

Growth, bone health & ambulatory status of boys with DMD treated With daily vs. intermittent oral glucocorticoid regimen

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2 **Growth, Bone Health & Ambulatory Status of Boys with**
3 **DMD Treated With Daily vs. Intermittent Oral**
4 **Glucocorticoid Regimen**

5
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31 **Short Title:** Bone health in DMD boys on different GC regimes

32

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42 **Abstract**

43 Oral glucocorticoids (GC) preserve muscle strength and prolong walking in boys with Duchenne
44 muscular dystrophy (DMD). Although vertebral fractures have been reported in boys taking GC,
45 fracture rates for different GC regimes have not been investigated. The aim of this pragmatic
46 longitudinal study was to compare growth, body mass, bone mineral density (BMD), vertebral
47 fractures (VF) and ambulatory status in boys with DMD on daily (DAILY) or intermittent
48 (INTERMITTENT), oral GC regimens.

49 A convenience sample of 50 DMD boys from two centres was included in the study; 25 boys each
50 were on the DAILY or INTERMITTENT regimen. Size adjusted lumbar spine BMD (LS BMAD), total
51 body less head BMD (TBLH), by DXA and distal forearm bone densities by pQCT, GC exposure, VF
52 assessment and ambulatory status were analysed at three time points; baseline, 1 and 2 years.

53 At baseline, there were no differences in age, GC duration or any bone parameters. However, DAILY
54 boys were shorter (height SDS DAILY= -1.4(0.9); INTERMITTENT= -0.8(1.0), $p=0.04$) with higher BMI
55 (BMI SDS DAILY= 1.5(0.9); INTERMITTENT= 0.8(1.0), $p=0.01$). Over 2 years, DAILY boys got
56 progressively shorter (delta height SDS DAILY= -0.9(1.1); INTERMITTENT= +0.1(0.6), $p<0.001$). At
57 their 2 year assessment, 5 DAILY and 10 INTERMITTENT boys were non-ambulant. DAILY boys had
58 more VFs than INTERMITTENT boys (10 versus 2; $\chi^2 p = 0.008$). BMAD SDS remained unchanged
59 between groups. TBLH and radius BMD declined significantly but the rate of loss was not different.

60 In conclusion, there was a trend for more boys on daily GCs to remain ambulant but at the cost of
61 more VFs, greater adiposity and markedly diminished growth. In contrast, boys on intermittent GCs
62 had fewer vertebral fractures but there was a trend for more boys to loose independent ambulation.

63 **Key words:** Glucocorticoids, Muscular dystrophy, vertebral fracture, ambulation, bone density,
64 growth

65

66

67 1. Introduction

68 Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder, which affects 1 in 3600–
69 6000 live male births [1]. DMD is caused by loss of function mutations in the *dystrophin* gene, which
70 encodes the dystrophin protein in muscle. Dystrophin deficiency results in necrosis of myofibres,
71 which in turn results in progressive deterioration of muscle function. The disease manifests usually
72 before 3 years of age with proximal muscle weakness and the inability to run and jump as their peers.
73 As the disease progresses, rising from the floor becomes difficult and eventually independent
74 ambulation is lost.

75 Currently there is no cure for DMD but the quality of life of patients can be improved by medical
76 treatment and supportive care. Long term treatment with oral glucocorticoids (GCs) slows down
77 deterioration of skeletal muscle function, prolonging ambulation by 2 to 5 years [2]. Moderate quality
78 evidence from RCTs demonstrates that GC treatment improves muscle function for about 12 months,
79 and muscle strength for up to two years [3]. Treatment with GCs along with improved
80 cardiorespiratory and orthopaedic supportive care, have led to improved survival of males with DMD.
81 The current standard of care is to treat with prednisolone 0.75 mg/kg/day or deflazacort 0.9mg/kg/day;
82 this treatment is initiated at the plateau of motor abilities between 4 and 6 years [1]. After loss of
83 ambulation, steroid treatment may be continued, usually without further weight-related dose increase,
84 or discontinued if side-effect burden is intolerable. Within the UK, GC treatment is given either daily or
85 intermittently (10 days on & 10 days off) [4].

