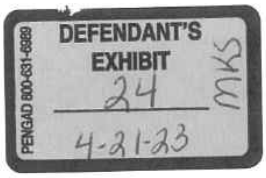
EXHIBIT 52



Hormonal Treatment in Young People With Gender Dysphoria: A Systematic Review

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abstract

CONTEXT: Hormonal interventions are being increasingly used to treat young people with gender dysphoria, but their effects in this population have not been systematically reviewed before.

OBJECTIVE: To review evidence for the physical, psychosocial, and cognitive effects of gonadotropin-releasing hormone analogs (GnRHa), gender-affirming hormones, antiandrogens, and progestins on transgender adolescents.

DATA SOURCES: We searched Medline, Embase, and PubMed databases from January 1, 1946, to June 10, 2017.

STUDY SELECTION: We selected primary studies in which researchers examined the hormonal treatment of transgender adolescents and assessed their psychosocial, cognitive, and/or physical effects.

DATA EXTRACTION: Two authors independently screened studies for inclusion and extracted data from eligible articles using a standardized recording form.

RESULTS: Thirteen studies met our inclusion criteria, in which researchers examined GnRHas ($n = 9$), estrogen ($n = 3$), testosterone ($n = 5$), antiandrogen (cyproterone acetate) ($n = 1$), and progestin (lynestrenol) ($n = 1$). Most treatments successfully achieved their intended physical effects, with GnRHas and cyproterone acetate suppressing sex hormones and estrogen or testosterone causing feminization or masculinization of secondary sex characteristics. GnRHa treatment was associated with improvement across multiple measures of psychological functioning but not gender dysphoria itself, whereas the psychosocial effects of gender-affirming hormones in transgender youth have not yet been adequately assessed.

LIMITATIONS: There are few studies in this field and they have all been observational.

CONCLUSIONS: Low-quality evidence suggests that hormonal treatments for transgender adolescents can achieve their intended physical effects, but evidence regarding their psychosocial and cognitive impact are generally lacking. Future research to address these knowledge gaps and improve understanding of the long-term effects of these treatments is required.

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REVIEW ARTICLE

Transgender is a term used to describe an individual whose inner gender identity differs from their sex assigned at birth. This mismatch can cause distress and functional impairment, resulting in gender dysphoria (GD) or what was previously termed "gender identity disorder" (GID).[1,2]

Several hormonal treatment options are available for GD, the appropriateness of which depends on developmental stage. For instance, puberty can frequently exacerbate GD because of the development of unwanted secondary sexual characteristics,[3] which can be reversibly suppressed by using gonadotropin-releasing hormone analogs (GnRHas).[4,5] In comparison, gender-affirming hormones (GAHs; also known as cross-sex hormonal therapy) allow individuals to actively masculinize or feminize their physical appearance to be more consistent with their gender identity. As GAHs are only partially reversible, they are generally used only once an individual reaches the legal age of medical consent, which varies across countries.[5] In addition, antiandrogens, such as spironolactone and cyproterone acetate, can be used to counter the effects of testosterone in birth-assigned male individuals,[6,7] whereas progestins, such as norethisterone and medroxyprogesterone, are often employed to suppress menses in younger birth-assigned female individuals.

Authors of multiple studies have investigated the physical and psychosocial effects of different hormonal interventions in adults with GD. GAHs have been examined most extensively, with authors of systematic reviews indicating that GAHs improve multiple aspects of psychosocial functioning,[8,9] although they also increase serum triglycerides and risk of cardiovascular disease (including venous thrombosis, stroke, myocardial infarction, and pulmonary embolism).[10–12] Studies of antiandrogens in transfemale adults have revealed that cyproterone acetate is able to reduce levels of testosterone, whereas spironolactone has a synergistic effect with estrogen in improving both physical and hormonal outcomes.[13]

In contrast, studies of different hormonal treatments in young people with GD are scarce, meaning that clinicians have often had to extrapolate from adult studies. This is problematic for several reasons. Firstly, adolescence is a period of rapid development across multiple domains,[14] and studies of hormonal treatments in adults with GD may not readily translate to adolescents. Secondly, some hormone treatments used in young people with GD (eg, GnRHas and progestins) are either not commonly used in adults with GD or are used in adults for different reasons (eg, GnRHas for prostate cancer).[15] Finally, hormonal dosing regimens in adolescents with GD are frequently different from those used in adults, which is likely to affect outcomes.

Our purpose in this systematic review is, therefore, to evaluate the currently available evidence about the physical, psychosocial, and cognitive effects of different hormonal therapies in transgender youth. By doing so, we can directly inform clinical practice involving this population and highlight existing knowledge gaps.

METHODS

Eligibility Criteria

Studies were considered eligible if participants were given hormonal treatment (GnRHas, GAHs, antiandrogens, or progestins) and if analysis of psychosocial, cognitive, and/or physical effects of these hormones were included. Participants had to be younger than 25 years of age and described as transgender or diagnosed with GD and/or GID according to the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition*; *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*; or *International Classification of Diseases* criteria. This age range was selected to be consistent with the definition of adolescence used by the recent *Lancet* Commission on Adolescent Health.[16] Studies were excluded if the effects of hormonal therapy could not be separated from gender-affirming surgery, which could cause potential issues related to interpretation of results. We included all published study designs in any language, but conference abstracts or studies in which researchers failed to report results at the group level with at least 10 individuals were excluded.

Study Identification

The Medline (Ovid) and Embase (Ovid) databases were searched for references from January 1, 1946, to June 10, 2017, by using thesauri and/or keywords. PubMed was searched by using keywords to retrieve electronic publications and items not indexed in Medline. The Medline search strategy was adapted for use in Embase and PubMed with the main search terms as follows: (GD or transsexualism or "sexual and gender disorders" or transgender persons or gender identity), (drug therapy or therapeutic use or [hormonal or hormone*] or *steroids or exp gestagen or exp antiandrogen), and (adolescen* or pediatric* or pediatric* or youth* or teen or teens or teenage*). Detailed search histories are available on request. Additional items were identified by manually searching reference lists of relevant retrieved articles. Two reviewers independently assessed all study titles and abstracts to determine inclusion, with the full text being subsequently retrieved for potentially eligible studies to assess final suitability. Any disagreements

were resolved with discussion and consensus was reached for final articles.

Data Extraction

Two reviewers, working independently and in duplicate, used a standardized form to extract methodological, demographic, and outcome data. Data extracted included reported youth characteristics (number of participants pre- and posttreatment, participant age range, diagnosis of GD, birth-assigned sex, and gender identity), hormonal therapy features (type, dose, route, duration of treatment), study design, and outcomes of interest (length of follow-up duration, follow-up outcome measures, and treatment effect on outcome measures).

Quality Assessment

Risk of bias in studies was assessed by 2 authors working independently using a modified version of the Quality in Prognosis Studies (QUIPS) tool from a previous study.[17] The original QUIPS tool[18] was modified because confounders or prognostic factors were not analyzed in this review and thus did not apply.

Review Protocol

A detailed protocol is available at PROSPERO (identifier 42017056670).

Statistical Analysis

Effect sizes were calculated for results with reported means and SDs,[19–27] according to a previous study.[28] Unadjusted effect sizes using the posttest SD were calculated for the majority of studies, with an adjusted effect size using the experimental SD calculated only for 1 study with comparison between groups.[24]

Meta-analysis

Meta-analysis was planned for outcomes examined by 3 or more studies but was unable to be conducted because individual outcome effect sizes were available for a maximum of 2 studies.

RESULTS

Study Selection

The study selection process is depicted in Fig 1. Eighty-three potentially relevant studies were retrieved, of which 13[19–27,29–32] met the inclusion criteria and were systematically analyzed. In Table 1, we summarize the main characteristics of these 13 studies, and their key physical, psychosocial, and cognitive findings are outlined in Tables 2, 3 and 4, respectively. Because research from the same cohort was described in 2 of the studies,[26,27] they were considered as 1 study.

Quality Appraisal

In all studies, there was a medium to high risk of bias (Table 5). In most studies, there were only small sample sizes (minimum of 21 and maximum of 201), with <50 participants in 38.5% of the studies. There were controls in only 2 studies, and all studies were conducted in clinical populations. There was often significant loss to follow-up, attributed partially to most studies being retrospective with missing data. Overall, the tools used to measure the specific outcomes were valid and reliable, although there was no blinding or randomization in any of the studies.

PHYSICAL EFFECTS

All relevant results are shown in Table 2.

Sex Hormones and Secondary Sexual Characteristics

GnRHas

GnRHas were successful in suppressing sex hormone secretion with significant decreases in gonadotropin,[29] estradiol, and testosterone[19,21,29] levels, although 1 study only revealed a significant decrease in transfemale adolescents (birth-assigned male individuals identifying as female individuals).[19] There was decreased testicular volume in transfemale adolescents[19,21,29] and cessation of menses in transmale adolescents (birth-assigned female individuals identifying as male individuals),[21] although the latter often occurred after a withdrawal bleed in postmenarchal individuals. Furthermore, GnRHas were shown to decrease luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[19,21]

Progestin

Researchers in 1 study examined the effects of the progestin lynestrenol[30] in transmale adolescents. Although there was no report of the efficacy of lynestrenol in stopping menses, there were significant reductions in levels of serum sex-hormone binding globulin (SHBG) and LH, in addition to a significant increase in free testosterone (fT). FSH, estradiol, testosterone, and anti-Mullerian hormone had nonsignificant decreases.[30]

Antiandrogen

In one study, researchers studied the effects of the antiandrogen cyproterone acetate alone in transfemale adolescents.[22] It was effective in significantly suppressing endogenous sex hormones with significant reductions in testosterone and dehydroepiandrosterone in addition to nonsignificant decreases in estradiol and fT after 12 months, with no significant changes in LH, FSH, and SHBG. Cyproterone acetate was associated with a marked increase in prolactin of ~2.5-fold that exceeded the normal reference range after 6 months but returned to the normal range after 12 months. No clinical consequences, including galactorrhea, were reported. Furthermore, 55.6% of participants

