DOI: 10.4274/jcrpe.galenos.2024.2024-2-18

Research Article

Frequency of Delayed Puberty in Boys with Contemporary Management of Duchenne Muscular Dystrophy

McCarrison S et al. Delayed Puberty in DMD

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What is already known on this topic?
Delayed puberty represents a documented clinical outcome associated with the prolonged administration of glucocorticoids in adolescents with Duchenne Muscular Dystrophy (DMD). Nevertheless, there exists a dearth of comprehensive data regarding the prevalence and severity of delayed puberty in individuals affected by DMD.

What this study adds?
Based on clinical assessments conducted by a paediatric endocrinologist, delayed puberty was observed in a striking 82% of boys with DMD who were receiving daily glucocorticoids. Our findings underscore the critical importance of implementing regular puberty monitoring and managing delayed puberty in adherence to international standards of care. Additionally, these results offer a valuable benchmark for evaluating the efficacy of newer treatment strategies designed to mitigate glucocorticoid-related side effects.

Abstract
Background: Delayed puberty is thought to be common in boys with Duchenne muscular dystrophy (DMD) treated with long term oral glucocorticoid. This study aims to report the frequency of delayed puberty in DMD from examination by a paediatric endocrinologist alongside detailed endocrine investigations.
Methods: All boys with DMD aged at least 14 years in January 2022 known to the paediatric neuromuscular service (2016-2022) were included in this study. Delayed puberty was defined based on testicular volume and genital staging in comparison to published puberty nomogram.
Results: Twenty-four out of 37 boys (65%) had evidence of delayed puberty, 23/24 (96%) of those with delayed puberty were on glucocorticoid therapy all of whom were on daily glucocorticoid. On the other hand, 7/13 (54%) of those with normal timing of puberty were on glucocorticoid; 2/7 (29%) were on the intermittent regimen. Of those who were on daily glucocorticoid therapy at the time of assessment of puberty, 23/28 (82%) had evidence of delayed puberty. In boys with delayed puberty, endocrine investigations showed low luteinizing hormone (LH) with undetectable testosterone levels, a pre-pubertal response with lutenizing hormone releasing hormone test and sub-optimal testosterone levels with prolonged human chorionic gonadotropin stimulation.
Conclusion: The frequency of delayed puberty in boys with DMD was 65%. Eighty-two percent of adolescent boys with DMD on daily glucocorticoid had evidence of delayed puberty. Biochemical investigations point to functional central hypogonadism in these adolescents. Our data supports the routine monitoring of puberty in boys with DMD.
Keywords: Delayed puberty; Hypogonadism; Glucocorticoid; Deflazacort; Prednisolone

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05.03.2024
18.06.2024

Published: 24.06.2024

Introduction
Duchenne muscular dystrophy (DMD) is a X-linked rare muscular dystrophy affecting approximately 1 in 3500 boys due to a mutation in the dystrophin gene. Despite advances in research in DMD, there is currently still no curative therapy for people with DMD (1). Long-term oral glucocorticoid is accepted worldwide as disease modifying therapy and has been standard of care for over two decades (2). Glucocorticoid is generally initiated between 4-7 years and continued indefinitely into adulthood with proven benefits on skeletal muscle, cardiorespiratory outcomes and may play a role in reducing early mortality (3). The commonest glucocorticoid regimens used worldwide are daily deflazacort, daily prednisolone and intermittent (10-days on/off) prednisolone. A recent international clinical trial confirmed the results of previous studies and smaller clinical trials, demonstrating the superiority of daily glucocorticoid (deflazacort and prednisolone) on skeletal muscle outcomes following three years of therapy compared with 10-days on/off prednisolone (4). However, published studies also demonstrate a higher glucocorticoid side effect burden in those treated with daily glucocorticoid, especially for endocrine consequences like growth failure, weight gain and bone fragility (5,6). Further information on the side effects of glucocorticoid in DMD is of great benefit for families to make the decision on risk versus benefits of long-term treatment.
Another known consequence of long-term glucocorticoid is its impact on pubertal development (7,8). Delayed puberty can contribute to the psychological distress in these adolescents who are already markedly different from their peers due to the short stature, obesity and cushingoid features in addition to the physical limitations. Fragility fractures are also very common; with clinical fractures reported in at least about 50% of glucocorticoid treated boys by the age of 11 years (9). With progression of normal puberty in healthy adolescents, bone accrual increases by about 40% (10). Therefore, delayed puberty in DMD is postulated to be an additional insult to the skeleton. However, there are very limited studies on pubertal delay in boys with DMD.
The aim of this study is to report on the frequency of delayed puberty in our whole clinic cohort of boys with DMD from clinical examination by a paediatric endocrinologist and biochemical assessments of the hypothalamic-pituitary-gonadal axis.

