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# Lean Body Mass in Boys With Prader-Willi Syndrome Increases Normally During Spontaneous and Induced Puberty

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## Abstract

**Context:** Prader-Labhart-Willi syndrome (PWS) is a rare genetic disorder characterized by intellectual disability, behavioural problems, and hypothalamic dysfunction combined with specific dysmorphisms. In PWS, growth hormone treatment is given primarily to improve body composition, yet lean body mass (LBM) does not normalize. Male hypogonadism is frequent in PWS and becomes evident during puberty. While LBM increases in normal boys during puberty, it is not known whether LBM and muscle mass concomitantly increase in PWS during spontaneous or induced puberty.

**Objective:** To describe the peripubertal increment in muscle mass in boys with PWS undergoing growth hormone treatment.

**Design:** Single-center, retrospective descriptive study, using data from 4 years before until 4 years after onset of puberty.

**Setting:** Primary referral centre for PWS.

**Patients:** Thirteen boys diagnosed with genetically proven PWS. The mean age at onset of puberty was 12.3 years; the mean observation period before (after) onset of puberty was 2.9 (3.1) years.

**Intervention:** Puberty was induced upon pubertal arrest. All boys received internationally standardized growth hormone treatment.

**Main Outcome Measure:** Lean mass index (LMI) determined by dual energy X-ray absorptiometry.

**Results:** LMI increased by 0.28 kg/m$^2$ per year before puberty and by 0.74 kg/m$^2$ per year after the onset of puberty. The time before puberty explained less than 10% of the variation in LMI, whereas the time after puberty onset explained about 25%.

**Conclusion:** Boys with PWS showed a recognizable increment in LMI during both spontaneous and induced puberty compared with the prepubertal phase, which was within the trajectories of normal boys. Therefore, timely testosterone substitution in the absence or at arrest of puberty during growth hormone treatment is important to optimize peak LBM in PWS.

**Key Words:** Prader-Willi syndrome, males, lean body mass, hypogonadism, testosterone

Prader-Labhart-Willi syndrome (PWS) is a rare genetic disorder resulting from the lack of expression of the paternally derived chromosome 15q11-q13. PWS is characterized by intellectual disability, behavioural problems, and hypothalamic dysfunction combined with specific dysmorphisms (1, 2). Over the past 3 decades, the phenotype of children with PWS has changed. Early growth hormone treatment, rigorous nutritional restriction, and enhanced physical activity has led to a taller stature, improved body composition, and an increase in motor strength (3-5). The changes in body composition and increased motor strength are the main reasons for initiating growth hormone treatment in children with PWS (4). For those children with PWS left untreated, their fat mass index (FMI) is increased and lean mass index (LMI) is decreased compared to children with nonsyndromic obesity (6).

During growth hormone treatment, FMI decreases, but LMI only increases during the initial 6 to 12 months (3, 5). Consequently, lean body mass (LBM) and muscle mass remain low despite growth hormone treatment, while increased fat mass persists, mostly because of habitually low physical activity, respectively insatiable hunger and increased food intake (7). This often results in sarcopenic obesity and decreased cardiovascular health (8). In healthy boys, normal development of muscle mass leads to normal bone mass (9) and positively influences physical activity, energy balance, and cardiovascular health (8). Lean body and muscle mass increase at an accelerated rate during puberty and peak by 30 years of age (10). Therefore, puberty is an important period of increasing muscle mass that might be a potential window of opportunity to improve body composition and health in boys with PWS. The potential benefit is unknown, since reports on the influence of puberty on LBM development in boys with PWS are limited (11). In most boys with PWS, gonadal failure becomes evident during puberty with a frequent arrest of puberty and the need for testosterone substitution (12). There is no consensus on when and how to dose sex

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steroid substitution (13). At our institution, we substitute testosterone as soon as gonadal failure becomes evident during puberty, yet we did not know whether this strategy leads to normal increases in LBM. There are no studies that have examined this specific question. Therefore, we initiated this retrospective study to describe the peripubertal increment in LBM in boys with PWS undergoing growth hormone treatment and timely induction of puberty and interpret the results using LMI trajectories from a population of healthy boys.