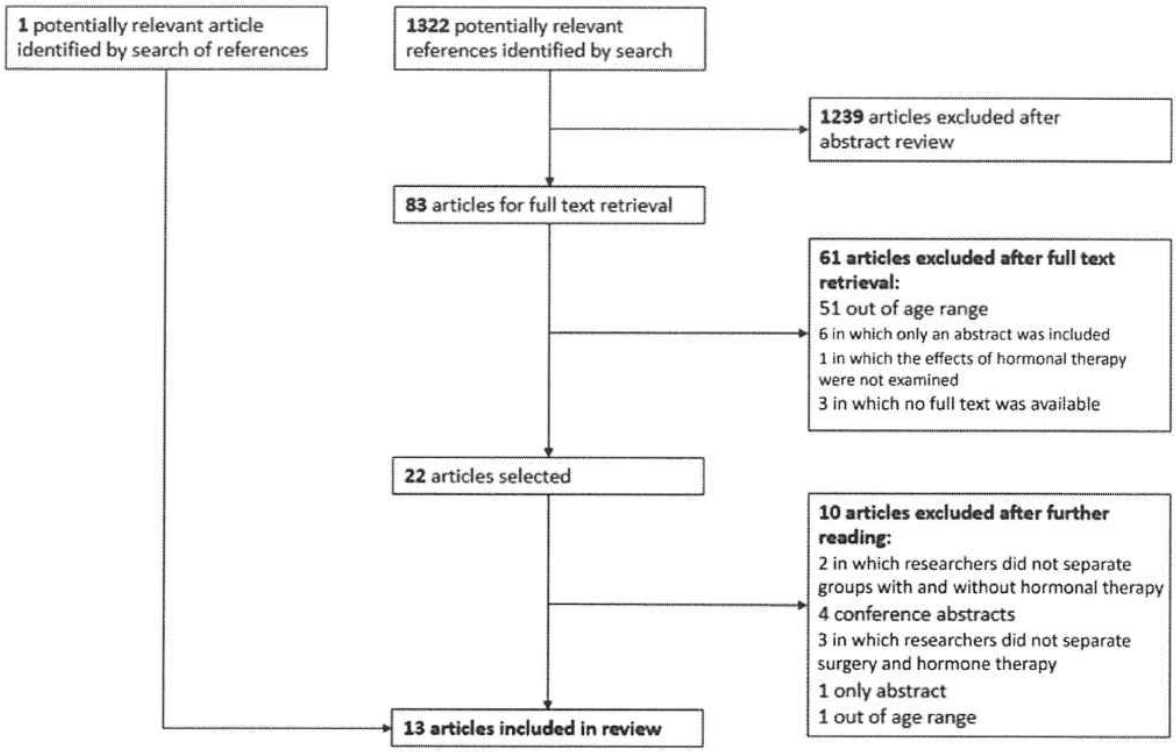

FIGURE 1
Flow diagram of study selection.

also reported decreased facial shaving frequency.[22]

Estrogen

Estrogen was successful in feminizing physical sex characteristics.[22,29] In 1 study, 66.7% of participants reached Tanner B3 stage (increase in breast and areola size), and 9.5% reached Tanner B4 (secondary mound created by areola and papilla) after treatment with cyproterone acetate and estrogen for at least 6 months.[22] However, breast development was found to be objectively dissatisfactory and subjectively less in size than expected for the majority.[22] There was a significant increase in serum estradiol after 6 months that reached the female reference range, whereas total testosterone decreased after 1 to 3 months to be outside of the male reference range.[22,32] Prolactin was unchanged.[22]

Testosterone

Testosterone resulted in virilization, including lower voice, clitoral enlargement, and body hair growth in a masculinized pattern.[29] Menses ceased in most transmale adolescents within 6 months, with an average time to cessation of 2.9 months.[20] Testosterone resulted in increased total testosterone and fT,[20,30,32] with most participants reaching levels within the normal male range after 6 months,[20,30] as well as significant decreases in LH and FSH.[30] This was accompanied by a decline in estradiol levels after 6 months,[20,30,32] which was statistically significant in 2 studies[20,30] but nonsignificant in 1 study.[32]

Side Effects

GnRHas

Hot flashes were a common side effect in transmale adolescents treated in late puberty (Tanner stages B4 and B5), although these decreased in frequency over time.[29] No other short-term side effects, including local reactions, were reported.

Progestin

Lynestrenol was evaluated as relatively safe, with the most common side effects being initial metrorrhagia (48.7%), headaches (12.1%), hot flashes (9.8%), and acne (which increased from 14.6% to 28.6%).[30]

Antiandrogens

Treatment with cyproterone acetate was evaluated to be relatively safe, with the most common side effect being fatigue (37%).[22]

Estrogen

Side effects reported with combined estrogen and cyproterone acetate

TABLE 1 Characteristics of 13 Studies on Hormonal Treatments in Transgender Youth

Study	Type of Study	Sample (*N*)	Gender Identity	Age, y ± SD	Loss to Follow-up, %	Effects Analyzed	Treatment	Duration of Treatment, y	Outcomes Examined
Delemarre-van de Waal and Cohen-Kettenis[29]	Prospective, longitudinal	21	11 transmale adolescents with GID, 10 transfemale adolescents with GID	Not mentioned	0	Physical	GnRHa	2 y or longer	Sex hormones and secondary sexual characteristics, safety profile, BMD, growth, and body composition
de Vries et al[27] (de Vries et al[26])[a]	Prospective, longitudinal	70 (55)	37 (33) transmale adolescents with GID, 33 (22) transfemale adolescents with GID	Baseline: 13.65 ± 1.85, at start of GnRHa: 14.75 ± 1.92, at start of GAH: 16.64 ± 1.90	Variable: 18–42[b]	Psychosocial	GnRHa, GAH (not assessed in de Vries et al[26])	GnRHa: average: 1.88 ± 1.05, range: 0.42–5.06	Psychological functioning, GD
Klink et al[19]	Retrospective, longitudinal	34	15 transfemale adolescents with GID, 19 transmale adolescents with GID	At start of GnRHa: transfemale adolescents: 14.9 ± 1.9, transmale adolescents: 5.0 ± 2.0; at start of GAH: transfemale adolescents: 16.6 ± 1.4, transmale adolescents: median of 16.4 and interquartile range of 2.3	Variable	Physical	GnRHa (only treatment studied), GAH	GnRHa: transfemale adolescents: average: 1.3, range: 0.5–3.8; transmale adolescents: average: 1.5, range: 0.25–5.2; GAH: transfemale adolescents: average: 5.8, range: 3–8; transmale adolescents: average: 5.4, range: 2.8–7.8	Sex hormones and secondary sexual characteristics, BMD, growth, body composition, and other physical effects
Olson et al[20]	Prospective, longitudinal	36	36 transmale transgender adolescents	18.7 ± 2.6	3	Physical	GAH (only testosterone)	Not mentioned	Sex hormones and secondary sexual characteristics, body composition, and other physical effects
Costa et al[25]	Prospective, longitudinal	201	124 transmale adolescents with GID, 77 transfemale adolescents with GID	Baseline: 15.52 ± 1.41, start of GnRHa: 16.48 ± 1.26	Variable: 0–65[a]	Psychosocial	GnRHa	Immediately eligible for GnRHa: average: 0.75 ± 0.59	GD-related discomfort, global psychosocial functioning

TABLE 1 Continued

Study	Type of Study	Sample (*N*)	Gender Identity	Age, y ± SD	Loss to Follow-up, %	Effects Analyzed	Treatment	Duration of Treatment, y	Outcomes Examined
Staphorsius et al[24]	Cross-sectional	116	22 transmale adolescents with GID, 18 transfemale adolescents with GID, 21 male control subjects, 24 female control subjects	Transmale adolescents: 15.8 ± 1.9, transfemale adolescents: 15.1 ± 2.4, male adolescents : 14.9 ± 1.5, female adolescents: 14.4 ± 1.8	28	Cognitive	GnRHa	GnRHa: average: 1.6 ± 1.0	Executive functioning
Burke et al[23]	Prospective, fMRI	62	21 transmale adolescents with GID, 20 male control subjects, 21 female control subjects	Transmale adolescents: 16.1 ± 0.8, control male subjects: 15.9 ± 0.6, control female subjects: 16.3 ± 1.0	8.1	Cognitive	GnRHa, GAH (testosterone)	GnRHa: average: 2, range: 0.17–4; testosterone: average: 0.83, range: 0.5–1.25	Mental rotation
Schagen et al[21]	Prospective, longitudinal	128	67 transmale adolescents with GID, 49 transfemale adolescents with GID	Transmale adolescents: 14.2, transfemale adolescents: 13.6	9	Physical	GnRHa	At least 0.25	Sex hormones and secondary sexual characteristics, growth, body composition, and other physical effects
Tack et al[50]	Retrospective, longitudinal	45	38 transmale adolescents with GID	15.8 at start of treatment	16	Physical	Androgenic progestin (lynestrenol), combination of androgenic progestin (lynestrenol) and GAH (testosterone)	Average of 10.5 for lynestrenol, average of 0.95 for lynestrenol and testosterone	Sex hormones and secondary sexual characteristics, safety profile, body composition, and other physical effects
Viot et al[51]	Retrospective, longitudinal	70	42 transmale adolescents with GID, 28 transfemale adolescents with GID	GnRHa at start of treatment: transmale adolescents: 15.1, transfemale adolescents: 13.5; GAH at start of treatment: transmale adolescents: 16.3, transfemale adolescents: 16.0	20	Physical	GnRHa, GAH (testosterone and estrogen)	Not mentioned	Bone turnover, BMD, and growth

TABLE 1 Continued

Study	Type of Study	Sample (*N*)	Gender Identity	Age, y ± SD	Loss to Follow-up, %	Effects Analyzed	Treatment	Duration of Treatment, y	Outcomes Examined
Jarin et al[52]	Retrospective, longitudinal	116	72 transmale adolescents with GD, 44 transfemale adolescents with GD	Transmale adolescents: average of 16 (range of 13–22) at start of treatment, Transfemale adolescents: average of 18 (range of 14–25) at start of treatment	Variable	Physical	GAH (testosterone and estrogen treatment)	Not mentioned	Sex hormones and secondary sexual characteristics, body composition, and other physical effects
Tack et al[22]	Retrospective, longitudinal	27	27 transfemale adolescents with GD	Antiandrogen: 16.5 at start of treatment, combination of antiandrogen and GAH: 17.6 at start of treatment	22.2 (variable)	Physical	Antiandrogen (cyproterone acetate), combination of antiandrogen (cyproterone acetate) and GAH (estrogen treatment)	Antiandrogen: minimum of 0.5 (mean of 1.0), combination of antiandrogen and GAH: minimum of 0.5 (mean of 1.3)	Sex hormones and secondary sexual characteristics, safety profile, growth, body composition, and other physical effects

Note that transmale adolescents are birth-assigned female individuals who identify as male individuals, whereas transfemale adolescents are birth-assigned male individuals who identify as female individuals.

[a] These 2 studies involved the same cohort and were therefore considered as 1 study. The values in parenthesis are used to indicate the results of the earlier study,[26] in which researchers examined a smaller subset of the cohort subsequently examined in de Vries et al.[27]

[b] Variable loss to follow-up depending on test.

