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Clinical Research Article

Back to the Future: Is GnRHa Treatment in Transgender and Gender Diverse Adolescents Only an Extended Evaluation Phase?

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Abstract

Context: The role of body modifications induced by gonadal suppression in transgender and gender diverse adolescents on psychological functioning has not yet been evaluated.

Objective: The main aim of the present study was to explore several hormone, physical and psychological functioning changes during gonadotropin-releasing hormone analog (GnRHa) treatment in transgender and gender diverse adolescents (TGDAs). The potential relationship between the physical and hormone effects of GnRHa and psychological well-being, along with its magnitude, was assessed for the first time.

Methods: This prospective multidisciplinary study included 36 TGDA (22 assigned female at birth, and 14 assigned male at birth) who received psychological assessment followed by triptorelin prescription after referring to the Florence Gender Clinic. This study consisted of 3 time points: first referral (T0), psychological assessment (T1); and treatment with intramuscular injections of triptorelin for 3 up to 12 months (T2). Psychometric questionnaires were administered at each time point, and clinical and biochemical evaluations were performed at T1 and T2.

Results: The following results were found: (1) GnRHa showed efficacy in inhibiting puberty progression in TGDAs; (2) an increase in psychopathology was observed before starting GnRHa (T1) compared with baseline levels; (3) during GnRHa treatment (T2), a significant improvement in psychological functioning, as well as decrease in suicidality, body uneasiness, depression, and anxiety levels were observed; (4) hormone and physical changes (in terms of gonadotropin and sex steroid levels, height and body mass index percentiles, waist–hip ratio, and acne severity) observed during triptorelin treatment significantly correlated with a reduction in suicidal ideation, anxiety, and body image concerns.

Conclusion: Psychological improvement in TGDA on GnRHa seems to be related to the objective body changes induced by a GnRHa. Therefore, the rationale for treatment with a GnRHa may not only be considered an extension of the evaluation phase, but also the start of a medical (even if reversible) gender-affirming path, especially in TGDAs whose puberty has already progressed.

Key Words: gonadotropin-releasing hormone analog (GnRHa), gonadal suppression, transgender and gender diverse adolescents, gender incongruence, psychological functioning

Abbreviations: AFAB, assigned female at birth; ALT, alanine transaminase; AMAB, assigned male at birth; AST, aspartate transaminase; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; BMI, body mass index; BP, blood pressure; BUT, Body Uneasiness Test; EDF, effective degrees of freedom; GAGS, Global Acne Grading Scale; FG, Ferriman–Gallwey; GD, gender dysphoria; FSH, follicle-stimulating hormone; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LH, luteinizing hormone; TGDA, transgender and gender diverse adolescent; GnRHa, gonadotropin-releasing hormone analog; MAST, Multi-Attitude Suicide Tendency Scale for Adolescents; YSR, Youth Self Report.

Transgender and gender diverse adolescents (TGDAs) may face a challenging phase of their lives at the onset or during puberty. Adolescence in transgender youth is often associated with high rates of depression, anxiety, eating disorders, suicidal thoughts and suicide attempts, and self-harming behaviors (1-7). Several reasons seem to be involved in explaining why TGDAs are a more vulnerable population than their peers. According to the minority stress model, chronic exposure to stigma and discrimination impacts strongly on psychological well-being (8, 9). In contrast, psychological functioning improves in inclusive and accepting environments after the start of specialized transgender care (10). However,

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the source of distress for some TGDAs at the onset of puberty may also be linked to pubertal body changes that are developing in an unwanted direction. International (7, 11, 12) and national (5) recommendations highlight the importance of multidisciplinary support for TGDAs seeking care. In particular, after the first phase, aimed at assessing if the pubertal body may be a source of distress according to the adolescent's gender identity and if specific criteria are met (11, 12), some TGDAs may receive a gonadotropin-releasing hormone analog (GnRHa, ie, triptorelin) to reversibly reduce gonadal steroids production and limit their possible gender-related physical effects. This step formerly known as the "extended evaluation phase" allows time to better think about further gender-affirming steps without the distress caused by unwanted pubertal body changes (13). Data about physical outcomes and the safety of GnRHa treatment in children with precocious puberty are widely available, but only a few studies have evaluated this medical therapy in TGDAs. Among the available literature in this field (14-17), several prospective studies have demonstrated the efficacy of GnRHas in terms of gonadal suppression in TGDAs, as well as their effects in terms of anthropometry and body composition (18-22). On the other hand, a study on a small sample of transgender boys reported an impact of GnRHa treatment on blood pressure, possibly leading to hypertension (23). Finally, a recent study has described a decrease in bone turnover markers in adolescents treated with GnRHa as an effect of sex steroid withdrawal (24). Follow-up studies on the use of GnRHa report an improvement in the psychological and global functioning of TGDA (25, 26). However, the role of body modifications induced by gonadal suppression on psychological functioning has not yet been evaluated. The present study aims to assess whether gender-physical and hormone modifications observed during GnRH treatment are related to changes in psychobiological functioning changes over time.

Materials and Methods

Participants

Youths referred to our Florence Gender Clinic from September 2014 to December 2020 were enrolled, provided they met the following criteria: (1) age under 18 years; (2) DSM 5 criteria for gender dysphoria (GD) (27); (3) Endocrine Society and World Professional Association for Transgender Health (WPATH) Standards of Care criteria, seventh version, for gonadotropin-releasing hormone analog (GnRHa) treatment (12, 28).

According to the existing guidelines at the time of the study (5, 12, 28), the first referral was followed by a psychological assessment, during which youths and families received support and counselling regarding the person's gender identity. When psychological criteria were met (5, 12, 28), the adolescents were referred to the endocrinologist for a GnRHa.

Study Design

This was a prospective quasi-experimental 1-group pretest–post-test study. In this design, the same group of participants is measured before (pretest) and after (post-test) a treatment or intervention is administered. Given that the group of participants who received the intervention was selected in a nonrandom way, it is considered a quasi-experimental design. The enrolled adolescents were evaluated at 3 different time points: at first referral, before receiving any kind of support for their gender identity issues (T0); after a first step of psychological assessment and just before starting the medical treatment with a GnRHa (T1); after being treated with a GnRHa for at least 3 months (T2). The maximum follow-up time was 12 months. In particular, gonadal suppression treatment consisted of the intramuscular administration of triptorelin 3.75 mg every 28 days, with interval adjustments based on clinical and laboratory data. No other gender-affirming hormonal treatments other than triptorelin (ie, no testosterone or estradiol therapy) were prescribed during the follow-up period. All participants underwent an initial period of psychological evaluation, during which criteria for GnRHas were assessed and which lasted a median of 7.0 (range 4.0-8.5) months. A further follow-up was carried out a median of 6.0 (range 6.0-12.0) months after the beginning of GnRHa. At each time point, adolescents were asked to complete several psychometric questionnaires; in addition, a medical assessment was performed at T1 and T2. It is important to remark that both an endocrinological and a psychological visit were required every 3 months as a requisite for treatment; in this regard, psychological support was part of the clinical protocol together with GnRHa. Participants and their parents gave their written informed consent for both medical treatment and participation in the study. The study design was approved by the Florence University Hospital ethics committee (2013/0016117).

A total of 154 adolescents were referred to our center from September 2014 to December 2020. Of these, 4 adolescents did not satisfy the DSM 5 criteria for GD; 14 were aged 18 by the time psychological assessment was completed and had started gender-affirming hormonal treatment, and 18 dropped out. In all, 36 adolescents were included in the final analysis; when the analysis was performed, 72 adolescents were still in the psychological assessment phase, and 10 were in the endocrinological assessment phase (for indications and contraindications for treatment). The slight overrecruitment compared with the initial power calculation was due to the need to compensate for the possibility of drop-outs. Given that, in the end, more participants completed the study approximately within the same time frame, 2 additional subjects were included beyond what was initially anticipated. Participants who dropped out had baseline psychometric characteristics similar to those who were included in the final analyses (see Table 1). Figure 1 reports the details of the participants in a flow chart.

In relation to the novel objective of this study, which was to investigate the relationship between physical and hormone changes induced by GnRHa treatment and psychometric measures, based on similar regression models concerning body uneasiness, carried out on an adult population of subjects with GD treated with hormonal therapy, medium effect sizes of $\beta = .45$ were hypothesized a priori, corresponding to an f^2 of approximately 0.25 (29). Power analysis for a linear regression model with $\alpha = .05$ indicates that a sample of at least 34 individuals is sufficient to identify a statistically significant effect size of this magnitude with a power of 0.80. Accordingly, the sample recruited for this study was considered adequate. The study was considered complete with the execution of the last planned follow-up, once the calculated sample size was reached.