## Methods

### Design

This study was approved by the local ethics committee (ethics committee of the Canton Zurich: BASEC 2021-322). Parents or the caregivers of patients provided written consent. For this retrospective study, we reviewed the medical records of male patients with PWS who were treated at our institution. The diagnosis of PWS had been established genetically in all patients. The main inclusion criteria were treatment at our institution during the prepubertal and pubertal phases and that puberty was reached in 2017 at the latest, to ensure a pubertal observation period of sufficient length at the time point of data analysis in 2021. An additional inclusion criterion of at least 1 dual energy X-ray absorptiometry (DXA) measurement within 4 years before the onset of puberty, 1 DXA measurement at the onset of puberty, and at least 2 DXA measurements within 4 years after the onset of puberty were required for evaluation. Patients were excluded from the evaluation if any of the aforementioned DXA measurements were missing. Induction of puberty and substitution of gonadal hormones was not standardized but based on the discretion of the primary physician in a single center. At our institution, in the period before 2010, we administered 500 to 1500 IU human chorionic gonadotropin (hCG) twice weekly, when pubertal arrest became evident. Since 2010 we administer testosterone enanthate with a starting dosage of 100 mg/4 weeks in this situation. The dosage is then increased to 250 mg/4 weeks after 1.5 years.

### Clinical Assessment

The onset of puberty was defined as the first consultation at which serum testosterone levels from blood samples taken early in the morning were $\geq 0.8$ nmol/L (14). The age interval of interest for data collection was defined as $\pm 4$ years around the onset of puberty.

Our primary outcome is the slope of prepubertal and pubertal course of the LMI, where LMI is the ratio of LBM in kilograms (kg) to height in square metres ($m^2$). LBM is a close approximate to muscle mass and was derived from DXA measurements. The FMI was calculated accordingly. We exported all available DXA measurements within the age interval of interest from the electronic patient files including LBM, fat mass, and bone mass. DXA measurements were, in principle, routinely made at our institution during the yearly standard consultations using Hologic QDR 2000 (7 cases) and later using Hologic Discovery Wi (5 cases) machines (Hologic; Bedford, MA). Array modes were used on both machines. As recommended, we determined regression equations for body composition parameters during the transition phase to the new machine using 51 test subjects. We found that the effect of transforming values from the old to the new device was negligible on our target outcome, the change in slope between prepubertal and pubertal phase. All within-person longitudinal measurements stemmed from the same DXA machine. We allowed 1 exception, where the device was changed for the pubertal phase assessment after the onset of puberty (Patient #2). We think a model change at this time point is acceptable, given the low case numbers for this rare disease.

Further variables extracted from the electronic patient files for the age interval of interest were height and weight measured using standard techniques (15), age, bone age (16), height velocity per year calculated from 6-monthly interval measurements, testicular volume expressed as the maximum of both sides using a Prader orchidometer, pubic hair according to Tanner stages, concentrations of testosterone and follicle-stimulating hormone (FSH) from morning serum,

