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Three years of alendronate treatment results in similar levels of vertebral microdamage as after one year of treatment

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37 Three years of daily alendronate treatment increases microdamage in vertebral bone but
38 does not significantly increase it beyond levels of microdamage found after 1 year of
39 treatment. This suggests microdamage accumulation peaks during the early period of
40 bisphosphonate treatment, and does not continue to accumulate with longer periods of
41 treatment.
42

43 Introduction: Clinically-relevant doses of alendronate increase vertebral microdamage by 4- to
44 5-fold in skeletally mature beagles after 1 year of treatment. The goal of this study was to
45 determine if microdamage would continue to accumulate with three years of alendronate
46 treatment in an intact beagle dog model.

47 Methods: One-year-old female beagles were treated with daily oral doses of vehicle (VEH, 1
48 ml/kg/day) or alendronate (ALN, 0.2 mg/kg/day or 1.0 mg/kg/day) for three years. These ALN
49 doses were chosen to approximate, on a mg/kg basis, those used to treat osteoporosis (ALN0.2)
50 and Paget's disease (ALN1.0). Microdamage accumulation, static and dynamic
51 histomorphometry, densitometry, and mechanical properties of lumbar vertebrae were assessed.
52 Comparisons were made among the three groups treated for three years, and also within each
53 treatment group, to animals treated under the same conditions for one year (Allen et al. Bone,
54 2006).

55 Results: Overall microdamage accumulation (crack surface density) was not significantly higher
56 in animals treated for three years with either dose of ALN, while crack density increased
57 significantly (100%; $p < 0.05$) with the higher dose of ALN when compared to VEH. Both ALN
58 doses significantly suppressed the rate of bone turnover (-60% versus VEH). There was no
59 difference among groups for any of the structural biomechanical properties - ultimate load,
60 stiffness, or energy absorption. However, when adjusted for areal bone mineral density ALN-
61 treated animals had significantly lower energy absorption (-20%) compared to VEH. Toughness,
62 the energy absorption capacity of the bone tissue, was significantly lower than VEH for both
63 ALN0.2 (-27%) and ALN1.0 (-33%). Compared to animals treated for one year, there was no
64 significant difference in microdamage accumulation for either ALN dose. VEH-treated animals
65 had significantly lower bone turnover (-58%) and significantly higher levels of microdamage (+
66 300%) compared to values in 1 year animals. Toughness was significantly lower in animals
67 treated for 3 years with ALN1.0 (-18%) compared to animals treated for 1 year while there was no
68 difference in toughness between the two treatment durations for either VEH or ALN0.2.

69 Conclusions: Although three years of alendronate-treatment resulted in higher microcrack
70 density in vertebral trabecular bone compared to control dogs, the amount of microdamage was
71 not significantly higher than animals treated for 1 year with similar doses. This suggests that
72 bisphosphonate-associated increases in microdamage occur early in treatment. Because
73 toughness continued to decline significantly over three years of treatment at the higher ALN dose,
74 decreases in toughness are probably not dependent on damage accumulation.
75

76 INTRODUCTION

77 Microdamage accumulates with age [1,2] and may play an important role in age-associated bone
78 fragility [3,4]. Microdamage formation occurs in response to mechanical loads [5-7],
79 preferentially at sites of increased tissue mineralization [8-10], and is removed by remodeling
80 [6,7]. The level of skeletal microdamage is determined by the balance between microdamage
81 formation and its removal. Therefore conditions that either increase microdamage formation, or
82 decrease its removal, can have a significant impact on the accumulation of microdamage and
83 bone fragility.

84 Bisphosphonates are efficacious for reducing fractures due to their suppression of bone
85 remodeling [11-13]. However, as reductions in remodeling are permissive for the accumulation of
86 microdamage, bisphosphonate treatment also increases skeletal microdamage. Numerous
87 animal studies have noted significant increases in microdamage following bisphosphonate
88 treatment [14-18]. This accumulation of microdamage occurs with alendronate and risedronate
89 doses comparable to those used for the treatment of post-menopausal osteoporosis, although the
90 accumulation is greater when higher doses are given (e.g. those approximating doses used for
91 treatment of Paget's disease) [18].

92 Whether microdamage accumulation continues or plateaus with extended bisphosphonate
93 treatment is not known, yet has significant implications as some patients now enter their second
94 decade of treatment. Studies to date have assessed microdamage at a single time point, most
95 often one year. Recently, Komatsubara et al. [16,17] reported that 3 years of daily incadronate,
96 at 2.5 or 5x the clinical dose, significantly increased the accumulation of microdamage in both the
97 vertebrae and rib of dogs. As no data are available concerning microdamage levels with shorter
98 term incadronate treatment (< 3 years), this study was not able to address whether microdamage
99 accumulation continues or plateaus with prolonged bisphosphonate treatment.