TABLE 2 Physical Effects of Hormonal Treatments in Transgender Youth

Study	Treatment	Outcome					
		Testosterone, Estradiol, and Gonadotropin Levels	Anthropometric Measurements	BMD	Body Composition	Safety Profile	Other Physical Effects
Delemarre-van de Waal and Cohen-Kettenis[28]	GnRHa	Decrease[a] in gonadotropin and sex hormone levels, decrease[a] in testicular volume in transfemale adolescents	Decrease[a] in height velocity, decrease[a] in height SDSs in youth who still have growth potential (related to bone age)	No change in bone density actual values but decrease[a] in standardized score (*z* score)	Increase[a] in fat mass percentage, decrease[a] in lean body mass percentage	Frequent hot flashes in transmale adolescents (when treated in late pubertal stages)	—
Delemarre-van de Waal and Cohen-Kettenis[28]	GAH (testosterone and estrogen)	Virilization of transmale adolescents (low voice, clitoris enlarged, facial and body hair growth) and transfemale adolescents (induced breast development)	Increase[a] in height (growth spurt) with androgen substitution therapy	Increase[a] in bone density (actual and *z* scores)	No effect on fasting glucose, insulin, cholesterol, HDL, and LDL levels	—	—
Klink et al[19]	GnRHa (only treatment studied), GAH	Decrease[b] in estradiol, decrease[b] in testosterone in transfemale adolescents with no change in transmale adolescents, decrease[b] in testicular volume in transfemale adolescents, decrease[c] in androstenedione, decrease[b] in LH and FSH	Increase[b] in height actual values, decrease[b] in height standardized values for transfemale adolescents, decrease[c] in height standardized values for transmale adolescents	Transfemale adolescents Lumbar spine: no significant changes in actual score and decrease[c] in *z* score Femoral nondominant: decrease[c] in actual and *z* scores Transmale adolescents Lumbar spine: decrease[c] in actual score and decrease[b] in *z* score Femoral nondominant: decrease[c] in actual and *z* scores	Increase[b] in wt for transfemale adolescents and transmale adolescents, increase[b] in BMI actual score for transfemale adolescents and transmale adolescents, nonsignificant changes in BMI SDSs for transfemale adolescents and transmale adolescents	—	—
Olson et al[20]	GAH (testosterone)	Increase[b] in total and fT levels, decrease[b] in normal and serum estradiol levels	—	—	Increase[b] in BMI, decrease[c] in total cholesterol	—	Increase[b] in Hb (but not to clinically significant levels), increase[b] in systolic BP and ALT (but not to clinically significant levels), decrease[c] in diastolic BP, increase[c] in AST

TABLE 2 Continued

Study	Treatment	Outcome					
		Testosterone, Estradiol, and Gonadotropin Levels	Anthropometric Measurements	BMD	Body Composition	Safety Profile	Other Physical Effects
Schagen et al[21]	GnRHa	Transmale adolescents: menses ceased; Transfemale adolescents: decrease[c] in testicular volume, decrease[c] in LH and FSH, and decrease[c] in gonadotropin, estradiol, and testosterone	Decrease[b] in height SDSs and increase[b] in height values in transfemale adolescents and transmale adolescents	—	Increase[b] in wt scores, increase[b] in BMI scores, increase[b] in BMI SDSs, increase[b] in fat percentage, decrease[b] in lean body mass percentage in transfemale adolescents and transmale adolescents	—	Decrease[b] in transmale creatinine levels; no significant change in γ-glutamyl transferase, AST, and ALT; and decrease[b] in ALP in transfemale adolescents and transmale adolescents
Tack et al[30]	Androgenic progestin (lynestrenol)	Decrease[b] in LH, decrease[c] in FSH, estradiol, testosterone and AMH; decrease[b] in SHBG; increase[b] in fT	—	—	Increase[b] in wt and BMI during first 6 mo but back to baseline after 12 mo, no significant changes in total cholesterol and triglyceride levels, no significant change in HbA1c and HOMA, decrease[b] in mean HDL, increase[c] in mean LDL	Metrorrhagia mainly reported in first 6 mo, increase[a] in acne, most common safety profile of headache and hot flashes	Increase[b] in mean Hb and Hct, increase[b] in ALT, increase[c] in creatinine, increase[b] in fT4, no significant changes in AST and thyrotropin
Tack et al[30]	Combination of androgenic progestin (lynestrenol) and GAH (testosterone)	Decrease[b] in LH and FSH, decrease[c] in SHBG, increase[b] in testosterone and fT (reaching levels within male reference ranges), increase[c] in estradiol	Increase[a] in height and wt	—	Increase[b] in wt and BMI; no significant changes in total cholesterol, triglyceride levels, HDL and LDL mean levels, HbA1c, glucose levels, insulin levels, or HOMA index	Few had fatigue; increase[a] in acne and menorrhagia	Increase[b] in mean Hb and Hct levels, increase[b] in ALT and AST (but remained within male reference range), increase[b] in creatinine, decrease[c] in thyrotropin, decrease[b] in fT4

TABLE 2 Continued

Study	Treatment	Outcome					
		Testosterone, Estradiol, and Gonadotropin Levels	Anthropometric Measurements	BMD	Body Composition	Safety Profile	Other Physical Effects
Viot et al[51]	GnRHa	—	Increase[c] in height and wt (significance level not reported)	Transmale adolescents Decrease[b] in bone density in hip for older bone age (actual and *z* scores) Decrease[b] in bone density in lumbar spine for older bone age (actual and *z* scores) Decrease[b] in bone density in lumbar spine for young bone age (*z* scores) Transfemale adolescents Decrease[b] in bone density in lumbar spine for young bone age (*z* scores)	—	—	—
Viot et al[51]	GAH (testosterone and estrogen)	—	Increase[a] in height and wt	Transmale adolescents Increase[b] in bone density in hip and lumbar spine (actual and *z* scores) Transfemale adolescents Increase[b] in bone density in lumbar spine (actual and *z* scores) No significant changes in bone density in hip	—	—	—
Jarin et al[52]	GAH (testosterone)	Increase[c] in total testosterone after 1–3 mo, decrease[c] in estradiol	—	—	Increase[c] in BMI (no results for height and/or wt); no significant changes in LDL, total cholesterol, triglycerides, triglyceride to HDL ratio, and HbA1c; decrease[b] in HDL	—	Increase[b] in Hct and Hb; no significant changes in SUN, creatinine, prolactin, or AST; decrease[c] in ALT after 4–6 mo but returned to baseline

TABLE 2 Continued

Study	Treatment	Outcome					
		Testosterone, Estradiol, and Gonadotropin Levels	Anthropometric Measurements	BMD	Body Composition	Safety Profile	Other Physical Effects
Jarin et al[32]	GAH (estrogen)	Increase[b] in estradiol levels, decrease[b] in testosterone levels	—	—	No significant change in BMI (no results for height and/or wt), no significant changes in LDL, HDL, total cholesterol, triglycerides, and triglyceride to HDL ratio	—	No significant changes in BP (systolic and diastolic); initial decrease in Hct and Hb but returned to baseline; no significant changes in SUN, creatinine, prolactin, AST, or HbA1c; decrease[b] in ALT
Tack et al[22]	Antiandrogen (cyproterone acetate)	No significant changes in LH and FSH, decrease[c] in SHBG, decrease[b] in testosterone, nonsignificant decrease[c] in estradiol and fT, decrease[b] in dehydroepiandrosterone, decreased facial shaving frequency (55.60%). Breast development: Tanner B2 (14.8%) and B3 (14.8%)	Increase[b] in height, decrease[b] in height compared with male peers	—	No clinically important or statistically significant changes in wt and BMI, decrease[b] in triglycerides, no significant changes in total cholesterol, HDL, and LDL	Breast tenderness (7.4%), emotionality (11.10%), fatigue (36%), hot flashes (5.7%)	Increase[b] in prolactin (no clinical galactorrhea); decrease[b] in creatinine, Hb and Hct, but not outside of reference ranges; no significant changes in AST and ALT; no significant change in thyrotropin and fT4
Tack et al[22]	Combination of antiandrogen (cyproterone acetate) and GAH (estrogen treatment)	Decrease[b] in LH, decrease[c] in FSH, increase[b] in SHBG, increase[b] in estradiol, decrease[b] in testosterone and fT, no significant change in dehydroepiandrosterone, decreased shaving need (71.40%). Breast development: Tanner B3 (66.7%) and B4 (9.50%)	Increase[b] in height, decrease[b] in height compared with male peers	—	Breast tenderness (57.1%), emotionality (28.60%), hunger (24%), fatigue (14%), hot flashes (14.3%)	Increase[b] in BMI after 6–12 mo but BMI still less compared with Flemish male peers, increase[c] in wt, no significant changes in LDL, total cholesterol, HDL, and triglyceride levels	No significant changes in Hb and Hct, increase[b] in creatinine after 12 mo, no significant changes in AST and ALT, no significant change in thyrotropin and free thyroxin, decrease[b] in prolactin

Note that transmale adolescents are birth-assigned female individuals who identify as male individuals, whereas transfemale adolescents are birth-assigned male individuals who identify as female individuals. AMH, anti-Mullerian hormone; SUN, serum urea nitrogen; —, not applicable.

[a] Indicates that a *P* value was not calculated.

[b] Indicates significant change ($P < .05$).

[c] Indicates nonsignificant change ($P > .05$).

TABLE 3 Psychosocial Effects of Hormonal Treatments in Transgender Youth

Study	Treatment	Outcome					
		Global Functioning	Depression	Anger and Anxiety	Behavioral and Emotional Problems		GD and Body Image
de Vries et al[27] (de Vries et al[26])[a]	GnRHa, GAH (not assessed)	Increase[b] (increase[c])	Decrease[b]	Decrease[c]	CBCL: decrease[b] in total and internalizing scores, decrease[b] (decrease[c]) in externalizing scores	YSR: decrease[b] in total and internalizing scores, decrease[b] (decrease[c]) in externalizing scores	No significant effect[d]
Costa et al[25]	GnRHa	Increase[e]	—	—	—	—	—

Although influential articles in this field, Cohen-Kettenis and Van Goozen[53] and Smith et al[54] were unable to be included in our study because of their focus on patients after sex reassignment surgery. CBCL, Child Behavior Checklist; YSR, Youth Self Report; —, not applicable.

[a] These 2 studies involved the same cohort and were therefore considered as 1 study. Parentheses are used to indicate the results of the earlier study[26] in which researchers examined a smaller subset of the cohort subsequently examined in the previous study.[27]

[b] Indicates significant change ($P < .05$).

[c] Indicates nonsignificant change ($P > .05$).

[d] It is important to note that the Utrecht Gender Dysphoria Scale that was used to measure GD in this study has various limitations, especially in relation to individuals who have already undergone social transition. Thus, the reported lack of improvement in GD here may reflect a lack of sensitivity in detecting psychological benefits. For example, it has been indicated in clinical experience that GnRHas help to satisfy the desire to prevent development of unwanted secondary sex characteristics (which is a criterion for GD under the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* in young adolescents), but the Utrecht Gender Dysphoria Scale does not have any items that address this issue.

