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ORIGINAL ARTICLE

Growth hormone and testosterone delay vertebral fractures in boys with muscular dystrophy on chronic glucocorticoids

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Abstract

Summary Glucocorticoid use in Duchenne and Becker muscular dystrophy prolongs ambulation but cause significant skeletal toxicity. Our analysis has immediate clinical implications, suggesting that growth hormone and testosterone have a stronger effect prior to first and subsequent vertebral fracture, respectively, relative to bisphosphonates alone in children with dystrophinopathies on chronic glucocorticoids.

Purpose Glucocorticoids prolong ambulation in boys with Duchenne muscular dystrophy; however, they have significant endocrine side effects. We assessed the impact of growth hormone (GH), testosterone, and/or zoledronic acid (ZA) on vertebral fracture (VF) incidence in patients with dystrophinopathies on chronic glucocorticoids.

Methods We conducted a longitudinal retrospective review of 27 males with muscular dystrophy. Accelerated failure time (AFT) models were used to estimate the relative time to VF while on GH, testosterone, and/or ZA compared to ZA alone. Results are reported as failure time ratio, where >1 indicates prolonged time versus <1 indicates shorter time to next VF.

Results The prevalence of growth impairment was 96% (52% utilized GH), pubertal delay was 86% (72% utilized testosterone), and low trauma fractures were 87% (72% utilized ZA). Multivariable analysis of the AFT models showed that participants on either GH or testosterone treatment relative to ZA alone experienced prolonged time to next VF (1.253, $P<0.001$), with GH being the significant contributor when analyzed independently from testosterone (1.229, $P<0.001$). Use of ZA with GH or testosterone relative to ZA alone resulted in prolonged time to next VF (1.171, $P<0.001$), with testosterone being a significant contributor (1.130, $P=0.033$).

Conclusion GH and testosterone each decreased VF risk in patients independent of or in combination with ZA, respectively.

Keywords Bone fragility · Glucocorticoids · Growth hormone · Muscular dystrophy · Puberty · Testosterone · Vertebral fracture

Introduction

The dystrophinopathies, Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (Becker MD), are X-linked recessive disorders caused by pathogenic variants in the gene that codes for the dystrophin protein. DMD and Becker MD affect approximately 4.78 and 1.53 out of 100,000 males, respectively [1]. Diagnosis is typically made around 4 years of age, due to calf pseudohypertrophy, leg weakness, and Gower's movement [2], and is confirmed through genetic testing [3]. DMD and Becker MD both lead to delayed gross motor skills due to severe muscle degeneration that progresses throughout life [4–6]. DMD and Becker MD are on a continuum; the diagnosis is made on predicted dystrophin quantity based on genotype or measured dystrophin quantity in muscle biopsy, with Becker MD having a milder phenotype compared to DMD.

Glucocorticoids (GCs) and respiratory support have extended the life expectancy of patients with DMD from their early 20s into their 4th decade [7]. High-dose GCs such

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as deflazacort and prednisone increase the muscle mass and strength of patients, which prolongs ambulation [4, 8]. However, GC use results in multiple endocrine complications including osteoporosis, linear growth failure, and delayed puberty [2, 9, 10]. Over 75% of patients with DMD have vertebral compression fractures (VF) resulting from a combination of chronic GCs and decreased mechanical loading [2, 4, 11–13]. GCs have negative effects on the bone both directly and indirectly. GCs impact bone reabsorption directly by decreasing osteoclastogenesis while inhibiting osteoclast apoptosis. GCs also impair bone formation, inhibiting osteoblastogenesis and increasing osteoblast apoptosis [14]. Chronic GCs indirectly affect bone through linear growth failure and delayed puberty [3, 15–18]. GCs suppress the release of growth hormone (GH) and insulin-like growth factor-1 which impairs bone acquisition, as evidenced by decreasing bone mineral density (BMD) Z-scores in males with DMD [14, 19–22]. Delayed puberty/hypogonadism contributes to decreased bone mass and increased bone fragility, as more than half of a person's bone mass is accrued during puberty [2, 10, 17, 19].

Multiple endocrine interventions have been used to treat osteoporosis, linear growth failure, and delayed puberty in patients with dystrophinopathies treated with chronic high-dose GCs. For osteoporosis, bisphosphonates have become the standard of care and may increase BMD, while reducing long bone and VF incidence [23–27]. With regard to linear growth failure, GH has been previously shown to increase the patients' growth velocity and causes no short-term neuromuscular or cardiopulmonary effects [28]. In terms of delayed puberty, testosterone (T) supplementation is the standard to induce males into puberty [3, 12, 16, 29, 30]. T is associated with a mild increase in body and muscle mass in patients with DMD and improved motor function in those who are ambulatory [29]. There is a major knowledge gap that results from the study of factors associated with outcomes via a cross-sectional study, since many patients are on multiple treatments at a given moment and it is therefore more challenging to understand the role of clinical variables in the evolution of longitudinal trajectories [16, 29–31]. Small sample size and short monitoring time also limit the generalizability of previous studies [23–25, 28, 29]. Physicians rely on evidence-based studies to guide safe medical interventions for patients with dystrophinopathies based on the patients' clinical scenario [15, 18, 32]. Longitudinal studies on endocrine interventions are needed to help improve the quality of life of patients experiencing endocrine complications. We conducted a long-term retrospective research study to describe the incidence of endocrine complications in patients with dystrophinopathies on chronic GCs and assessed the impact of GH, T, and/or bisphosphonates on VF incidence. We used accelerated time failure (AFT) models rather than a standard Cox proportional hazards model to take advantage of a larger sample size using vertebral event data rather than being limited to patient numbers.

Methods

Study design

A retrospective longitudinal study on patients with DMD or severe Becker MD was conducted on a cohort of individuals treated at the Johns Hopkins University (JHU) and the Kennedy Krieger Institute (KKI) in Baltimore, Maryland. To be eligible, participants must have had a diagnosis of DMD or severe Becker MD, be on chronic GC treatment, and have been referred to an endocrinologist. Participants did not necessarily have osteoporosis at enrollment. The study was approved by both the JHU and KKI Institutional Review Boards. Informed consent was obtained from all participants included in the study.