86 Long-term GC treatment in DMD boys is associated with obesity, short stature, pubertal delay, and an
87 increased risk of long bone and vertebral fractures (VFs) [5]. Fractures are associated with low bone
88 mineral density (BMD) [6-12] and mainly caused by progressive muscle weakness and GC use[13].
89 Furthermore, GC-related growth deceleration and pubertal delay might also contribute to bone fragility
90 in DMD boys [5]. Long bone fractures may precipitate permanent loss of mobility [8]. VFs may be
91 asymptomatic or associated with severe localised back pain. The role of chronic GC therapy in the
92 causation of VFs in DMD is emphasized by their absence in boys who are GC naïve [10]. Although
93 VF have previously been reported in DMD boys taking GCs [7, 9-12, 14], fracture rate for different GC
94 regimes has not been investigated.

95 Clinical observation suggests that DMD boys on continuous GC regimes may be more prone to VFs
96 compared to those on intermittent regimes. However, to the best of our knowledge bone health
97 outcomes have not been compared in DMD boys on different GC regimes. Thus, the aim of this study
98 was to compare longitudinal growth, body mass, BMD, VFs and ambulatory status in boys with DMD
99 on daily or intermittent, oral GC regimens.

100

101 **2. Materials and Methods**

102 This was a pragmatic study to compare the two different GC regimes. The majority of children
103 from Manchester are treated using the continuous GC regime (DAILY), whilst the majority of children
104 from Birmingham are treated using the intermittent 10 days on-10 days off regime (INTERMITTENT).
105 The decision to commence GC therapy was made by the local neuromuscular consultant according to
106 the guidelines used by the NorthStar network [4].

107 **2.1. Subjects**

108 A retrospective convenience sample of boys with established DMD was included, managed in two UK
109 centres between 2006 and 2014. At both centres, boys were excluded if they were not on the daily or
110 intermittent GC regime, for e.g. alternate day or weekend only regimes. Additionally, boys were
111 excluded if they had had exposure to oral or intravenous bisphosphonate treatment at baseline bone
112 assessment. At Manchester Children's Hospital, 25 boys were selected who had undergone annual
113 "bone health" assessments on at least 3 consecutive time points irrespective of their age (i.e. had at
114 least 2 years follow up). The same number of children was then identified from Birmingham Children's
115 Hospital DMD bone database in order to match as far as possible with those in Manchester, by age
116 and imaging modality. At both centres, bisphosphonate treatments were initiated when there was
117 evidence of a VF and associated pain. This information was extracted from the boys' medical records.

118 At both centres, boys are routinely given advice to optimise their dietary calcium intake, preferably
119 from low fat dairy products; they also receive vitamin D supplements and 25-hydroxy vitamin D levels
120 are monitored annually.

121 **2.2. Ethics**

122 Since this project was undertaken as a service / therapy evaluation between two UK centres, it did not
123 require ethical approval [15].

124 **2.3. Cumulative Glucocorticoid Exposure**

125 Cumulative GC exposure was calculated using information recorded in the subjects' medical records.

126 **2.4. Anthropometric measurements**

127 Height and weight were measured and body mass index calculated as weight/height² (kg/m²). Once
128 the child was unable to stand, arm span or supine length were used as surrogates for height. Height,
129 weight and BMI measurements were transformed to standard deviation scores using the 1990 British
130 growth reference data [16]. At both centres, anthropometric and bone assessments were repeated
131 approximately annually.

132 **2.5. Ambulation Status**

133 At each bone imaging time-point, the subject's mobility status was recorded, categorised as
134 independently ambulant or requiring wheelchair assistance.

135 **2.6. Bone Health Assessments**

136

137 **2.6.1. Vertebral Fracture Assessment (VFA)**

138

139 The VFA was undertaken, at each measurement point, using all available spine imaging (e.g. CT
140 scout views, anterior and lateral whole spine radiographs and Dual-Energy X-ray Absorptiometry
141 (DXA). Until recently vertebral fracture assessment, (VFA) by DXA was considered inferior to plain X-
142 rays for the diagnosis of vertebral fracture. However, with the development of new higher-resolution
143 scanners, VFA by DXA is proving to be an attractive imaging tool for children. Compared to lateral
144 radiographs, it affords the child a significant (approximately threefold) reduction in radiation dose and
145 is available at the time of routine DXA scanning [17, 18].