[e] Indicates that a *P* value was not calculated.

TABLE 4 Cognitive Effects of Hormonal Treatments in Transgender Youth

Study	Treatment	Outcome	
		Executive Functioning	Mental Rotation
Staphorsius et al[24]	GnRHa	No significant effect on Tower of London performance scores except for decrease[a] in accuracy in suppressed transfemale adolescents (but this was thought to be chance finding because of small sample size), no significant change in overall global functioning, exaggerated sex-typical brain activation of regions of interest	—
Burke et al[23]	GnRHa, GAH (testosterone treatment)	—	Inferred effect of GnRHa (transmale adolescents) At baseline, showed masculinized mental rotation–associated brain activation Testosterone treatment (transmale adolescents) Increase[b] in performance in mental rotation tasks, similar to control girls; increase[a] in bilateral parietal and left frontal activation

Note that transmale adolescents are birth-assigned female individuals who identify as male individuals, whereas transfemale adolescents are birth-assigned male individuals who identify as female individuals. —, not applicable.

[a] Indicates significant change ($P < .05$).

[b] Indicates that a *P* value was not calculated.

included breast tenderness (57.1%), emotionality (28.6%), hunger (23.8%), fatigue (14.3%), and hot flashes (14.3%).[22]

Testosterone

Few side effects were reported with testosterone treatment, with localized injection reactions (5.6%)[20] and fatigue (8%)[30] all relatively uncommon. However, acne (37.5%) and menorrhagia (25%) were common complaints.[30]

Bone Mineral Density

GnRHas in Transfemale Adolescents

Lumbar spine bone mineral density (BMD) *z* scores decreased after treatment with GnRHa monotherapy,[19,29,31] and this reduction was statistically significant in all[29,31] but 1 study.[19] When results were stratified by bone age, the mean reduction in *z* score was only significant (1.32) for individuals with a bone age <15 years.[31] Absolute lumbar spine BMD did not change over time, and thus the decrease in *z* scores after GnRHas likely reflects a failure to accrue BMD compared with age-matched peers. In 2 studies, researchers also examined BMD at the hip and femoral regions, which

TABLE 5 Risk of Bias for Studies of Effects of Hormonal Treatments in Transgender Youth

Study	Study Participation (Overall)	Study Attrition (Overall)	Outcome Measures (Overall)
Delemarre-van de Waal and Cohen-Kettenis[29]	High	High	Medium
de Vries et al[26,27]	High	High	Medium
Klink et al[19]	Medium	High	Medium
Olson et al[20]	Medium	High	Medium
Costa et al[25]	Medium	Medium	Medium
Staphorsius et al[24]	Medium	High	Medium
Burke et al[23]	Medium	Medium	Medium
Schagen et al[21]	High	High	Medium
Tack et al[30]	High	High	Medium
Vlot et al[31]	Medium	High	Medium
Jarin et al[32]	High	High	Medium
Tack et al[22]	Medium	High	Medium

A modified version of the QUIPS tool was used to assess risk of bias according to 3 domains of bias, with each domain having 3 potential ratings of low, medium, or high.[18] These domains of bias included study participation (study sample adequately represents population of interest), study attrition (available study data adequately represents the study sample), and outcome measurement (outcomes of interest are measured in a similar way for all participants). Use of the QUIPS tool has been described previously.[17]

revealed nonsignificant decreases in absolute and *z* scores for BMD.[19,31] However, the duration of treatment varied significantly in these studies, being unknown in 1 study[31] and at least 1 year[19] and 2 years[29] in the others.

GnRHas in Transmale Adolescents

There was a greater reduction in BMD in transmale adolescents treated with GnRHas than transfemale adolescents. Two studies revealed a significant decrease in absolute and *z* scores for lumbar spine BMD,[19,31] whereas another study revealed a significant reduction in only *z* scores.[29] In 1 study, researchers quantified the reduction in BMD *z* scores as being 0.79 for individuals with a bone age <14 years and 0.56 for individuals with bone ages ≥14 years.[31] Two studies also revealed statistically significant reductions in BMD *z* scores at the hip and femoral regions in transmale adolescents.[19,31]

Estrogen

Estrogen monotherapy was associated with significant increases in both absolute BMD and *z* scores in the lumbar spine,[29,31] but not the hip,[31] of transfemale adolescents previously treated with GnRHas. Furthermore, their *z* scores after 2 years of estrogen were still below that of age- and birth-assigned sex-matched norms.[31] Specifically, *z* scores in the spine were −1.10 and −0.66 in those with younger (<15 years) and older (≥15 years) bone ages, respectively.

Testosterone

Testosterone monotherapy led to a significant increase in both absolute BMD and *z* scores in the lumbar spine[29,31] and hip[31] of transmale adolescents, who had previously been on GnRHas. However, their *z* scores did not reach that of age- and birth-assigned sex-matched controls, aside from the *z* scores in the hip of individuals with older bone ages. Specifically, *z* scores in the spine and hip were −0.15 and −0.37, respectively, in those with younger (<15 years old) bone ages and −0.06 and 0.02, respectively, in those with older (≥15 years old) bone ages.

Growth and Body Composition

GnRHas

Growth velocity decreased during treatment with GnRHas[29] in all transgender youth compared with pubertal-matched peers.[21] In particular, younger individuals, who had greater growth potential, had significantly lower height standard deviation scores (SDSs) after treatment.[21,29] One study revealed significantly lower height standardized values for transfemale adolescents only.[19] No researchers have examined whether individuals given GnRHas achieved their predicted final height after GAHs. After 1 year on GnRHas, individuals had a significant increase in body fat percentage[29] and BMI,[19,21] which was accompanied by a decrease in lean body mass.[29]

Progestins

Lynestrenol resulted in significant increases in weight and BMI absolute and *z* scores during the first 6 months with a return to baseline after 12 months of treatment.[30]

Antiandrogens

Cyproterone acetate resulted in a decrease in growth velocity compared with age-matched peers,[22] with a final height after 12 months of treatment also being significantly lower than age-matched peers with a mean standardized score of −0.309. There were no clinically significant changes in body weight and BMI after 12 months.

Estrogen

It is unclear whether differences in the pubertal stage and bone age at which estrogen was commenced contributed to the variable outcomes found because these data were not collected.[29] Estrogen in combination with cyproterone acetate resulted in reduced growth compared with age-matched peers in 1 study,[22] whereas another study revealed no change in growth velocity after estrogen.[29] Total BMI was significantly increased after estrogen in 1 study,[22,32] although another revealed that total BMI did not change after 6 months.[32]

Testosterone

Testosterone monotherapy resulted in increased growth velocity compared with age-matched peers in 1 study,[29] but the impact on final

height (nor the age and pubertal stage at commencement) was not specified. Testosterone was also associated with weight gain,[30] resulting in a significantly raised absolute BMI[20,30] from an average baseline of 20.7 to 22.4 within 6 months.[20] This increase in BMI was less than that of age-matched male adolescents.[32]

Other Physical Effects

GnRHas

After 1 year of GnRHa, there were no changes in carbohydrate or lipid metabolism as measured by fasting glucose, insulin, cholesterol, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) levels.[29] In 1 study, researchers observed that alkaline phosphatase (ALP) was decreased as a likely secondary result of decreased bone turnover, whereas all other liver enzymes were unchanged.[21] In this same study, researchers also reported lower levels of creatinine and hypothesized that this might be due to reduced muscle mass but found that there was no correlation between change in muscle mass and creatinine.[21]

Progestin

Progestins were associated with an adverse lipid profile, with a significant decrease in HDL cholesterol by an average of 0.46 mmol/L and an elevation of LDL cholesterol by 0.37 mmol/L after 1 year.[30] There were no significant changes in hemoglobin A1c (HbA1c), glucose levels, insulin levels, or homeostasis model assessment (HOMA) index.[30] Alanine aminotransferase (ALT) increased after 12 months, but this was not clinically significant.[30] Mean hemoglobin (Hb) and hematocrit (Hct) levels increased in the first 6 months and subsequently remained stable.[30]

Antiandrogens

Cyproterone acetate was associated with a significant reduction in only triglycerides, but total cholesterol, LDL cholesterol, HDL cholesterol, HbA1c, glucose, insulin, and HOMA index were unaffected.[22] There was no change in liver enzymes or thyrotropin.[22] There was a slight decrease in Hb and Hct after 12 months, but this was not clinically significant.[22]

Estrogen

Apart from 1 study in which a significant decrease in HDL after 4 to 6 months was observed,[32] estrogen had no effect on carbohydrate and lipid metabolism.[22,29,32] Similarly, no significant changes in liver enzymes,[22,32] thyrotropin, or free thyroxine (fT4)[22] were noted with estrogen treatment. A significant increase in serum creatinine was seen after 12 months with combined estrogen and cyproterone acetate treatment.[22] Hb and Hct were found to have decreased initially but returned to baseline after approximately 6—12 months.[32] However, when estrogen was used in combination with a progestin, Hb and Hct levels did not change any further after 12 months.[22] Blood pressure (BP) was unchanged after 6 months of estrogen.[32]

Testosterone

Testosterone had no significant effect on carbohydrate and lipid metabolism.[29,30,32] Although in 1 study researchers observed raised liver enzymes (aspartate aminotransferase [AST] and ALT) after a year,[30] another study revealed no significant change in AST and a decrease in ALT after 4 to 6 months.[32] Testosterone treatment decreased thyrotropin and fT4 to be outside of the normal reference ranges, although these changes were not clinically relevant because there was no clinical or biochemical hypo- or hyperthyroidism in participants.[30]

Serum creatinine increased after 6 months of testosterone with no subsequent change thereafter and was thought to reflect an increase in muscle mass.[30] Hb[20] and Hct[30] were increased after 6 months but remained stable during the next 6 months within the male reference ranges,[20,30] whereas another study revealed no significant change in these parameters at any stage.[32] Systolic BP was elevated in treated individuals, with an average rise of 5 mm Hg after 6 months.[20]

PSYCHOSOCIAL EFFECTS

All relevant results are shown in Table 3.

GnRHas

GnRHa treatment was associated with significant improvements in multiple psychological measures, including global functioning,[25–27] depression,[26,27] and overall behavioral and/or emotional problems.[26,27] The effects of GnRHas on anger and anxiety remain unclear with conflicting results.[26,27] Moreover, GnRHa treatment had no significant effect on symptoms of GD,[26,27] with researchers in 1 study observing a nonsignificant increase in GD and body image difficulties.[26]

Progestin, Antiandrogens, Estrogen, and Testosterone

Critically, no researchers have examined the psychosocial effects of these hormonal therapy types in transgender youth.

COGNITIVE EFFECTS

All relevant results are shown in Table 4.