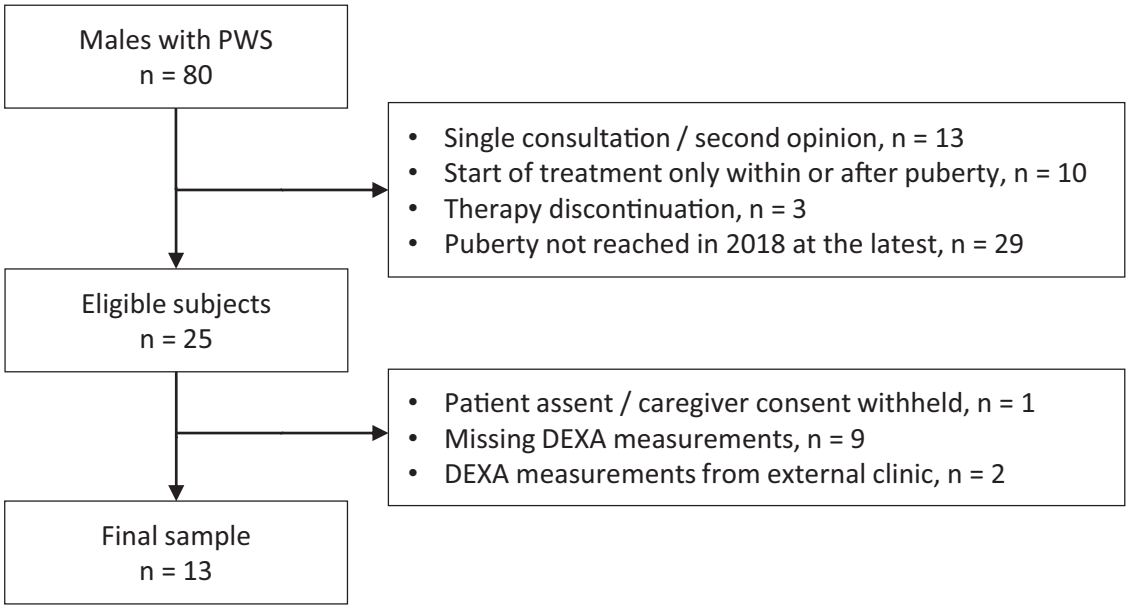


**Figure 1.** Inclusion flow chart.

and, if applicable, the beginning and type of gonadal substitution.

## Hormone Measurements

Serum FSH was determined using Access FSH Kit (Beckman Coulter; catalog no. 33520, RRID:AB_2750983) with a detection limit of 0.2 U/L and intra- and interassay coefficients of variation of less than 10%.

Serum testosterone was determined using Architect$^{TM}$ 2nd Generation Kit (Abbott; catalog no. 2P13, RRID: AB_2895254) with a detection limit of 0.05 nmol/L and intra- and interassay coefficients of variation of less than 8%.

## Statistical Methods

We were interested in the average effect of time and FMI on LMI in boys with PWS in the prepubertal and pubertal phases. For statistical analysis, we chose 2-level linear mixed models. The average effect of both variables on LMI was expressed by the regression coefficients (slopes) for the so-called fixed effects calculated in the models. Furthermore, we considered interindividual differences regarding LMI at $t = 0$ (the so-called random intercept) and interindividual responses of LMI change to time (random slope) in the model. We separated the time around puberty into 2 periods and calculated respective models: 1 for the prepubertal period and 1 for the pubertal period. Time was centered to the onset of puberty as defined previously. The models were estimated using the restricted maximum likelihood method and nloptwrap optimizer. LMI for both periods showed a moderate skewness ($\leq$0.85), and FMI was highly skewed ($\geq$0.97). Before the inclusion of FMI values into the models, we reduced the skewness by using a log10 transformation.

In our first approach, we fitted the models to predict LMI with time only as a fixed effect and time and subject as random effects [Model A formula: LMI ~ time + (time | subject)].

As a second approach, FMI_log10 was added as a fixed effect to test for influences on LMI [Model B formula: LMI ~ time + FMI_log10 + (time | subject)]. We used the Akaike and the Bayesian information criterion to measure the model

**Table 1. Sample description at the onset of puberty**

| Parameter | n | Mean ± SD | Quartiles (25., 50., 75.) | min | max | CV | Shapiro-Wilk *P* |
|---|---|---|---|---|---|---|---|
| Age (years) | 13 | 12.3 ± 1.4 | 11.6<br>12.7<br>13.0 | 9.4 | 14.4 | 0.11 | .58 |
| Body height (cm) | 13 | 155.7 ± 10.1 | 148.3<br>153.0<br>161.2 | 143.7 | 178.1 | 0.06 | .25 |
| Body weight (kg) | 13 | 50.6 ± 14.1 | 42.1<br>45.9<br>52.4 | 34 | 81.8 | 0.28 | .02 |
| Testicular volume (mL) | 13 | 3.8 ± 2.6 | 2.5<br>3.0<br>4.5 | 0 | 10 | 0.68 | .37 |
| Bone age, Greulich-Pyle (years) | 11 | 13.3 ± 1 | 13.0<br>13.2<br>13.7 | 11 | 15 | 0.08 | .45 |
| Height velocity (cm/year) | 13 | 5.5 ± 1.6 | 4.8<br>5.6<br>6.2 | 1.3 | 7.8 | 0.29 | .11 |
| Lean mass (kg) | 13 | 31.1 ± 5.7 | 27.3<br>29.4<br>32.6 | 23.9 | 42.1 | 0.18 | .24 |
| Fat mass (kg) | 13 | 17.6 ± 8.4 | 12.5<br>15.6<br>19.3 | 8.2 | 36.7 | 0.48 | .03 |
| Bone mass (kg) | 13 | 1.4 ± 0.3 | 1.3<br>1.4<br>1.6 | 1.1 | 1.9 | 0.21 | .71 |
| Lean mass index (kg/m$^2$) | 13 | 12.8 ± 1.3 | 11.7<br>12.5<br>13.3 | 11.3 | 15.5 | 0.10 | .28 |
| Fat mass index (kg/m$^2$) | 13 | 7.0 ± 2.6 | 5.5<br>7.1<br>7.8 | 4.0 | 13.5 | 0.37 | .09 |
| FSH (IU/mL) | 9 | 6.0 ± 6.0 | 2.0<br>3.1<br>13.0 | 0.6 | 15.2 | 1.00 | .01 |