100 Animal studies documenting increased microdamage with bisphosphonate-treatment
101 have consistently shown increases in vertebral bone strength and stiffness, leading to questions
102 regarding the implications of increased microdamage with bisphosphonates. However, in all but
103 one of these studies [16] bisphosphonate treatment reduced bone toughness, the energy

104 absorption capacity of the bone tissue [14,15,17,18]. Furthermore, when normalized for
105 increases in BMD, energy absorption capacity at the whole bone (structural) level was
106 significantly compromised following 1 year of alendronate treatment [19]. If microdamage
107 continues to accumulate with prolonged bisphosphonate treatment it is possible that this could
108 lead to further reductions in work to failure and toughness.

109 The goal of this study was to test the hypothesis that microdamage continues to accumulate
110 throughout the duration of bisphosphonate treatment and that this continued accumulation is
111 accompanied by a progressive decline in energy absorption and toughness. We have recently
112 documented that clinically-relevant doses of alendronate reduce vertebral bone turnover by more
113 than 70%, increase microdamage by 4- to 5-fold and non-significantly reduce vertebral toughness
114 by 14-17% in skeletally mature beagles after 1 year of treatment [18]. The current study reports
115 results from animals treated for three years with the same doses of alendronate used in the one
116 year study. This allows both an across-treatment analysis at the three-year time point (vehicle
117 versus alendronate) as well as a within-treatment analysis across time points (1 versus 3 years).

118

119 MATERIALS AND METHODS

120 Animals

121 All procedures were approved prior to the study by the Indiana University School of Medicine
122 Animal Care and Use Committee. Thirty-six female beagles (1-2 years old upon arrival) were
123 purchased from LBL (Reelsville, IN). Upon arrival, lateral X-rays of all dogs were obtained to
124 confirm skeletal maturity (closed proximal tibia and lumbar vertebra growth plates). Animals were
125 housed two per cage in environmentally controlled rooms at Indiana University School of
126 Medicine's AALAC accredited facility and provided standard dog chow and water. Two dogs
127 (both in the ALN 0.2 group) developed hernias, both in year 2, that required surgery. One of
128 these animals developed a second hernia which progressed to the point of needing to be
129 terminated early (month 34 of treatment); this animal was still included in all analyses. All other
130 animals completed the 36 month treatment without serious complication.

131

132 Experimental Design

133 Following two weeks of acclimatization, animals were assigned to treatment groups (n=12/group)
134 by matching body weights. All dogs were treated daily for 3 years with oral doses of vehicle
135 (saline, 1 ml/kg/day) or alendronate sodium (0.20 or 1.00 mg/kg/day; Merck and Co., Inc).
136 Alendronate doses were chosen to approximate, on a mg/kg basis, the doses used for treatment
137 of post-menopausal osteoporosis and Paget's disease, respectively. Alendronate was dissolved
138 in saline and administered to the dogs orally with a syringe. Vehicle-treated animals received 1
139 ml/kg/day of saline. Dosing was performed each morning after an overnight fast and at least 2
140 hours prior to feeding.

141 Prior to necropsy, animals were injected with calcein (0.20 mL/kg, IV) using a 2-12-2-5
142 labeling schedule. Animals were euthanized by intravenous administration of sodium
143 pentobarbital (0.22mg/kg Beuthanasia-D Special). After death, lumbar vertebrae were dissected
144 and saved for analyses. The second and third lumbar vertebrae were fixed in 10% neutral
145 buffered formalin while the fourth lumbar vertebra was wrapped in saline-soaked gauze and
146 frozen (-20°C). All tissue preparation, processing, and analyses were similar to those used for
147 dogs treated for one year [18].

148

149 Histology (Static, dynamic, and microdamage)

150 Static and dynamic histomorphometric measures of trabecular bone were obtained on second
151 lumbar vertebrae (L2). Bones were embedded undecalcified in methyl methacrylate (MMA;
152 Aldrich). Mid-sagittal (4 µm) sections were cut using a Reichert-Jung 2050 microtome (Magee
153 Scientific, Inc) and stained with McNeal's tetrachrome for static histomorphometry. Mid-sagittal (8
154 µm) sections were cut and left unstained for dynamic histomorphometry and wall thickness
155 measures.