146 Images from both centres were jointly reviewed by 2 experienced investigators (JEA & NJC) to arrive
147 at a consensus on fracture identification. Fractures were defined if there was $\geq 25\%$ loss in height of
148 anterior, mid or posterior vertebral body, in relation to the adjacent unaffected vertebrae [19]. This

149 threshold was based on a UK based survey which suggested that $\geq 25\%$ loss in vertebral height was
150 the most likely level to prompt bisphosphonate treatment [20]. In addition, the identification of VFs
151 with less than 25% vertebral height loss is unreliable both by plain radiography and DXA based VFA
152 [17, 18].

153 **2.6.2. Long bone Fractures**

154 Data on long bone fractures was collected from review of hospital records and confirmed where
155 possible from radiological review.

156 **2.6.3. Dual-Energy X-ray Absorptiometry (DXA)**

157 DXA scans of the lumbar spine (L1-4) and total body were performed on either a GE Lunar iDXA™ or
158 Prodigy (GE Lunar Corp. Madison, WI, USA) (Birmingham) or a Hologic Discovery (QDR 4500
159 Discovery, Hologic Inc. Bedford, MA) (Manchester) scanner, according to standard protocol. Results
160 are presented as lumbar spine bone mineral apparent density [L1-L4 BMAD (g/cm^3)] [21] and total
161 body less head BMD [TBLH BMD (g/cm^2)]. Age, gender and machine specific Z-scores were
162 calculated using UK reference data [22].

163 **2.6.4. Peripheral quantitative computed tomography (pQCT)**

164 At both sites, pQCT scans were acquired at the distal radius (4% of radial length) of the non-dominant
165 forearm using a Stratec XCT-2000 scanner (Stratec, Pforzheim, Germany). Outcome variables
166 included trabecular and total volumetric BMD (g/cm^3). Age- and gender-specific Z-scores were
167 calculated using the manufacturer's reference data [23].

168 **2.7. Statistical Analysis**

169 Analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp, Armonk, NY).
170 Parameter differences from baseline to follow-up were evaluated using General Linear Model
171 Repeated Measures analyses, with time as the within-subject factor and GC regime (DAILY or
172 INTERMITTENT) as the between-subjects factor. If the sphericity assumption was not met, the
173 Huynh-Feldt correction was applied. A level of $p < 0.05$ was used to denote statistical significance.
174 Results are presented as mean and standard deviation unless otherwise stated.

175

177 **3. Results**

178 **3.1. Baseline Characteristics**

179 By design, subjects from both GC regimes did not differ in age (mean (SD) 8.3 (2.4), age of GC
180 therapy initiation (6.3 (1.9) years) or duration of GCs 2.0 (1.9) years). However, boys on the DAILY
181 regime had greater cumulative GC exposure and were significantly shorter (height SDS -1.4 (1.0) vs. -
182 0.8 (1.0) $p=0.04$) with greater BMI (BMI SDS 1.5 (0.9) vs. 0.8 (1.0) $p=0.01$). In contrast, there were no
183 differences in any of the measured bone density parameters between treatment regimens whether we
184 included or excluded (data not shown) boys who subsequently went on to receive bisphosphonate
185 therapy (Table 1). One boy from the DAILY group and 3 boys from the INTERMITTENT group had
186 suffered a long bone fracture. In contrast, 1 of the DAILY boys had radiological evidence of a
187 vertebral fracture, whereas none of the INTERMITTENT boys had evidence of vertebral fracture.

188 **3.2. Follow-up Data**

189 **3.2.1. Growth and body mass**

190 After 2.5 (0.9) years, height SDS decreased significantly in the DAILY boys but remained constant for
191 the INTERMITTENT boys (delta height SDS -0.9 (1.1) vs. +0.1 (0.6); $p<0.001$). Weight SDS
192 increased significantly in both the DAILY boys and INTERMITTENT boys (delta weight SDS +0.3 (0.8)
193 vs. +0.7 (0.7); $p=0.05$). In contrast, BMI SDS increased at the same rate for both DAILY and
194 INTERMITTENT boys (mean delta BMI SDS = +0.8 (0.8)). Consequently, BMI SDS for DAILY boys
195 remained significantly higher at the final assessment (BMI SDS DAILY = +2.3 (0.7) vs.
196 INTERMITTENT = +1.5 (1.0); $p=0.001$).