Clinical data of the participants was manually abstracted from JHU and KKI electronic medical records into a REDCap database. Participants were typically seen every 6–12 months for follow-up through a multidisciplinary clinic. The initial clinic encounter with an endocrinologist was defined as the baseline encounter, not the time at which consent was obtained. During the baseline encounter, past medical and family history was abstracted, along with type and duration of medication use including GCs, T, GH, and bisphosphonates. GCs were initiated when symptoms were noted, generally at diagnosis. The target dose of deflazacort was 0.9 mg/kg/day (maximum dose 36 mg/day), and prednisone target was 0.75 mg/kg/day (maximum dose 30 mg/day), with the actual prescribed dose adjusted to feasible dose based on tablet size. Weekend dosing of deflazacort was 3.15 mg/kg/day on Saturday and Sunday only. The endocrinologist assessed pubertal status and diagnosed delayed puberty if he remained Tanner Stage 1 at 14 years based on gonad size. The participants' standing height was measured from vertex to feet. Arm span has been used as a surrogate to standing height in patients with severe spine deformity [33] and in oncologic patients who are unable to stand [34] and has been found to be proportional to standing height. Therefore, in our study, we measured arm span and assumed an equal measurement to standing height for non-ambulatory participants. The height Z-score was calculated in the electronic medical record per CDC growth charts. Cardiac complication was defined as changes in cardiac medication prescriptions in response to blood pressure and echocardiogram findings. Pulmonary function testing was performed by spirometry. Spine X-rays and dual-energy X-ray absorptiometry (DXA) scans were performed every 1–2 years to assess skeletal

health [2, 10, 29] with frequency based on current guidelines [3].

Endocrine interventions

GH was discussed with participants who had either height Z-score < –2 or low growth velocity for age and pubertal status. The risks and benefits of GH were discussed by the endocrinologist with the patient and their family. GH was dosed according to FDA-approved guidelines at 0.3 mg/kg/week. GH dose was titrated to maintain insulin-like growth factor type 1 concentration within the normal range. Delayed puberty was discussed with participants with the option to begin T if the participant remained prepubertal by 14 years of age. Puberty was induced by administering T ethanoate 100 mg intramuscularly once monthly for 6 months, then observed for 6 months. In participants who did not continue to progress through puberty, T ethanoate was resumed at 100 mg intramuscular monthly for six months, increasing by 100 mg monthly every 6 months. For bone health, all participants were counseled on the recommended daily amount of calcium and vitamin D for age. Calcium supplementation was adjusted based on dietary intake and laboratory evaluation for hypercalciuria (urinary calcium/osmolality ratio > 0.025 mg/dl per mOsm/kgH_2O [33]). Vitamin D supplementation was adjusted to maintain 25(OH) vitamin D concentration > 30 ng/ml. Intravenous zoledronic acid (ZA) was discussed and prescribed when the participant satisfied the clinical diagnosis of osteoporosis: significant fracture history with or without low BMD. The initial dose of ZA (0.0125 mg/kg intravenous) was given twice, one month apart. Subsequent doses of 0.025 mg/kg were administered every 6 months until BMD Z-score and/or fracture frequency were stable (no fractures in the prior 2 years). Once stable, infusion frequency was decreased to 0.025 mg/kg annually.

Radiographic analysis

To assess the prevalence of VFs in participants, spine X-rays were analyzed using Genant's semi-quantitative method [34, 35]. The research team was trained by a Johns Hopkins musculoskeletal radiologist to interpret X-rays to ensure consistency and discuss how to mediate discrepancies. Anterior, posterior, and middle heights of vertebrae T4–L4 were first quantitatively measured in millimeters using a ruler on the spine radiograph as demonstrated in the representative images of the lateral thoracic and lumbar spine (Fig. 1a, b). Each vertebral height was initially measured by two researchers (EL, JC, and JS) independently; discrepancies of greater than 5% were resolved by a third reader (EL, JS, JC, or SB). If the discrepancy was unable to be resolved, the vertebral height was labeled as "unreadable." Potential vertebral fractures were identified if there was a >20% height difference between the minimum height of the vertebrae being assessed and the maximum height of the vertebrae being assessed as well as the maximum height of the vertebrae immediately above or below. All potential VFs were qualitatively checked by SB and JC to ensure that benign pathology such as Schmorl's nodes or Cupid's bow was not being erroneously identified as a vertebral fracture. Confirmed VFs were then classified in terms of severity using the modified Genant semi-quantitative method, where mild is height loss of 20–25%, moderate 25–40%, and severe >40%. Incident VF was defined as a new VF, meaning that the immediate prior x-ray did not have a height loss of >20%. To assess VF severity, the spine deformity index (SDI) was calculated by assigning a grade of 0, 1, 2, or 3 for no VF or mild, moderate, or severe VF, respectively, and summing the VF grades of all vertebrae (T4–L4). Due to technical limitations of x-rays with unreadable vertebrae, we standardized SDI per vertebrae by taking the calculated SDI and dividing by readable T4–L4 vertebrae, such that range of SDI per vertebrae is 0–3.

BMD of each participant was measured by DXA (Hologic Inc., Horizon A, S/N 100164) and abstracted into REDCap. BMD Z- and height adjusted Z-scores (HAZ) were calculated based on the BMD in Childhood Study to account for race, sex, and age [36]. Radiographic data from participants ranged from at most 1 year prior to enrollment to the most recent follow-up. Height-adjusted BMD Z-score and SDI across intervention categories were analyzed using the last DXA or spine X-ray of the participant while on the specific treatment regimen. As participants could change treatment interventions over time, a participant may be represented in more than one intervention category. While "none" was an intervention category, there was limited longitudinal data for this group because participants were enrolled at the first endocrinology visit at which time interventions were often initiated.