156 Third lumbar vertebrae (L3) were processed for microdamage assessment by bulk staining in
157 basic fuchsin as previously described [18,20]. Using 1% basic fuchsin dissolved in increasing
158 concentrations of ethanol, specimens were stained according to the following schedule: 8 hours
159 80% (with one change to fresh 80% after 4 hours), overnight in 95% (with one change to fresh

160 95%), 8 hours in 100% (with one change to fresh 100% after 4 hours). Bones were placed under
161 vacuum (20 in Hg) for all stages during the day and left on the bench top overnight. Following
162 staining, bones were washed in 100% ethanol and embedded undecalcified in MMA. Mid-sagittal
163 (80-100 μm) sections were cut using a diamond wire saw (Histosaw; Delaware Diamond Knives).

164 Histological measurements were made using a semiautomatic analysis system (Bioquant
165 OSTEO 7.20.10, Bioquant Image Analysis Co.) attached to a microscope equipped with an
166 ultraviolet light source (Nikon Optiphot 2 microscope, Nikon). A 5 x 5 mm region of interest,
167 located 1 mm below the cranial plateau, was used for sampling. Static and dynamic variables
168 were measured and calculated in accordance with ASBMR recommended standards [21].
169 Microdamage was assessed using UV fluorescence as previously described [22]. Measurements
170 included crack length (Cr.Le, μm) and crack number (Cr.N), with calculations of crack density
171 (Cr.Dn, $\#/\text{mm}^2$; Cr.N / bone area) and crack surface density (Cr.S.Dn, $\mu\text{m}/\text{mm}^2$; Cr.N * Cr.Le /
172 bone area).

173

174 Densitometry

175 Areal bone mineral density (aBMD, g/cm^2) of the fourth lumbar vertebra (L4), without the posterior
176 elements or cranial/caudal endplates, was quantified using a PIXImus II densitometer (Lunar
177 Corp.). Volumetric bone density and geometry of the L4 vertebra was quantified using a Norland
178 Stratec XCT Research SA+ pQCT (Stratec Electronics). One slice (0.07 X 0.07 x 0.50 mm voxel
179 size) was taken at three locations (25, 50 and 75% of total vertebra height). Total, trabecular,
180 and cortical volumetric bone mineral density (vBMD, mg/cm^3) and cross-sectional area (CSA,
181 mm^2) were obtained for each slice and then averaged together to obtain a single representative
182 value for each specimen.

183

184 Biomechanical Testing

185 The biomechanical properties of L4 vertebrae were quantified using a servohydraulic testing
186 system (MTS Bionix, MTS Corporation). Compression to failure was carried out on saline soaked
187 specimens using displacement control mode (20 mm/min). Load vs displacement data were

188 digitally recorded at a sampling rate of 10Hz. Plots were analyzed for determination of ultimate
189 force (F), stiffness (k) and work to ultimate force (w). Apparent material-level properties ultimate
190 stress (σ_{ult}), modulus (E), and toughness (U) were estimated using the following equations: $\sigma_{ult} =$
191 $(F / CSA) / BV/TV$; $E = (k * (height / CSA)) / BV/TV$; $U = (w / (height * CSA)) / BV/TV$, where cross
192 sectional area (CSA) is from pQCT, height measured using digital calipers, and BV/TV from L2
193 histomorphometry.

194

195 Statistics

196 All statistical tests were performed using SAS software (SAS Institute, Inc.). To determine
197 whether variables were different among treatment groups after 3 years, data were evaluated
198 using a one-way analysis of variance (ANOVA) with Fisher's protected least-significant difference
199 (PLSD) post-hoc tests. Strength-density and energy absorption-density relationships from three-
200 year treated animals were compared between VEH and ALN treatments using analyses of
201 covariance with least square means (LSM) used to determine differences in parameters after
202 accounting for aBMD. To determine whether changes occurred within treatment groups across
203 time, t-tests were used to compare data from animals treated for three years with results from an
204 earlier study in our lab which treated animals under the same conditions for one year [18]. For all
205 tests, $p \leq 0.05$ was considered statistically significant. All data are presented as mean \pm standard
206 error.

207

208 RESULTS

209 At the conclusion of the study there was no significant difference in body mass among the three
210 groups (VEH: 12.6 ± 0.6 kg; ALN0.2: 12.4 ± 0.5 kg; ALN1.0: 11.6 ± 0.7 kg; $p = 0.492$).

211 Crack density, the number of microcracks per mm bone tissue, was significantly higher
212 than VEH for ALN1.0 (+100%, $p = 0.01$), but not ALN0.2 (+50%; $p = 0.12$)(Figure 1A). Mean
213 crack length was significantly smaller in both ALN-treated groups compared to VEH (-20% for
214 both) (Figure 1B). Crack surface density, the product of crack density and crack length, was not
215 significantly different among groups (Figure 1C).