Sociodemographic and Psychometric Evaluations

TGDAs and their parents completed a structured interview to collect sociodemographic characteristics at the time of the first

Table 1. Comparisons between participants who dropped out and those who completed the study and were included in the final analyses, performed using analysis of covariance (with age and Tanner stage as covariates)

	Dropouts (n = 18)	Treatment completers (n = 36)	F	*P*
YSR Total Score	65.50 ± 6.72	62.94 ± 10.07	0.29	.593
YSR Externalizing	56.00 ± 5.39	55.75 ± 8.91	0.03	.863
YSR Internalizing	68.00 ± 8.54	67.33 ± 11.61	0.01	.921
YSR Aggressive Behavior	54.83 ± 3.31	57.75 ± 6.52	0.92	.342
YSR Rule-Breaking Behavior	55.11 ± 4.34	55.84 ± 6.26	0.48	.492
YSR Attention Problems	62.11 ± 9.16	62.44 ± 9.26	0.04	.842
YSR Thought Problems	61.00 ± 8.19	61.28 ± 11.67	0.04	.842
YSR Social Problems	63.33 ± 4.92	62.59 ± 9.02	0.02	.888
YSR Somatic Complaints	63.33 ± 10.58	66.38 ± 12.18	0.41	.523
YSR Withdrawn/Depressed	65.44 ± 7.02	66.75 ± 13.13	0.15	.700
YSR Anxious/Depressed	67.56 ± 10.26	66.31 ± 10.97	0.07	.792
MAST Attraction to Death	2.60 ± 0.98	2.67 ± 1.24	0.07	.792
MAST Repulsion by Life	2.98 ± 0.69	2.88 ± 0.67	0.04	.842
MAST Attraction to Life	2.90 ± 0.52	3.49 ± 1.10	1.33	.254
MAST Repulsion by Death	2.54 ± 1.72	2.52 ± 1.56	0.01	.921
BUT-A GSI	3.03 ± 1.00	2.98 ± 0.90	0.01	.921
BDI	22.67 ± 14.83	17.52 ± 11.52	0.73	.397
BAI	24.44 ± 14.65	18.13 ± 10.93	0.74	.394

No comparison was statistically significant (all $P > .05$).
Abbreviations: BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; BUT, Body Uneasiness Test; MAST, Multi-Attitude Suicide Tendency Scale; YSR, Youth Self Report.

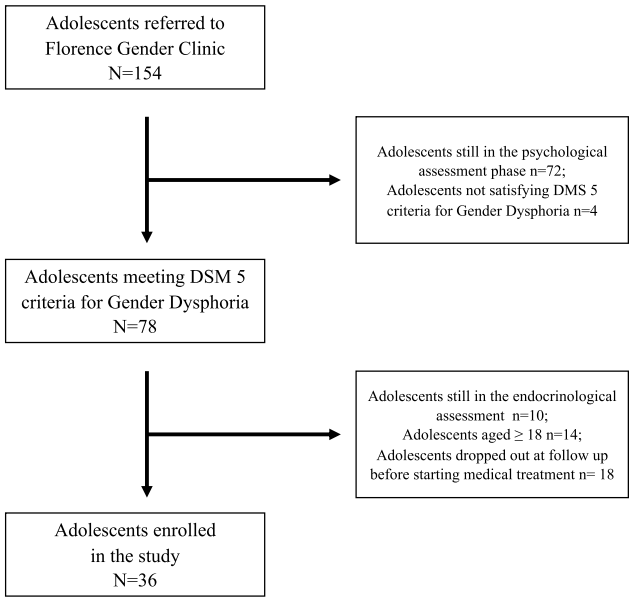

Figure 1. Flow chart reporting details of the participants.

referral. At the same time, TGDAs were asked to complete several psychometric questionnaires, including the Youth Self Report (YSR) (30, 31), the Body Uneasiness Test (BUT) (32, 33), the Multi-Attitude Suicide Tendency Scale for Adolescents (MAST) (34, 35), the Beck Depression Inventory (BDI)-II (36), and the Beck Anxiety Inventory (BAI) (37).

A description of the aforementioned questionnaires is reported in Table 2.

Physical Assessment and Laboratory Measurements

Adolescents on GnRHas attended an endocrinological visit every 3 months; the visit included a physical examination for systolic and diastolic blood pressure (BP) (mean of 3 measurements 5 minutes apart, in a sitting position, with a standard sphygmomanometer), height, weight, waist circumference, hip circumference, body and face hair distribution using the Ferriman–Gallwey score (FG score) (38), acne severity using the Global Acne Grading Scale (GAGS) (39), and Tanner stage. Tanner stage evaluation was based on breast growth in assigned female at birth (AFAB) adolescents and on genital development and testicular volume in assigned male at birth (AMAB) adolescents (40). Height was measured using a wall-mounted stadiometer and weight with a digital floor scale. Height and weight were used to calculate body mass index (BMI; kg/m^2). The mean BP was calculated as (diastolic BP + (1/3×difference between systolic and diastolic BP) (41).

Blood tests were performed at least 3 weeks before each consultation, and laboratory measurements included gonadotropins, sex steroids, liver function parameters, fasting glucose, glycated hemoglobin (HbA1c), lipid profile test; a biochemical assessment was performed in the morning, in fasting conditions, to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17β-estradiol (using the chemiluminescence method; DIMENSION VISTA System, Siemens), testosterone (by liquid chromatography mass spectrometry; Agilent Technologies, Santa Clara, CA), HbA1c, glucose, liver enzymes aspartate transaminase (AST), alanine transaminase (ALT), cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol

Table 2. Descriptions of questionnaires used in the study

Youth Self Report
The Youth Self Report (YSR) is a self-rating scale evaluating emotional and behavioral functioning of adolescents through a 3-point scale (from 0 = not true to 2 = very true). It consists of about 100 items grouped in 8 syndrome scales according to a dimensional approach (anxiety and depression, withdrawal and depression, somatic complaints, social problems, problems of thought, problems of attention, rule transgression behavior, aggressive behavior) ,and 3 general scales (total problem, internalizing, and externalizing scale). Raw scores are transformed into T scores based on normative data in relation to norms for their age and gender.
Body Uneasiness Test
The Body Uneasiness Test (BUT) is a self-rating scale evaluating different areas of body-related psychopathology, such as weight phobia, avoidance, compulsive control behavior, experiencing separation and strangeness from the body, and specific worries about certain body parts or characteristics. Subjects rate 34 different body image experiences (BUT A), reporting how often they happen to dislike each experience. Higher scores indicate greater body uneasiness.
Multi-Attitude Suicide Tendency Scale for adolescents
The Multi-Attitude Suicide Tendency Scale for adolescents (MAST) is a self-rating scale evaluating suicidal tendency which reflect 4 types of attitudes: attraction to life, attraction to death, repulsion by life and repulsion by death. In particular, the "repulsion by life" component reflects such experiences as stress and pain; "attraction to death" represents religious convictions or perceptions that death is a superior way of being; "attraction to life" is based on the level of satisfaction with life and a sense of well-being; and "repulsion by death" indicates fears of death. Each item is rated on a 5-point scale (from 1 = strongly agree to 5 = strongly disagree).
Beck Depression Inventory
The Beck Depression Inventory (BDI-II) is a self-rating scale that measures depressive symptoms in emotional, cognitive, and somatic dimensions. Each item is rated on a 4-point Likert scale (from 0 to 3), taking into consideration the past 2 weeks as time frame. Higher scores indicate greater levels of depressive symptoms. Scores may range from 0 to 63 with the following cut offs: 0-13 = minimal depression; 14-19 = mild depression; 20-28 = moderate depression and 29-63 = severe depression.
Beck Anxiety Inventory
The Beck Anxiety Inventory (BAI) is a self-rating scale evaluating the intensity of physical and cognitive anxiety symptoms considering the past week as time frame. It consists of 21 items rated on a 4-point Likert scale (from 0 = not at all to 3 = severely). Higher scores indicate greater levels of anxiety. Scores may range from 0 to 63 with the following cut offs: 0-7 = minimal anxiety; 8-15: mild anxiety; 16-25: moderate anxiety and 26-63: severe anxiety.

(HDL-C), and triglycerides (by routine clinical chemistry methods). To estimate LDL-C the Friedewald equation was used: LDL-C = total cholesterol − (HDL cholesterol + triglycerides/5) (42). All the above-mentioned psychological, biochemical, and physical assessment tools were part of the standard clinical protocol applied to all youths referred to the gender clinic.

Statistical Analysis

Tanner stage was reported using the median and interquartile range (IQR). Continuous data were reported as mean and SD, with the exception of endocrinological characteristics expected to be near 0 in at least 1 group at at least 1 time point (gonadotropins, sex steroid), or parameters with a skewed evaluation and hence possibly 0 inflated (like GAGS and FG scores), which were reported using median and IQR. The comparisons of psychometric variables between participants who completed the treatment and those who dropped out were conducted using analysis of covariance, with age and Tanner stage as covariates. The F values were reported, corresponding to the ratio of the variance between groups to the variance within groups (larger F values indicates that the between-group variation is larger than the within-group variation), along with their corresponding *P* values.