Statistical analysis

We used accelerated failure time (AFT) regression models for time-to-event data to estimate the relative time to VF while on treatment with GH or T alone, or ZA with GH and/or T, compared to ZA alone. The use of AFT models allowed for a larger effective sample size, with 34 incident vertebral fracture events, and greater degrees of freedom for multiple regression analysis of our small cohort, compared to a more conventional Cox proportional-hazards model, which is limited to number of participants. AFT models estimated the relative change in time to VF in exposed versus unexposed participants (failure time ratio) [37, 38] for each risk factor investigated. In terms of fractures, if the relative failure time ratio was greater than 1; then, the analyzed treatment lengthens time to next VF. However, if the relative failure time

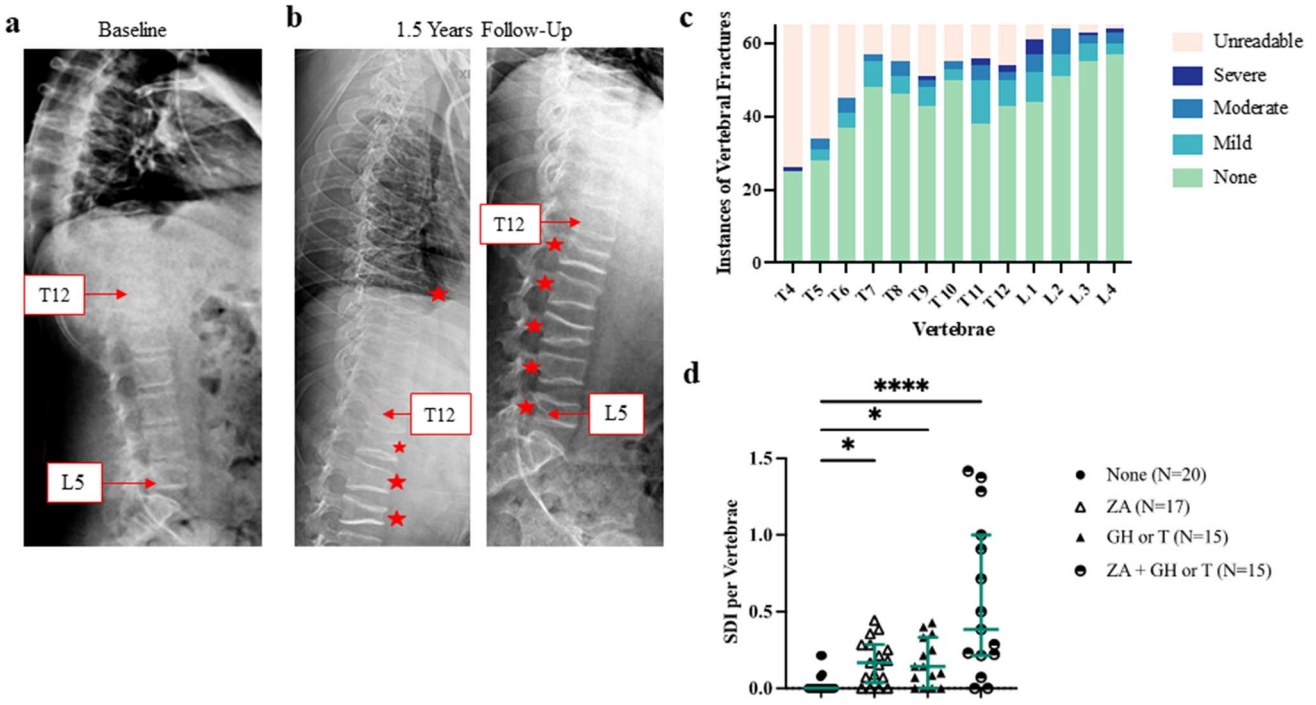

Fig. 1 Vertebral fracture data. **a** Representative lateral X-ray view of the thoracic and lumbar spine of a participant at their initial endocrine evaluation. **b** Lateral X-ray view of the thoracic and lumbar spine of the same participant after 1.5 years of follow-up. T12 and L5 were labeled on the X-rays. Red stars represent vertebral compression fractures. **c** Total instances of vertebral fractures stratified as unreadable and Genant classification of none, mild, moderate, or severe of all X-rays completed during observation period. **d** Spine deformity index (SDI) plotted as median with interquartile range, adjusted for each readable vertebrae in the X-ray and stratified per intervention group: none, zoledronic acid (ZA), growth hormone (GH) and/or testosterone (T), and ZA + GH or T. Abbreviations: SDI, spine deformity index; ZA, zoledronic acid; GH, growth hormone; T, testosterone

ratio was less than 1; then, the analyzed treatment shortens time to next VF. Repeated observations and recurrent events for participants were included in each model. Robust standard errors were used for statistical inference to account for within subject correlations. Interval censoring for asymptomatic VF times was used to adjust for the lack of knowing the precise date the VF occurred using dates between spine X-rays. Differences between groups were analyzed using bivariable AFT regression models and visualized using Kaplan–Meier survival curves. Multivariable AFT regression models were fitted to estimate independent associations between treatments and time to the next VF. The following covariates were included based on *a priori* association of these variables with VF risk: long bone fracture history, VF history, including SDI per vertebrae, years of GC use, age at enrollment, height Z-score, BMI Z-score, and total body less head (TBLH) BMD HAZ.

We used linear regression models to estimate the average change in vertebral height while on treatment with GH or T alone, or ZA with GH and/or T, compared to ZA alone (Δheight). Multivariable linear regression models included length of follow-up as well as the covariates included in the AFT models. Models were fitted by generalized estimating equations to account for correlations within vertebrae and subjects over time. We fitted separate models for anterior, middle, and posterior vertebral height and corrected for multiple testing across vertebrae positions to control the false discovery rate [39].