Abbreviations: CV, coefficient of variation given as a ratio of the standard deviation to the mean; FSH, follicular-stimulating hormone; max, maximum; mean, arithmetic mean; min, minimum.

**Table 2. Model fit summary and explanatory power of the prepubertal and pubertal Models A and B**

| Period | Model[a] | AIC | BIC | Conditional $R^{2,b}$ | Marginal $R^{2,c}$ | $P^d$ |
|---|---|---|---|---|---|---|
| Prepubertal | A | 125.00 | 137.36 | 0.88 | 0.07 | |
| | B | 124.70 | 139.11 | 0.88 | 0.07 | .89 |
| Pubertal | A | 157.86 | 169.90 | 0.91 | 0.23 | |
| | B | 154.31 | 168.36 | 0.88 | 0.30 | .06 |

Abbreviations: AIC, Akaike information criterion; BIC, Bayesian information criterion (both are used for comparison of model candidates; smaller values describe a better model), *P*.
[a]Model A formula: Lean mass index ~ time + (time | subject), Model B formula: Lean mass index ~ time + fat mass index_log10 + (time | subject).
[b]Conditional $R^2$ = the fixed and random effects variance divided by the total variance, indicating how much of the model variance is explained by the complete model.
[c]Marginal $R^2$ = the fixed effects variance divided by the total variance, indicating how much of the model variance is explained by the fixed effects part of the model.
[d]Probability value from the likelihood ratio test, to see if Model B explains more variability than Model A.

fit, with smaller values indicating a better fit. The fit of Models A and B were compared using the likelihood ratio test and 95% CIs, and *P*-values were computed using the Wald approximation. Additionally, we visually inspected Q-Q-plots and histograms of the residuals to check the assumptions of normality and homogeneity of variances (homoscedasticity) as a precondition for the use of linear mixed models with our data.

We expected models of the prepubertal and pubertal periods to differ in LMI slope with the pubertal period showing a steeper slope.

After identifying the best models for the prepubertal and pubertal periods, longitudinal change of LMI was visualized for individual patients over time with their model predictions and individual data points. For interpretation purposes, we added the 25th and 75th percentiles of LMI for healthy European boys (17).

Descriptive statistics were used to report patient demographics, and CIs were calculated at the 95% level. The correlation between lean mass and bone mass was calculated using the Pearson correlation coefficient (r). Analyses were conducted using R statistical language (version 4.0.3) (18) and the packages lme4 [version 1.1.27 (19)] and lmerTest [version 3.1.3 (20)].

## Results

The inclusion criteria were met by 25 subjects from a total of 80 male PWS patients documented in our center (Fig. 1). Twelve cases had to be excluded because DXA measurement data were either absent or derived at an external institution or parent/guardian consent was withheld. The final sample for this evaluation included 13 patients, who were all undergoing growth hormone treatment during the study period.

On average, the boys were 12.3 years old at the onset of puberty with a bone age of 13.3 years (Table 1). Height velocity and testicle size were heterogeneous, and the frequencies of Tanner stages were 2 stage I, 2 stage II, 6 stage III, 2 stage IV, and 1 stage V. The variation in FMI was almost 4-fold larger than the variation in LMI. Lean mass correlated strongly with bone mass at the onset of puberty [r = 0.91, 95% CI (.73, .97)]. The mean observation periods before and after onset of puberty were 2.9 years and 3.1 years, respectively.