GnRHas

In one study, researchers examined the effect of GnRHas on executive functioning using the Tower of London test, which is used to assess mental planning ability.[24]

After GnRHa treatment, there was significantly reduced accuracy in transfemale adolescents.[24] There was also exaggerated regional brain activation typical of birth-assigned sex on functional magnetic resonance imaging (fMRI).[24] However, given the small sample size (8 participants), these results should be interpreted cautiously.

In another study, researchers examined the effect of GnRHa treatment on mental rotation in transmale adolescents,[23] exploring whether rotated pairs of three-dimensional shapes were identical images of each other.[35,36] Because men significantly perform better on this task compared with women, this result has also previously been suggested as evidence for the classic theory of the organizational and activational effects of sex hormones on the brain.[37,38] Interestingly, GnRHa suppression in transmale adolescents was associated with male brain activation patterns, with reduced activity in the right frontal area.[23]

Progestin, Antiandrogens, and Estrogen

No researchers have examined the cognitive effects of these treatments.

Testosterone

In the same study, researchers also examined the effects of testosterone in transmale adolescents on mental rotation tasks, in which they observed moderate to strong improvements in accuracy and reaction time.[23] Similar to control boys, treated transmale adolescents also demonstrated increased activation of brain regions implicated in mental rotation on fMRI.[23]

DISCUSSION

This is the first systematic review of the effects of hormonal treatment in transgender youth; authors of previous systematic reviews in this field, including those commissioned by the recent Endocrine Society Clinical Practice Guidelines, focused on the use of GAHs in adults.[8–11,15]

GnRHas successfully suppressed endogenous puberty, consistent with the primary objective of this treatment, although there was only a single study in which researchers actually recorded these data.[29] GnRHas were observed to be associated with significant improvements in global functioning,[25–27] depression,[26,27] and overall behavioral and/or emotional problems[26,27] but had no significant effect on symptoms of GD. The latter is probably not surprising, because GnRHas cannot be expected to lessen the dislike of existing physical sex characteristics associated with an individual's birth-assigned sex nor satisfy their desire for the physical sex characteristics of their preferred gender. Like GnRHas, the antiandrogen cyproterone acetate effectively suppressed testosterone in transfemale adolescents,[22] but its potential psychosocial benefits remain unclear. Meanwhile, GAHs increased estrogen and testosterone levels and thus induced feminization and masculinization, respectively, of secondary sex characteristics.[22,29] However, in the case of breast development, the outcomes were subjectively less in size than expected in the majority of recipients,[22] and the potential psychosocial benefits of GAHs remain unknown. Finally, although the use of the progestin (lynestrenol) has been studied in transmale adolescents,[30] its effects were predominantly examined in the context of potential adverse effects, so the therapeutic impact of progestins for menses suppression and psychosocial outcomes cannot be understood from the current literature.

Overall, hormonal treatments for transgender youth were observed to be relatively safe but not without potential adverse effects. For GnRHas, a significant concern in clinical practice is their potential effects on BMD accrual; their use was associated with a significant reduction in BMD,[19,29,31] which appeared to be worse for transmale adolescents[19,31] and is consistent with previous studies of nontransgender youth[39,40] and adults[41] who received GnRHas. However, given the relatively short follow-up duration of the studies reviewed here, it will be important for future researchers to better establish if this reduction in bone density is long-lasting or transient, as observed in nontransgender youth after GnRHa cessation.[39,40] It is notable that BMD increased after estrogen and testosterone, which suggests potential compensation by GAHs. However, for estrogen treatment, the BMD of those who had previously received GnRHas still remained lower than age-matched peers 2 years after estrogen treatment,[31] so compensation may only be partial. Furthermore, there is a lack of reporting of pubertal stage at treatment commencement, which makes interpretation of some changes difficult, especially BMD.

Clinically, patients who receive GnRHas and still have significant growth potential are counseled about the risk of the treatment affecting their final height. Although researchers in 2 studies have now examined growth and height characteristics in transgender youth receiving GnRHas,[21,29] their relatively short follow-up times (≤3 years) precluded determination of the effects of GnRHas on final height, and future researchers should address this knowledge gap. Another clinical concern in the use of GnRHas is the induction of menopausal-like symptoms due to the withdrawal of sex steroids, especially in postpubertal individuals. GnRHas were commonly observed to cause hot flashes in transmale adolescents in late puberty, but these decreased in frequency over time.[29] For

potentially similar reasons, one of the main complaints after cyproterone acetate administration in transmale adolescents was fatigue.

Hormonal treatment of transgender adults is known to be associated with various metabolic and cardiovascular effects.[10–12] GnRHas significantly increased both body fat percentage[29] and BMI[19,21] while decreasing lean body mass.[29] Similarly, testosterone significantly increased both body fat and BMI.[20,29] Although lynestrenol also increased BMI, this was transient, with BMI returning to baseline after 12 months.[30] Cyproterone acetate was not associated with any changes in BMI.[22] In terms of lipid metabolism, neither testosterone nor estrogen had any observable impact, but lynestrenol was associated with lower HDL and higher LDL cholesterol after 1 year,[30] whereas cyproterone acetate significantly reduced triglycerides.[22]

The findings from this review are subject to limitations. Firstly, the current literature has a limited number of studies in which the different hormonal treatments in transgender youth is examined. Secondly, for any given class of hormonal treatments, there is a variety of different agents, formulations, and administration routes that are being used clinically in transgender youth. For example, the physical effects of 1 antiandrogen and 1 progestin have been studied in only 1 study each, with no confirmation of results or further exploration. Thirdly, in existing studies there is a medium to high risk of bias, given small sample sizes, retrospective nature, and lack of long-term follow-up. In this regard, although randomized controlled trials are often considered gold standard evidence for judging clinical interventions, it should be noted that, in the context of GD in which current guidelines highlight the important role of hormonal treatments,[15] conducting such trials would raise significant ethical and feasibility concerns. Fourthly, authors of existing studies have neglected several key outcomes. These include the following: psychological symptoms related to GD, which is a critical knowledge gap given the high rates of mental health problems observed in transgender youth and justification of these treatments as treating GD[42]; the impact of hormonal treatments on fertility, which is an integral part of the counseling recommended by current guidelines[15]; and potential adverse effects such as arterial hypertension, which was reported in a recent case series in association with GnRHas.[43] Finally, there are no known studies to date in which researchers have reported the rates and circumstances under which transgender youth cease their hormonal therapy in an unplanned manner or the risk of subsequent regret, which would be of great clinical utility.

Notwithstanding these limitations, collectively, the studies reviewed provide qualified support for the use of GnRHas, GAHs, cyproterone acetate and, to a lesser extent, lynestrenol in transgender youth. Overall, these hormonal treatments appear to provide some therapeutic benefits in terms of physical effects and are generally well-tolerated on the basis of current evidence.

CONCLUSIONS

Looking ahead, it will be essential for future researchers to reassess and expand on the findings of the existing studies. Large, prospective longitudinal studies, such as have been recently initiated,[44] with sufficient follow-up time and statistical power and the inclusion of well-matched controls will be important, as will the inclusion of outcome measures that investigate beyond the physical manifestations.

ABBREVIATIONS

ALT: alanine aminotransferase
AST: aspartate aminotransferase
BMD: bone mineral density
BP: blood pressure
fMRI: functional magnetic resonance imaging
FSH: follicle-stimulating hormone
fT: free testosterone
fT4: free thyroxine
GAH: gender-affirming hormone
GD: gender dysphoria
GID: gender identity disorder
GnRHa: gonadotropin-releasing hormone analog
Hb: hemoglobin
HbA1c: hemoglobin A1c
Hct: hematocrit
HDL: high-density lipoprotein
HOMA: homeostasis model assessment
LDL: low-density lipoprotein
LH: luteinizing hormone
QUIPS: Quality in Prognosis Studies
SDS: standard deviation score
SHBG: sex-hormone binding globulin

Ms Chew screened studies for inclusion and exclusion, conducted the data extraction, conducted the analyses, drafted the initial manuscript, and revised the manuscript; Dr May screened studies for inclusion and exclusion, conceptualized and designed the study, and reviewed and revised the manuscript; Dr Anderson conducted the data extraction, conducted the analyses, and revised the manuscript; Prof Williams reviewed and revised the protocol and manuscript; Dr Pang conceptualized and designed the study and reviewed and revised the manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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ORIGINAL ARTICLE

Consensus Parameter: Research Methodologies to Evaluate Neurodevelopmental Effects of Pubertal Suppression in Transgender Youth

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Abstract

Purpose: Pubertal suppression is standard of care for early pubertal transgender youth to prevent the development of undesired and distressing secondary sex characteristics incongruent with gender identity. Preliminary evidence suggests pubertal suppression improves mental health functioning. Given the widespread changes in brain and cognition that occur during puberty, a critical question is whether this treatment impacts neurodevelopment.

Methods: A Delphi consensus procedure engaged 24 international experts in neurodevelopment, gender development, puberty/adolescence, neuroendocrinology, and statistics/psychometrics to identify priority research methodologies to address the empirical question: is pubertal suppression treatment associated with real-world neurocognitive sequelae? Recommended study approaches reaching 80% consensus were included in the consensus parameter.

Results: The Delphi procedure identified 160 initial expert recommendations, 44 of which ultimately achieved consensus. Consensus study design elements include the following: a minimum of three measurement time points, pubertal staging at baseline, statistical modeling of sex in analyses, use of analytic approaches that account for heterogeneity, and use of multiple comparison groups to minimize the limitations of any one group. Consensus study comparison groups include untreated transgender youth matched on pubertal stage, cisgender (i.e., gender congruent) youth matched on pubertal stage, and an independent sample from a large-scale youth development database. The consensus domains for assessment includes: mental health, executive function/cognitive control, and social awareness/functioning.

Conclusion: An international interdisciplinary team of experts achieved consensus around primary methods and domains for assessing neurodevelopmental effects (i.e., benefits and/or difficulties) of pubertal suppression treatment in transgender youth.