Methods

Patients

Since January 2016, all boys with DMD who were at least 13 years of age managed in the paediatric neuromuscular clinic in the Royal Hospital for Children, Glasgow were referred to the paediatric endocrine clinic for assessment of puberty, if not already known to the endocrine service. All eligible adolescents were reviewed in the paediatric endocrine clinic. Thirty-eight boys were aged 14 years or older in January 2022 and had examination of puberty at least twice. One boy was excluded due to a concurrent diagnosis of neurofibromatosis type-1 with optic glioma where early puberty can occur. A total of 37 boys are included in this report.

Pubertal assessment

Assessment of puberty was performed by a single paediatric endocrinologist (SCW), which included clinical evaluation of testicular volume and genitalia stage (11). Pubertal assessment was performed when the young person was recumbent, where possible. Based on testicular volume and genitalia stage in comparison with published puberty nomogram each boy was classified as having evidence of delayed puberty (< -2.0 SD below the mean for age) or "normal" timing of puberty (12). We utilised this method of classification rather than the traditional definition of delayed puberty (testicular volume < 4 ml by 14 years) as not all boys were reviewed exactly at 14 years of age. For boys who were treated with testosterone therapy, clinical examination prior to initiation of therapy was used for purposes of classification. For boys who did not receive testosterone therapy, either the latest clinical examination or when testicular volume was first noted to be □ 4 ml was used for purposes of classification. For boys with bilateral impalpable testes, testicular ultrasound volume in comparison with published paediatric reference data was used for classification, with boys classified as having evidence of delayed puberty if testicular ultrasound volume was < -2.0 SD below the mean for age (13). For boys with single testis or bilateral testes that were impalpable on two occasions despite attempts to milk into the scrotum, testicular ultrasound was performed.

Biochemical evaluation of testicular function

Blood samples were performed which included non-timed luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone in accordance with the 2018 international standards of care for DMD (2). Blood samples were mostly conducted in the morning (but not necessarily at 9 am) coinciding with clinical review. Testosterone was measured using liquid chromatography-tandem mass spectrometry via the Xevo TQS Tandem Mass Spectrometer (Waters Corporation, Milford, MA) with an assay sensitivity of 0.5 nmol/L. Plasma samples were extracted using Biotage supported liquid extraction (SLE), automated on the CTC PAL (MicroLiter Analytical Supplies Inc, Suwanne, Georgia), followed by ultra-performance liquid chromatographic separation. For this, assay sensitivity was 0.1 nmol/L and the intra- and inter-assay CV for this assay was < 8%. FSH and LH were measured by two-step Chemiluminescent microparticle immunoassay (CMIA) on the Abbott Architect (Abbott Laboratories, IL, USA). The functional sensitivities were 0.2 mU/L and the intra – and inter-assay coefficients were <5%.

Luteinizing hormone releasing hormone (LHRH) and prolonged human chorionic gonadotrophin (HCG) stimulation

Between 2016 and 2017, boys with DMD with delayed puberty underwent evaluation of the hypothalamic-pituitary-testicular axis which included luteinizing hormone releasing hormone (LHRH) stimulation test and prolonged human chorionic gonadotrophin stimulation (HCG) test (to evaluate the ability of the Leydig cells of the testes to produce testosterone). These were carried out prior to consideration of testosterone as part of clinical care to guide the duration of testosterone therapy in boys with DMD based on published report suggesting that the combined LHRH and prolonged HCG test may discriminate patients with central hypogonadism and self-limiting delayed puberty (14). In addition, the prolonged HCG test has also been used to attempt to facilitate testicular descent in boys with inguinal testes.

