Breast Development in Transwomen After 1 Year of Cross-Sex Hormone Therapy: Results of a Prospective Multicenter Study

Christel Josefa Maria de Blok,[1] Maartje Klaver,[1] Chantal Maria Wiepjes,[1] Nienke Marije Nota,[1] Annemieke Corine Heijboer,[2,3] Alessandra Daphne Fisher,[4] Thomas Schreiner,[5] Guy T'Sjoen,[6] and Martin den Heijer[1]

[1]Department of Endocrinology and Center of Expertise on Gender Dysphoria, VU University Medical Center, 1007 MB Amsterdam, The Netherlands; [2]Department of Clinical Chemistry, Endocrine Laboratory, VU University Medical Center, 1007 MB Amsterdam, The Netherlands; [3]Laboratory of Endocrinology, Academic Medical Center, 1105 AZ Amsterdam, The Netherlands; [4]Sexual Medicine and Andrology Unit, Department of Experimental, Clinical, and Biomedical Sciences, University of Florence, 50139 Florence, Italy; [5]Department of Endocrinology, Oslo University Hospital, N-0424 Oslo, Norway; and [6]Department of Endocrinology and Center for Sexology and Gender, Ghent University Hospital, 9000 Ghent, Belgium

Context: Breast development is a key feature of feminization and therefore important to transwomen (male-to-female transgender persons). It is not exactly known when breast development starts after initiating cross-sex hormone therapy (CHT) and how much growth may be expected.

Objective: To investigate breast development in transwomen during their first year of CHT and whether clinical or laboratory parameters predict breast development.

Design: This study was performed as part of the European Network for the Investigation of Gender Incongruence, which is a prospective multicenter cohort study.

Setting: Gender clinics in Amsterdam, Ghent, and Florence.

Participants: Transwomen who completed the first year of CHT (n = 229).

Intervention: CHT.

Main Outcome Measures: Breast development in centimeter and cup size.

Results: The median age of the included transwomen was 28 years (range, 18 to 69). Mean breast-chest difference increased to 7.9 ± 3.1 cm after 1 year of CHT, mainly resulting in less than an AAA cup size (48.7%). Main breast development occurred in the first 6 months of therapy. Serum estradiol levels did not predict breast development after 1 year of CHT (first quartile, 3.6 cm [95% confidence interval (CI), 2.7 to 4.5], second quartile, 3.2 cm [95% CI, 2.3 to 4.2], third quartile, 4.4 cm [95% CI, 3.5 to 5.3], and fourth quartile, 3.6 cm [95% CI, 2.7 to 4.5]).

Conclusion: This study shows that, after 1 year of CHT, breast development is modest and occurs primarily in the first 6 months. No clinical or laboratory parameters were found that predict breast development. (***J Clin Endocrinol Metab*** **103: 532–538, 2018)**

Persons diagnosed with gender dysphoria can be treated with cross-sex hormones to reduce distress and induce desired physical changes. In transwomen (male-to-female transgender persons) treatment consists of antiandrogens and estrogens. Physical changes induced by this treatment include breast development, reduced sperm production,

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Abbreviations: BMI, body mass index; CHT, cross-sex hormone therapy; CI, confidence interval; CV, coefficient of variation; ENIGI, European Network for the Investigation of Gender Incongruence; LC-MS/MS, liquid chromatography tandem mass spectometry; LH, luteinizing hormone; LOQ, limit of quantification; RIA, radioimmunoassay; SD, standard deviation.

reduced number of spontaneous erections, and changes in body hair and body composition (1, 2), leading to feminization.

Breast development is a key feature of feminization and therefore important to transwomen. The Clinical Practice Guidelines of the Endocrine Society indicate that breast development starts 3 to 6 months after start of cross-sex hormone therapy (CHT). The maximum effect may be expected after 2 to 3 years of CHT (1). However, because these time frames are estimated and not based on clinical data, it is not exactly known when breast development starts after induction of CHT. Besides, the literature is inconsistent about how much growth may be expected from CHT alone. The few studies addressing this issue showed modest breast development after CHT (3–5). Moreover, because of anatomical differences in the male chest compared with the female chest, breast size may appear smaller than the actual objectively measured volume (6). Consequently, 60% to 70% of all transwomen seek additional breast augmentation besides CHT (7, 8). Because a small portion of women with silicone breast implants have local and/or systemic health complaints (9), it is important to gain more knowledge about breast development in transwomen to be able to reduce the number of breast augmentations in this population.