197 **3.2.2. Ambulation, fractures and bone density**

198 There was a non-significant trend for more boys in the INTERMITTENT regime to be non-ambulant.
199 At their final assessment, mean age 10.7 (2.7) years, 10 boys on the INTERMITTENT regime (40%)
200 and 5 in the DAILY group (20%) were non ambulant (Figure 1a).

201 During follow-up, one DAILY boy sustained a minimally displaced metaphyseal fracture of the distal
202 tibia. None of the INTERMITTENT boys sustained any long bone fractures. Over the same period, 10
203 (40%) DAILY and 2 (8%) INTERMITTENT boys suffered VFs ($\chi^2=7.018$; $p=0.008$) (Figure1b). Six of
204 the 10 DAILY boys with symptomatic VF prior to the 2 year assessment were commenced on
205 intravenous bisphosphonate therapy. As such, bone density data from these 6 boys were excluded
206 from follow-up comparisons.

207 LS BMAD Z-scores did not change over time and did not differ between the treatment groups. In
208 contrast, Z-scores for TBLH BMD, TotBMD and TrabBMD at the 4% distal radial site decreased
209 significantly ($p<0.05$) with time. However, there was no statistical interaction between GC regime and
210 time for any of the bone parameters (Table 2).

211 Similar differences were observed combining GC regimes and grouping according to fracture. There
212 were no significant differences in LS BMAD or TBLH BMD Z-scores between those that did or did not
213 suffer a VF. However, TotBMD and TrabBMD Z-scores at the 4% distal radius were significantly lower
214 in boys who fractured ($p<0.05$), and declined ($p<0.05$) over time in both groups (Table 3 & Figure 2b).

215

216 **4. Discussion**

217 This study found that boys on the DAILY GC regime suffered significantly more VFs than those on the
218 INTERMITTENT GC regimen but more tended to be ambulant at the end of the observation period.
219 DAILY boys were also shorter, grew less and had greater BMI.

220 Despite the striking differences in VFs there were no differences in BMAD between DAILY and
221 INTERMITTENT GC regimes either at baseline or over the duration of follow-up. Similarly, in the
222 whole group, there were also no differences in BMAD between boys with VFs and those without. Thus
223 BMAD, in DMD boys treated with GC, does not appear to be a predictive marker of vertebral fracture.
224 This supports observations in children with nephrotic syndrome [24] but contrasts findings from the
225 STOPP study in children with acute lymphoblastic leukaemia, nephrotic syndrome and rheumatoid
226 arthritis, where low areal BMD was a significant predictor of VFs [25, 26]. The lumbar spine BMAD
227 may not be useful for differentiating boys with and without VFs in DMD.

228 Distal radial total and trabecular bone density measured by pQCT were not different between GC
229 regimes at baseline. However, in contrast to BMAD, the pQCT density (g/cm^3) Z-scores decreased
230 over time. We postulate that the differences between BMAD and pQCT distal radial densities may be
231 due to the higher amount of trabecular bone in the pQCT measured distal radial parameters and the
232 greater sensitivity of pQCT to detect trabecular changes. Adult studies indicate that GC have a
233 predilection to affect the more metabolically active trabecular bone [27]. If this holds true for children,
234 it would explain why reductions in trabecular rich sites as measured by pQCT are more sensitive to
235 change than the lumbar spine.

