small degree (24). The use of spironolactone for the treatment of hirsutism in cisgender women is well described, and spironolactone can be given as a part of oral contraceptives as drospirenone (4th generation oral contraceptive) (36).

In transgender women, spironolactone is administered orally as tablets in 25, 50, or 100 mg doses. Few studies evaluated the feminizing effects of spironolactone treatment in transgender women. In a recent non-randomized Australian study (38 transgender women treated with spironolactone), the average prescribed dosage of spironolactone was 100 mg (interquartile range) (87.5–200 mg). Serum testosterone levels during spironolactone treatment were significantly higher compared to CPA (2.0 vs 0.8 nmol/L); however, data regarding feminizing physical characteristics and treatment satisfaction were not available (24, 26). As discussed by the authors, the mechanisms of actions differ between CPA and spironolactone and the level of total testosterone concentration may not be an applicable marker of feminization (24).

Several studies supported that spironolactone could improve metabolic risk compared to CPA. HDL levels

increased (25) during spironolactone without any significant changes in prolactin (25, 26, 37) and potassium levels (26, 38).

GnRH-a

GnRH-a inhibits the pituitary-gonadal axis and leads to suppressed gonadal sex hormone levels, whereas adrenal androgen production is kept intact. In children, GnRH-a is used for the treatment of central precocious puberty. In adults, GnRH-a is applied as a part of fertility treatment, treatment of endometriosis, and prostate cancer. GnRH-a is usually administered by depot injections, and the cost of treatment is relatively high. In many countries, GnRH-a is commonly prescribed to suppress endogenous puberty in transgender adolescents, whereas GnRH-a treatment is not commonly applied for treatment in adult transgender women (1) due to the high cost and other available treatments (1). As a result, the use of GnRH-a has mainly been studied in adolescent study populations. One study in transgender girls reported effective gonadal suppression after 3 months of GnRH-a treatment, resulting in nearly undetectable gonadotropins levels and decreased testosterone from 9.1 nmol/L to 1.0 nmol/L (39). The main concern is the long-term health consequences of gonadal suppression (40). Bone mineral density Z-scores remained below zero in transgender girls 3 years after treatment with combined GnRH-a and E2 (41). We are not aware of long-term studies on fracture risk following the suppression of puberty. Limited data are available in adult transgender female study populations regarding study outcomes of previous puberty blocking such as cardiovascular risk. A Trans Youth Care Research Network was established in the US with the aim to perform a longitudinal observational study in young transgender individuals starting puberty blockers compared to individuals starting GAHT without GnRH-a (42). Data from this study cohort showed that transgender women treated with GnRH-a + estrogen needed lower doses of estrogen to achieve desired physiologic changes compared to transgender women not using GnRH-a (43). However, the number of transgender women treated with GNRH-a was so far limited to only six, and spironolactone was used as an androgen blocker in the comparison group, which could have affected study outcomes (43). Administration of GnRH-a in early puberty will lead to underdevelopment of the penis, which can compromise future vaginoplasty (44). Furthermore, termination of puberty will lead to underdeveloped testes and semen production, which will compromise options for fertility preservation (45).

5α-reductase inhibitors

Finasteride and dutasteride are 5α-reductase inhibitors, which inhibit the conversion of testosterone to the more active dihydrotestosterone. Furthermore, 5α-reductase inhibitors block the conversion of progesterone to dihydroprogesterone and deoxycorticosterone to dihydrodeoxycorticosterone (46). The use of 5α-reductase inhibitors is prescribed in cisgender men for the treatment of benign prostate hypertrophia and sometimes for the treatment of androgenic hair loss, whereas the use of 5α-reductase inhibitors in transgender women is controversial (46). Transgender women may request 5α-reductase inhibitors to improve the anti-androgen effects of feminizing treatment, but no clinical studies supported their use. Especially when testosterone levels are already suppressed, as seen during CPA or spironolactone treatment, the benefit of 5α-reductase inhibitors will probably be negligible. As recently discussed, testosterone levels could increase during treatment with 5α-reductase inhibitors (46, 47). We are not aware of studies in transgender women regarding the effect of 5α-reductase inhibitors on secondary female characteristics. In conclusion, treatment with 5α-reductase inhibitors in transgender women is considered to be of no clinical benefit and is, therefore, not recommended.