Regarding longitudinal analyses, 3 different approaches were adopted. Tanner stage evolution over time before and after GnRHa therapy was investigated with the Wilcoxon signed rank test, a nonparametric paired-samples test for ordered variables. Regarding laboratory measurements, a nonlinear variation was expected over time after administration of the GnRHa, and preliminary analyses confirmed this hypothesis. Therefore, generalized additive mixed models with random intercepts were used in order to capture nonlinear trends in the longitudinal context. In all generalized additive mixed models, time was entered as a smooth term based on thin plate regression splines, which are considered optimal (43); a modified smoothing penalty was used in order to allow the smooth term to be shrunk to 0 and avoid overfitting (43). The models for FSH and LH were adjusted for Tanner stage, while those for waist and hip circumferences were adjusted for BMI. Moreover, assigned sex at birth was entered as a fixed effect, with individual time smooth terms computed for each group to differentiate between AMAB and AFAB. In accordance with common guidelines for statistical reporting, the F value and its corresponding *P* value, as well as effective degrees of freedom (EDF), have been provided for all smooth terms. In this context, EDF can be used as a proxy for measuring the nonlinearity of the relationship between the variables in the model: a value less than or equal to 1 indicates a substantial linear trend of the dependent variable over time, while a value greater than 1 indicates an increasingly curved longitudinal trend. For significant time smooth terms, the first derivatives of the fitted trend and their respective 95% CI were computed using standard theory: a time interval where the CI on the first derivative did not include 0 was considered to be a period of statistically significant change (44). Finally, linear mixed models with random intercepts (with Tanner stage and age as covariates) were used to investigate the longitudinal trend of all psychometric measurements, given that they did not show nonlinear trends in preliminary analyses. The time variable was entered into the model as a 3-level polytomous independent variable, where each patient's longest follow-up was considered for the GnRHa treatment period. F tests for the fixed effect time were reported for each model. For every statistically significant F test, which indicates at least 1 variation over time different from 0, was identified; post hoc pairwise tests were performed to identify the periods of change.

To test the additional effect of GnRHa administration on psychometric variables compared with psychological support alone, moderation models were also performed in which time, the presence of GnRHa (before GnRHa vs after GnRHa) and their interaction were included as predictors; a statistically significant time × GnRHa interaction indicated a different longitudinal trend between the 2 periods and was consequently probed using simple effects analysis.

Finally, linear regression analyses were used to test whether physical or endocrinological changes over time predicted variations in psychometric scores, adjusting for Tanner stage and

Table 3. Physical and endocrinological characteristics of the sample before (T1) and after (T2) the beginning of GnRHa, divided by assigned sex at birth

	AMAB (n = 14)		AFAB (n = 22)	
	Before GnRHa (T1)	After GnRHa (T2)	Before GnRHa (T1)	After GnRHa (T2)
SBP (mmHg)	114 ± 11	114 ± 9	111 ± 10	110 ± 10
SPB percentile	55.4 ± 26.4	53.1 ± 26.0	51.3 ± 27.7	46.14 ± 28.4
DBP (mmHg)	70 ± 7	74 ± 7	71 ± 7	70 ± 9
DBP percentile	65.1 ± 23.1	74.4 ± 22.4	64.8 ± 19.6	63.2 ± 24.9
Mean BP (mmHg)	85 ± 8	87 ± 6	84 ± 8	84 ± 9
Weight (kg)	58.9 ± 12.4	60.6 ± 11.4	63.5 ± 17.5	67.2 ± 16.2
Height (cm)	167.3 ± 9.4	169.3 ± 8.3	161.9 ± 5.6	162.8 ± 5.1
Height percentile	63.9 ± 34.4	56.2 ± 35.1	58.0 ± 26.4	58.9 ± 26.1
BMI percentile	62.2 ± 39.0	63.9 ± 27.3	69.8 ± 29.5	78.1 ± 22.4
Waist (cm)	79.0 ± 8.9	76.9 ± 8.0	85.6 ± 14.2	87.4 ± 14.2
Hip (cm)	91.5 ± 6.8	92.0 ± 6.6	98.0 ± 13.6	101.5 ± 12.1
Ferriman–Gallwey (FG) score	8.0 (6.3-13.3)	4.5 (3.0-7.5)	6.0 (2.3-8.8)	3.50 (2.3-6.8)
Global Acne Grading System (GAGS)	7.5 (0.0-16.8)	0.0 (0.0-7.3)	8.0 (0.3-21.5)	4.5 (0.0-9.5)
LH (mUI/mL)	3.1 (2.3-4.0)	0.8 (0.3-1.2)	4.6 (3.6-6.2)	0.5 (0.3-1.0)
FSH (mUI/mL)	4.9 (4.1-5.9)	0.6 (0.3-1.0)	4.4 (3.1-5.1)	2.8 (1.8-3.4)
Testosterone (nmol/L)	18.4 (15.6-21.0)	1.0 (0.4-2.9)	1.2 (0.8-1.8)	0.7 (0.7-1.1)
Estradiol (pmol/L)	77.2 (59.6-110.2)	36.8 (15.0-70.0)	114.0 (70.0-228.4)	18.4 (15.0-36.8)
HbA1C (mmol/mol)	33.1 ± 3.8	34.7 ± 4.0	33.6 ± 8.2	34.2 ± 6.7
AST (UI/L)	20.3 ± 6.6	20.4 ± 7.5	17.5 ± 4.1	23.6 ± 11.1
ALT (UI/L)	19.9 ± 11.7	17.4 ± 6.7	14.9 ± 6.5	19.4 ± 8.1
Total cholesterol (mg/dL)	152.0 ± 24.0	158.4 ± 30.2	155.3 ± 22.7	165.3 ± 26.4
Triglycerides (mg/dL)	60.2 ± 23.4	66.6 ± 32.6	76.0 ± 26.8	80.4 ± 39.2
HDL cholesterol (md/dL)	56.6 ± 14.4	63.5 ± 13.0	54.6 ± 9.3	55.6 ± 9.0
LDL cholesterol (mg/dL)	79.1 ± 14.9	76.3 ± 24.3	86.7 ± 17.1	93.8 ± 24.5

Between-group comparisons were not performed as they were considered irrelevant to the objectives of the study.
Abbreviations: AFAB, assigned female at birth; ALT, alanine transaminase; AMAB, assigned male at birth; AST, aspartate transaminase; BMI, body mass index; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; SBP, systolic blood pressure; TGNA, transgender and gender nonconforming adolescents.

taking into account the moderating role of sex assigned at birth. Among the available laboratory variables, LH and FSH were included in these analyses (considered the main outcomes of the effectiveness of GnRHa treatment), while among the physical variables, only those that were estimated to have a significant impact on psychological well-being were considered: height, weight, BMI, waist/hip circumference, and FG and GAGS scores. As for the psychometric variables, to limit the number of associations to be tested, the MAST ideation scores and the measures of body uneasiness (BUT-A GSI), depression (BDI), and anxiety (BAI) were included.

Data were analyzed using R Statistical Software v. 4.1.2 (45) and the following packages: ggplot2, gratia, mgcv, nlme, reghelper, sjPlot (43, 46-50).

Results

A total of 36 adolescents were enrolled in this study, of which 14 were AMAB and 22 were AFAB, with an average age of 14.19 ± 1.88 years; the age range was 11-15 years old and 9-17 years old for AMAB and AFAB adolescents, respectively. Tanner stage ranged from 3 to 5, with a median of 5 (IQR 4-5).

Endocrinological Evaluation

All endocrinological characteristics of the sample are reported in Table 3, before and after GnRHa therapy.

After GnRH therapy, a total of 9 patients (25.0%) reported a reduction in Tanner stage at the final evaluation. This transition towards lower Tanner stages was statistically significant, as evidenced by the Wilcoxon signed rank test ($P = .005$); Fig. 2 shows the violin plots at the 2 time points. In all the AFAB adolescents who had menarche (n = 20, 90.01%), menses stopped at T3. Nonlinear longitudinal analyses are reported in Table 4. On average, participants of both groups grew taller, whereas a significant increase in total weight was observed only in the AFAB group (Table 4 and Fig. 3A and 3B, respectively). These changes were basically linear, as indicated by the EDFs, which were close to 1 (Table 4), and the analyses of the first derivatives of the fitted splines confirmed that they were significantly different from 0 over the entire follow-up period (Fig. 3A and 3B). AMAB individuals experienced a progressive reduction in height percentiles (Table 4 and Fig. 3C), whereas a significant increase in BMI percentiles was observed in AFAB individuals, but only from the third month of therapy and only for a few months (Table 4 and Fig. 3D). Both hair growth and acne