All analyses were conducted using R version 4.2.0, AFT models were fitted using the survival package, and linear regression models were fitted using the geepack package. We used GraphPad Prism version 9.4.1 to conduct ordinary one-way ANOVA analysis with post hoc Tukey's on SDI per vertebrae and BMD HAZ results.

Results

Participant characteristics

Twenty-seven research participants were included in the study (Table 1) who experienced 34 incident VF events and provided >400 measures of vertebral height during the follow-up period. Baseline endocrine evaluation occurred at a median age of 13.5 years (interquartile range (IQR) 11.3–14.9) with a median follow-up time of 27.6 months (IQR 14.3, 37.5). All

Table 1 Baseline characteristics

	Baseline endocrine evaluation ($n = 27$) (n (%)) or (median [IQR])	Most recent endocrine evaluation ($n = 27$)
Demographics		
Age (years)	13.5 [11.3, 14.9]	15.8 [14.0, 16.6]
Follow-up time (months)		27.6 [14.3, 37.5]
Race/Ethnicity		
White	21 (77.8%)	
Black	0 (0.0%)	
Asian	5 (18.5%)	
Native American	1 (3.7%)	
Hispanic	0 (0.0%)	
Family history		
Diabetes	18 (66.7%)	
Osteoporosis	3 (11.1%)	
Short stature	0 (0.0%)	
Delayed puberty	7 (33.3%)	
Glucocorticoid history		
Participants on treatment	27 (100.0%)	25 (92.6%)
Age started (years)	4.5 [3.1, 6.7]	
Duration (years)	8.5 [6.1, 10.6]	11.5 [8.5, 13.4]
Dosing regimen (daily or weekend)		
Deflazacort (% prescribed daily)	20/22 (90.9%)	20/24 (83.3%)
Prednisone (% prescribed daily)	5/5 (100.0%)	3/3 (100.0%)
Complications		
Ambulatory status		
Fully ambulatory	9 (33.3%)	5 (18.5%)
Ambulates short distances	9 (33.3%)	8 (29.6%)
Unable to ambulate independently	9 (33.3%)	14 (51.9%)
Cardiac complications	23/27 (85.2%)	27/27 (100.0%)*
Pulmonary complications		
Use of BIPAP and/or cough assist	7 (25.9%)	13 (48.1%)
FVC (percent predicted)	90.0 [77.0, 103.5]	83.0 [63.0, 96.0]
FEV1 (percent predicted)	78.0 [62.5, 95.5]	71.0 [61.0, 87.0]
FEV1/FVC	0.89 [0.776, 0.960]	0.89 [0.787, 1.000]

IQR interquartile range, *BIPAP* bilevel positive airway pressure, *FVC* forced vital capacity, *FEV1* forced expiratory volume. *Eleven participants initiated or intensified cardiac medications

participants were on GCs at the initial endocrine encounter. The mean age at GC initiation was 4.5 years, and the median duration of GC treatment at most recent follow-up was 11.5 years. At baseline, twenty-two participants were using deflazacort, of whom twenty (91%) were on daily dosing and two (9%) were on weekend dosing. The remaining five participants were taking daily prednisone. At the most recent visit, twenty-four participants were taking deflazacort, with twenty (83%) on daily and four (17%) on weekend dosing. The other three participants were on daily prednisone. Similar to prior reports, our participants had progression of neuromuscular, cardiac, and pulmonary complications [40].

Incidence of endocrinopathies

Participants were evaluated for growth impairment, delayed puberty, and osteoporosis (Table 2). Short stature (height Z-score less than −2.25) was observed in twenty-three participants (85.2%). Twelve (44.4%) participants were treated with GH. Delayed puberty (sexual maturity rating gonad 1 at ≥14 years of age) was observed in seventeen of twenty-one participants (80.9%). Twelve (57.1%) participants were treated with T. Regarding the diagnosis of osteoporosis, nine participants had sustained at least one VF, eight had sustained a long bone fracture, and four had sustained a small bone fracture at

Table 2 Endocrine complications

	Baseline endocrine evaluation ($n = 27$) (n (%)) or (median [IQR])	Most recent endocrine evaluation ($n = 27$)
Growth impairment		
Height SD < −2.25	21/27 (77.8%)	23/27 (85.2%)
Growth hormone use	0/27 (0.0%)	12/27 (44.4%)
Pubertal status		
Delayed puberty*	10/13 (76.9%)	17/21 (80.9%)
Testosterone use	1/13 (7.7%)	12/21 (57.1%)
Bone health		
VF incidence		
Participants with VF	9/24 (37.5%)	21/24 (87.5%)
Number of VF/participants	2.0 [1.0, 3.0]	3.0 [2.5, 6]
Long bone fractures		
Participants with fracture	8/27 (29.6%)	13/27 (48.1%)
Number of fractures/participants	2.0 [1.0, 2.0]	2.0 [1.0, 3.0]
Small bone fractures		
Participants with fracture	4/27 (14.8%)	4/27 (14.8%)
Number of fractures/participants	1.0 [1.0, 1.0]	1.0 [1.0, 1.2]
Bone health interventions		
Current calcium supplementation	12 (44.4%)	17 (63.0%)
Current vitamin D supplementation	25 (92.6%)	27 (100.0%)
BP therapy		
Participants on treatment	10/27 (37.0%)	14/27 (51.9%)
Age started	11.3 [9.4, 13.0]	
Duration (yrs)	2.4 [1.7, 3.8]	4.2 [2.5, 7.2]
Intravenous versus oral		
Intravenous	6/10 (60.0%)	14/14 (100.0%)
Oral	5/10 (50.0%)	5/14 (35.7%)

VF vertebral fracture, *SD* standard deviation, *IQR* interquartile range, *BP* bisphosphonate. *Delayed puberty status only includes prepubertal participants older than 14 years

baseline (Table 2). Over the course of the study, eleven participants experienced a new VF, increasing the prevalence of VF to 87% (Table 2). Ten participants (37%) had a history of bisphosphonate use at baseline and four participants began ZA during follow-up, increasing the prevalence to fourteen (52%). Of the participants who had a history of bisphosphonate use, five had been treated with oral bisphosphonates and six had been treated with intravenous bisphosphonates, with one participant having been treated with both oral and intravenous bisphosphonates. All participants were converted to ZA during follow-up.