Flutamide

Flutamide acts as a selective antagonist of the androgen receptor (AR) and is used for the treatment of prostate cancer. Testosterone levels are unchanged during flutamide treatment and due to the risk of hepatotoxic side effects, flutamide is not recommended for GAHT (1).

Progesterone

In themenstrual cycle, progesterone levels surge after ovulation, and the measurement of serum progesterone in the late menstrual cycle is applied to determine ovulatory cycles. The stimulatory effect of progesterone on female breast development is well described and progesterone promotes alveologenesis and ductal side branching (48). Progesterone (progestogen) therapy can be prescribed as the natural hormone or synthetic as progestins. Natural progesterone is used as a part of fertility treatment, and progestins are used in oral contraceptives.

In transgender women, the mechanism for a feminizing effect of progestogens is the inhibition of the hypothalamic-pituitary-gonadal axis and possibly

improved breast development. As earlier discussed, feminizing treatment with CPA imply a considerable progestin effect. Therefore, a reduction of CPA dosage to a minimum may lead to a higher request for additional progesterone treatment. Breast development during progesterone treatment in transgender women has been investigated as a part of lactation induction, where transgender women were treated with high dosage progesterone and domperidone on top of usual GAHT (49). GAHT treatment protocols using medications with progestational properties did not result in a difference in the request for mammoplasty compared to treatment protocols without progestins (50). Due to limited evidence regarding breast development, progesterone treatment is currently not recommended in guidelines (51, 52).

The risk of side effects during progesterone treatment is debated. Information regarding the proliferative/anti-proliferative effects of progesterone in breast cancer is conflicting (48). According to a recent review in transgender women, half of the diagnosed breast cancers were hormone-sensitive with positive progesterone receptor status in 5 of the 14 tested transgender women (53). Natural progesterone treatment may have fewer adverse effects on breast cancer risk than progestin (54). Mental health could be associated with progesterone/estradiol levels (55, 56). Low luteal phase progesterone levels were associated with the peri-menstrual syndrome in cisgender women (57). Progestin treatment was associated with a higher risk of depression (58), whereas progesterone treatment decreased postpartum depression (59). Whether the progesterone/estradiol ratio is associated with mental health in transgender women is undetermined.

The risk of VTE is considered to be increased during progestogen treatment (60). Progestogens affect the tissue factor-dependent pathway of the coagulation cascade and could increase the levels of protein S and fibrinogen (60). However, progestogens also affect the concentration of fibrinolytic factors, which questions the overall effect of progestogens on the turnover of fibrin (60). The effect of natural progesterone on the hemostatic system is sparsely examined (61). Fluid retention, weight gain and higher blood pressure are well-described side effects of progesterone treatment (62). Progesterone has a high affinity for the mineralocorticoid receptor and acts as a mineralocorticoid receptor antagonist (62, 63). Furthermore, inhibition of the enzyme 11beta-hydroxysteroid dehydrogenase 2 (11beta-HSD2) by progesterone and its metabolites results in decreased inactivation of cortisol and hence increased mineralocorticoid receptor binding by cortisol (62). In contrast, progesterone has been shown to suppress 11beta-HSD1 activity in cultured human hepatocytes (63), which will decrease cortisol concentrations. Because of these contrasting effects of progesterone in different tissues, it is clear that only experimental testing can elucidate the *in vivo* effect of progesterone on mineralocorticoid signaling. We are not aware of studies regarding the possible effects of progesterone treatment on blood pressure, renal salt retention, renin/aldosterone ratio and cortisol metabolites in study populations of transgender women.