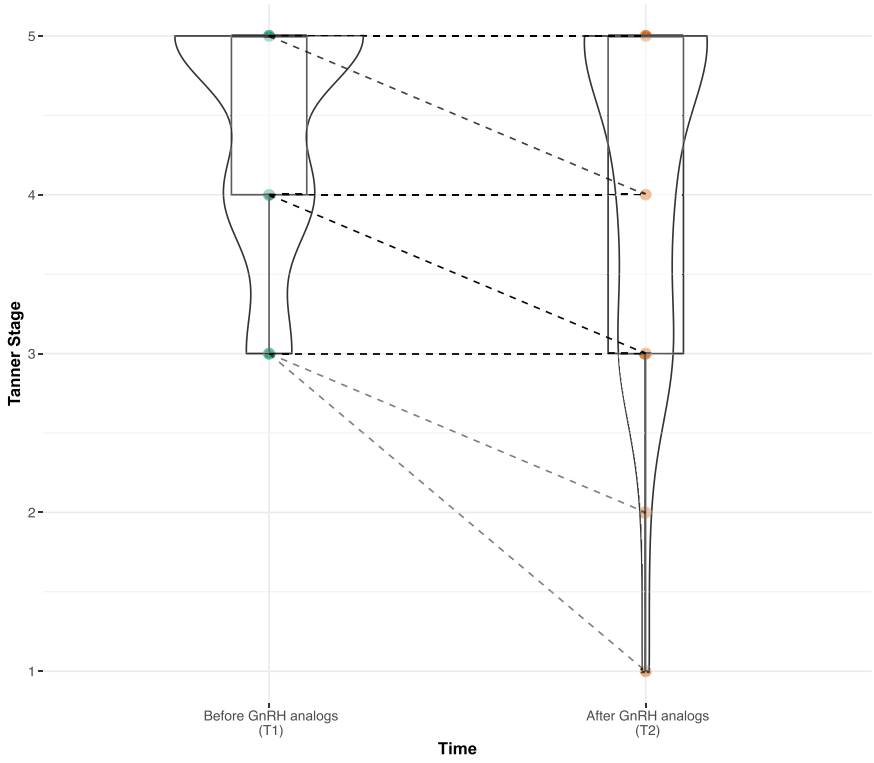

Figure 2. Violin plot of Tanner stages before (T1) and after (T2) the beginning of GnRHa treatment. Each "violin" in the plot corresponds to a specific time point, with its width indicating the frequency of Tanner stages at that time. The wider sections of the violin plot represent a higher density of data points. The individual points within each violin represent the actual data for the Tanner Stage at each time point. The lines connecting the points illustrate the progression of Tanner stages over time. The rectangles within each violin represent the interquartile range of the data.

severity were significantly reduced in all participants after initiation of GnRHa, especially in AMAB individuals (Table 4 and Fig. 3E and 3F).

The data analyses confirmed a general reduction in levels of gonadotropins; in particular, FSH levels decreased nonlinearly and faster over the first months of GnRHa treatment in AMAB individuals, while the same trend was observed for LH in AFABs (Table 4 and Fig. 3G and 3H). In the AMAB group alone, testosterone levels fell in the first half of the observation period under GnRHa, and then stabilized in the second half at levels similar to those in the AFAB group (Table 4 and Fig. 3I). Estradiol levels decreased significantly and approximately linearly in AFAB individuals during the first 10 months of GnRHa treatment (Table 4 and Fig. 3I). No statistically significant changes were observed in either AMAB or AFAB with regards to BP, waist and hip circumferences, HbA1c, AST, ALT, and lipid levels, with the exception of a slight elevation of HDLs in AMAB individuals (Table 4 and Fig. 3K).

Psychometric Evaluation

All psychometric characteristics of the sample at all time points are reported in Table 5. Longitudinal analyses are reported in Table 6. After GnRHa therapy, a significant reduction in both externalizing and internalizing problems was observed (Table 6). In particular, participants reported lower scores on scales related to thought and social problems, somatic complaints, and anxious–depressive symptomatology (Table 6). Almost every improvement was observed after initiation of GnRHa therapy, with the exception of YSR Withdrawn/Depressed (Table 6). Conversely, the overall YSR score significantly worsened during the initial assessment follow-up period, whereas an amelioration was observed after GnRHa therapy (Table 6). A similar trend was observed for body uneasiness and repulsion by life which, after an initial (although not statistically significant) slight increase, significantly improved after GnRHa therapy compared with the previous follow-up (Table 6). Figure 4 shows the longitudinal trends of YSR total score, BDI, BAI, MAST Repulsion by Life, and BUT-A GSI.

In order to confirm that GnRHa therapy had a different effect on psychopathological domains than psychological assessment alone, moderation analyses were performed taking into account the duration of treatment, while adjusting for age and Tanner stage. As indicated by the statistically significant interaction effect ($b_{Time*GnRHa} = -1.08$, $P < .001$), the longitudinal course of YSR total score exhibited an inverted tendency over time following GnRHa treatment (indicating a moderating effect), with adolescents experiencing an increase in psychopathology before ($b_{Before\ GnRHa} = 0.54$, $P = .006$) and a progressive amelioration after initiation of therapy ($b_{After\ GnRHa} = -0.54$, $P = .015$). A similar trend was observed on MAST Repulsion by Life ($b_{Time*GnRHa} = -0.08$, $P = .028$; $b_{Before\ GnRHa} = 0.01$, $P = .675$; $b_{After\ GnRHa} = -0.07$, $P = .010$), body uneasiness ($b_{Time*GnRHa} = -0.07$, $P = .001$; $b_{Before\ GnRHa} = 0.01$, $P = .468$; $b_{After\ GnRHa} = -0.06$, $P < .001$), and BAI scores ($b_{Time*GnRHa} = -0.61$, $P = .049$; $b_{Before\ GnRHa} = -0.15$, $P = .449$; $b_{After\ GnRHa} = -0.76$, $P = .001$). These moderation effects are shown in Fig. 5.

Correlations between endocrinological and psychometric modifications

The total reduction in LH levels observed at follow-up after GnRHa therapy was significantly associated with the

Table 4. Longitudinal analysis of physical and endocrinological measurements

		AMAB TGDA			AFAB TGDA		
	Group fixed effect (AFAB vs AMAB TGDA)	EDF	F (smooth term)	Period of significant change (months)	EDF	F (smooth term)	Period of significant change (months)
SBP (mmHg)	−3.61, $P = .205$	0.00	0.00, $P = .531$	—	0.00	0.00, $P = .381$	—
SPB percentile	−5.62, $P = .465$	0.00	0.00, $P = .503$	—	0.26	0.12, $P = .262$	—
DBP (mmHg)	−1.37, $P = .558$	0.76	0.98, $P = .058$	—	0.61	0.48, $P = .138$	—
DBP percentile	−5.63, $P = .411$	0.70	0.73, $P = .087$	—	0.44	0.25, $P = .204$	—
Mean BP (mmHg)	−2.16, $P = .352$	0.07	0.03, $P = .316$	—	0.50	0.32, $P = .181$	—
Weight (kg)	5.77, $P = .262$	0.50	0.32, $P = .177$	—	1.03	**5.95, $P < .001$**	0.00-12.00
Height (cm)	**−5.86, $P = .014$**	1.10	**10.60, $P < .001$**	0.00-12.00	0.95	**3.15, $P = .002$**	0.00-12.00
Height percentile	−1.79, $P = .858$	1.04	**5.89, $P < .001$**	0.00-11.46	0.00	0.00, $P = .682$	—
BMI percentile	11.09, $P = .193$	0.00	0.00, $P = .917$	—	0.85	**1.53, $P = .025$**	2.99-8.43
Waist (cm)	0.72, $P = .696$	0.74	0.92, $P = .068$	—	0.00	0.00, $P = .427$	—
Hip (cm)	1.02, $P = .554$	0.00	0.00, $P = .872$	—	0.42	0.24, $P = .212$	—
Ferriman Gallwey (FG) score	−2.76, $P = .160$	1.04	**6.83, $P < .001$**	0.00-12.00	0.85	**1.61, $P = .022$**	1.53-7.54
Global Acne Grading System (GAGS)	1.91, $P = .510$	0.97	**3.61, $P = .001$**	0.00-12.00	0.90	**2.15, $P = .010$**	0.00-9.45
LH (mUI/mL)	0.56, $P = .506$	0.99	**3.34, $P = .004$**	0.00-10.21	1.86	**18.80, $P < .001$**	0.00-6.51
FSH (mUI/mL)	0.77, $P = .383$	1.99	**12.45, $P < .001$**	0.00-4.98	0.93	**2.18, $P = .016$**	0.00-7.82
Testosterone (nmol/L)	**−9.28, $P < .001$**	2.36	**85.20, $P < .001$**	0.00-6.63	0.00	0.00, $P = .626$	—
Estradiol (pmol/L)	39.30, $P = .301$	0.00	0.00, $P = .435$	—	0.98	**3.02, $P = .003$**	0.00-9.93
HbA1C (mmol/mol)	0.01, $P = .997$	0.68	0.68, $P = .094$	—	0.00	0.00, $P = .738$	—
AST (UI/L)	−0.02, $P = .995$	0.00	0.00, $P = .662$	—	0.76	0.83, $P = .082$	—
ALT (UI/L)	−1.72, $P = .499$	0.00	0.00, $P = .361$	—	0.05	0.02, $P = .325$	—
Total cholesterol (mg/dL)	5.19, $P = .524$	0.76	0.99, $P = .059$	—	0.66	0.60, $P = .115$	—
Triglycerides (mg/dL)	13.80, $P = .138$	0.00	0.00, $P = .331$	—	0.00	0.00, $P = .722$	—
HDL cholesterol (md/dL)	−4.10, $P = .230$	0.96	**3.37, $P = .002$**	0.00-12.00	0.00	0.00, $P = .781$	—
LDL cholesterol (mg/dL)	12.21, $P = .077$	0.00	0.00, $P = .975$	—	0.74	0.85, $P = .075$	—

Group fixed effects represent the average difference in the outcome variable associated with being a member of the AFAB group compared with the AMAB group, across all time points. Nonlinear time effects are reported as F values (for the smooth terms) with their respective effective degrees of freedom (EDF) and *P* values. EDF can be used as a proxy for measuring the nonlinearity of the relationship between the variables in the model: a value less than or equal to 1 indicates a substantial linear trend of the dependent variable over time, while a value greater than 1 indicates an increasingly curved longitudinal trend. For significant effects, the period of significant change is also reported. Bold values denote statistical significance at the $P < .05$ level.
Abbreviations: AFAB, assigned female at birth; ALT, glutamic-pyruvic transaminase; AMAB, assigned male at birth; AST, glutamic-oxaloacetic transaminase; BMI body mass index; DBP, diastolic blood pressure, HbA1c, glycosylated hemoglobin; SBP, systolic blood pressure; TGDA, transgender and gender diverse adolescents.