We analyzed the VF severity by vertebral level to determine if there was a specific vertebra most prone to fracture. Interpretation of X-rays were limited by quality of X-rays. The readability of T4–T6 was worst, around 30–70%. Readability improved to 80–90% for T7–T12 and was greater than 90% of lumbar spine (L1–L4). Fractures were noted in all levels of the spine, with most fractures being mild. Most VFs occurred at T11, T12, and L1, with seventeen, eleven, and seventeen total VFs, respectively (Fig. 1c). The SDI per vertebrae was stratified per intervention group (Fig. 1d). All participants treated with at least GH and/or T had a significantly higher SDI compared to those on no endocrine interventions ($P = 0.03$). Similarly, participants treated with ZA coupled with GH and/or T had a significantly higher SDI compared to participants treated with nothing ($P < 0.0001$).

Skeletal response to ZA, T, and GH

Fracture risk in healthy children is reported to be most greatly associated with spine and TBLH BMD [41]. Participants were grouped based on most recent DXA results while on each specific treatment regimen. The median HAZ for lumbar spine BMD was normal in all groups. There was a significant increase in lumbar spine HAZ between participants on ZA alone versus treatment with GH or T alone ($P = 0.039$) (Supplemental Fig. 1a). The median HAZ for TBLH BMD was normal in the ZA only group and low in all other groups. GH and/or T, regardless of ZA, had the

lowest TBLH BMD HAZ relative to none or ZA alone (Supplemental Fig. 1b).

Given the limitations of BMD assessments in predicting future fractures in individuals with DMD, we also analyzed the time to next VF of T4–L4 using bivariable AFT linear regression models and Kaplan–Meier survival curves (Fig. 2a–c, Supplemental Table 1). Relative to ZA alone, GH and/or T with or without ZA significantly prolonged the time to next VF. Specifically, survival plot analysis revealed that the age at follow-up in which 50% of participants had sustained a VF was longer in GH and/or T with or without ZA relative to ZA alone groups (Fig. 2a). To determine if GH or T contributed more to the prolonged time to VF, we stratified survival curves by GH or T. When analyzing GH independently, GH had a modest impact of delaying time to VF (Fig. 2b). Analyzing T independently, the combination of T with or without ZA resulted in the oldest age to sustain a new VF (Fig. 2c).

To account for variables known to affect fracture risk, we evaluated the association between time to next VF (T4–L4) while using GH, T, and ZA with a multivariable AFT model (Table 3). A failure time ratio >1 indicates a longer time to

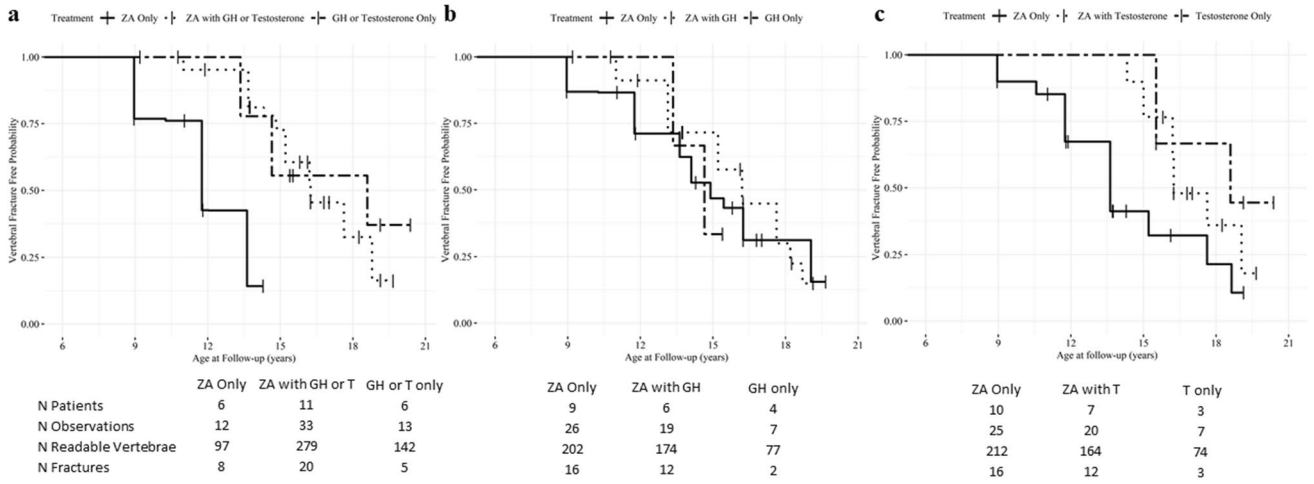

Fig. 2 Survival plot of time to vertebral fracture stratified by zoledronic acid, growth hormone, and/or testosterone use. **a–c** The survival plot of time (months since enrollment) to vertebral fracture (VF) stratified by: zoledronic acid (ZA) with or without either growth hormone (GH) and/or testosterone (T) **(a)**, disregarding T and stratified by ZA and/or GH **(b)**, or disregarding GH and stratified by ZA and/or T **(c)**. Each tick represents the last follow-up visit from each participant in their respective group. Abbreviations: ZA, zoledronic acid; GH, growth hormone; T, testosterone

Table 3 Multivariable AFT regression evaluating associations between time to a new vertebral fracture and GH/T or combined ZA plus GH/T use versus ZA use only, adjusted for other participant characteristics