Therefore, this study aims to prospectively examine the absolute breast development in centimeters and bra cup sizes in transwomen during their first year of CHT. Furthermore, it will be studied whether age, weight change, body mass index (BMI), smoking, treatment regimen, or serum hormone levels influence breast development.

Subjects and Methods

Study population

This study was performed as part of the European Network for the Investigation of Gender Incongruence (ENIGI) study (10), a collaboration of four centers (Amsterdam, Florence, Ghent, and Oslo), to investigate the effects of CHT on different parameters such as breast development. A detailed description of the study design and protocol is described by Dekker *et al.* (10). Briefly, persons were eligible for participation in the ENIGI study if they were age 18 years or older, had a diagnosis of gender dysphoria confirmed by a psychiatrist or psychologist, and were about to start CHT. Persons were not eligible if they had ever used or were currently using cross-sex hormones, if they were treated following a different protocol, or if there was insufficient knowledge of the nation's language. Treatment of transwomen consisted of antiandrogens, with either cyproterone acetate (10 to 100 mg daily) or spironolactone (100 to 150 mg daily), combined with administration of estradiol valerate (2 to 6 mg daily), estradiol patches (50 to 100 mcg/24 hours twice weekly), or estradiol gel (0.75 to 3 mg daily). Those older than age 40 were advised to use estradiol transdermally to reduce the slightly increased risk of thrombosis (11).

All transwomen included in the ENIGI study between 2010 and April 2016, who completed the first year of CHT and for whom baseline breast and chest measurements were available, were eligible for analyses. Because there were no breast and chest measurements performed in the Oslo clinic, their participants were omitted for analyses in this study. There were no data available on serum hormone levels from the Florence clinic; therefore, their patients were omitted for analyses including laboratory data.

The overall study protocol was approved by the ethical review board of Ghent University Hospital, Belgium. Of the other participating centers, the local ethical review boards approved participation to this study. Informed consent from participants was obtained following institutional guidelines.

Data collection

In accordance with the study protocol, all persons were evaluated every 3 months at the different outpatient clinics. During physical examination, different circumference and length measurements in centimeters and weight in kilograms were registered. Breast circumference was measured in centimeters with a tape measure horizontally placed around the thorax over the fullest part of the bare breasts. Chest circumference was measured with a tape measure horizontally placed around the thorax in the inframammary fold (Fig. 1).

Laboratory tests

Blood samples were drawn at baseline, after 3 months, and after 12 months of CHT. In Amsterdam, serum estradiol levels were measured using a competitive immunoassay (Delfia; PerkinElmer, Wallac Oy, Turku, Finland) with an interassay coefficient of variation (CV) of $< 13\%$ and a lower limit of quantitation (LOQ) of 20 pmol/L^{-1} until July 2014. From July 2014, estradiol levels were measured using liquid chromatography tandem mass spectometry (LC-MS/MS; VUmc, Amsterdam, The Netherlands) with an interassay CV of $<7\%$ and an LOQ of 20 pmol/L^{-1}. For analyses, Delfia values were converted to LC-MS/MS values using the following formula: LC-MS/MS = 1.60 × Delfia − 29. Until January 2013, serum testosterone levels were measured using a radioimmunoassay (RIA) (Coat-A-Count, Siemens, Los Angeles, CA; interassay CV, $<10\%$; LOQ, 1 nmol L^{-1}). After January 2013, testosterone levels were measured using a competitive immunoassay (Gen III, Architect, Abbott, Abbott Park, IL) with an interassay CV $<10\%$ and an LOQ of 0.1 nmol/L^{-1}. For analyses, RIA values were converted to Architect values using the following formulas: Architect = 1.1 × RIA + 0.2 for levels <8 nmol/L^{-1} and Architect = 1.34 × RIA − 1.65 for levels >8 nmol/L^{-1}. Luteinizing hormone (LH) was measured using an immunometric assay (Architect) with an interassay CV $<6\%$ and an LOQ of 2 nmol/L^{-1}.