Keywords: expert consensus; Delphi; puberty blockers; GnRHa; transgender; adolescents

Introduction

Standards of care established by the World Professional Association for Transgender Health[1] and the Endocrine Society[2] recommend pubertal suppression for gender dysphoric transgender youth during early puberty (i.e., Tanner stages 2–3).[3,4] Pubertal suppression is achieved through administration of gonadotropin-releasing hormone agonists (GnRHa). When administered in early puberty, GnRHa suppress endogenous sex hormone production and prevent the development of undesired and irreversible secondary sex characteristics, thereby minimizing distress associated with pubertal development incongruent with gender identity.[5] For youth who later decide to initiate estrogen/testosterone (gender-affirming hormones [GAH]) treatment to induce development of the desired secondary sex characteristics, pubertal suppression may minimize the need for more invasive, surgical interventions (e.g., facial and chest surgery). For youth who decide not to pursue GAH treatment, discontinuing GnRHa will reactivate the hypothalamic-pituitary-gonadal axis and endogenous puberty will resume.[6]

Three longitudinal studies have examined psychosocial outcomes in GnRHa-treated transgender youth; two (conducted by the same research group) followed a single cohort over time, immediately before initiating GAH ($N=70$)[7] and later in early adulthood after surgery for gender affirmation ($N=55$).[8] The third study compared groups of GnRHa-treated ($n=35$) and untreated ($n=36$) youth longitudinally.[9] Findings across these studies include significant reductions in depressive symptoms and improvement in overall psychosocial functioning in GnRHa-treated transgender youth. A fourth cross-sectional study compared adolescents diagnosed with gender dysphoria (GD), who were treated with GnRHa and close to starting GAH treatment ($n=178$), adolescents newly referred for GD evaluation ($n=272$), and cisgender adolescents recruited from the general population ($n=651$) on self-reported internalizing/externalizing problems, self-harm/suicidality, and peer relationships.[10] Before medical treatment, clinic-referred adolescents reported more internalizing problems and self-harm/suicidality and poorer peer relationships compared to age-equivalent peers. GnRHa-treated transgender adolescents had fewer emotional and behavioral problems than clinic-referred, untreated adolescents and had comparable or better psychosocial functioning than same-age

cisgender peers. In addition to studies of youth, the 2015 U.S. Transgender Survey included questions about past gender-affirming medical treatment, including pubertal suppression. These questions were asked retrospectively and linked to reported current and lifetime mental health.[11] Individuals who received pubertal suppression treatment ($n = 89$), when compared to those who wanted pubertal suppression, but did not receive it ($n = 3405$), had lower odds of endorsing lifetime suicidal ideation on the survey. Given these five studies and the presumed reversibility of GnRHa treatment, pubertal suppression is increasingly offered to early pubertal transgender youth. It is important to note that there has been only one longitudinal report of adult outcomes,[8] and questions remain regarding the potential for both positive *and* disruptive effects of pubertal suppression on neurodevelopment.[12–14]

The pubertal and adolescent period is associated with profound neurodevelopment, including trajectories of increasing capacities for abstraction and logical thinking,[15] integrative thinking (e.g., consideration of multiple perspectives),[16,17] and social thinking and competence.[18,19] During this period, there is a developmental shift toward greater exploration and novelty seeking,[20,21] salience of peer perspectives and interactions,[22] and accelerated development of passions/interests and identities.[23] These developments lay the groundwork for adult functioning.[18,24] At the level of the brain, several primary neurodevelopmental processes unfold during adolescence, including myelin development[25] and changes in neural connectivity[26]; synaptic pruning[27] and gray matter maturation[28,29]; changes in functional connectivity[30]; and maturation of the prefrontal cortex[31] and the "social brain" network.[19] Adolescent neurodevelopmental processes underlie mental health risks, resilience, and outcomes.[32,33]

Considerable research has addressed the effects of puberty-related hormones on neurodevelopment, including hormone manipulation studies in nonhuman animals and observational studies in humans. Animal studies demonstrate pubertal hormones exert broad neuronal influence, including effects on neurogenesis, differentiation, apoptosis, dendritic branching, spine density, and regional gray and white matter volumes.[30,34] Androgen and estrogen receptors are found in high density within the hypothalamus and amygdala, and are also present in the hippocampus, midbrain, cerebellum, and cerebral cortex of the rodent and monkey.[35–37] This widespread receptor distribution in rodents may explain the diverse effects of pubertal hormones on both reproductive and nonreproductive behaviors, including anxiety, scent-marking, and food guarding.[34] In human studies, pubertal progression has been linked to developmental changes in reward,[38] social,[39] and emotional processing[40] as well as cognitive/emotional control.[41] However, consensus regarding pubertal impacts at the neural level—such as puberty-associated changes observed in magnetic resonance imaging (MRI) measures—has been more difficult to achieve.[42] Distinct puberty-related neurodevelopmental trajectories have been differentiated by sex.[43]

The combination of animal neurobehavioral research and human behavior studies supports the notion that puberty may be a *sensitive* period for brain organization:[44–46] that is, a limited phase when developing neural connections are uniquely shaped by hormonal and experiential factors, with potentially lifelong consequences for cognitive and emotional health. Studies have linked early life adversity to early puberty onset[47] and early puberty onset to poorer mental health.[48] There is also some evidence to suggest that delayed puberty onset predicts slightly poorer adult functional outcomes.[49] Taken as a whole, the existing knowledge about puberty and the brain raises the possibility that suppressing sex hormone production during this period could alter neurodevelopment in complex ways—not all of which may be beneficial.

Two small studies have assessed impacts of pubertal suppression on neural and cognitive functioning in peripubertal transgender youth. Staphorsius et al. compared brain and behavioral responses of GnRHa-treated (8 transgender girls [birth-assigned male] and 12 transgender boys [birth-assigned female]) and untreated transgender youth (10 of each sex) during an executive function task.[50] No group differences were found in task load-related brain activation; GnRHa-treated transgender girls demonstrated poorer performance compared with untreated transgender boys and cisgender controls. Schneider et al. evaluated a single pubertal transgender girl undergoing GnRHa with MRI scans of white matter and cognitive assessments at baseline (before GnRHa initiation) and at 22 and 28 months of pubertal suppression treatment.[51] During follow-up, white matter fractional anisotropy (i.e., a measure of axonal diameter, fiber coherence, and myelination) did not increase in the manner otherwise expected during puberty. By 22 months of pubertal suppression treatment, working memory scores dropped by more than half a standard deviation.

Larger-scale, longitudinal studies are required to understand possible neurodevelopmental impacts of pubertal suppression over time in transgender youth. Suppressing puberty may reduce dysphoria and diminish risks for poor mental health in this population, thereby exerting neuroprotective effects. If pubertal suppression disrupts aspects of neurodevelopment, it is possible these effects are temporary, with youth "catching up" developmentally after transitioning to GAH treatment or discontinuing GnRHa. However, pubertal suppression may prevent key aspects of development during a sensitive period of brain organization. Neurodevelopmental impacts might emerge over time, akin to the "late effects" cognitive findings associated with certain oncology treatments.[52] In sum, GnRHa treatment might produce a myriad of *varied* impacts, both positive and disruptive.

The goal of this study was to develop a framework in which these questions could be asked, and ultimately answered. We identify priority research methodologies that can be used to address the empirical question of how pubertal suppression in transgender youth may affect neurodevelopment and real-world functioning. Given the complexity of neural development during the pubertal period and the novelty of developmental research with transgender youth, this study employed a Delphi consensus method to leverage international expertise in neurodevelopment, gender development, puberty/adolescence, neuroendocrinology, and statistics/psychometrics. By engaging a community of experts in an iterative consensus-building procedure, this study aimed to advance thinking about efficacious designs by moving beyond individual research efforts and single-discipline approaches.

Methods

The Delphi procedure is a reliable iterative research method for establishing expert agreement,[53,54] and has been used extensively to address health-related questions, particularly in emerging fields of clinical care.[55–57] In the first round of a two-round Delphi procedure, a key question is presented to experts, who remain anonymous to one another throughout the Delphi process. Each expert provides responses/solutions to the question, which are then combined and organized by the study team. In the Delphi round two, experts rate each proposed statement/solution according to the level of agreement. Responses reaching the *a priori* consensus criterion are included as consensus statements. Given its anonymous iterative nature, the Delphi method avoids problems of typical expert work groups (e.g., adhering to the perspectives of more senior workgroup experts, inflexibly defending ideas) and allows for interaction among larger groups of experts from diverse locations and disciplines through asynchronous communication.[58–60]

We employed a two-round Delphi procedure to obtain expert consensus regarding the most efficacious research design elements to address the following research question: *What, if any, real-world impact does pubertal suppression have on transgender children's cognitive and neural development?* International experts in relevant research fields were identified and invited as follows:

1. An independent advisory panel consisting of five experts across key disciplines (see Acknowledgments section) was formed to identify international experts who, based on knowledge and experience, could best propose a research design to assess neurodevelopmental impacts of pubertal suppression in transgender youth.
2. Thirty-two recommended experts were vetted for their expertise; all met required criteria (i.e., a minimum of 10 first-author publications in relevant fields).
3. These experts were invited to participate in the Delphi procedure and were informed they would be invited to consider being a co-author of the resulting article. Twenty-eight experts responded: 20 agreed to participate, 4 declined due to lack of time, and 4 declined due to self-reported lack of expertise in this research area. Snowball sampling identified an additional 16 recommended experts, who were vetted (as described above) for their experience. Eight met criteria and were invited. Five of these experts participated, yielding a total of 25 experts agreeing to participate, 24 of whom completed the Delphi process. See Table 1 for academic institution locations and areas of expertise represented in the expert panel.

The Ann & Robert H. Lurie Children's Hospital of Chicago Institutional Review Board found that an expert Delphi consensus initiative did not require informed consent since the experts were direct partners in the research product. The first round of Delphi survey was distributed through the REDCap online survey platform and presented an overview of the research question with the following prompt for

Table 1. Institutional Representation and Self-Reported Areas of Expertise

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Location of academic institution	
United States	16
The Netherlands	3
Belgium	2
Canada	1
Norway	1
Sweden	1
Self-endorsed areas of expertise[a]	
Brain development	13
Adolescent development	12
Neuroendocrinology	11
Neuroimaging	11
Neuropsychology	8
Cognitive development	7
Developmental assessment	4
Expert in GnRHa	2
Other (write in)	4
Developmental social neuroscience	1
Transgender health	1
Genetics of sex chromosomes	1
Gender development	1

[a]Experts endorsed as many areas of expertise as applicable.
GnRHa, gonadotropin-releasing hormone agonists.

respondents: "What methods and tools should we use to identify clinically meaningful neurodevelopmental impacts of pubertal suppression? What type of longitudinal design and follow-ups are both practical and appropriate? What comparison groups might we consider?" This initial process yielded 131 distinct research design considerations; multiple descriptions of the same concept were collapsed into single statements. In the second Delphi round, each first-round research design consideration was presented back to the experts and rated as follows: a priority idea/approach or not a priority idea/approach. Experts could also select, "cannot rate due to lack of expertise." The first Delphi round also yielded lists of potential comparison groups and assessment domains (29 items). In the second Delphi round, participants were asked to rank order these items according to priority. For the priority rankings of comparison groups, the top-rated comparison group by each expert was given a value of 2 and the second rated comparison group was given a value of 1. A mean was calculated for each comparison group option based on these values and these mean scores were used to identify the overall priority rankings. For the list of priority domains to measure, a parallel approach was taken with the top 6 domains ranked by each expert.

All experts participated in the second Delphi round. Twenty-two of the Delphi experts participated in the construction of the resulting article and are co-authors listed in reverse alphabetical order by last name (authors 5–26). The Results section contains the exact statements endorsed as a "priority" approach by 80% or more of the Delphi panel.