Prior to adminisation of Gonadorelin 100 microgam intravenously, blood samples for LH and FSH levels were obtained at 9 am and repeated at 30 and 60 minutes. Prolonged HCG was performed as previously described (15). Intramuscular injection of HCG 1500 IU was administered with blood samples collected at baseline and at day 4. Two further HCG injections were administered each week in the following two weeks with the final blood sample collected on day 22. From 2018, investigations prior to consideration of testosterone therapy were performed in accordance with the 2018 international standards of care for DMD, which did not include LHRH and prolonged HCG stimulation tests (2).

Radiographs: bone age and lateral thoracolumbar spine radiographs for vertebral fracture assessment

Bone age was calculated using the automated BoneExpert 3.0 software (Visiana, Denmark) in accordance with the Tanner-Whitehouse TW2 method (16,17). Annual lateral thoracolumbar spine radiographs were performed since 2015, and information on vertebral fracture was based on clinical radiological reports by consultant paediatric radiologists. For purposes of this report, lateral spine imaging within six months of evaluation of puberty was used.

Conduct of study and consent

This report was conducted as a service evaluation and clinical audit against the 2018 international standards of care for DMD in the area of monitoring and management of puberty (2). All investigations were performed as part of routine clinical care, and anonymised data was collected. Formal ethical approval and written informed consent was not required in line with regulations laid out by the United Kingdom National Health Service Health Research Authority (18). This evaluation of service was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Statisical analysis

Continuous data was presented as median (minimum and maximum). Discrete variables were reported as frequency in percentages (with 95% confidence intervals). Patients were divided into groups based on the presence or absence of delayed puberty for the purpose of comparison based on the puberty nomogram. Delayed puberty was defined as -<2 SDS as previously defined (12). Comparison of continuous variables between the group with delayed puberty and normal timing of puberty was performed using the Mann-Whitney test. Comparison of categorical outcomes between the two groups was using the Fishers-exact test. $p < 0.05$ was accepted as statistically significant. Statistics were performed using IBM SPSS Statistics (Version 29).

Results

Based on testicular volume in comparison to the puberty nomogram on clinical examination or testicular ultrasound and genital staging in comparison to the puberty nomogram, 24/37 [65%; 95% confidence interval (CI): 48% to 80%] had evidence of delayed puberty (12).

Clinical status (Table 1)

Table 1 shows the clinical characteristics of boys with delayed puberty and those with normal timing of puberty at the time of assessment of puberty. Median age of the 24 boys with delayed puberty was 14.3 years (13.6, 16.7) at assessment of puberty. Median age of the 13 boys with normal timing of puberty was 14.0 years (11.8, 16.8).

At assessment of puberty, 29% with delayed puberty were still ambulant whereas this was only noted in 8% of those with normal timing of puberty [p= 0.216]. Eight percent in the delayed puberty group required assisted ventilation with nocturnal bilevel positive airway pressure (biPAP) due to an obstructive picture. On the other hand, 15% of those with normal timing of puberty were on nocturnal biPAP all due to due to neuromuscular weakness [p= 0.601]. Only one boy in the delayed puberty group had signficiant scoliosis (defined as Cobbs angle > 20 degrees or had required surgery), whereas this was noted in 39% of those with no evidence of delayed puberty [p= 0.014] (19). Sixty-seven percent in the delayed puberty group had evidence of vertebral fracture, whereas this was only noted in 23% of those with normal timing of puberty [p= 0.017]. Ninety-six percent (23/24) in the delayed puberty group were still alive in January 2022, whereas this was noted in 77% (10/13) of those with normal timing of puberty [p= 0.115].