reduction in suicide ideation as measured by the MAST Repulsion by Life subscale, but only in AMAB individuals ($b_{\Delta LH*Group} = -0.96$, $P < .001$; $b_{AMAB} = 0.93$, $P < .001$; $b_{AFAB} = -0.03$, $P = .727$) (Fig. 6A). The same effects on suicide ideation were observed for the reduction in FSH levels ($b_{\Delta FSH*Group} = -0.65$, $P = .001$; $b_{AMAB} = 0.53$, $P < .001$; $b_{AFAB} = -0.13$, $P = .316$) (Fig. 6B). Similarly, the reduction in body uneasiness was associated with that of LH levels in AMAB individuals ($b_{\Delta LH*Group} = -0.40$, $P = .006$; $b_{AMAB} = 0.28$, $P = .025$; $b_{AFAB} = -0.13$, $P = .078$) (Fig. 6C) and of FSH levels in all adolescents ($b_{\Delta FSH} = 0.19$, $P = .031$; $b_{\Delta FSH*Group} = -0.05$, $P = .635$) (Fig. 6D). Additionally, lower FSH levels after GnRHa correlated with lower anxiety levels, with no significant differences between AMAB and AFAB adolescents ($b_{\Delta FSH} = 2.56$, $P = .030$; $b_{\Delta FSH*Group} = -1.86$, $P = .218$) (Fig. 6E).

Considering the relationship between physical changes and psychopathology, the reduction in waist circumference was associated with a reduction in suicidal risk in AMAB individuals in terms of attraction to death ($b_{\Delta Waist*Group} = -0.11$, $P = .015$; $b_{AMAB} = 0.09$, $P = .015$; $b_{AFAB} = -0.03$, $P = .322$) and repulsion by life ($b_{\Delta Waist*Group} = -0.23$, $P = .030$; $b_{AMAB} = 0.24$, $P = .005$; $b_{AFAB} = 0.01$, $P = .957$). Similarly, in the same group, the reduction in the waist–hip ratio correlated with the reduction in repulsion by life ($b_{\Delta Waist/Hip*Group} = -24.86$, $P = .017$; $b_{AMAB} = 21.68$, $P = .009$; $b_{AFAB} = -3.18$, $P = .588$) and anxiety levels ($b_{\Delta Waist/Hip*Group} = -210.43$, $P = .004$; $b_{AMAB} = 121.99$, $P = .025$), whereas in AFAB individuals a reduced waist–hip ratio resulted in higher anxiety ($b_{AFAB} = -88.44$, $P = .035$) (Fig. 6F). Improved acne severity was associated with reduced suicidal risk (MAST Repulsion by Life) in both groups ($b_{\Delta GAGS} = 0.15$, $P = .18$; $b_{\Delta GAGS*Group} = -0.09$, $P = .260$). Finally, in AMAB individuals lower body uneasiness levels were associated with reductions in weight ($b_{\Delta Weight*Group} = -0.21$, $P = .007$; $b_{AMAB} = 0.15$, $P = .011$; $b_{AFAB} = -0.06$, $P = .145$) and BMI percentile

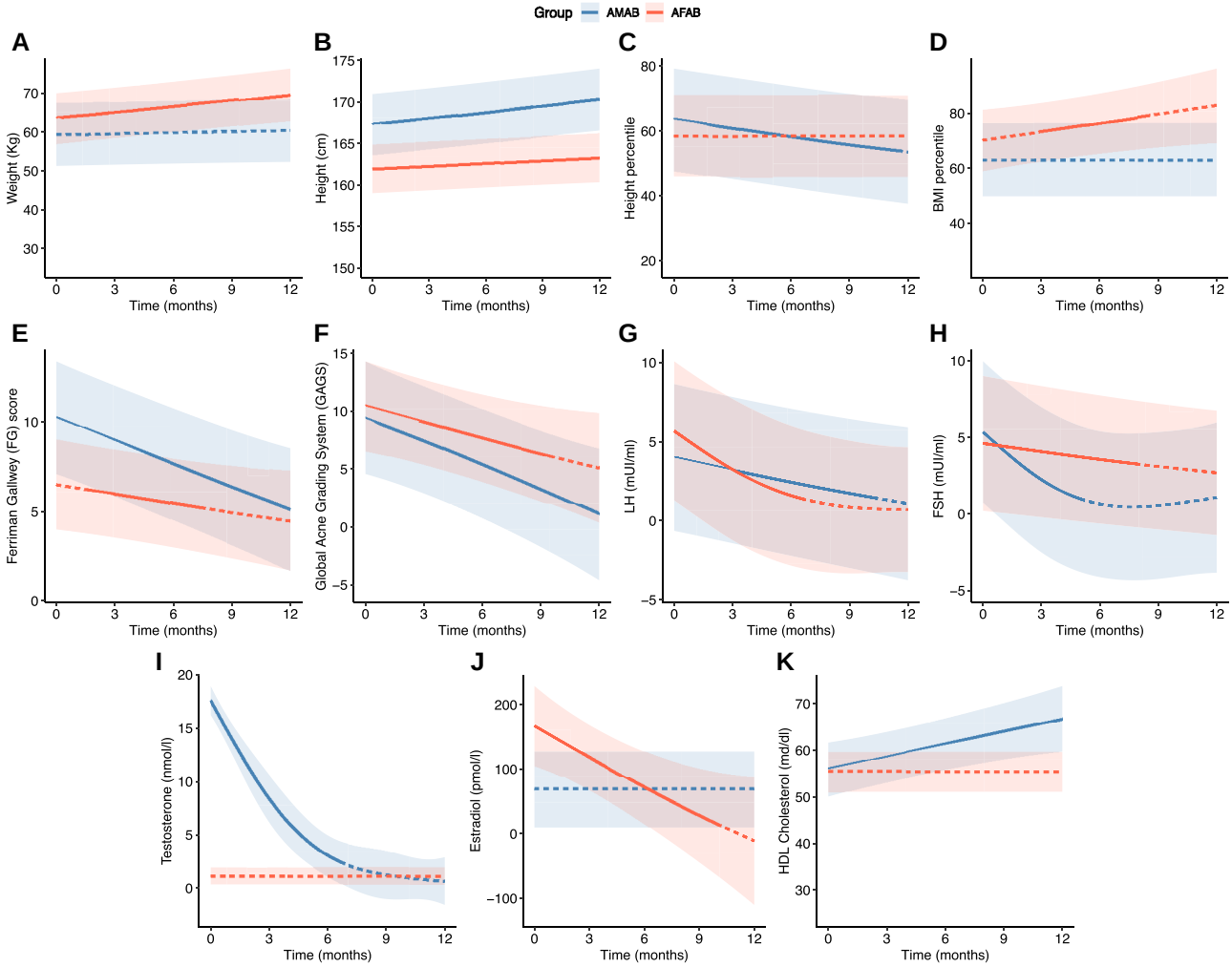

Figure 3. Physical and endocrinological characteristics over time after the beginning of GnRHa treatment. The lines represent generalized additive mixed model-based predicted values, whereas ribbons illustrate the range corresponding to 2 standard errors from such values. To allow for the complete depiction of the ribbons, the Y-axis has been permitted to extend below 0.

($b_{\Delta BMIperc*Group} = -0.03$, $P = .041$; $b_{AMAB} = 0.02$, $P = .024$; $b_{AFAB} = -0.01$, $P = .460$), whereas all adolescents reported greater body uneasiness with decreasing percentile height ($b_{\Delta HeightPerc} = -0.05$, $P = .031$; $b_{\Delta HeightPerc*Group} = 0.01$, $P = .956$).

Discussion

This is the first follow-up study exploring the impact of the possible correlation between GnRHa-induced physical or hormone changes and psychological well-being in TGDAs. The strength of the present study is in its multidisciplinary prospective design, which evaluates both the psychological and endocrinological aspects of GnRHa treatment. The size of the relationship between psychological and endocrinological effects of this treatment is also assessed. Furthermore, the psychobiological changes associated with GnRHa treatment have been systematically evaluated for the first time in a sample of Italian TGDAs. The main results of the present study were as follows: (1) GnRHa treatment was followed by a significant inhibition of puberty progression in TGDAs; (2) during GnRHa treatment, a significant improvement in psychological functioning as well as a decrease in suicidal ideation and body uneasiness, and depression and anxiety levels were observed; (3) a significant improvement in psychopathological domains was observed during GnRHa treatment (T2), while an increase in psychopathology was observed before starting GnRHa treatment (T1) compared with baseline levels; (4) physical and hormone changes observed during triptorelin treatment showed a correlation with changes in psychological functioning, suicidal risk, anxiety, and body image concerns.