Feminizing effects of GAHT

Breast development

Breast development during different treatment regimens in transgender women was evaluated in few clinical studies. The European Network for the Investigation of Gender Incongruence (ENIGI) study is an ongoing collaboration of four centers (Amsterdam, Florence, Ghent, and Oslo) (64). Breast development was investigated in 329 transgender women (average age 28 years) after 1 year of feminizing treatment (65). Feminizing treatment included E2 tablets (2 to 6 mg daily), E2 patches (0.05 to 0.1 mg/24 h twice weekly), or E2 gel (0.75 to 3 mg daily) combined with CPA (10 to 100 mg daily) or spironolactone (100 to 150 mg daily). Transdermal E2 was advised in transgender women > 40 years. The study reported modest breast development (mean change in breast circumference +3.7 cm), which occurred primarily within the first 6 months (65). Transdermal E2 resulted in faster increase in breast–chest difference until 6 months after initializing treatment. However, breast development after 1 year of feminizing treatment was comparable between oral and transdermal E2. No clinical or laboratory parameters predicted breast development (65). Most recently, de Blok *et al.* applied 3D imaging during 3 years of feminizing treatment in 69 transgender women and reported no associations between increase in breast volume and treatment regimen, serum E2 levels, age, BMI, and tobacco use (66). These results are in line with a controlled, retrospective case audit in transgender women seeking mammoplasty after at least 2 years of feminizing treatment (67). Transgender women were 39 years of age and had been treated with different hormonal regimens for 10 years in average. The type of estrogen use did not affect the request for mammoplasty (67).

Body composition

The aim of GAHT is to obtain female body shape with fat redistribution from central to peripheral fat. A recent meta-analysis included 21 studies and showed that GAHT was associated with decreased waist-to-hip ratio, a rise in gynoid fat, and gain of total fat mass (68). Lean body mass (68) and muscle strength (69) were decreased during the initiation of GAHT, but muscle strength still remained higher compared to cisgender women during the first 3 years of GAHT (69). Interestingly, transgender women had lower lean body mass and higher fat mass than control males even before initiating GAHT (68). Several studies supported that GAHT could increase insulin resistance, but long-term studies are missing (68).

Terminal hair growth (hirsutism)

Quality of life is inversely associated with hirsutism in transgender women (70), but clinical studies regarding terminal hair growth during GAHT are limited. One study reported decreased hair growth and sebum production during GAHT and the decrease in hair shaft diameter reached its maximum at 4 months (71). Facial hair growth continued during GAHT, but at a slower rate (71). In accordance, it was reported that more than 90% of transgender women had a wish for hair removal (72).

Other treatment effects of GAHT

Patient reported outcomes, depression and aggression

The prevalence of depression and low quality of life is high in transgender study populations (73) and one of the more important aims of GAHT is to improve the quality of life. GAHT is considered beneficial for the quality of life and reduction of depression (74), but high-quality data are limited (75). Importantly, suicide rates remain high in transgender persons after GAHT (76). We are not aware of studies comparing individual treatment regimens regarding patient-reported outcomes. Changes in patient-reported outcomes including mental health could be a valuable tool to determine and validate the effects of GAHT. Depression is more than twice as prevalent in cisgender women compared to cisgender men (77), and the interaction between estradiol and mental health has been investigated in cisgender study populations. A national Danish register-based study reported that the use of hormonal contraceptives in cisgender women was followed by increased prescription of antidepressants (58), but all oral contraceptives contained some form of progesterone. A Swedish register-based study found that the use of oral contraceptives was associated with a higher risk of suicidal behavior (78). The risk of depression could be mediated by fluctuating hormone levels as seen during puberty, post-partum, at perimenopause and after initiating oral contraceptives (77, 78). In analogy, in transgender women, the risk of depression could be highest after the initiation of feminizing treatment, and if dose reduction of E2 is necessary during aging or occurrence of intercurrent disease. Some transgender women may be more susceptible to psychiatric side effects of estrogen fluctuations (77). Monitoring of depressive symptoms and psychological support could be warranted during short-term changes in feminizing treatment regimen, whereas long-term stable treatment seems safer. Fluctuations of testosterone levels could also affect psychological wellbeing in transgender women in line with the findings in cisgender women, where irritability during the premenstrual phase was associated with testosterone levels (79). However, in long-term studies, testosterone levels were not associated with aggression in transgender study populations (80, 81).

Sexual health

In a questionnaire-based cross-sectional study in 214 transgender women, 62% reported decreased sexual desire after feminizing treatment and 73% never or rarely experienced spontaneous or responsive sexual desire (82). Four to six years after entering the ENIGI study, 26% of the transgender women reported difficulties in initiating and seeking sexual contact and 28% had difficulties achieving an orgasm (83). There were no significant differences in study outcomes between participants with or without intentions for genital surgery (83).