Glucocorticoid therapy (Table 1)
In the group with delayed puberty, 23/24 (96%) boys were on glucocorticoid, all of whom were on daily glucocorticoid. 19/23 (83%) were on daily deflazacort with 4/23 (17%) on daily prednisolone. One boy had discontinued glucocorticoid in the previous 4 years. On the other hand, 7/13 (54%) boys with normal timing of puberty were on glucocorticoid: 5 were on daily glucocorticoid. 3/7 (43%) were on daily deflazacort, 2/7 (29%) were on daily prednisolone and 2/7 (29%) were on intermittent (10 days on/10 days off prednisolone). Six of the thirteen boys (46%) with normal timing of puberty were not on glucocorticoid at assessment of puberty, having discontinued glucocorticoid for a median of 2.9 years (1.0, 6.8). The percentage of boys on glucocorticoid at time of assessment of puberty was significantly higher in the group with delayed puberty compared with the group with normal timing of puberty [96% vs 54%, p= 0.004].
Glucocorticoid dosing (Table 1)
In the group with delayed puberty and who were on glucocorticoid, median dose of glucocorticoid was 0.24 mg/kg/day (0.13, 0.67) in prednisolone equivalent. Of those who were on glucocorticoid in the group with normal timing of puberty, median dose of glucocorticoid was 0.26 mg/kg/day (0.08, 0.57) in prednisolone equivalent. In the group with delayed puberty, median duration of glucocorticoid exposure was 9.3 years (4.0, 11.5) whereas median glucocorticoid exposure was 7.0 years (3.7, 7.3) in the group with normal timing of puberty [p= 0.002]. In the group with delayed puberty, 7/23 (30%) were on daily glucocorticoid since initiation whilst the others (16/23 - 70%) were previously on the 10 days on/10 days off regimen. In the group with normal timing of puberty, 2/5 (40%) were on daily glucocorticoid since initiation whilst the others (3/5, 60%) were previously on the 10 days on/10 days off regimen. Median duration of exposure to daily glucocorticoid was 5.2 years (0.8, 10.0) in the boys with delayed puberty whereas median duration of exposure to daily glucocorticoid was 4.0 years (3.7, 6.0) in the boys with normal timing of puberty (p=0.07).
Delayed puberty in boys on daily glucocorticoid and 10 days on/10 days off glucocorticoid
Of the 28 on daily glucocorticoid at the time of assessment of puberty, 23 [82%; 95% CI: 63% to 94%] had evidence of delayed puberty. Of the 2 boys on 10 days on/10 days off glucocorticoid at time of assessment of puberty, neither boy had evidence of delayed puberty. Both boys were on 10 days on/10 days off prednisolone. The percentage of boys on daily glucocorticoid was significantly higher in the group with delayed puberty compared with the group with normal timing of puberty [100% vs 71%, p= 0.048].
Delayed puberty in boys on daily deflazacort and daily prednisolone (Table 1)
Of the 21 boys on daily deflazacort, 19 [91%; 95% CI: 70% to 99%] had evidence of delayed puberty. Of the 6 boys on daily prednisolone, 4 [67%; 95% CI: 22% to 96%] had evidence of delayed puberty.
Pubertal assessment in boys with delayed puberty
Twenty-two of the 24 boys (88%) with delayed puberty had testicular volume < 4 ml (combined testicular volume < 8 ml). Sixteen of these 21 (76%) were aged 14 years or older. Two out of the 24 boys (8%) in the delayed puberty group had clinical signs of puberty with genitalia stage 2, pubic hair stage 2, testicular volume 4 ml (combined testicular volume 8 ml) at 15.6 and 16.7 years, respectively, but were classified as having delayed puberty in accordance to the definition adopted for this study based on the puberty nomogram (12). Three boys (13%) with delayed puberty had bilateral testes or single testis in a non-scrotal location: bilateral inguinal testes (n,2), and left inguinal testis (n,1). There was no past history of neonatal and childhood undescended testes or family history of undescended testes. Twenty three out of the 24 boys were initiated on testosterone therapy at a median of 14.3 years (13.6, 16.7) in line with the 2018 international standards of care (2). All 23 boys were initiated on an escalating regime of testosterone therapy in accordance with the British Society of Paediatric Endocrinology and Diabetes testosterone replacement therapy guidance (20). Twelve boys were treated with intramuscular testosterone, eight with topical testosterone and three with oral testosterone. A total of six of the 23 boys were initiated on testosterone < 14 years at a median age of 13.7 years (13.6, 13.8), all of whom were pre-pubertal in line with the current 2018 international standards of care which state that testosterone can be considered from the age of 12 years (2). One boy with delayed puberty was not treated as the decision was made to discontinue glucocorticoid treatment when delayed puberty was identified on clinical examination.
Pubertal assessment in boys with normal timing of puberty
In the group with normal timing of puberty (median age 14.0 years), 9/13 boys (69%) were in early to mid-puberty: genitalia stage 2 or 3 and testicular volume of 4-10 ml (combined testicular volume 8-20 ml), whereas four others were in late puberty: genitalia stage 4 or 5 and testicular volume of 12-20 ml (combined testicular volume 24-40 ml). Median testicular volume was 6 ml (4, 20) with median combined testicular volume of 12 ml. All boys in this group had bilateral testes in the scrotum on clinical examination. In ten boys, follow-up clinical examination of puberty was available at median of 15.8 years (14.0,18.8). All ten boys showed further progression of puberty. Seven out of 10 achieved adult external virilisation for genitalia and pubic hair staging (i.e. genitalia stage 5, pubic hair stage 5) at follow-up examination. At follow-up examination, median testicular volume for the ten boys was 15 ml (8,20) with median combined testicular volume of 30 ml. Of the seven who reached adult external virilization at follow-up clinical examination of puberty, all had testicular volume of at least 15 ml.
Endocrine investigations
Twenty boys in the delayed puberty group and ten boys in the normal timing of puberty group has LH, FSH and testosterone measured within six months of clinical evaluation of puberty. Nineteen out of 20 (95%) boys with delayed puberty had undetectable LH levels (< 0.2 U/L) with one boy in this group with a measurable LH of 0.4 IU/L. All 10 boys with normal timing of puberty had detectable LH level- median of 0.8 (0.2, 4.2). There was a statistically significant difference between the proportion of boys with undetectable LH in the group with delayed puberty and normal timing of puberty [p< 0.0001]. Median FSH in the boys with delayed puberty was 1.1 IU/L (0.2,4.2) whereas this was 3.0 IU/L in the group with normal timing of puberty [p=0.03]. All 20 boys with delayed puberty had undetectable testosterone levels (<0.5 nmol/L), whereas all 10 in the group with normal timing of puberty had detectable testosterone levels - median of 2.4 nmol/L (0.6, 13.4). There was a statistical significant difference between the proportion of boys with undetectable testosterone in the group with delayed puberty and normal timing of puberty [p< 0.0001].
Six boys with delayed puberty had LHRH stimulation test and prolonged HCG test and results are presented in Figure 1. All boys had an undetectable LH level at baseline which rose to a peak of 1.4 IU/L (0.7, 4.4). Peak FSH level was higher than peak LH level in all six boys. All had undetectable testosterone levels at baseline of HCG test which rose to a median of 3.0 nmol/L (1.5, 5.3) on day 4. Three out of the 6 had testosterone levels > 3.5 nmol/L at day 4. By day 22, 4/6 had undetectable testosterone levels. The fourth boy had testosterone level of 2.3 nmol/L at day 4 and 0.5 nmol/L at day 22. The sixth boy had testosterone level of 3.6 nmol/L at day 4 and 5.6 nmol/L at day 22. In both boys with bilateral inguinal testes, prolonged HCG stimulation did not lead to testicular descent.