236 It is generally assumed that BMAD reflects predominantly trabecular bone density of the spine;
237 however, because DXA measures the whole projection, at the lumbar spine it invariably includes the
238 cortical spinous processes. The combination of trabecular bone in the vertebral body with the cortical
239 bone from the spinous processes may mask any changes within the trabecular compartment arising
240 from GC and progressive immobility of the DMD boys. Although DXA remains the preferred method of
241 bone health assessment in children [28], it is well known that areal bone mineral density, as
242 measured by DXA, is significantly affected by bone size. Children with reduced stature and hence
243 reduced bone size will have spuriously low areal bone mineral density [29]. In our group of boys the
244 mean Z score for height, in both groups at the start of the assessment was reduced. However, boys
245 on the DAILY regime were already significantly shorter at baseline and grew less than the intermittent
246 group. To overcome the known pitfall of bone size in DXA imaging, we used the bone mineral
247 apparent density (BMAD) to assess their bone changes [28]. However, even though BMAD reduces
248 the size influence it is only an approximation for true volumetric bone density. As such, quantitative
249 computed tomography (QCT) of the lumbar spine may be a more appropriate lumbar spine bone
250 density technique to apply in children with DMD. We speculate that volumetric BMD by spine QCT
251 may a better predictor of vertebral fracture. Longitudinal studies are needed to answer this question
252 [30].

253 Our pragmatic study has a number of shortcomings. The data on ambulatory status was gathered
254 from review of patient's records. Since patients were reviewed in at roughly 6 monthly intervals at
255 both centres accurate documentation of exact time of loss of ambulation was not always possible and
256 would often rely on parent recalling this information. Again the information on GC dose was estimated

257 from patient records. There were some differences in the annual bone health assessments. Most
258 notably bone density measures were acquired on different bone densitometers. However, the
259 machine differences were minimised by using Z-scores from a robust reference dataset [22]. There
260 were also differences in the imaging type from which vertebral fractures were assessed annually but
261 again these differences were minimised by reviewing the images and consensus reporting by 2
262 investigators experienced in vertebral morphometry (JEA & NJC). It is feasible that the assessment of
263 bone density may be affected by differences in adiposity. Experimental work with phantoms has
264 shown that increasing adiposity increases the detected bone area and consequently results in
265 artificially lower bone density values [31]. However, these measurement errors have not been
266 quantified in children and as such it is difficult to assess the true impact they have on our different
267 treatment groups with statistically different levels of adiposity. Consequently, further work on the
268 relationship between lumbar spine BMAD and vertebral fracture is required as this study was not
269 powered to statistically evaluate changes in bone density.

270 **5. Conclusion**

271 In summary, boys on a daily GC regimen tend to remain ambulant longer but at the cost of
272 significantly more VFs, greater adiposity and markedly diminished growth. In contrast, boys on the
273 intermittent GC regimen had fewer fractures but tended to lose ambulation earlier. In both groups, LS
274 BMAD was a poor predictor of VFs. The high prevalence of VF, and the limited value of DXA to
275 predict VF, suggests the need for VF screening as part of the boys routine bone health assessments.
276 Finally, the decline in volumetric bone density as measured by peripheral QCT may be a more
277 sensitive measure of bone loss and vertebral fracture risk.

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290

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406

407 **Table 1** Baseline characteristics of the DMD boys in each GC regimen (Mean (SD))

	DAILY Steroid Regime (n = 25)	INTERMITTENT Steroid Regime (n = 25)	Difference p value
Age (years)	8.1 (2.3)	8.5 (2.7)	NS
Height SDS	-1.4 (0.9)	-0.8 (1.0)	0.04
Weight SDS	0.3 (0.9)	0.1 (1.1)	NS
BMI SDS	1.5 (0.9)	0.8 (1.0)	0.01
Age started glucocorticoids	6.0 (1.9)	6.5 (1.8)	NS
Duration of Steroids (years)	2.1 (1.6)	2.0 (2.2)	NS
Cumulative glucocorticoid dose (mg)	11278 (7248)	6397 (7543)	0.02
Lumbar Spine BMAD (g/cm ³) Z-Score	-0.6 (0.7)	-0.3 (1.0)	NS
Total Body Less Head BMD (g/cm ²) Z-Score	-2.3 (1.2)	-1.8 (0.8)	NS
4% Distal Radius Total Density (g/cm ³) Z-Score	-0.5 (1.5)	-0.8 (1.2)	NS
4% Distal Radius Trabecular Density (g/cm ³) Z-Score	-1.1 (1.5)	-1.3 (1.2)	NS
Number of boys with long bone fractures	1	3	
Number of boys with vertebral fractures	1	0	