In Ghent, serum estradiol levels were measured using E170 Modular (E2 Gen II, Roche Diagnostics, Mannheim, Germany), until 19 March 2015. Thereafter, serum estradiol levels were measured using E170 Modular (E2 Gen III, Roche Diagnostics) with an interassay CV of 3.2% and an LOQ of 92 pmol/L^{-1}. For analyses, values measured before 19 March 2015 were converted to Gen III values using the following formula: Gen III = 6.687940 + 0.834495 × Gen II. Serum testosterone levels were measured using E170 Modular (E2 Gen II) with an interassay CV of 2.6% and an LOQ of 0.4 nmol/L^{-1}. Serum LH

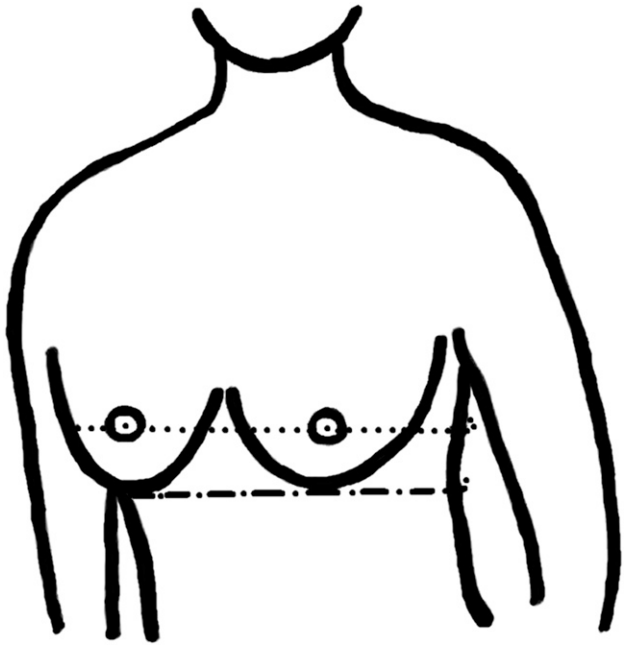

Figure 1. Placement of the tape measure for circumference measurements. For breast circumference measurement, the tape measure was placed around the thorax over the fullest part of the breast (dotted line) and for chest circumference measurement in the inframammary fold (dashed-dotted line).

levels were measured using E170 Modular (Roche Diagnostics) with an interassay CV of 2.2% and an LOQ of 0.1 mIU/mL^{-1}.

Statistical analysis

First, the database was screened for missing, inconsistent, and unreliable breast and chest values. Because of the overstated inverted pyramid shape of the thorax, a greater chest than breast circumference is highly unlikely. Consequently, all breast and chest values resulting in a negative breast minus chest value were replaced by a missing value assuming these were input mistakes (3.1%). Subsequently, all population- and individual-level breast and/or chest circumference measurement outliers were identified and replaced by a missing value (3.1%). Population outliers were defined as mean ± 3 times the standard deviation (SD). Individual-level outliers were identified using the method described by Welch *et al.* (12), with a standardized residual cutoff point of ±5. Last, all transwomen with unknown baseline breast or chest measurements were removed from the database.

Baseline data are presented as mean with SD for normally distributed data, and median with range for nonnormally distributed data. Data on tobacco use and alcohol use are presented as percentage of users. To examine the breast growth, chest circumference in centimeters was subtracted from breast circumference in centimeters separately for all visits. To analyze the effect of CHT on breast growth during the first year of therapy, mixed-model analyses were performed with observations clustered within patients within center (13). Nonnormally distributed data were log-transformed before mixed-model analyses were performed. Analyses were stratified to study whether age, weight change, differences in BMI, tobacco use, treatment regimen, and serum hormone levels influenced breast development during the first year of CHT. Age was analyzed in quartiles and weight change was divided in three groups (weight loss, no weight change, and weight gain). Differences in BMI were defined as normal weight (BMI < 25 kg/m^2), overweight (25 kg/m^2 $\geq$ BMI > 30 kg/m^2), and obese (BMI ≥ 30 kg/m^2). Analysis for tobacco use was stratified into smokers vs nonsmokers. Because most transwomen (99%) in this study received cyproterone acetate as antiandrogen treatment, no analyses on treatment regimen other than oral vs transdermal estradiol administration route could be performed. Persons were included in this analysis if they had received the same administration route for at least 9 months. Analysis for serum estradiol levels was stratified for center-specific quartiles. Analyses for testosterone and LH levels were stratified in suppressed (testosterone levels ≤ 2 nmol/L, LH levels ≤ 5 IU/L) vs nonsuppressed (testosterone levels > 2 nmol/L, LH levels > 5 IU/L) based on local female reference values. All data are presented as mean change in centimeters with 95% confidence interval (CI). Breast-chest differences after 1 year of CHT were translated into bra cup sizes following the method described by Greenbaum *et al.* (14).