	GH/T or combined ZA plus GH/T		Any GH or Any GH plus ZA		Any T or any T plus ZA	
	Failure time ratio (95% CI)	*P*	Failure time ratio (95% CI)	*P*	Failure time ratio (95% CI)	*P*
GH/testosterone only vs. ZA only	1.253 (1.144, 1.373)	<0.001	1.229 (1.105, 1.367)	<0.001	1.110 (0.968, 1.273)	0.135
ZA plus GH/testosterone vs. ZA only	1.171 (1.104, 1.242)	<0.001	1.042 (0.972, 1.116)	0.244	1.130 (1.010, 1.264)	0.033
Long bone fracture	1.033 (0.937, 1.138)	0.520	1.031 (0.951, 1.117)	0.461	1.049 (0.947, 1.161)	0.36
Vertebral fracture	0.968 (0.912, 1.028)	0.291	0.969 (0.914, 1.028)	0.295	0.954 (0.881, 1.033)	0.247
SDI per vertebrae	1.043 (0.949, 1.147)	0.381	1.078 (1.001, 1.160)	0.047	1.036 (0.928, 1.157)	0.53
TBLH BMD HAZ (SDS)	0.992 (0.983, 1.001)	0.076	0.985 (0.974, 0.997)	0.011	0.983 (0.965, 1.001)	0.068
GC exposure (years)	1.031 (1.014, 1.048)	<0.001	1.036 (1.022, 1.051)	<0.001	1.046 (1.025, 1.068)	<0.001
Enrollment age (months)	1.004 (1.002, 1.006)	<0.001	1.002 (1.000, 1.004)	0.032	1.004 (1.003, 1.005)	<0.001
Height Z-score (SDS)	1.010 (0.981, 1.039)	0.514	0.967 (0.935, 1.000)	0.05	1.015 (0.962, 1.071)	0.586
BMI Z-score (SDS)	0.994 (0.956, 1.033)	0.756	0.983 (0.939, 1.029)	0.459	1.016 (0.949, 1.089)	0.644

GH growth hormone, *T* testosterone, *ZA* zoledronic acid, *CI* confidence interval, *SDI* spine deformity index, *TBLH* total body less head, *BMD* bone mineral density, *SDS* standard deviation score, *GC* glucocorticoid, *BMI* body mass index

next VF, whereas a failure time ratio <1 indicates a shorter time to next VF. We first conducted a combined GH or T AFT model to increase our sample size and statistical power. We next stratified the sample based on GH or T treatment to determine which was the greater contributor to the initial result. Treatment with GH or T prolonged time to next VF relative to ZA only (1.253, $P<0.001$) with GH only showing statistical significance when analyzed independently from T (1.229, $P<0.001$). Once participants were being treated with ZA, the addition of GH or T was protective, prolonging time to next VF compared to ZA only (1.171, $P<0.001$). This combination was significant for T combined with ZA in increasing the time to next VF (1.130, $P=0.033$). Of the assessed confounders, GC exposure and enrollment age independently altered time to next VF. An increase in enrollment age was associated with a longer time to next VF when treated with ZA and/or GH or T (1.004, $P<0.001$). The effect of GC exposure and enrollment age persisted with GH alone (1.031, $P<0.001$ and 1.002, $P=0.032$, respectively) and T alone (1.046, $P<0.001$ and 1.004, $P<0.001$, respectively). An increase in TBLH BMD HAZ and height Z-score was associated with a shorter time to next VF with participants treated with GH and/or ZA (0.985, P and 0.967, $P=0.05$, respectively).

Given the observation of VF reshaping in the pediatric population treated with bisphosphonates [42], we analyzed the change in anterior, middle, and posterior T4–L4 vertebrae height based on endocrine intervention using a similar multivariable analysis as VF (Tables 4 and 5). After adjustment for multiple testing across vertebrae positions, GH only treatment significantly increased vertebral anterior (2.31 mm, $P=0.022$), middle (4.32 mm, $P=0.001$), and posterior (2.76 mm, $P=0.008$) heights. In contrast, GH and ZA combined treatment trended to increase anterior and middle

Table 4 Differences in vertebral heights when treated with GH and ZA, adjusted for other participant characteristics

	Anterior vertebral height		Middle vertebral height		Posterior vertebral height	
	ΔHeight (95% CI)	*P*	ΔHeight (95% CI)	*P*	ΔHeight (95% CI)	*P*
Any GH vs. ZA only	2.31 (0.33, 4.30)	0.022	4.23 (1.71, 6.74)	0.001	2.76 (0.71, 4.81)	0.008
ZA plus any GH vs. ZA only	1.01 (−0.03, 2.06)	0.058	1.12 (−0.13, 2.38)	0.078	1.14 (0.31, 1.97)	0.007
Long bone fracture	−1.03 (−2.29, 0.22)	0.105	0.67 (−0.78, 2.12)	0.365	0.29 (−0.99, 1.57)	0.657
Vertebral fracture	−0.48 (−1.88, 0.93)	0.507	−0.45 (−2.26, 1.36)	0.627	−0.72 (−2.01, 0.57)	0.274
GC exposure (years)	0.05 (−0.43, 0.53)	0.838	0.22 (−0.35, 0.78)	0.452	0.09 (−0.35, 0.53)	0.696
Enrollment age (months)	0.02 (−0.04, 0.07)	0.549	−0.02 (−0.09, 0.04)	0.477	−0.02 (−0.07, 0.04)	0.546
Length of follow-up (months)	0.02 (−0.01, 0.04)	0.149	0.01 (−0.02, 0.05)	0.452	0.03 (0.00, 0.06)	0.030
BMI Z-score (SDS)	1.07 (0.19, 1.95)	0.017	1.13 (0.21, 2.04)	0.015	1.09 (0.21, 1.97)	0.016
Height Z-score (SDS)	0.14 (−0.85, 1.14)	0.779	−0.11 (−1.34, 1.12)	0.865	−0.30 (−1.22, 0.63)	0.532
TBLH BMD HAZ (SDS)	−0.99 (−1.83, −0.16)	0.020	−0.77 (−1.74, 0.19)	0.116	−1.36 (−2.20, −0.52)	0.001