Arterial cardiovascular disease (CVD)

Epidemiological studies in transgender women reported an increased risk of acute myocardial infarction and stroke during feminizing treatment, whereas long-term, controlled studies regarding feminizing treatment and the risk of arterial CVD are lacking (84). In contrast, a systematic review and meta-analysis of cardiovascular outcomes in transgender individuals did not find an increased risk of myocardial infarction or stroke in transgender women, owing to the lack of reported outcomes from 29 eligible

studies (85). Feminizing hormone therapy was associated with increased serum triglyceride levels of 31.9 mg/dL (95% CI, 3.9 to 59.9) in transgender women treated for >24 months with no changes in serum low-density lipoprotein or high-density lipoprotein (85). Of note, CVD is the main cause of death in cisgender persons, but, in general, coronary heart disease occurs years later in women compared to men (86). Premenopausal women are relatively protected against arterial CVD compared to age-matched men and high E2 levels were negatively associated to CVD in postmenopausal women (86). These findings suggest that endogenous and exogenous estrogens influence the risk of CVD in cisgender women. Findings regarding the higher risk of acute myocardial infarction and stroke during feminizing treatment confirm that CVD risk in transgender women is influenced by the known risk factors such as high BMI, smoking, sedentary lifestyle, hypertension, hypercholesterolemia or type 2 diabetes, but also the 'natal sex atheroma burden' (84). Cisgender men will tend to have a larger 'atheroma burden' at a given age compared to cisgender women. Therefore, the age of initiation of feminizing treatment in transgender women is important regarding the duration of exposure to potential risk factors. Furthermore, age and timing are important with respect to estrogen therapy, as estrogens may be vasoprotective in women without atherosclerosis as opposed to women with later stages of atherosclerosis (84). Hypertension is an important risk factor for CVD, which may be relatively easily modified (86), and E2 protected against hypertension in experimental animal studies (87).

Venous thromboembolism (VTE)

Risk of VTE occurrence in transgender women is likely increased given the known prothrombotic actions of estrogen (84). In accordance, the incidence of VTE was increased in transgender women with 2- and 8-year risk differences of 4.1 (95% CI, 1.6–6.7) and 16.7 (6.4–27.5) per 1000 persons relative to cisgender men and 3.4 (1.1–5.6) and 13.7 (4.1–22.7) relative to cisgender women (88). Measurement of coagulation markers confirmed procoagulant changes during the initiation of GAHT (89). Most transgender women need life-long E2 therapy, and aging is a major risk factor for VTE development (90, 91). Other risk factors for VTE include BMI > 25 kg/m^2, genetic factors and previous VTE (90, 91). The route of estrogen administration affects the risk of VTE (89, 91). Oral E2 changes hemostatic variables in a pro-thrombotic direction in contrast to transdermal E2, which was similar to placebo in a recent meta-analysis (91). Hepatic first-pass effect of oral E2 negatively impacts the synthesis of coagulation proteins, but other factors play a role (91, 92). At present, oral E2 is not recommended in transgender women with a high risk of VTE, and a change to dermal E2 administration should be discussed around the age of 40 years in all transgender women (1). Adding synthetic progesterone, including CPA, to E2 therapy further increases the risk of VTE (see below) (91). Anticoagulation therapy following a thrombotic event in transgender women can be considered (1), but there are no long-term studies to guide GAHT in high-risk transgender women and following a thrombotic event.