Discussion

This current report identified that delayed puberty was noted in about 65% (upper limit of 95% CI of 80%) of our entire cohort of boys with DMD and in 82% (upper limit of 95% CI of 94%) on daily glucocorticoid. Our results provide strong supportive evidence for routine monitoring of puberty as recommended by the 2018 international standards of care (2). Our results and methods for classification of delayed puberty could be used to study the impact of newer treatment strategies in DMD and pubertal delay, especially those that may be postulated to have less glucocorticoid associated side effects. Our study also identified abnormal testes location in a small number of boys but only in the group with delayed puberty (13%) - the reasons for this are unclear but could point to a state of functional central hypogonadism although further studies are needed.
Current published studies show that 50-97% of adolescents with DMD are pre-pubertal (9,21,22). However, some of these studies included younger boys (5-9 years) where onset of puberty is not expected (21,22). Information on puberty in one study was from self-assessment,

which has never been validated in this group of boys (22). Our previous retrospective report focusing on fractures in boys with DMD in all neuromuscular centres Scotland managed up to December 2015 identified that 79% (11/14) of adolescents aged 14 years or older had evidenced of delayed puberty (9). However, in that previous report, only 48% (14/29) of eligible boys aged 14 years or older had examination of puberty following referral to paediatric endocrinology due to clinical concerns. In our present study, we report puberty in the whole cohort of boys with DMD from the clinic in Glasgow following a clinical pathway of routine assessment of puberty during adolescence since 2016, thereby allowing information on frequency of delayed puberty (65%) in the neuromuscular clinic in Glasgow.