In 4 subjects, the onset of puberty was related to the start of gonadal substitution (1 boy received testosterone enanthate; 3 boys received hCG). For the remaining 9 subjects, puberty started spontaneously, whereby 5 received gonadal substitution initiated within 2 years after the onset of puberty (2 boys received testosterone enanthate; 3 boys received hCG).

**Table 3. Parameter estimates for the final Model A**

| Period | Parameter | Beta[a] | Beta 95% CI | Random effect[b] (SD) |
|---|---|---|---|---|
| Prepubertal | Intercept[c] | 12.70 | [12.01; 13.39] | 1.18 |
| | time[d] | 0.28 | [0.16; 0.40] | 0.09 |
| Pubertal | Intercept[c] | 12.72 | [12.03; 12.43] | 1.14 |
| | time[d] | 0.74 | [0.52; 0.97] | 0.30 |

[a]The predicted change in the dependent variable (here: lean mass index) when a predictor (here: time) is increased by one unit while holding all other predictors constant.
[b]Interindividual differences, variability not explained by model parameters.
[c]Constant, is the predicted value when time is set to 0.
[d]Predictor variable.

The total explanatory power of the prepubertal and pubertal A models was substantial (prepubertal conditional $R^2$ = 0.88; pubertal conditional $R^2$ = 0.91). The part related to the fixed effect "time" alone (marginal $R^2$) was 0.07 for the prepubertal phase and 0.23 for the pubertal phase (Table 2). The likelihood ratio test revealed that the B models, which considered "time" and "FMI" as fixed effects, were no better than the A models ($P > .05$). Also, the values of the Akaike and the Bayesian information criterion from Models A and B were close.

The intercept, corresponding to the onset of puberty, coincided with a LMI of 12.7 [95% CI (12.0, 13.4)] for both models (Table 3). Within the prepubertal phase, the effect of time on LMI, represented by the coefficient "beta," was statistically significant and positive. Beta quantifies the increase of LMI (slope) with 0.28 kg/m$^2$ per year [95% CI (.16, .40), $t(52) = 4.87$, $P < .001$]. Within the pubertal phase, the effect of time on LMI was stronger, with an increase of 0.74 kg/m$^2$ per year [95% CI (.52, .97), $t(49) = 6.76$, $P < .001$]. It should be noted that the CIs of the prepubertal and pubertal slopes are not overlapping, indicating that they are really different. Q-Q-plots and histograms of the residuals of both A models [see Supplemental Data (21)] confirmed our assumptions of normality and homogeneity of variances, thus justifying the use of the linear mixed models for the analysis.