GH growth hormone, *ZA* zoledronic acid, *CI* confidence interval, *GC* glucocorticoid, *BMI* body mass index, *TBLH* total body less head, *BMD* bone mineral density, *HAZ* height adjusted Z-score

Table 5 Differences in vertebral heights when treated with T and ZA, adjusted for other participant characteristics

	Anterior vertebral height		Middle vertebral height		Posterior vertebral height	
	ΔHeight (95% CI)	*P*	ΔHeight (95% CI)	*P*	ΔHeight (95% CI)	*P*
Any T vs. ZA only	−1.38 (−4.71, 1.95)	0.416	1.17 (−2.61, 4.96)	0.544	−0.11 (−3.23, 3.01)	0.944
ZA plus any T vs. ZA only	−0.22 (−0.96, 0.53)	0.569	0.52 (−0.42, 1.47)	0.276	0.04 (−0.61, 0.68)	0.912
Long bone fracture	−0.92 (−2.19, 0.35)	0.157	0.57 (−0.89, 2.02)	0.446	0.30 (−0.99, 1.58)	0.654
Vertebral fracture	−0.68 (−1.99, 0.62)	0.307	−1.00 (−2.60, 0.59)	0.218	−1.21 (−2.44, 0.02)	0.053
GC exposure (years)	0.14 (−0.33, 0.60)	0.558	0.17 (−0.40, 0.74)	0.562	0.15 (−0.30, 0.60)	0.501
Enrollment age (months)	0.03 (−0.02, 0.08)	0.198	−0.02 (−0.08, 0.04)	0.451	−0.01 (−0.06, 0.03)	0.595
Length of follow-up (months)	0.03 (−0.01, 0.06)	0.109	0.01 (−0.03, 0.06)	0.501	0.03 (−0.00, 0.06)	0.055
BMI Z-score (SDS)	0.93 (0.18, 1.69)	0.016	0.88 (0.11, 1.64)	0.024	0.85 (0.09, 1.61)	0.028
Height Z-score (SDS)	0.31 (−0.68, 1.31)	0.536	0.18 (−1.02, 1.37)	0.771	−0.22 (−1.08, 0.64)	0.615
TBLH BMD HAZ (SDS)	−0.96 (−1.78, −0.14)	0.021	−1.01 (−1.89, −0.13)	0.025	−1.38 (−2.19, −0.56)	0.001

HA anterior vertebral height, *HM* middle vertebral height, *HP* posterior vertebral height, *T* testosterone, *ZA* zoledronic acid, *CI* confidence interval, *GC* glucocorticoid, *BMI* body mass index, *TBLH* total body less head, *BMD* bone mineral density, *HAZ* height adjusted Z-score

vertebral height, while significantly increasing posterior height (1.14 mm, P=0.007) compared to ZA only. Other factors that influenced vertebral height comparing GH with or without ZA to ZA alone included follow-up time, BMI Z-score, and TBLH HAZ. Treatment with T significantly reduced middle vertebral height (−1.01 mm, P=0.025), a phenomenon not observed with GH treatment. When treated with T, a history of vertebral fractures trended to increase posterior vertebral height. We also observed a previously undescribed phenomenon, where some VF simultaneously both improved and worsened, changing from a wedge to concave fracture or vice versa. This observation was not at a high enough frequency for statistical analysis.

Discussion

GCs prolong ambulation and life span in DMD and Becker MD but also cause significant endocrine complications, including osteoporosis, poor linear growth, and delayed puberty. This study demonstrated a high prevalence of growth impairment (85%), pubertal delay (81%), and osteoporosis (87%). Endocrine interventions were pursued based on discussion of potential benefits and risks of medications, with 52% of those with growth failure electing to try GH, 72% of those with delayed puberty taking testosterone, and 72% with osteoporosis utilizing ZA. 77% of the cohort sustained VFs during the observation period, mostly concentrated in T11, T12, and L1, highlighting extreme skeletal fragility of especially the lower vertebrae that sustain the weight of the upper vertebrae. Relative to prior studies, we found a similar prevalence of osteoporosis [4] and pattern of VFs [21], However, this study extends previous results by including a longer observation period of interventions and collection of additional clinical information for multiple regression analyses.

Bisphosphonates are the standard of care for patients who have a significant fracture history [23–25, 27]. In DMD, there is limited data regarding the efficacy of osteoporosis medications in treatment or prevention of VFs or decreased BMD. In a randomized placebo-controlled trial, treatment with ZA showed descriptively lower incidence of VF [43]. In our cohort, patients with vertebral compression fractures were treated with ZA during the study period. Outcomes assessing efficacy of bisphosphonates in DMD show limitations of BMD assessments in predicting future fractures. One limitation is related to BMD height-adjustments, as people with muscular dystrophies are often extremely short. Two studies showed that bisphosphonates increased lumbar spine BMD Z-score significantly [24, 25], with only the first adjusting for height, while another study showed no significant increase in lumbar spine BMD HAZ [23]. We found that most participants with DMD had normal lumbar spine BMD HAZ, with only participants trending outside the normal range if they were treated with GH or T. There was no change in lumbar spine BMD HAZ when comparing the effect of ZA to either no treatment or in addition to GH or T. A similar but more pronounced pattern of change was noted in TBLH BMD HAZ. Overall, TBLH BMD HAZ was low among all treatment groups and significantly less when participants were treated with either GH or T, but no change was seen with ZA. There are two potential opposing explanations for this observation. One is a concern about a potential detrimental effect of GH or T on BMD HAZ [44, 45]. Differently, the HAZ calculations may be overcorrecting BMD in the case of extreme short stature. We used HAZ from the Bone Mineral Density in Childhood Study, which included normal healthy children who fell within the normal height range of the general population [29, 31, 46]. No studies, including ours, has adjusted BMD for delays in puberty. As this was a retrospective study, we had insufficient bone age X-rays to correct BMD for pubertal age. Additionally, bone ages of children on GC are incongruent, with bones of carpals, metacarpals, and phalanges often differing by multiple years. Overall, we did not find BMD HAZ to be predictive of VF in any treatment group aside that a higher TBLH BMD HAZ was associated with a shortened time to next VF when treated with ZA, which likely reflects that prior VF increases the chance of ZA treatment resulting in increased BMD and is a significant predictor of future VF.