216 Activation frequency (Ac.f) was significantly lower than VEH in both ALN0.2 (-59%) and
217 ALN1.0 (-60%) treated animals. The reduction in Ac.f resulted from significant suppression of
218 both mineral apposition rate (MAR) and mineralizing surface (MS/BS), with no change in wall
219 thickness. MAR was 17% lower than VEH for both doses of ALN while MS/BS was -51% and -
220 62% for ALN0.2 and ALN1.0 groups, respectively (Table 1).

221 Structural biomechanical properties – ultimate load, stiffness, and energy to ultimate load
222 – were not significantly different among the treatment groups (Table 2). When normalized for
223 aBMD, there was no difference in the strength-density relationship between VEH- and ALN-
224 treated animals (Figure 2A). The slope of the energy absorption-density relationship was similar
225 between treatments yet at a given aBMD the energy absorption capacity was significantly lower in
226 vertebrae from ALN-treated animals (-20%, $p = 0.01$) compared to VEH (Figure 2B). For both the
227 strength-density and energy absorption-density relationships the two doses of ALN were pooled
228 as the results were similar when doses were assessed separately.

229 Toughness, the energy absorption capacity of the bone tissue, was significantly lower in
230 both ALN0.2 (-26%) and ALN1.0 (-33%) groups compared to VEH (Table 2). There was no
231 difference among groups for the other two material-level properties, ultimate stress and modulus.

232 Vertebral aBMD was not significantly different among groups while vBMD tended to be
233 higher ($p=0.056$) in both ALN0.2 and ALN1.0 groups (both +7%) versus VEH (Table 3).
234 Trabecular vBMD, cortical vBMD, and cross-sectional area were not different among the three
235 treatment groups. Trabecular bone volume, assessed by histology, was significantly greater in
236 both ALN0.2 (+23%) and ALN1.0 (+31%) treatment groups compared to VEH (Table 3).

237 After three years of treatment Ac.f. was significantly lower in ALN0.2 (-40%, $p = 0.01$), but
238 not ALN1.0 (-30%, $p = 0.30$), compared to similar treatment groups at 1 year (Figure 3a). The
239 level of microdamage (both Cr.Dn and Cr.S.Dn) was not significantly different at 3 years
240 compared to 1 year for either ALN group (Figure 3b, 3c). Compared to 1-year of treatment,
241 ALN0.2 had higher ultimate load (+21%), stiffness (+55%) and modulus (+65%) at 3 years while
242 ALN1.0 had significantly higher stiffness (+42%) and modulus (+30%) and lower toughness (-
243 18%) (Table 4 and Figure 3). VEH-treated animals had significantly lower Ac.f. (-58%), higher

244 microdamage accumulation (+301%), and higher structural- and material-level strength and
245 stiffness at 3 years compared to VEH-treated animals after 1 year (Table 4 and Figure 3).

246

247 DISCUSSION

248 Animal studies have consistently documented higher levels of microdamage in bisphosphonate-
249 treated animals [14-18] yet it has remained unclear whether microdamage accumulation
250 continues or plateaus with extended bisphosphonate treatment. Recently, we have documented
251 that clinically-relevant doses of alendronate increase microdamage by 4- to 5-fold in skeletally
252 mature beagles after 1 year of treatment [18]. We now present data to show that the level of
253 microdamage in vertebral trabecular bone does not significantly increase with an additional two
254 years of alendronate treatment (3 years total treatment duration) at doses approximating those
255 used to treat post menopausal osteoporosis or Paget's disease.

256 As remodeling is necessary to remove microdamage [6,7], bisphosphonate-treatment would
257 be expected to allow accumulation of damage due to turnover suppression. While the degree of
258 turnover suppression is correlated to the degree of microdamage accumulation [15,17,18], even
259 mild suppression of turnover (~40%) with bisphosphonate-treatment is sufficient to allow
260 significant increases in microdamage [18]. The current study shows that the initial suppression of
261 turnover with bisphosphonate treatment has the greatest influence on microdamage
262 accumulation. Following one year of ALN-treatment, vertebral bone turnover is suppressed by
263 ~70%, associated with a 4 to 5-fold increase in microdamage [18]. With an additional 2 years of
264 treatment, and a continued decline in turnover (-30 to -40% compared to values in 1 year
265 animals), microdamage was not significantly increased (1.3- and 1.6-fold higher than VEH). The
266 most plausible explanations for this finding are 1) microdamage can be controlled at a new
267 equilibrium level even with only 30% of normal bone turnover and/or 2) there is a reduced
268 formation of microdamage. The latter could result from the lowering of trabecular strains due to
269 the 20-30% increase in bone volume (Table 3).