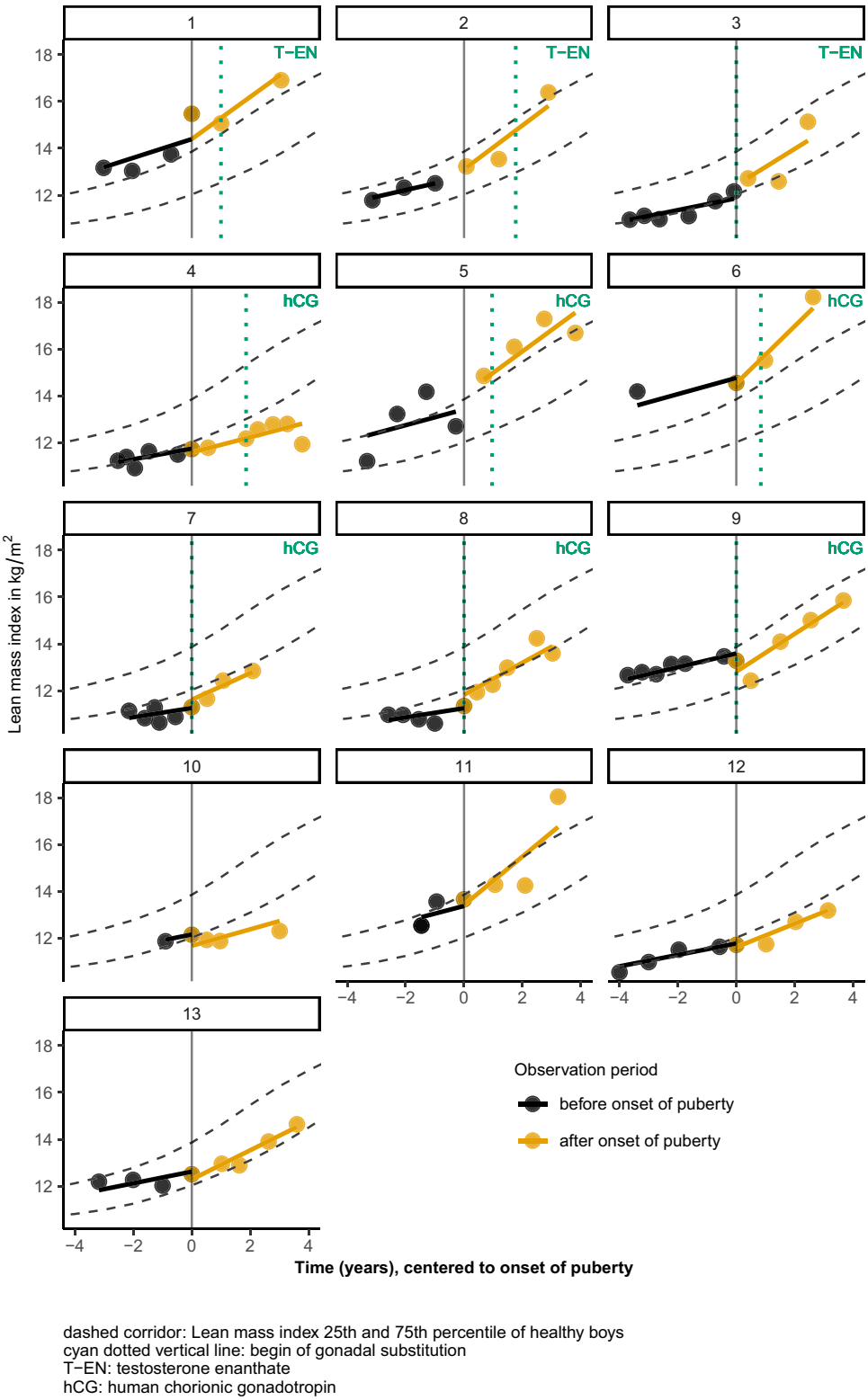


**Figure 2.** Lean mass index in the 13 individual patients over time with their model A predictions and respective data points. NB: Different DXA equipment was used for patient #2 DXA at time = 0.

Abbreviations: DXA, dual energy X-ray absorptiometry.

Figure 2 shows the evolution of LMI in the 13 individual patients over time with their Model A predictions and individual data points. Figure 3 shows the estimated slopes of the individual patients before and after onset of puberty grouped by treatment method.

## Discussion

The presented data clearly show that LBM increases during puberty in boys with PWS and is comparable to the trend reported in normal boys. By evaluating LMI, we considered and corrected for the increase in body height. We were able to