All analyses were performed using STATA Statistical Software, version 13.1 (Statacorp, College Station, TX).

Results

Until April 2016, 926 persons were included in the ENIGI study, of which 53.8% were transwomen. Persons who were still in their first year of CHT, who were lost to follow-up, or who had unknown baseline breast or chest measurements were excluded for analyses. Finally, a total of 229 transwomen were included for analyses (Fig. 2). The median age before start of CHT was 28 years (range, 18 to 69 years). At baseline, the mean breast-chest difference was 4.1 ± 2.9 cm. All characteristics of the study group are summarized in Table 1. Persons who were excluded for analyses did not differ in baseline characteristics from those who were included.

Breast development predominantly occurred in the first 6 months of CHT, with an increase of 1.8 cm (95% CI, 1.4 to 2.3) over the first 3 months, and 1.3 cm (95% CI, 0.9 to 1.8) over the following 3 months. The change in breast-chest difference over the last 6 months was only 0.5 cm [0.3 cm (95% CI, −0.2 to +0.8) between 6 and 9 months of CHT and 0.2 cm [95% CI, −0.3 to +0.7] over the last 3 months (Fig. 3)]. Mean breast-chest difference increased 3.7 cm (95% CI, 3.3 to 4.2) from 4.1 ± 2.9 cm at baseline to 7.9 ± 3.1 cm after 1 year of CHT. Mean breast development did not change after adjustment for baseline breast-chest difference [4.1 cm (95% CI, 3.4 to 4.7)]. After calculating mean breast-chest difference into bra cup sizes, almost half of the transwomen (48.7%) had a bra cup size of less than an AAA cup after 1 year of CHT (Fig. 4). Furthermore, 52 transwomen (26.4%) had an AAA cup, 28 (14.2%) an AA cup, 14 (7.1%) an A cup, and only 7 transwomen (3.6%) gained a bra cup size larger than an A cup. As shown in Fig. 5(a) and 5(b), breast development did not

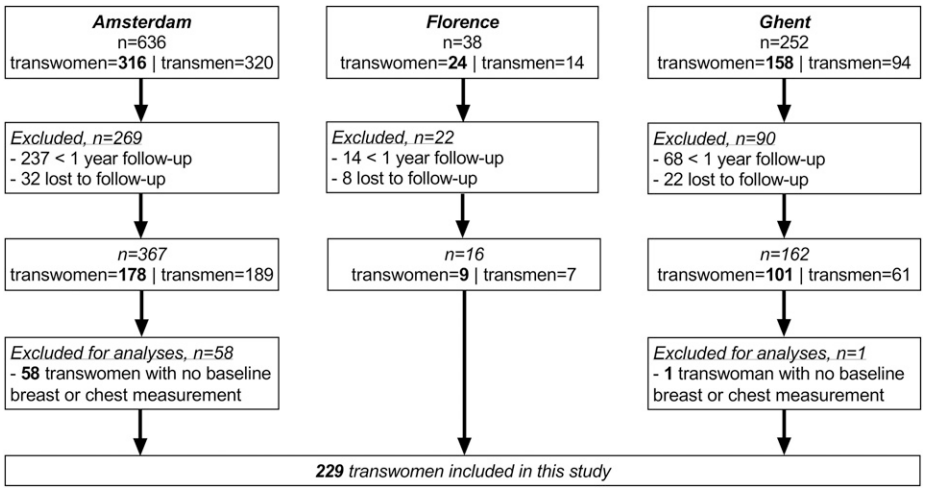

Figure 2. Flowchart of the inclusion of participants in the current study. Abbreviations: transmen, female-to-male transgender persons; transwomen, male-to-female transgender persons.

vary in different age groups or between transwomen who had no weight change, weight loss, or weight gain over the study period. Although obese transwomen had a greater breast-chest difference in the first 6 months of CHT, total breast-chest difference after 12 months did not differ among normal weight, overweight, and obese transwomen [Fig. 5(c)]. There was no difference in breast development between smokers and nonsmokers [Fig. 5(d)]. Transwomen treated with transdermal estradiol had a faster increase in breast-chest difference until 6 months after initializing treatment. However, breast development after 1 year of treatment did not differ between oral and transdermal estradiol administration [Fig. 5(e)].