Medical Efficacy of GnRHa

As expected, significant inhibition of the hypothalamus–pituitary–gonadal axis was observed after the start of GnRHa treatment, as confirmed by endocrinological assessments. Furthermore, we here report reductions in levels of gonadotropins and sex hormones. This is clinically transduced in a partial regression of the Tanner stage, as well as in a reduction of body hair growth and of the severity of acne in both TGDAs. To the best of our knowledge, no previous studies have assessed the effects of GnRHa treatment on dermatologic outcomes in TGDAs. On average, subjects of both groups, reported a statural increase. However, a reduction in height growth was observed in AMAB adolescents according to

Table 5. Psychological characteristics of the sample at baseline (T0), before (T1) and after (T2) the beginning of GnRHa, divided by assigned sex at birth

	AMAB (n = 14)			AFAB (n = 22)		
	Baseline (T0)	After psych. assessment, before GnRHa (T1)	After GnRHa (T2)	Baseline (T0)	After psych. assessment, before GnRHa (T1)	After GnRHa (T2)
YSR Total Score	63.08 ± 9.73	71.00 ± 9.48	63.15 ± 11.14	62.84 ± 10.55	71.72 ± 14.48	66.83 ± 14.92
YSR Externalizing	52.62 ± 9.38	53.50 ± 8.54	49.57 ± 10.57	57.89 ± 8.14	57.07 ± 13.69	53.00 ± 11.39
YSR Internalizing	65.62 ± 10.98	68.75 ± 9.66	59.08 ± 8.11	68.45 ± 12.14	65.50 ± 10.82	59.00 ± 11.08
YSR Aggressive Behavior	57.58 ± 7.96	57.25 ± 6.62	55.75 ± 5.67	57.85 ± 5.71	57.73 ± 6.83	54.87 ± 7.09
YSR Rule-Breaking Behavior	52.92 ± 3.94	53.33 ± 5.26	52.00 ± 3.13	57.68 ± 6.82	60.47 ± 8.55	56.40 ± 6.42
YSR Attention Problems	62.67 ± 5.65	60.67 ± 9.59	58.10 ± 10.51	62.30 ± 11.02	63.80 ± 13.13	59.40 ± 12.02
YSR Thought Problems	57.42 ± 7.55	59.25 ± 10.55	54.33 ± 5.90	63.60 ± 13.20	61.33 ± 10.39	54.93 ± 7.60
YSR Social Problems	61.83 ± 9.00	63.92 ± 10.40	55.92 ± 6.01	63.05 ± 9.24	61.00 ± 10.73	57.29 ± 10.34
YSR Somatic Complaints	64.83 ± 12.44	64.69 ± 9.05	57.50 ± 6.89	67.30 ± 12.25	64.70 ± 13.03	59.40 ± 7.43
YSR Withdrawn/Depressed	63.25 ± 13.42	62.67 ± 8.71	56.00 ± 6.61	68.85 ± 12.84	62.47 ± 10.17	63.60 ± 13.45
YSR Anxious/Depressed	67.25 ± 10.95	70.25 ± 12.35	60.33 ± 9.94	65.75 ± 11.22	63.36 ± 12.88	58.18 ± 9.91
MAST Attraction to Death	2.99 ± 1.67	2.37 ± 0.87	2.30 ± 0.82	2.44 ± 0.80	2.51 ± 0.79	2.29 ± 0.82
MAST Repulsion by Life	2.68 ± 0.53	3.31 ± 2.35	2.31 ± 0.69	3.01 ± 0.74	2.96 ± 0.70	2.45 ± 0.96
MAST Attraction to Life	3.54 ± 1.61	2.99 ± 1.02	3.63 ± 0.75	3.45 ± 0.58	3.24 ± 0.80	3.58 ± 0.79
MAST Repulsion by Death	2.43 ± 1.76	2.57 ± 1.28	2.14 ± 1.28	2.59 ± 1.46	2.29 ± 2.19	1.83 ± 1.01
BUT-A GSI	2.96 ± 1.05	3.19 ± 0.94	2.48 ± 0.80	3.00 ± 0.82	3.18 ± 0.82	2.78 ± 0.96
BDI	17.23 ± 11.99	19.25 ± 13.62	9.31 ± 7.03	17.70 ± 11.52	17.47 ± 10.46	11.33 ± 8.64
BAI	17.69 ± 10.44	20.75 ± 15.64	12.29 ± 9.11	18.47 ± 11.60	16.53 ± 12.57	10.47 ± 10.72

Between-group comparisons were not performed as they were considered irrelevant to the objectives of the study.

Table 6. Longitudinal trend of psychological measurements

	T0	T1	T2	Time Effect (F)
YSR Total Score	62.94 ± 10.07	71.41 ± 12.36[a]	65.29 ± 13.39[b]	**9.80, $P < .001$**
YSR Externalizing	55.75 ± 8.91	55.48 ± 11.62	51.50 ± 11.00[a]	**4.51, $P = .015$**
YSR Internalizing	67.33 ± 11.61	67.00 ± 10.23	59.03 ± 9.79[a,b]	**14.65, $P < .001$**
YSR Aggressive Behavior	57.75 ± 6.52	57.52 ± 6.61	55.26 ± 6.39	2.71, $P = .077$
YSR Rule-Breaking Behavior	55.84 ± 6.26	57.30 ± 8.01	54.44 ± 5.60	2.38, $P = .104$
YSR Attention Problems	62.44 ± 9.26	62.41 ± 11.59	58.88 ± 11.23	2.19, $P = .123$
YSR Thought Problems	61.28 ± 11.67	60.41 ± 10.31	54.67 ± 6.78[a,b]	**7.45, $P = .002$**
YSR Social Problems	62.59 ± 9.02	62.30 ± 10.48	56.65 ± 8.48[a,b]	**12.92, $P < .001$**
YSR Somatic Complaints	66.38 ± 12.18	64.70 ± 11.47	58.69 ± 7.06[a,b]	**9.44, $P < .001$**
YSR Withdrawn/Depressed	66.75 ± 13.13	62.56 ± 9.37[a]	60.22 ± 11.43[a]	**7.86, $P = .001$**
YSR Anxious/Depressed	66.31 ± 10.97	66.54 ± 12.87	59.07 ± 9.80[a,b]	**11.82, $P < .001$**
MAST Attraction to Death	2.67 ± 1.24	2.45 ± 0.81	2.30 ± 0.81	2.17, $P = .124$
MAST Repulsion by Life	2.88 ± 0.67	3.12 ± 1.62	2.39 ± 0.85[b]	**3.71, $P = .031$**
MAST Attraction to Life	3.49 ± 1.10	3.13 ± 0.90	3.60 ± 0.76	2.26, $P = .115$
MAST Repulsion by Death	2.52 ± 1.56	2.42 ± 1.82	1.96 ± 1.12	1.48, $P = .238$
BUT-A GSI	2.98 ± 0.90	3.18 ± 0.86	2.66 ± 0.89[b]	**5.06, $P = .010$**
BDI	17.52 ± 11.52	18.26 ± 11.76	10.48 ± 7.95[a,b]	**10.45, $P < .001$**
BAI	18.13 ± 10.93	18.41 ± 13.90	11.24 ± 9.96[a,b]	**5.27, $P = .008$**

F values for time effects are reported together with their respective *P* values. Statistically significant effects are reported in bold.
[a]Significantly different from T0.
[b]Significantly different from T1.