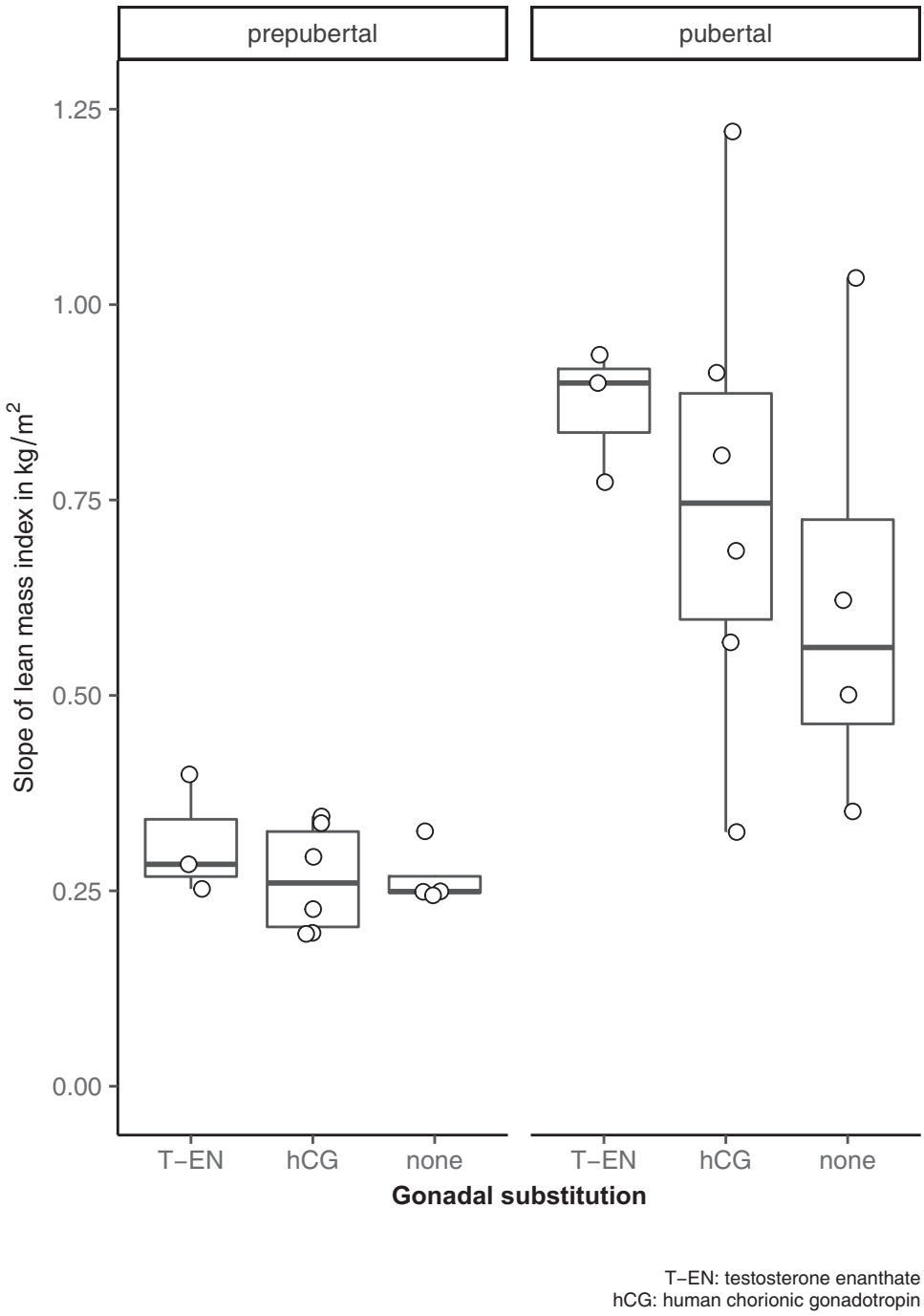


**Figure 3.** Lean mass index slope estimates for the 13 individual patients before and during pubertal phase. Patients are grouped by type of gonadal substitution during pubertal phase.

show that the increase in LBM after either spontaneous or induced onset of puberty was significantly different compared to the increase in the prepubertal period. Before puberty, time explained less than 10% of the variation in LMI, whereas time explained about 25% of the variation in LMI after the start of puberty. That still leaves room for other factors to influence LMI. We tested the influence of the amount of fat, which had no additional effect on the development of LBM, probably because of the lack of physical activity typically observed in these patients. The pubertal increase in LBM occurred when morning serum testosterone levels increased above 0.8 nmol/L. This level corresponds to the threshold commonly used to biochemically define the onset of puberty in boys (14). The timing of this event around the age of 12 in our sample represents an early normal age for entering puberty. Throughout the follow-up period, most boys received testosterone or hCG substitution to maintain pubertal progression; LBM increased further during this treatment.

Based on a case study focused on the induction of puberty with hCG in 6 adolescent males with PWS, it was suggested that muscle mass increases after the induction of puberty (11). The current study confirms this hypothesis. In a later study, an increase in LBM after 24 months of testosterone

treatment in adult men who had never received substitution with sex steroids was observed (22); most of these cases attained spontaneous onset of puberty that was nonetheless superseded by pubertal arrest. Long-term observations are missing.