Mean serum estradiol levels in the center-specific quartiles were 110 pmol/L^{-1} (first quartile), 184 pmol/L^{-1} (second quartile), 271 pmol/L^{-1} (third quartile), and 452 pmol/L^{-1} (fourth quartile) in Amsterdam, and 142 pmol/L^{-1} (first quartile), 206 pmol/L^{-1} (second quartile), 299 pmol/L^{-1} (third quartile), and 567 pmol/L^{-1} (fourth quartile) in Ghent. Serum estradiol levels did not predict breast development after 1 year of CHT [first quartile: 3.6 cm (95% CI, 2.7 to 4.5); second quartile: 3.2 cm (95% CI, 2.3 to 4.2); third quartile: 4.4 (95% CI, 3.5 to 5.3); and fourth quartile: 3.6 cm (95% CI, 2.7 to 4.5); Fig. 5(f)]. Because CHT induced suppression of serum testosterone and LH levels in almost all transwomen (testosterone: not suppressed in 7.9%; LH: not suppressed in 1.3%), no analyses could be performed on these data. Transwomen with an A cup size or larger did not differ in baseline characteristics from transwomen with a cup size smaller than an A cup.

Table 1. Characteristics of 229 Transwomen Before CHT

Age, y	**28 (18–69)**
Ethnicity, % Caucasian	95.6
Height, cm	178.6 ± 6.8
Weight, kg	72.3 (49.9–137.2)
BMI, kg/m^2	22.7 (16.5–37.7)
Smoking, % yes	25.3
Alcohol use, % yes	46.0
Breast circumference, cm	92.9 ± 9.3
Chest circumference, cm	88.8 ± 8.9
Breast-chest difference, cm	4.1 ± 2.9

Data are presented as mean ± SD, median (range), or percentages.

Discussion

The current study shows a modest increase in breast-chest difference after 1 year of CHT, mainly resulting in less than an AAA bra cup size. Age, weight change, smoking, BMI, serum estradiol levels, and estrogen administration route did not predict total breast development after 1 year of CHT.

This large multicenter cohort study shows slightly less breast development than shown in previous studies performed (3, 4, 11, 15). However, these previous studies used other measurement methods (*e.g.*, hemicircumference measurements) and bra cup sizes were estimated or self-reported rather than translated or calculated from circumference measurements. Moreover, CHT duration in these studies is not always clearly reported and varies widely (from 3 to 24 months) (3–5), which makes comparison of these studies with the current study difficult. This study found a bra cup size of less than an AAA cup in 48.7% of transwomen. Only 10.7% had an A cup or larger after 1 year of CHT. Because only breast and chest circumference were measured and converted into bra cup sizes, it was impossible

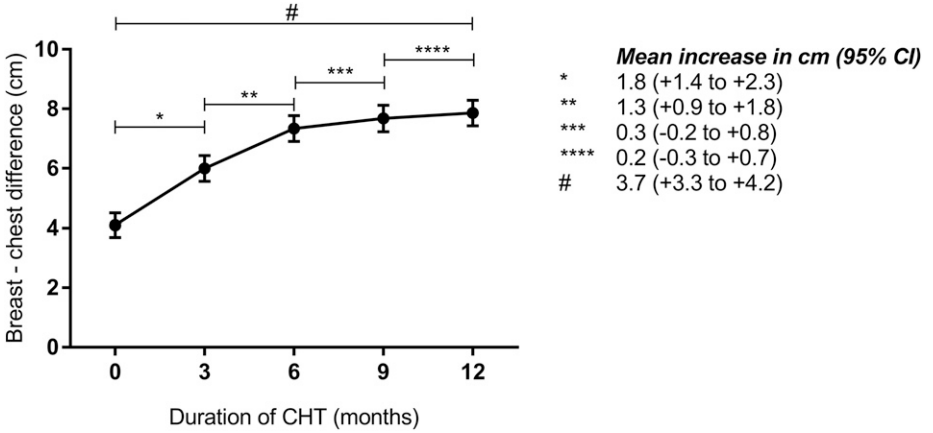

Figure 3. Mean breast-chest difference in centimeters over duration of CHT in months. For each time point, the mean increase in centimeters (dot) with 95% CI (bars) is shown. Data in the legend are shown as mean increase with 95% CI.

to calculate breast volumes in this study. Because cup size consists of more than thorax circumferences alone and breast shapes vary widely between different people, cup sizes found in this study were probably underestimated.