The impact of glucocorticoid regimen on pubertal development in DMD has not been previously evaluated. Published evidence shows that skeletal morbidity, growth failure and weight gain are commoner in boys with DMD treated with daily glucocorticoid in comparison with those on intermittent therapy (5,6). Our study shows the significant side-effect toxicity of daily glucocorticoid on pubertal development as just over 80% of boys with delayed puberty were on daily glucocorticoid therapy, whereas all on 10 days on/10 days off glucocorticoid had no evidence of delayed puberty. On the other hand, a report in four boys with DMD managed with daily prednisolone for the first two weeks followed by alternate day prednisolone for three years, before changing to alternate day deflazacort reported delayed puberty (23). Larger studies on the impact of intermittent glucocorticoid regimen and puberty are needed. Other factors like glucocorticoid type, dose and duration of glucocorticoid treatment may also impact on puberty. Whilst our results show that 91% of boys on daily deflazacort had delayed puberty in comparison to 67% of boys on daily prednisolone, the proportionately larger number of boys on daily deflazacort in our study do not allow us to make firm conclusions but this also requires further study.

The underlying endocrine abnormalitiy of the hypothalamic-pituitary-gonadal axis in adolescents with chronic conditions on long-term glucocorticoid is speculated to be due to a functional state of hypothalamic (central) hypogonadism (8,24). Our endocrine investigations provide supportive evidence of that in DMD. LH levels were suppressed (or low) in those with delayed puberty. LH response to LHRH stimulation indicated no evidence of activation of the hypothalamic-pituitary-gonadal axis and was associated with undetectable or low testosterone levels. In our report, with prolonged HCG stimulation, 3/6 (50%) had testosterone levels of >3.5 nmol/L at day 4 but none had testosterone level > 9.5 nmol/L at day 22. Previous publications have defined testosterone level of > 3.5 nmol/L at day 4 and > 9.5 nmol/L at day 22, following HCG stimulation as acceptable response (25). It is known that testosterone response to HCG stimulation can be poor in people with central hypogonadism (26). Five out of the six boys (83%) in our report had testosterone levels which were lower at day 22 than day 4 including four boys with undetectable testosterone levels at day 22. Previous reports using the same protocol of HCG stimulation in children with undescended testes showed that 2/16 (13%) and 2/12 (17%) had lower testosterone levels at day 22 than day 4 levels although all these children in the two previous reports still had detectable level of testosterone at day 22 (25,27). We speculate that the pattern of testosterone levels with prolonged HCG could be due to an abnormality of testosterone production due to prolonged gluccorticoid exposure.

Study Limitations

There are limitations to our current study which include the relatively small sample size. Due to the heterogeneity of glucocorticoid treatment, we are unable to conclusively clarify issues like glucocorticoid regimen, dose and type and their impact on delayed puberty. Future studies are needed including a larger group of boys on intermittent glucocorticoid regimen. Future studies should also address pubertal progression in boys with DMD in a standardized research study with regular timing of longitudinal assessments. Nevertheless, our study is the largest report of pubertal delay in adolescents with DMD with complete case ascertainment of all eligible boys in our neuromuscular clinic.

Conclusion

In summary, we report a high frequency of delayed puberty in adolescent boys with DMD especially those on daily glucocorticoid, and therefore routine examination of puberty from late childhood in boys with DMD should be mandatory, in line with the 2018 international standards of care (2).