What can we learn from the current observations? During spontaneous and induced puberty and testosterone substitution in boys with PWS, LBM increases in a physiological manner. There is no consensus on how to induce puberty and supplement testosterone in boys and men with PWS (13), but this is an important issue as hypogonadism is prevalent in PWS (12, 23, 24) and our data show that puberty is a sensitive phase for the development of LBM. At our institution, we administer testosterone enanthate with a starting dosage of 100 mg/4 weeks at a mean age of 13.5 (12.5-15.5) years, when pubertal arrest becomes evident in males. The dosage is then increased to 250 mg/4 weeks after 1.5 years. We administer 750 to 1000 mg testosterone enanthate every 12 weeks in adult men. Before 2010 we administered 500 to 1500 IU hCG twice weekly. Now, testosterone enanthate is considered the treatment of choice in boys with PWS because PWS-associated hypogonadism is a combination of primary and secondary hypogonadism (23). Six boys in the current study received hCG substitution during this earlier period. Three boys received testosterone enanthate according to the current treatment regimen. Four boys received no substitution because of their endogenous testosterone production. The differences in LMI increase between these groups (Fig. 3) are too small to draw conclusions given the observed high variance and small sample. However, the findings tend to confirm testosterone enanthate as treatment of choice for gonadal substitution in PWS. Our data show that LBM increases in a normal way during the first 3 to 4 years of puberty regardless of whether this stage of physical maturation occurs spontaneously or is induced. This is an important finding. As shown in our cohort, LBM strongly correlates with bone mass, and it can be assumed that the development of bone mass will also be enhanced after the induction of puberty and testosterone substitution. Furthermore, LBM and the amount of muscle mass positively influence energy balance, physical activity, insulin sensitivity, and cardiovascular health. With increased fat mass in almost all cases of PWS, optimal development of LBM is particularly important. Optimal peak LBM and muscle mass concomitantly mitigate sarcopenic obesity and increase cardiovascular fitness (8). Optimum muscle mass may also have a positive effect by lessening the development of scoliosis, a condition frequently associated with PWS and probably due to decreased muscle mass and hypotonia (25). During growth hormone treatment, LBM remains low in PWS (5, 26). Thus, appropriate and timely substitution of testosterone is of utmost importance in boys with PWS to achieve an optimal, albeit still lower, muscle mass. Differences in physical activity explain differences in attained peak LBM in normal individuals (10). Therefore, physical training is of added importance to further optimize peak LBM during growth and puberty.

The current study is accompanied by some limitations. The study has a retrospective design, and the treatment protocol was not standardized, although the latter was based on the discretion of the primary physician in a single center. The number of patients is rather small, which is expected for this rare disease. The testosterone measurements were determined using a chemiluminescence and not by mass spectrometry, which could have influenced precision of the measurements in the lower range of the measurement. Two different DXA devices were used, although consistency per patient was ensured for all but 1 patient, where the device was changed for the pubertal period.

Based on the current evidence, the next step would be to explore the effects of varied (pubertal) testosterone substitution regimens and physical training on peak LBM during growth hormone treatment in a prospective study. Ultimately, we would like to maximize the development of peak LBM in PWS during growth hormone treatment by optimizing the timing and dosing of testosterone substitution. This would further improve body composition and health in PWS. A further step should also focus on enhancing the monitoring of LBM; DXA measurements that are dependent on specific and rather expensive equipment could be replaced by more frequent and accessible clinical measurements of LBM.

## Conclusion

Boys with PWS who receive growth hormone treatment showed an increase in LMI during spontaneous and age-appropriate induced puberty that is comparable to normal boys. Appropriate and timely substitution of testosterone during growth hormone treatment is of utmost importance for boys with PWS to achieve optimal muscle mass, which can foster positive health effects in these patients.

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## Disclosures

The authors have nothing to disclose.

## Data Availability

For the purposes of patient confidentiality, restrictions apply to the availability of some or all data generated or analyzed during this study. The corresponding author can detail the restrictions and any conditions under which access to some data may be provided upon request.

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