Bone health

Sex hormones are essential for osteoblast function, and eugonadal men and premenopausal women are relatively protected against osteoporosis. Testosterone levels will decrease during feminizing treatment, but sufficient treatment with E2 could maintain bone health (93). A recent meta-analysis included 13 studies (392 transgender women) (94). Bone mineral density at the lumbar spine significantly increased after the initiation of feminizing treatment at 12 months (0.04 g/cm^2; 95% CI 0.03–0.06 g/cm^2) and at 24 months (0.06 g/cm^2; 0.04–0.08 g/cm^2), which is in the range considered clinically significant (94). Hip bone mineral density was unchanged (94). The review included studies until 2015 and various estrogen regimens were applied, which could have affected study results (94). More recently, the ENIGI study addressed bone turnover markers during a contemporary E2 regimen in 121 transgender women (95). Bone resorption markers decreased by 10–11% after 1 year of treatment, which supported protected bone health (95). One recent study reported higher fracture risk in older transgender women (>50 years) compared to age-matched reference cisgender men, but fracture risk resembled age-matched cisgender women (96). However, transgender women aged <50 years tended to have a higher fracture risk compared with age-matched reference cisgender women (96). More prospective data on bone mineral density and fractures in transgender women are awaited. Importantly, a high percentage of low bone mineral density and hypovitaminosis D was found prior to GAHT (97). Therefore, evaluation of bone mineral density and 25OH vitamin D before starting the feminizing treatment may be considered in high-risk individuals (93, 94). It

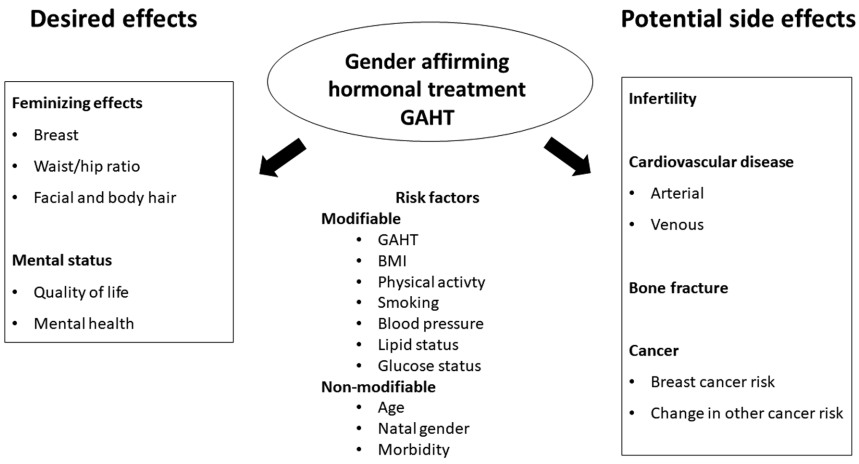

Figure 1
Feminizing treatment. Balance between desired effects and potential side effects and a list of most important risk factors (modifiable and non-modifiable).

is important to recognize that suboptimal medicine compliance in transgender women is associated with low bone mass (97).

Cancer risk

GAHT could affect the risk of hormone-sensitive cancer types including breast cancer and prostate cancer. Furthermore, the gender difference is described for several other cancer types in cisgender people, which suggests an impact of sex hormones. In a recent register-based study, cancer diagnosis at later stages in transgender persons and worse survival for many cancer types was partly explained through existing barriers to access the healthcare system (98).

Breast tissue has estrogen receptors and the risk of breast cancer is higher in cisgender women compared to cisgender men. Incidence rates of breast cancer in transgender women were lower than in cisgender women (incidence ratio 0.3) in a recent retrospective Dutch study of 2260 transgender women (median duration of feminizing treatment, 13 years) (32). The risk of breast cancer increased toward cisgender female levels during feminizing treatment and the characteristics of breast cancer resembled a female pattern (32, 99). These results suggest that breast cancer screening guidelines for cisgender women are sufficient for transgender women on feminizing treatment (32). The study included the whole spectrum of estrogen treatments and data could not be split up into subgroups of treatment modalities (32). The median age at breast cancer diagnosis was 50 years (interquartile range 43–55 years). However, a few breast cancer cases were diagnosed at age 30 years (32), which indicates that transgender women at particular risk (such as BRCA positive persons) should attend specific screening programs in accordance with those for cisgender women (99).

Prostate cancer is affected by testosterone levels and data support that antiandrogen treatment in transgender women results in a much lower risk of prostate cancer (standardized incidence ratio 0.20, 95% CI 0.08–0.42) (100). However, the presence of estrogen receptor α cells in a case report of prostate cancer raised concern for a possible contributing role of exogenous estrogen therapy in tumorigenesis (101).