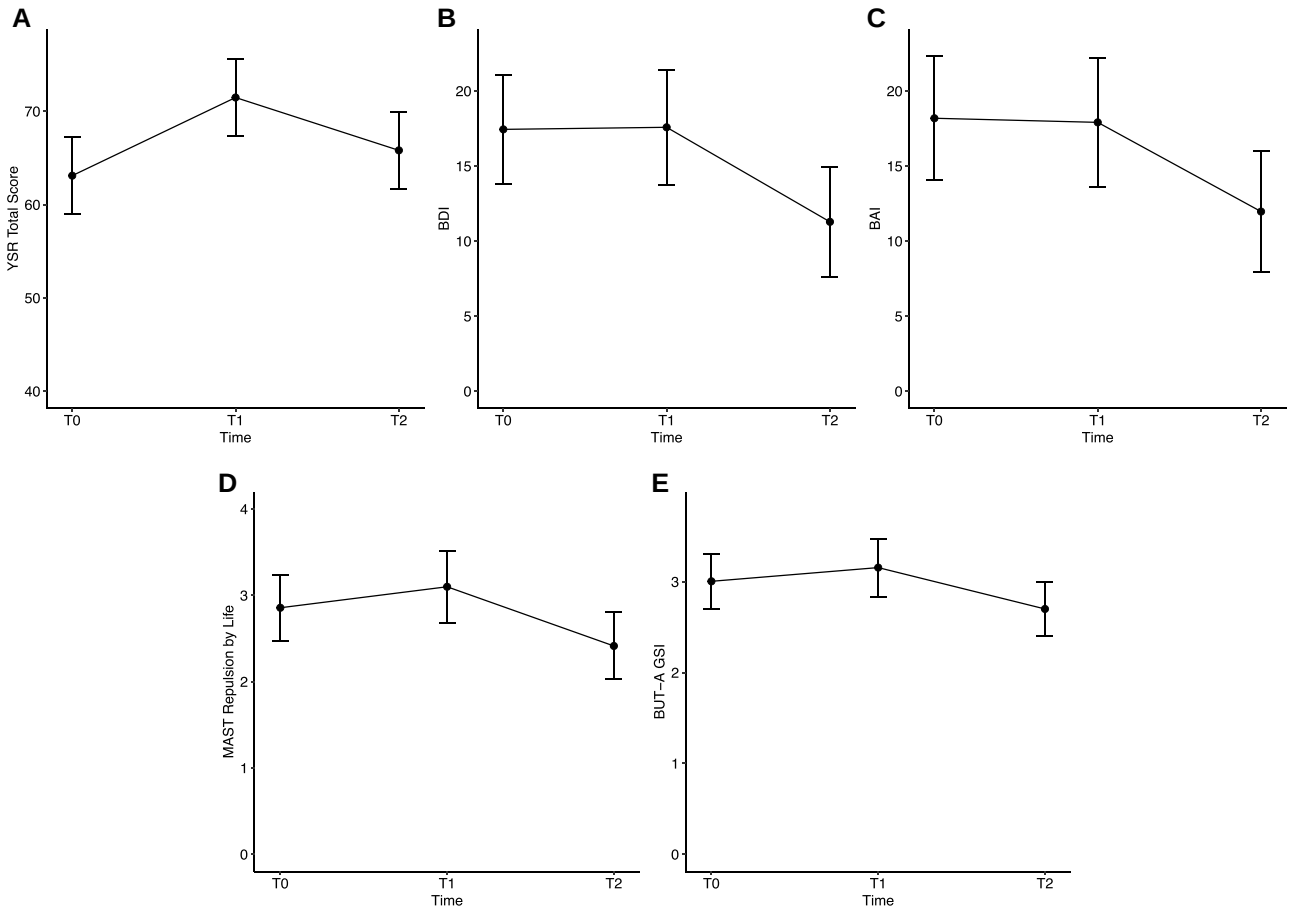

Figure 4. Longitudinal trends of YSR total score, BDI, BAI, MAST Repulsion by Life, and BUT-A GSI. Bars illustrate the range corresponding to 2 standard errors from predicted values.

growth percentile curves. A reduction in growth rate is a known effect of GnRHa treatment; however, this is expected to be temporary, considering the central role of sex hormones in the induction of the pubertal spurt, as demonstrated by recent studies both in AMAB and AFAB adolescents (20, 21). Significant weight and BMI increases were found in the AFAB group. Previous studies reported transient weight gain during GnRHa treatment (22, 51). There were no changes in blood tests in terms of HbA1c, transaminase, and lipid structure, except for a slight increase in HDL in AMAB subjects. Likewise, no significant changes in BP and waist and hip circumference were found. Other previous studies show similar data, except for HDL values which were found to be slightly reduced (22, 52).

Psychological Effects of GnRHa

Regarding psychological functioning, a reduction in both internalizing and externalizing problems was found upon GnRHa therapy, as shown by significant differences in psychological functioning (YSR) and depression (BDI) before and after the start of treatment. In particular, on average, internalizing YSR scored under the clinical cut off at T2. In the present study we also provide evidence for the first time that anxiety (BAI) and body image (BUT) significantly changed with GnRHa treatment. These findings are in disagreement with previous longitudinal studies (25, 26) in which anxiety and body image levels remained stable after 2 years of GnRHa use. Our results can be explained by the different aspects explored by the psychometric tools used. Indeed, the BAI (37) was used to assess State Anxiety, which refers to psychological and physiological transient reactions related to adverse triggering situations in a specific moment. In contrast, de Vries et al (25, 26) assessed a personality trait of anxiety (Trait Anxiety). We can speculate that State Anxiety could be more representative on how anxiety might work with TGDAs and, in particular, the decrease in anxiety could be explained with TGDAs being less worried about pubertal body changes while on GnRHa treatment. As far as body dissatisfaction is concerned, the unexpected changes observed after GnRHa treatment might be explained by the effect of treatment on body image concerns explored by the BUT. In fact, while satisfaction for individual body parts was not affected by GnRHa in previous studies using the Body Image Scale (25, 26), according to our results TGDAs were less worried about body changes once they started this medical treatment. It should also be considered that the BUT scale assesses the intimate relationship with one's own body than the distress caused by how one may appear to others (53, 54). These data stress the role of GnRHa treatment in reducing psychological distress and social difficulties when puberty moves in an unwanted direction (5, 12, 25, 55). Furthermore, the significant reduction in the "Repulsion to Life" MAST scale following the start of GnRHa treatment appears to be in line with previous literature, reporting an inversely proportional relationship between suicidal ideation and access to treatment

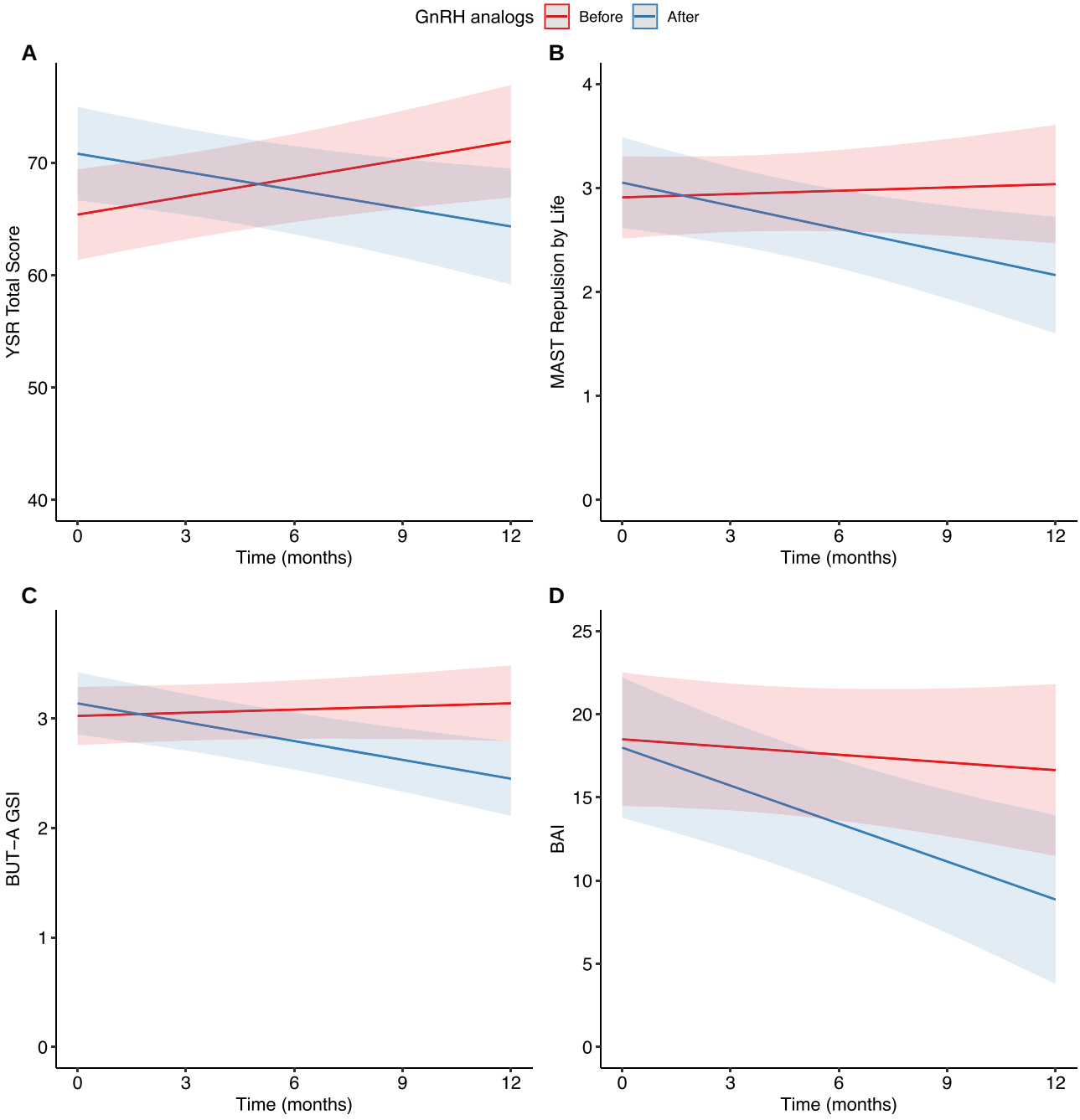

Figure 5. Longitudinal trends of psychopathological domains before and after GnRHa treatment. Ribbons illustrate the range corresponding to 2 standard errors from predicted values.

(56). Of particular interest is the initial statistically significant worsening in the total YSR score highlighted here. This seems to suggest an overall worsening of psychological functioning before the start of GnRHa treatment, then followed by a marked improvement after the start of therapy. The initial worsening could be explained as an exacerbation of GD in the peripubertal period, considering that in the absence of a GnRHa the bodily changes associated with puberty can gradually become more evident. We should also consider that adolescents present on average with an advanced Tanner stage and, therefore, with a strong desire to change some physical features. In line with this, psychological improvement—also of suicide risk (MAST), body uneasiness (BUT), and anxiety (BAI)—after the start of the GnRHa may be associated with the perception of having really started a medical gender-affirming path.