Breast tissue consists of both epithelial components, with ducts and lobules, and stromal elements, consisting of adipose and fibrous connective tissue. The latter accounts for the majority of the total breast volume. The fat distribution of transwomen after 1 year of CHT is still developing and is not yet similar to that of ciswomen (women with the birth-assigned sex of a female who identify themselves as female) (16). This may contribute to the modest breast development measured in this study. Another explanation for the modest breast development found could be that exposition to high testosterone during male puberty induces some structural changes that inhibit breast development. There were transwomen in this study who had substantial breast development during the first year of therapy and had passed normal male puberty.

An interesting finding is that main breast development occurred in the first 6 months of CHT. The flattening of the breast development curve in this study suggests no further increase in breast-chest difference after the first year of CHT. However, the maximum effect of CHT on breast development may be expected after 2 to 3 years, as also seen during pubertal development in girls (1, 5, 17). Because the follow-up of the current study is 2 year, no conclusions can be drawn on final breast development induced by CHT. Moreover, a recent study by Fisher *et al.* showed breast development to Tanner stage 3 after 2 years of CHT, which emphasize the need for studies with longer follow-up (18).

Levy *et al.* state that transwomen with a higher BMI seem to have better breast development (15). Moreover, Coltman *et al.* showed that breast volumes in overweight or obese ciswomen were greater compared with ciswomen with a normal weight BMI (19). However, the current study found no differences in breast development in different BMI categories, which may be due to the small number of overweight (18.1%) and obese (8.3%) persons in this study. Another possible explanation may be that the overweight and obese transwomen in this study were encouraged to lose weight during the first year of CHT in view of surgical treatment thereafter. Furthermore, this study did not find any differences in characteristics, treatment regimen, or serum estradiol levels in transwomen with a breast development of an A cup or larger compared with transwomen with a development of smaller than an A cup. Consequently, no clinical or laboratory parameters were found to be predictive for breast development in this study. Although an explanation for the difference in breast development between persons may be genetic variation (20, 21), further studies are required.

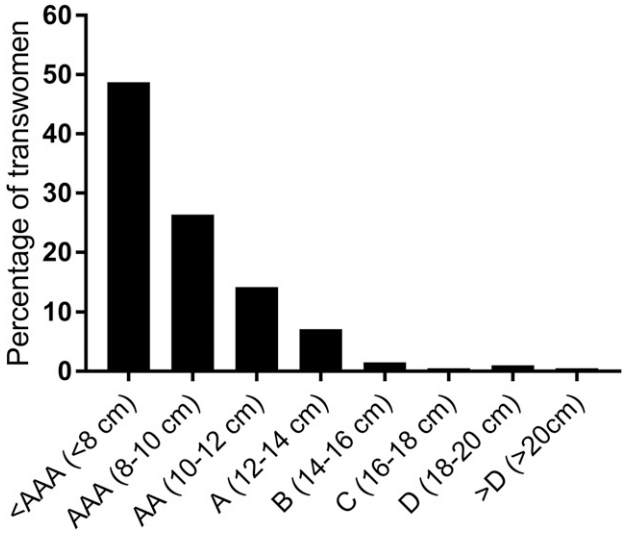

Figure 4. Gained bra cup sizes in 197 transwomen after 1 year of CHT. Data are shown as percentage of transwomen per cup size.