Colorectal cancer is more prevalent in men compared to women. In postmenopausal women, E2 replacement therapy and the consumption of soy reduced the risk of colorectal cancer (102). The protective effect of E2 on colorectal cancer is mediated by the estrogen receptor subtype β (102). The putative protective effect of estrogen therapy on the risk of colon cancer in transgender women is undetermined.

Esophageal adenocarcinoma has a male to female ratio of 9:1, and the time of diagnosis is postponed for a median of 16 years in women compared to men (103). This suggests that estrogens may protect and/or testosterone exposure may increase the risk of esophageal adenocarcinoma. However, men with higher testosterone levels had a significantly lower risk of esophageal adenocarcinoma and E2 levels were not associated with cancer risk (104). Age, BMI and smoking are important risk factors for esophageal adenocarcinoma (103) and several other cancer types.

Obesity and smoking are relative contraindications for GAHT, and lifestyle intervention is often a part of good clinical practice of transgender care. Therefore, the individual role of exogenous estrogen for cancer risk in transgender women will be difficult to determine.

Monitoring GAHT in transgender care

Objective measurement of breast development (breast–chest difference or 3D imaging) may not be routinely performed in the clinic, and more research is needed to determine the best marker for feminization in study cohorts of transgender women. Instead, serum E2 is often used to monitor the feminizing effect of GAHT. According to recent guidelines for hormonal treatment in transgender persons, the goal of feminizing treatment is to reach a physiologic serum level of E2 and testosterone for similar-aged cisgender women, that is, serum E2 level in the upper follicular range (0.4–0.6 mmol/L) and a low serum testosterone (3, 4). Unfortunately, there are no general recommendations regarding the timing of blood sampling in relation to E2 administration (105). Timing of blood sampling should reflect the average level of E2, which implies that sampling should be performed halfway between two E2 administrations. Contamination of the blood sample by dermally applied hormone should be avoided. Ideally, the timing of blood sampling and drug administration should be similar across different sampling points to ensure that longitudinal hormone results are minimally influenced by absorption, metabolism, and user-related discrepancies. In daily clinics, this will be at least challenging or often impossible. Furthermore, the gold standard for E2 measurement, mass spectrometry, is not widely available. The use of serum E2 as a marker of feminization contrasts studies reporting no significant association between E2 levels and breast development (65, 66, 67). However, serum E2 was measured by different hormone assays, and the timing of blood sampling after E2 administration was not standardized, which could have affected study results (65, 66, 67). According to Table 1, E1 measurements could be relevant during oral E2 treatment, but this hypothesis remains to be tested. Recently, polymorphisms of the estrogen receptor α gene were associated with gender incongruence in transgender men, whereas no association was found in transgender women (106). In postmenopausal women, variations in the estrogen receptors affected individual variation in treatment effects of E2 (107), and further studies regarding variations in the estrogen receptor are requested in transgender study populations.

Contraindications and reservations for E2 treatment in transgender women

Long-term risks regarding cardiovascular health and cancer should be discussed with the patient before initiating E2 treatment (Fig. 1). Clinical and laboratory workup at baseline should assess BMI, blood pressure, lipid profile, and HbA1c. BMI and smoking are important modifiable risk factors for cardiovascular disease and several cancer types. Obesity and smoking could be considered relative contraindications for GAHT, but cut-off levels for BMI are not available. Of course, lifestyle intervention is considered part of good clinical practice if needed. In these patients, the preferred use of dermal E2 above the age of 40 years is discussed previously. But, overall, feminizing treatment can be considered safe, and prospective metabolic risk screening can follow current guidelines for cisgender persons (108).

Conclusion

GAHT for transgender women should be tailored to the individual patient. In normal-weight younger persons, the *a priori* general health is usually good and the choice of treatment can be done quite freely. However, as age, BMI and cardio-metabolic risk factors increase, feminizing treatment should be prescribed under consideration of risk factors. Lifestyle factors should always be discussed as a part of the outpatient consultation. There is a need for clinical studies exploring the optimal treatment of transgender women. Overall, feminization treatment can be considered as safe, but hormonal treatment may need adaptations if health risk factors develop over time. Future studies should add patient-reported outcomes including satisfaction with breast development as these outcomes may not correlate with serum measures of E2 and testosterone levels.

Declaration of interest
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