In healthy children, skeletal mass increases rapidly with the pubertal growth spurt, doubling during adolescence [47]. Prior studies suggested a potential benefit of T as boys with DMD, osteoporosis, and delayed puberty treated with T had increased bone mass and bone accrual rate [29, 30]. Therefore, we focused on VF outcomes using the AFT model to assess the impact that GH and/or T had on time to a new VF, relative to ZA. The probability of not sustaining a VF for participants was highest and most prolonged when participants were treated with GH or T coupled with ZA. Prior to the initiation of ZA, GH alone prolonged the time to first VF, whereas the addition of T to ZA significantly prolonged time to next VF relative to ZA alone. Overall, our results suggest that induction of puberty is important for skeletal health, particularly in patients who have a significant fracture history and are being treated with ZA.

We analyzed SDI when on distinct endocrine interventions. A previous study highlighted that GCs place patients with DMD at an extremely high risk of sustaining VFs, with the lower spine being more likely to sustain more severe VFs [21]. Another study found that oral bisphosphonates did not increase the prevalence or severity of VFs for participants who already sustained a VF [27]. We found no significant difference in SDI for participants treated with only ZA compared to no treatment. However, treatment with GH and/or T was associated with an increased SDI. We are unable to

assess causation in this study design, but is an important observation for future prospective studies to determine if VF severity is related to endocrine complication or endocrine intervention. Studies regarding long-term outcomes of GH versus none or T versus none in patients with DMD of equal short stature and delayed puberty will be necessary to determine if the short stature and delayed puberty versus GH and T interventions associate with SDI.

When evaluating for reshaping of vertebrae after a VF was diagnosed, we noted an interesting phenomenon where a mild VF in T5 underwent anterior height reshaping after initiation of ZA, but loss of middle height, transitioning from a wedge-shaped fracture to a concave-shaped VF. Future studies are needed to understand if specific factors promote this phenomenon. We noted an increased vertebral height if participants were treated with GH relative to ZA only. Combined with the protective effect on time to next VF with GH alone and trend of increasing vertebral height of GH plus ZA relative to ZA, longer studies evaluating vertebral body reshaping are necessary to determine if GH may help restore vertebral height after a VF.

We analyzed multiple factors associated with time to next VF to adjust for potential confounders for our observations with GH, T, and ZA. In the bivariable AFT models, there was a protective effect of a family history of osteoporosis, possibly because these families are more likely to seek early guidance about skeletal health. Nearly all participants were on appropriate vitamin D and calcium supplementation such that no effects were noted on VF risk. Age, height, and weight all had unique effects of time to next VF. The BMI Z-score of the population decreased over time, particularly when the height Z-score increased. The increase in height Z-score was associated with a reduced time to next VF, suggesting a detrimental effect with increasing height. However, older age and loss of ambulation, with lower fall risk, appeared to be protective. Prior studies have suggested that older age and loss of ambulation was associated with increased risk of fracture [48], while more recent studies have reported either no difference or decreased incidence of fracture, likely related to reduced fall risk and changes in the mechanical loading patterns to the spine [21, 49]. These conflicting views may be due to the age of the cohorts studied and differences in timing of the fracture. For example, patients can sustain a fracture and consequently lose ambulation [21]. On the contrary, loss of skeletal mass from decreased mechanical loading with loss of ambulation may increase fracture risk. Additional contrasting factors on fracture risk include longer duration of GC use increasing fracture risk, but the association of GCs prolonging time of ambulation reducing fracture risk.

There are limitations in the study. A major weakness of the study is the inclusion bias of being referred to an endocrinologist, such that all participants had at least one endocrine complication from prolonged GC exposure and were started on an intervention shortly after enrollment. Thus, there was limited longitudinal data of a completely untreated group for statistical comparison. Similarly, conclusions drawn from GH intervention are biased with data of predominantly ambulatory participants, since they are, typically, the ones most interested in increasing height. Differences in spinal alignment and mechanical loading from ambulatory and non-ambulatory status may confound these results. Further observational studies with inclusion of all patients with a dystrophinopathy on chronic GCs would be needed to fully evaluate the effect of GH and T alone on VF in the absence of ZA. The study also has the same limitation as previous studies with a small sample size [23–25, 28, 29, 31]. However, the AFT model has been shown to have increased power compared to the more widely used Cox proportional hazards model [37], and there were more than 400 observations in our analyses of vertebral height. Each participant followed a different regimen, and the study was further limited by missing data due to the restrictions of the COVID-19 pandemic. This resulted in high variability in follow-up time, including when DXAs and spine X-rays were done. Additionally, older participants whose growth plates already closed or were still experiencing delayed puberty may have different outcomes than prepubescent patients who sought preventative care, which we were not able to control for given the small sample size. Future studies would benefit from recruiting a more diverse patient population and minimizing the variability in follow-up times. Moreover, a larger sample size would allow separation of patient group by endocrine intervention and analysis of the efficacy and complications of each treatment option.

Conclusion

People with DMD and Becker MD endure a range of endocrine complications due to chronic, high-dose GC treatment, including osteoporosis, delayed puberty, and impaired linear growth. Studies that explore the endocrine complications along with prevalence of VFs are useful for providing doctors with tangible results to educate the patients and their families on intervention efficacy, along with complications that could arise from treatment [25, 28, 29]. Different studies point to the potential efficacy of GH and/or T as treatment options but recognize the lack of knowledge in the field about long-term effects on bone strength and fragility [3, 17, 30]. We, for the first time, provide supportive data that combination therapy with GH and/or T and ZA is more protective against VF than ZA alone.

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Data availability Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Declarations

Statement of human rights The study has been approved by the Johns Hopkins University institutional review board and has been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Conflict of Interest None.

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