Correlation Between of Body Changes and Psychological Functioning

The longitudinal course of general psychological functioning, suicidal risk, and body uneasiness was positively associated with the physical effects and the hormone changes (height, BMI, hair growth, acne severity, reduction in plasma LH

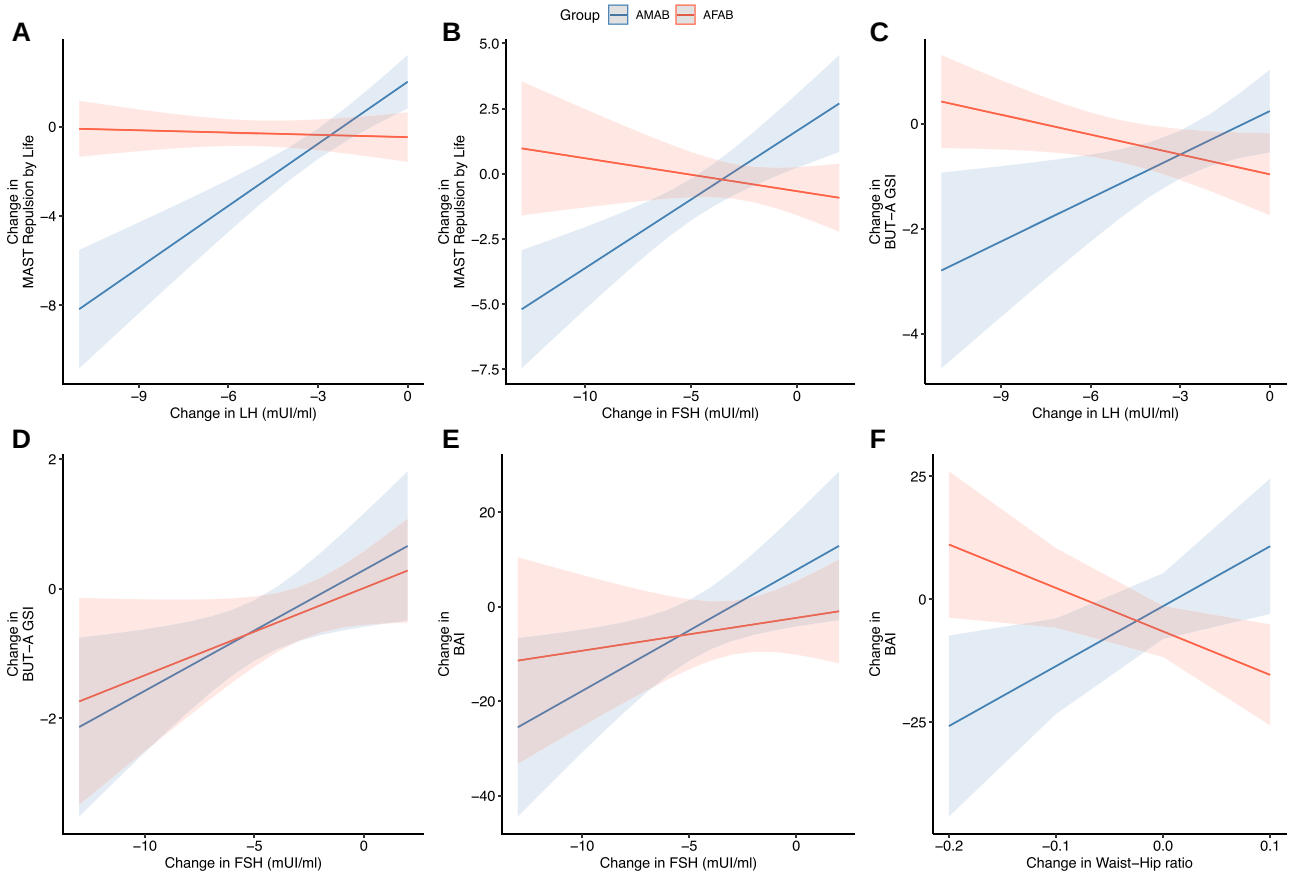

Figure 6. Correlations between endocrinological and psychometric modifications during GnRHa treatment. Ribbons illustrate the range corresponding to 2 standard errors from predicted values.

and FSH levels, waist circumference) of the GnRHa. These effects could be considered as being gender related, and the improvement in psychological functioning could be explained by those body changes being perceived as gender affirming, with the changes in gonadotropin levels being markers of an effectiveness in inducing such physical changes. In particular, reductions in both suicidal ideation and body uneasiness were more evident in the AMAB adolescent group. This could be explained as TGDA girls having more pressure to adhere to physical gender stereotypes or as a consequence of transphobic stigma that seems to affect TGDA girls more than TGDA boys. Previous studies had already documented the improvement in psychological functioning after body changes induced by gender-affirming hormonal treatment (57) and gender-affirming surgery (58, 59). This study underlines how this may also be happening regarding GnRHa treatment and may question the original rationale of this treatment that was originally developed as an extended evaluation phase. In particular, the use of a GnRHa was aimed at providing time for adolescents to think more calmly about their gender identity and about further gender-affirming steps (11, 12, 28). However, it has been recently reported that some TGDAs experience GnRHa treatment as the first formally necessary step of a seemingly clear trajectory towards further gender-affirming irreversible interventions (60). Also, the majority (93%) of TGDAs on a GnRHa requested to proceed with gender-affirming hormones later (61). In line with and considering that GnRHa treatment induces some body changes perceived as gender affirming, their use could represent the start of a medical gender-affirming path, especially in adolescents in the later stages of puberty.

Limits

The present study has several limitations. First of all, the small sample size (36 adolescents) could affect the statistical significance of the results, as multiple testing corrections were not considered feasible for this study. Furthermore, adolescents reported an advanced Tanner stage that could go beyond the rationale of the GnRHa as an extended evaluation phase. Indeed, GnRHa treatment was used in late puberty in order to stop menses in AFAB trans adolescents and to prevent further facial hair growth in trans AMAB adolescents, even though no regression of other physical sex characteristics was expected. On the other hand, one of the main findings of our study (even though it involved a very limited number of participants) is that some slight physical modifications induced by GnRHa treatment (ie, changes in waist–hip ratio) were associated with favorable outcomes in psychological functioning and body image in trans adolescents. However, most adolescents in late puberty would start hormonal treatment shortly after the start of GnRHa treatment, limiting concerns regarding a detrimental effect on bone health. Moreover, most AMAB adolescents were treated with a GnRHa as an antiandrogen therapy together with estradiol treatment in adulthood, in line with recent guidelines.

For late adolescents, there are no data to state whether and for what duration GnRHa therapy can be administered as a monotherapy without an impact on bone health (11, 62). Oral or injectable progestins (which are currently a second-line therapy, when GnRHas are not available or not indicated) might be a valid alternative to GnRHas in late puberty, especially in AFAB TGDAs. However, data about psycho-biological effects of progestins on TGDAs are still lacking. Moreover, depot medroxyprogesterone use in AFAB adolescents is associated with detrimental effects on healthy bone (63).

The assessment was based mainly on self-administered questionnaires that could affect the reliability of the answers: subjects may not have correctly understood the questions and, even if unintentionally, given the wrong answers. In interpreting the results of the YSR, MAST, and BUT-A measures in this population, it is important to note that there are no widely accepted guidelines or consensus to define what constitutes clinically meaningful changes for these psychometric outcomes. Thus, the significant changes observed in these measures should be assessed considering the study's specific context, population, and complementary findings rather than assuming a common point of clinical significance. Future research and expert consensus are needed to establish these benchmarks for clinical significance. The maximum follow-up duration was 12 months. Future studies with longer evaluations will be necessary to confirm these results and investigate the maintenance of the benefits obtained in the long term. Finally, for ethical reasons no comparison with a control group (TGDAs who are not given a GnRHa) was made, thus the study could not establish causality between the body changes provided by medical treatment and the psychological outcomes of the study participants. However, a randomized control study would raise ethical issues: transgender adolescent would have to face pubertal development regardless their psychological well-being, putting their psychological and physical health at risk.

Conclusions

This study shows the effectiveness of body changes induced by GnRHa treatment in alleviating psychological distress secondary to gender incongruence in a sample of Italian TGDAs. Interestingly, this study underlines how psychological functioning improves only following the first physical and hormone changes associated with the effects of GnRHa treatment. This seems to suggest that GnRHa treatment could be considered not only as an extended evaluation phase, but also as the start of a gender-affirming path (even if reversible).

Further studies on larger samples and with longer follow-ups are needed in order to evaluate the long-term effects of the use of a GnRHa. This study highlights once again the need for a multidisciplinary approach to the care of transgender health (11-13).

Author Contributions

A.D.F. and J.R. equally contributed to the manuscript. A.D.F. and J.R. contributed to the study conception and design. Material preparation, data collection and analysis were performed by A.R., E.C., F.M., C.C., M.P., M.M., V.R., M.M., L.V., G.C.. The first draft of the manuscript was written by A.R., E.C., F.M., C.C. The manuscript was revised critically for important intellectual content by A.D.F., J.R., M.P., M.M., V.R., M.M., L.V., G.C. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. A.D.F. and J.R. had directly accessed and verified the underlying data in the manuscript. S.D.A. reviewed the statistical analysis.

Disclosures

The authors declare that they have no conflict of interest.

Data Availability

Original data generated and analyzed during this study are included in this published article.

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