Author Contributions

All authors contributed to the conception and design of this study; SM designed the tables and figures and assisted in drafting of the manuscript; MD collected the data; SCW drafted the manuscript and supervised the study; all authors critically revised the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

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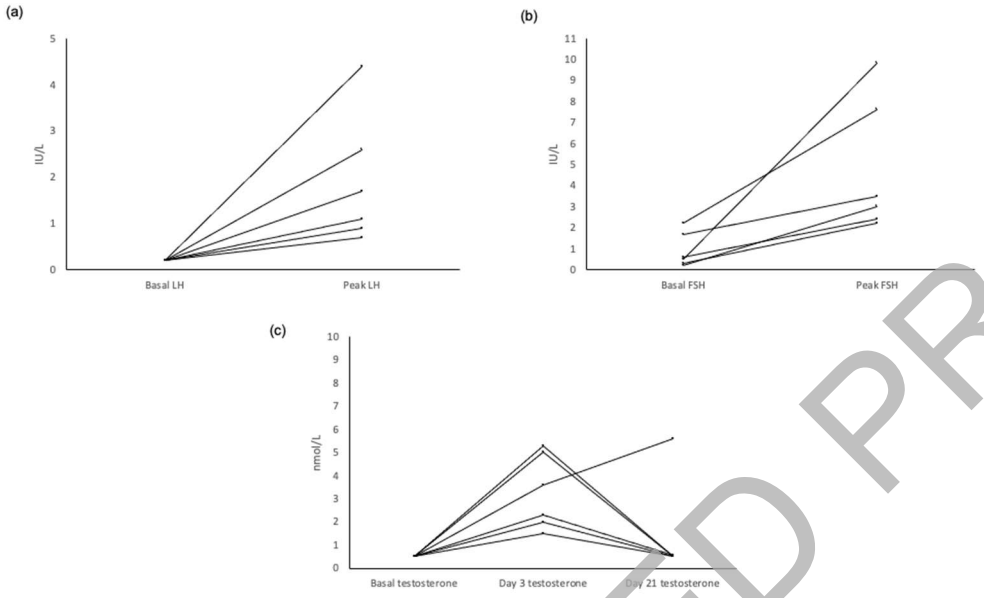
***Table 1**: Clinical characteristics in boys with delayed puberty and those with normal timing of puberty.*

	Delayed puberty (n, 24)	**Normal timing of puberty (n, 13)**	**p-value**
Age (years)	14.3 (13.6, 16.7)	14.0 (11.8, 16.8)	0.07
Ambulant	7/24 (29%)	1/13 (8%)	0.216
On oral glucocorticoid	23/24 (96%)	7/13 (54%)	0.004*
Daily glucocorticoid	23/23 (100%)	5/7 (71%)	0.048*
- Daily deflazacort	19/23 (83%)	3/7 (43%)	0.06
- Daily prednisolone	4/23 (17%)	2/7 (29%)	0.60
10 day on/10 days off prednisolone	0/23	2/7 (29%)	0.048*
Duration of glucocorticoid therapy (years)	9.3 (4.0, 11.5)	7.0 (3.7, 7.3)	0.002*
Duration of daily glucocorticoid therapy (years)	5.2 (0.8, 10.0)	4.0 (3.7, 6.0)	0.07
Dose of glucocorticoid in prednisolone equivalent (mg/kg/day)	0.24 (0.13, 0.67)	0.26 (0.08, 0.57)	0.61
Severe scoliosis [a]	1/24 (4%)	5/13 (39%)	0.014*
Vertebral fractures [b]	16/24 (67%)	3/13 (23%)	0.017*
Bone age (years)	10.7 (6.6, 14.9)	13.3 (9.4, 17.1)	0.046*

[a] Severe scoliosis is defined as Cobbs angle > 20 degrees and/or if required surgery; [b] Vertebral fracture is diagnosed by lateral thoracolumbar spine radiographs within 6 months of assessment of puberty.
Data expressed as median (range).

Figure legends

Fig. 1: LHRH and prolonged HCG stimulation test in six boys with DMD.
1a: LH response to LHRH stimulation test.
1b: FSH response to LHRH stimulation test.
1c: Testosterone response to prolonged HCG stimulation test.



LHRH: luteinizing hormone-releasing hormone; HCG: human chorionic gonadotrophin; DMD: Duchenne muscular dystrophy: LH; luteinizing hormone; FSH: follicle stimulation hormone.

LHRH stimulation test: A peak LH < 5 IU/L to LHRH stimulation and peak FSH > peak LH denotes a pre-pubertal response.

HCG stimulation test: Testosterone levels > 3.5 nmol/L at day 4 and > 9.5 nmol/L at day 22 denotes normal testosterone production.