Results

Four of the 131 individually presented statements were excluded from analyses because fewer than 15 experts rated them. Of the remaining 127 statements, 44 met the 80% or higher criterion for consensus and inclusion (see Table 2 for endorsement rates by statement). The average endorsement rate of included statements was 89.4%.

Consensus parameter

Study design considerations. A multicenter design with more than a single clinic will be necessary to recruit a sufficient sample size, as the effect size will likely be small. Meaningful effect sizes must be determined to ensure sufficient recruitment to power multiple expected comparisons accounting for attrition in a longitudinal design. Three time points of measurement are the absolute minimum. It will be necessary to manage the effects of repeated testing with a particular focus on minimizing the practice effects of a longitudinal design with multiple time points. For cognitive assessments, standardized batteries should be employed as: (1) there may be a larger database of norms available that the cohort could be compared to, in addition to a local comparison (control) group(s), (2) general composite scores within test batteries tend to provide more reliable and stable scores than individual tests, and (3) tasks within a category may be swapped in case of worries for learning effects. In any study of cognitive change based on serial assessments, reliability of measures is paramount (the consensus in the field is that tests should have a minimum test-retest reliability of >0.70). It may be pragmatic to use measures and methods from large representative studies, such as the Adolescent Brain Cognitive Development (ABCD) Study.

All processes being studied (e.g., gender identity, mental health, neural structure, and function) display considerable heterogeneity, and methods that fail to capture this will provide distorted findings and lead to biased clinical recommendations. Analyses based on group means (e.g., regression or ANOVAs) are unlikely to generalize to all individuals being treated. Therefore, it is necessary to collect enough data per person to characterize individual trajectories of change over time.

Table 2. Consensus Priority Recommendations Ordered by Consensus Ratings Within Categories

Study design considerations		
1	It would be helpful to follow these youth through and beyond initiation of cross-sex hormone treatment. Some aspects of human adolescent brain development are more related to pubertal hormone status than age *per se*, and to the extent that pubertal suppression may also put some features of brain development on hold; it would be good to know whether these features "catch up" once cross-sex hormone treatment has begun or whether a sensitive window for hormone-dependent brain development has closed.	22/22
2	Follow cohort after GnRHa treatment ends—collect data after the youth transition to GAH (when they complete their GnRHa treatment).	22/23
3	Any neurocognitive effect of GnRHa pubertal suppression may be complicated by the psychosocial and affective aspects of the transgender experience. This means that you would have to include multivariate models of both cognitive and psychosocial functioning.	22/23
4	Need to determine meaningful effect sizes and ensure sufficient statistical power for multiple expected comparisons with attrition.	21/22
5	Across the course of the study, three assessment points is the absolute minimum.	20/21
6	Need to use a multicenter design (not just one clinic).	21/23
7	Effects of GnRHa may not appear for several years. Any difference in brain structure due to GnRHa is likely to be seen over time (long term), rather than immediately.	20/22
8	Social and affective learning process may be affected by pausing puberty. These social and affective learning processes might cause subtle short-term differences that could ultimately cause clinically impactful and meaningful longer-term effects.	17/19
9	Of particular interest would be to also monitor the impact of hormonal therapy. One could then ask, "Does the trajectory change in response to cross-sex hormonal therapy or do they stay on the same trajectory as when they were on GnRHa?"	16/18
10	Assess target and comparison groups before puberty.	20/23
11	Need to manage the effects of repeated testing (i.e., minimize the practice effect of a longitudinal design with multiple time points).	19/22
12	The effect size will likely be small—therefore, you would need a large sample size.	19/23
13	The research design will need to account for the differences between youth who are assumed male versus assumed female as biological sex is differentially related to rate and pattern of cognitive development, connectome distinctiveness, and timing of peak brain volume.	19/23
14	All processes being studied (e.g., gender identity, mental health, and neural structure and function) display huge amounts of heterogeneity, and research methods that fail to capture this will provide distorted findings and lead to biased clinical recommendations. Analyses based on mean levels of these processes are unlikely to generalize to all individuals being treated (e.g., regressions or ANOVAs that compare groups with a slew of covariates). It is, therefore, necessary that enough data are collected per person to capture personalized trajectories of change across time. And the data need to be modeled in ways that reflect the heterogeneity of individual characteristics and trajectories.	18/22
Comparison groups and recruitment		
15	At least one control group should be cisgender participants as this area of research (i.e., hormones and the adolescent brain) is still rather new and more data are needed on all youth during this stage.	20/22
16	Critical to match the groups carefully to allow for evaluation of the effects of repeated testing (practice effects).	20/22
17	Comparison groups should be matched for pubertal stage.	19/21
18	Recruit all gender dysphoric youth across the pubertal age range, including those who are treated with GnRHa and those who are not.	18/21
19	This is not dissimilar from issues of discerning differences in cognitive trajectories in normal aging versus neurodegenerative disorders. The basic question involves cognitive growth curves among cisgender and transgender children overtime. There have been large-scale large-sample studies that have produced trajectories of brain development during the pre-pubertal, pubertal, and adolescent periods that could treated like a "brain growth curve."	15/18
20	Need more than one comparison group to minimize the limitations of any one comparison group (no single comparison group is ideal).	18/22
Pubertal staging/measurement		
21	Measure gonadal hormone levels.	23/23
22	Collect information on menstrual cycle and contraceptive use for female adolescents involved in the study.	23/23
23	Measure Tanner staging (i.e., secondary sex characteristics).	21/23
24	Measure height/weight.	18/22
Domains to measure		
25	Use white matter microstructure scans (diffusion tensor imaging)—and use a longitudinal imaging pipeline (which exists) for processing these data with scientific rigor.	15/15
26	A pragmatic methodological implication is to consider: (1) not only relying solely on measures of performance and behavior but also measures of learning and motivation, and (2) not only relying solely on measures of cognitive capacities but also on social, affective, and value-based learning processes.	19/20
27	If MRI is included, consider imaging approaches focused on the following domains: social-emotional processing, executive functioning, risk and reward processing, and self-concept.	20/22
28	Studies in laboratory rodents show that testosterone, acting during puberty, programs the ability to adapt behavior as a function of social experience—therefore, include instruments that evaluate social proficiency.	19/21
29	Use diffusion tensor imaging to analyze white matter at the microstructural level.	17/19

(*continued*)

Table 2. (Continued)

	Study design considerations	
30	Studies in laboratory rodents show that ovarian hormones, acting during puberty, program cognitive flexibility by exerting long-lasting effects on excitatory-inhibitory balance in prefrontal cortex—so include instruments that evaluate behavioral flexibility.	18/21
31	Examine white matter development, which is important for processing speed.	17/20
32	Important to measure emotional functioning because it is bidirectionally related to executive functioning.	16/19
33	Look at white matter characteristics since they seem to develop during puberty under the influence of sex hormones.	15/18
34	One cannot study everything or study everything well. It will be critical to identify the priorities in such a study, as there is a danger of doing too much here. Consider the outcomes that matter most and the hypothesized mediating mechanisms. Focus on the outcomes of interest.	19/23
35	There is no clear evidence that progressing through puberty later than peers is associated with delayed maturation of abstract reasoning, executive function, and social capacities.	18/22
36	Use structural MRI (T1/T2)—and use a longitudinal imaging pipeline (which exists)—for processing these data with scientific rigor.	13/16
37	There is an emerging shift in thinking about the increase in reward sensitivity and sensation-seeking during puberty as related to social value learning. Dopamine release is not primarily a "reward" signal, but rather a learning signal (e.g., prediction error signal)—the natural increased salience of social learning (status, prestige, being admired, respected, liked, etc.) These pubertal changes may have small effects on immediate behavior, yet that could contribute to changes in patterns of behavior over time, which could lead to large individual differences in developmental trajectories for people, such as if they had blocked puberty.	13/16
	Measurement approaches	
38	In any study of cognitive change based on serial assessments, reliability of the measure is paramount. The consensus in the field is that tests should have a minimum test-retest reliability of >0.70.	20/20
39	Behavioral measurements should include standardized measures appropriate for repeated assessment with high test-retest reliability.	21/22
40	Match acquisition parameters between imaging sites.	17/18
41	Consider implementing measures and methods from large representative protocols, such as the ABCD.	17/18
42	Neuroimaging should parallel the behavioral study—neural measures should be linked to neurocognitive and behavioral measures.	19/22
43	For cognitive assessment, use a standardized battery for two reasons: (1) there might be a larger database of norms available that the cohort could be compared to, in addition to the likely to be small comparison ("control") group, and (2) tasks within a category may be swapped in case of worries for learning effects.	18/21
44	Use "test batteries" that provide a general composite score as well as specific composites. By virtue of being composites, scores tend to be more reliable and stable than individual test scores.	17/20

The proportion represents the number of experts endorsing an item as a "priority" out of the total number of experts who rated the item as "priority" or "not priority." The denominator represents the number of experts rating an item as a "priority" or "not priority" (as opposed to "cannot rate due to lack of expertise" or skipping the item).

ABCD, Adolescent Brain Cognitive Development Study; GAH, gender-affirming hormones; MRI, magnetic resonance imaging.

Any GnRHa-induced neurocognitive effect may be complicated by psychosocial and affective aspects of the transgender experience. Therefore, multivariate models of both cognitive and psychosocial functioning should be included. Accounting for differences between birth-assigned male youth versus birth-assigned female youth is important, as sex is differentially related to the rate and pattern of cognitive development, connectome distinctiveness, and timing of peak brain volume. Assessments should begin before puberty in both treatment and comparison groups. The effects of pubertal suppression may not appear for several years. Any GnRHa-related difference in brain structure is likely to be observed over the long term, rather than immediately. Shifts in social and affective learning processes might cause subtle short-term differences that could ultimately result in clinically impactful longer-term effects. Therefore, studies should follow GnRHa-treated youth over time, including the time period after GnRHa treatment ends and/or when GAH commence. Some aspects of human adolescent brain development are more related to pubertal hormone status than age *per se*. To the extent that pubertal suppression may also put some features of brain development on hold, it is critical to know whether these features "catch up" (either once GAH treatment is initiated or if the adolescent elects to stop GnRHa and resume endogenous puberty), or whether a sensitive window for hormone-dependent brain development has closed. One way to measure this is to assess whether neurodevelopment shifts in response to initiating GAH following pubertal suppression: Do GnRHa-treated youth stay on the same neurodevelopmental trajectory as when puberty was suspended or does this trajectory change?

Comparison groups. To assess neurodevelopmental trajectories associated with GnRHa treatment, more than one comparison group is needed to minimize the limitations of any one comparison group. No single comparison group is ideal for this study question.