408

409 **Note:**

410 **Abbreviation:** BMAD Bone Mineral Apparent Density

411

412 **Table 2** Mean (SD) Bone density Z-scores of DMD boys on DAILY and INTERMITTENT steroid
 413 regimen (excluding 6 boys who started bisphosphonate treatment due to symptomatic vertebral
 414 fractures)

415

Z-Scores	DAILY Steroid Regime			INTERMITTENT Steroid Regime		
	Baseline n = 19	Year 1 n = 19	Year 2 n = 19	Baseline n = 25	Year 1 n = 25	Year 2 n = 25
Lumbar Spine BMAD	-0.6 (0.7)	-0.6 (0.7)	-0.4 (0.9)	-0.3 (1.0)	-0.1 (1.0)	-0.1 (1.1)
*Total Body Less Head BMD	-2.3 (1.2)	-2.4 (1.5)	-3.1 (1.3)	-1.8 (0.8)	-1.9 (0.9)	-2.0 (0.9)
*4% Distal Radius Total Density	-0.2 (1.3)	-1.0 (1.4)	-1.0 (1.4)	-0.8 (1.2)	-1.2 (1.0)	-1.4 (1.2)
*4% Distal Radius Trabecular Density	-0.9 (1.4)	-1.2 (1.0)	-1.9 (1.3)	-1.3 (1.2)	-1.5 (1.1)	-1.8 (1.2)

416 **Note:**

417 **Abbreviation:** BMAD Bone Mineral Apparent Density

418 * Significant change over time ($p < 0.05$) but no significant interaction between GC regime and bone
 419 density over time.

420

421

422

423 **Table 3** Mean (SD) Bone density Z-scores of boys with and without vertebral fractures at the end of
 424 the study (excluding 6 boys who started bisphosphonate treatment due to symptomatic vertebral
 425 fractures)

426

	No Vertebral Fractures			Vertebral Fractures		
Z-Scores	Baseline (n = 38)	Year 1 (n = 38)	Year 2 (n = 38)	Baseline (n = 6)	Year 1 (n = 6)	Year 2 (n = 6)
Lumbar Spine BMAD	-0.4 (0.8)	-0.3 (0.8)	-0.2 (0.9)	-0.4 (1.4)	-0.6 (1.4)	-0.7 (1.6)
Total Body Less Head BMD	-1.8 (0.9)	-2.0 (1.1)	-2.3 (1.2)	-2.7 (1.0)	-2.7 (0.9)	-3.2 (1.1)
*4% Distal Radius Total Density	-0.5 (1.3)	-0.9 (0.7)	-1.1 (1.3)	-1.2 (0.7)	-2.2 (0.7)	-2.3 (0.6)
*4% Distal Radius Trabecular Density	-1.0 (1.2)	-1.3 (1.1)	-1.7 (1.2)	-2.1 (1.2)	-2.0 (0.8)	-2.6 (1.3)

427 **Note:**

428 **Abbreviation:** BMAD Bone Mineral Apparent Density

429 *Significantly different at baseline and significant change over time ($p < 0.05$) but no significant
 430 interaction between fracture and bone density over time.

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434

435 **Figure Legends**

436

437 **Figure 1** Changes in ambulation over time (a). There was a trend for more boys on the DAILY
438 GC regime (20%) (Black boxes) to remain ambulant after two years than boys on the INTERMITTENT
439 (40%) (Grey boxes). Occurrences of vertebral fracture (b). Significantly more vertebral fractures were
440 reported in boys on the DAILY regime (40%) (Black boxes) than the INTERMITTENT regime (8%)
441 (Grey boxes) (χ^2 ; $p = 0.008$).

442

443 **Figure 2** Bone density differences between the vertebral fracture group (n= 6, black circles)
444 and non-vertebral fracture group (n= 38 grey squares). (a) Demonstrates no significant difference in
445 LSBMAD Z-scores between groups (n= 38). In contrast, (b) demonstrates the reduction in baseline
446 TotBMD Z-scores at the 4% distal radius for DMD boys who suffered a vertebral fracture and their
447 greater drop in TotBMD Z-scores over time, as measured by pQCT.

448