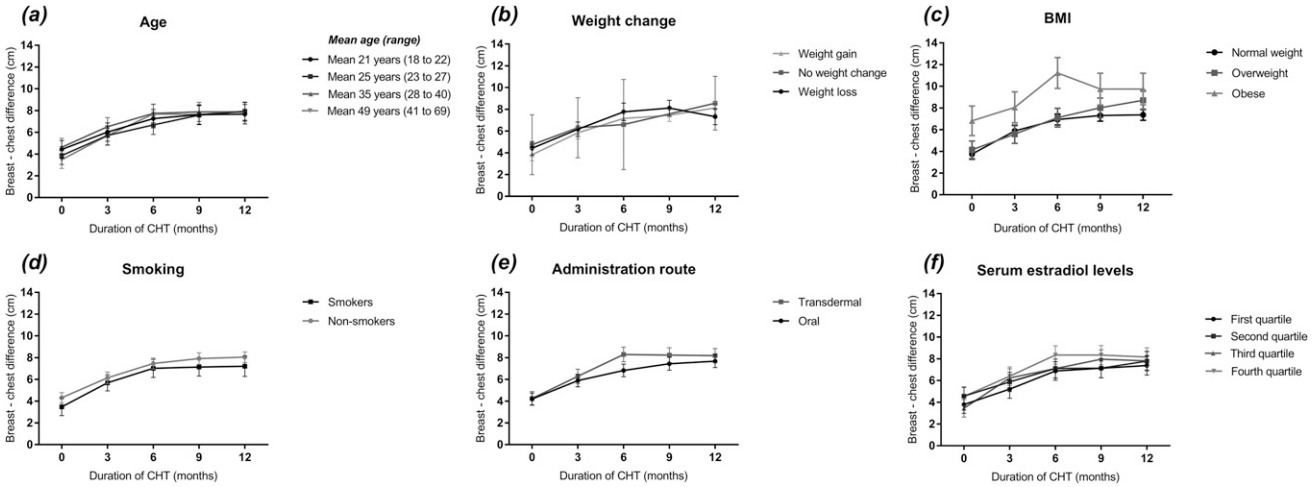

Figure 5. Mean breast-chest difference in centimeter over duration of CHT in months, stratified for age in four groups. (a) Weight change in three groups (b), normal weight (BMI < 25 kg/m^2), overweight (25 kg/m^2 $\geq$ BMI > 30 kg/m^2), and obese (BMI ≥ 30 kg/m^2) BMI (c), smokers vs nonsmokers (d), oral vs transdermal estradiol administration route (e), and four quartiles of serum estradiol levels (f). Mean serum estradiol levels in the center-specific quartiles were 110 pmol/L^{-1} (first quartile), 184 pmol/L^{-1} (second quartile), 271 pmol/L^{-1} (third quartile), and 452 pmol/L^{-1} (fourth quartile) in Amsterdam, and 142 pmol/L^{-1} (first quartile), 206 pmol/L^{-1} (second quartile), 299 pmol/L^{-1} (third quartile), and 567 pmol/L^{-1} (fourth quartile) in Ghent. For each time point, the mean increase in centimeters (dot) with 95% CI (bars) is shown.

This study provides more insight in how much breast development can be expected of CHT during the first year of therapy. Other strengths are the prospective, multicenter design of the study and the large number of participants with a wide range of age. Moreover, this study had fixed measurement moments every 3 months with relatively few missing values (12.1% of all breast and/or chest measurements). However, this study has some limitations as well. First, thorax circumferences were used for estimation of breast development, which probably provide a less reliable reflection of the actual breast development compared with volume measurements. Pechter suggested a hemicircumference measurement method to calculate bra cup sizes (22), which may be a better estimator than thorax circumference measurements (23). Furthermore, McGhee *et al.* showed that respiration affects circumference measurements and, consequently, cup size estimations up to four sizes discrepancy (23). Because respiration was not standardized in this study, measurements were likely to be affected by the respiration of the transwomen measured and thereby it probably affected the accuracy of the measurements. On the other hand, because of the lack of standardization, some values will be overestimated and some underestimated resulting in an overall reliable mean. Second, this study was performed during regular care; as a consequence, participating centers were not always able to collect all data needed because of limited resources. Nevertheless, we had access to a large sample with relatively few missing values. Third, all data regarding other medication use, comorbidities, tobacco use, and alcohol use were self-reported and therefore might be incomplete. Last, this study only reported objective outcomes of breast development. This however says nothing about the satisfaction of transwomen with the gained breast development. Van de Grift *et al.* (24) showed that body satisfaction increased with hormone treatment, but that in transwomen who received breast augmentation surgery, body satisfaction was higher. Moreover, transpersons with high overall body dissatisfaction at baseline showed persistent higher dissatisfaction after treatment. Future studies should incorporate body and/or breast satisfaction scales to relate the clinical observed breast development to the satisfaction of the transwomen themselves.

In conclusion, this large multicenter cohort study shows modest breast development after 1 year of CHT in transwomen. No predictive markers in clinical and laboratory parameters, including serum estradiol levels, were identified. It would be interesting for future studies to search for other parameters and study whether these predict breast development in transwomen during CHT. Studies with longer follow-up are needed to determine the final breast development gained with CHT alone. Furthermore, it would be worthwhile for future studies to use volume measurements for examining hormonally induced breast development in transwomen.

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Correspondence and Reprint Requests: Martin den Heijer, MD, PhD, Department of Internal Medicine, Section Endocrinology, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands. E-mail: m.denheijer@vumc.nl.

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