A rank order of possible comparison groups is provided in Table 3. Groups should also be well matched, given the effects of a repeated testing design (e.g., practice effects). Matching for pubertal/developmental stage will be critical, including Tanner staging, gonadal hormone levels, height and weight, and, among youth assigned female at birth, menstrual cycle and contraceptive use. A primary comparison should be between GnRHa-treated transgender youth and untreated transgender youth, but it will also be important to include comparisons with cisgender samples as research on hormones and the adolescent brain is still novel and emerging and more data are needed on all youth during this developmental period. One way to accomplish the latter is to employ existing large-scale databases from studies of brain development during the pre-pubertal, pubertal, and later-adolescent periods, treating them as brain growth curves for comparisons. This approach is similar to the differentiation of cognitive trajectories in normal aging versus neurodegenerative disorders. The basic research question involves comparing cognitive growth curves over time.

Domains to assess. It will be critical to prioritize assessment domains based on hypothesized mediating mechanisms, with the most important domains to measure as follows: mental/behavioral health, pubertal stage, executive function/control, gender identity/dysphoria, and social awareness/functioning. See Table 4 for a complete list of ranked domains. Although we (the Delphi experts) identify executive function/control and social functioning as key domains to measure, it is important to note that there is no clear evidence that progressing through puberty later than peers is associated with delayed maturation of abstract reasoning, executive function, and social capacities. Executive function and emotional functioning are bidirectionally related, and for this reason, the two should be integrated in models/analyses. In addition, cognitive/behavioral flexibility, a component of executive functioning, should be measured, given that studies in rodents show ovarian hormones, acting during puberty, program cognitive flexibility by exerting long-lasting effects on excitatory-inhibitory balance in the prefrontal cortex.[61] Studies in rodents also demonstrate that testosterone, acting during puberty, programs the ability to adapt behavior as a function of social experience.[34] Measurement approaches should extend beyond cognitive capacities alone, embedding social, affective, and value-based learning processes. There is an emerging shift in thinking about increases in reward sensitivity

Table 3. Rank Order of Priority Comparison Groups

Rank order of priority	Comparison group
1	Transgender youth who do not take GnRHa matched on pubertal status at the beginning of the study
2	Cisgender typically developing adolescents matched on pubertal status at the beginning of the study
3	Use a standardized battery and/or a large existing database of norms to compare to (in addition to a smaller comparison group)
4	Transgender youth who commence GnRHa treatment earlier compared to later in puberty
5	Siblings of transgender youth enrolling in the study (to serve as genetic and shared environmental controls)
6	Mixed clinical group of adolescents presenting for MH assessment/treatment in an outpatient setting matched on pubertal status
7[a]	Peers with mood disorders (to control for the overoccurrence of mental health distress in transgender youth) matched on pubertal status
7[a]	Youth with precocious puberty who are given GnRHa to delay puberty

This priority sequence was based on participants' top 2 ranked comparison groups, where the top rated comparison group was given a value of 2 and the second rated comparison group was given a value of 1. A mean score was derived for each comparison group based on participants' ratings and ordered from highest to lowest.

[a]Comparison groups received the same mean score in the ranking.

Table 4. Rank Order of Priority Domains of Characterization and Assessment

Rank order of priority	Domains of characterization and assessment
1	Mental/behavioral health (including suicidality/hopelessness)
2	Pubertal stage/development (Tanner staging/hormone levels)
3	Executive function/control and attention
4	Gender identity/dysphoria
5	Social awareness/functioning
6	Quality of life
7	Brain/functional connectivity
8	Brain structure/volume
9	Emotional awareness/functioning
10	Physical health symptoms and outcomes (especially in adulthood)
11	Adaptive/independence skills
12	General cognitive functioning (IQ)
13	Sensation seeking, risk taking, reward sensitivity, and motivation
14	Genetics (i.e., possible impacts of GnRHa on DNA and RNA expression)
15	Academic functioning
16	Processing speed
17	Memory systems

This priority sequence was based on participants' top 6 ranked domains to measure, where the top rated domain was given a value of 6 and the second rated comparison group was given a value of 5, and so on. A mean score was derived for each domain based on participants' ratings and ordered from highest to lowest.

and sensation-seeking during puberty as related to social-value learning.[18] Dopamine release is not primarily a "reward" signal, but rather a learning signal (e.g., prediction error signal)—the natural increased salience of social learning (e.g., status and prestige, being admired, respected, and liked). The effects of suspending puberty on the salience of social-value learning might produce small near-term effects, but could contribute to changes in patterns of behavior over time, leading to large individual differences in developmental trajectories for GnRHa-treated youth.

If neuroimaging is included, imaging approaches should focus on the following domains: social/emotional processing, executive functioning, risk and reward processing, and self-concept. Neuroimaging should parallel behavioral assessment. Neural measures should be linked to neurocognitive and behavioral measures. Acquisition parameters should be matched between imaging sites. Investigation of white matter development is important as myelination progresses during puberty, likely under the influence of sex hormones,[62] and is related to cognitive processing speed. Both structural MRI and diffusion tensor imaging approaches should be used for white matter imaging and analyzed using a longitudinal imaging pipeline for processing these data with scientific rigor.

Discussion

Puberty suppression has become an increasingly available option for transgender youth, and its benefits have been noted, particularly in the area of mental health. However, puberty is a major developmental process and the full consequences (both beneficial and adverse) of suppressing endogenous puberty are not yet understood. The experts who participated in this procedure believe the effects of pubertal suppression warrant further study, and this Delphi consensus process develops a framework from which future research endeavors can be built.

Expert consensus emphasized a minimum of three measurement time points, inclusion of multiple comparison groups to minimize the limitations of any one group, precision pubertal staging at baseline, accounting for sex in design and analysis, and the use of designs that capture heterogeneity in processes being studied. Focus on longer-term trajectories and outcomes was emphasized, given that effects of pubertal suppression on various processes may not be evident in the near term, and responses to delayed receipt of gonadal hormones may not be comparable to initial potentially organizing effects. Experts also highlighted that accounting for the psychosocial aspects of the transgender experience itself on development will require models that integrate both cognitive and psychosocial functioning. The highest endorsed measurement priorities were mental and behavioral health, executive function/cognitive control, and social awareness/functioning. The importance of interrelations between domains that mature during puberty/adolescence was also emphasized, including bidirectional relationships between cognitive and emotional control and links between reward sensitivity and social value learning. Regarding neuroimaging, experts stressed the importance of linking neural signatures to cognitive and behavioral measures, with attention to white matter development. Notably, while there was consensus in this approach to neuroimaging, there were divergent views as to whether a neuroimaging protocol should be prioritized in a study with limited resources. Some experts noted that insufficient work has been done on neural development during puberty in general and expending resources on an expensive neuroimaging protocol for this subset of youth may be premature, while others felt that defining underlying brain mechanisms by neuroimaging was important. Furthermore, at the final review of the article, four co-authors noted a concern with this specific Delphi consensus recommendation: "Accounting for differences between birth-assigned male youth versus birth-assigned female youth is important, as sex is differentially related to the rate and pattern of cognitive development, connectome distinctiveness, and timing of peak brain volume." The four authors felt that instead of "peak brain volume," a more appropriate measurement concept might be that of "structural brain metrics" (e.g., thickness and regional volumes).

Twelve different comparison groups were proposed in the first round of the Delphi and 8 of the 12 groups were rated as either first or second priority by at least 1 expert in the second Delphi round. This heterogeneity underscores the complexity of selecting comparison groups for this research and lends support to the experts' recommendation to engage more than one comparison group. The highest rated comparison groups were untreated transgender youth matched on pubertal stage, cisgender youth matched on pubertal stage, and a sample from a large-scale quasi-normative database (e.g., from the ABCD study) used as a "brain growth curve." These comparison groups are not without weaknesses. Untreated transgender youth may differ in their

intensity or experience of GD, level of parent support (e.g., are the parents against GnRHa treatment?), and socioeconomic status of the family and access to treatment (e.g., insurance coverage). A cisgender comparison group would lack gender-minority experience and associated stress.

Some statements approached, but did not reach consensus. For example, many experts suggested continuing assessments of transgender youth through young adulthood (mid-20s) when prefrontal development is near completion. Assessing adaptive functioning (everyday skills) over time due to the bidirectional link between executive functioning and adaptive behaviors was also often endorsed.

Not all relevant study considerations were raised by the Delphi panel. Neurodevelopmental impacts of pubertal suppression in transgender youth with neurodevelopmental differences/diagnoses (e.g., attention deficit/hyperactivity disorder and autism spectrum disorder) were not specifically addressed by the experts. Yet, evidence suggests an overoccurrence of neurodiversity characteristics (especially related to autism) among gender-referred youth.[55,63–66] The neurodevelopmental impacts of pubertal suppression on neurodiverse gender-diverse youth might well be different than in neurotypical gender-diverse youth, given variations in neurodevelopmental trajectories observed across neurodevelopmental conditions.[67–69]

This study included experts from a range of relevant disciplines—a strength and also a possible limitation. The varied disciplines allowed for a broader range of ideas and perspectives, but some specialized recommendations might not have been sufficiently understood by Delphi experts from other disciplines. It is possible that some useful recommendations were lost in the process because few experts had backgrounds relevant to them. In fact, four recommendations were dropped from consideration because more than nine experts indicated they could not rate the item or skipped the item. These four items included topics related to advanced growth curve modeling, impact of GnRHa on immune system functioning, multifactorial relationships between GD and neurodevelopment, and challenges associated with using alternative forms of measures in longitudinal designs. The Delphi team included experts across the fields of neuroscience, neurodevelopment, developmental measurement, and gender development; however, most were not specialized in clinical transgender care *per se*. This reflects the dearth of transgender care clinicians/specialists with research productivity in adolescent neurodevelopment. Thus, the experts could comment with authority on neurodevelopment, including gender development/dysphoria aspects of study design, but the real-world clinical care considerations may well be underdeveloped in the proposed research design. For example, the everyday lived experience of transgender youth seeking gender-affirming medical care would be unfamiliar to most neurodevelopmental researchers. After the Delphi procedure was completed, one panelist commented that pubertal hormones might play a role in organizing neurodevelopmental gender-related trajectories, including identity itself, which would be important to consider for a developmental study of gender diverse youth.

Despite these limitations, an international expert team successfully completed an iterative Delphi procedure achieving consensus around priority research design elements to study neurodevelopmental impacts of pubertal suppression in transgender youth. The resulting consensus parameter addresses broad design issues, including comparison groups, longitudinal design, neurodevelopmental targets for assessment, and measurement approaches. While it may not be possible to incorporate all consensus methodologies into a single study, this parameter may serve as a roadmap for a range of research initiatives investigating pubertal suppression treatment in transgender youth.

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Abbreviations Used

ABCD = Adolescent Brain Cognitive Development
GAH = gender-affirming hormones
GD = gender dysphoria
GnRHa = gonadotropin-releasing hormone agonists
MRI = magnetic resonance imaging