270 Consistent with the relationship between turnover suppression and microdamage
271 accumulation, animals treated for 3 years with vehicle had significantly lower turnover (-58%) and

272 significantly higher levels of microdamage (+300%) compared to those treated for 1 year. These
273 data highlight that microdamage accumulation is not due to bisphosphonates, per se, but rather
274 the reduction in turnover brought about by bisphosphonate treatment.

275 Toughness, the energy absorption capacity of the material, is consistently reduced in
276 bisphosphonate-treated animals [14,15,17,18]. This change has often been attributed to
277 microdamage accumulation although a cause and effect has yet to be established. The current
278 results provide two pieces of evidence to suggest microdamage accumulation is not 'causing'
279 reduced toughness in bisphosphonate-treated bone. First, despite higher levels of microdamage
280 in VEH-treated animals after three years (compared to 1 year), there was no change in bone
281 toughness. Second, despite no significant difference in microdamage accumulation between
282 animals treated for one and three years with either dose of ALN, animals treated with ALN1.0 had
283 significantly lower bone toughness at three years compared to one year. While these data do not
284 disprove a cause/effect relationship they strongly suggest bisphosphonate-associated reductions
285 in bone toughness extend beyond simply the accumulation of microdamage.

286 Structural biomechanical properties – ultimate load, stiffness, and energy absorption – were
287 not significantly different than VEH after 3 years of ALN treatment. These results differ from
288 those at one year, where both doses of ALN significantly increased vertebral stiffness [18] and
289 the higher dose of ALN increased strength [15]. The absence of difference among these groups
290 treated for 3 years is likely the result of significant increases in VEH-treated animals, which had
291 significantly higher ultimate load (+24%) and stiffness (+68%) compared to values in 1 year
292 treated animals. These higher structural-level mechanical properties in 3-year VEH-treated
293 animals compared to 1-year VEH-treated animals likely result from age-associated periosteal
294 expansion. Vertebral cross-sectional area, which plays a significant role in determining structural
295 parameters and results from continued periosteal expansion, was significantly higher (+16%) in
296 the 3-year VEH group compared to the 1 year group. Material-level properties – ultimate stress
297 and modulus – were also higher in VEH-treated animals at 3 years compared to one year. We
298 have recently documented increases in collagen cross-linking and collagen maturity of vertebrae
299 that are attributable to turnover suppression [23]. As the organic matrix is known to affect

300 material properties, we hypothesize that the reduction in turnover between 1 year and 3 years in
301 vehicle-treated animals (-58%) results in an increase in collagen cross-linking and maturity which,
302 in conjunction with other parameters such as mineralization and microdamage, determine
303 material-level biomechanical properties [24].

304 An alternative approach to investigate the effects of bisphosphonate treatment on
305 biomechanical properties is to compare the relationships between bone density and
306 biomechanical properties. Proposed by Hernandez and Keaveny [25], these relationships allow
307 the determination of changes in bone strength or energy to fracture that are not accounted for by
308 a change in bone mass (aBMD). ALN-treated animals had 20% lower energy absorption capacity
309 at a given aBMD, indicating that an increase in BMD is necessary with alendronate treatment to
310 maintain energy absorption capacity at a level comparable to non-treated bone. This result is
311 consistent with the 22% lower energy absorption at a given aBMD following one year of treatment
312 with doses of ALN approximating those used to treat osteoporosis [19].

313 Given the invasive nature of both microdamage and biomechanical property measures, it
314 proves difficult to determine if the changes noted in the current study extend to humans treated
315 with bisphosphonates. Higher levels of microdamage exist in bisphosphonate-treated women
316 [26], although there is no data to support whether there exists a similar treatment-duration
317 accumulation pattern as noted in the current study. Bisphosphonates have clear anti-fracture
318 efficacy suggestive of improved biomechanical properties [11-13]. However, given the multi-
319 factorial nature of fractures it remains possible that reduced toughness or lower energy
320 absorption at a given aBMD could exist even in light of an overall population reduction in fracture
321 risk with bisphosphonates. Indeed both toughness and energy absorption are compensated for
322 by the increased bone density that routinely occurs with bisphosphonate treatment, but the
323 material properties of the tissue are nevertheless compromised.

324 In conclusion, three years of alendronate-treatment resulted in higher microcrack density in
325 vertebral trabecular bone of intact beagle dogs, yet the amount of microdamage was not
326 significantly higher than in animals treated with equivalent doses for 1 year. This suggests that
327 increased skeletal microdamage associated with turnover suppression occurs early in treatment,

328 and does not progress with longer treatment duration. Because toughness continued to decline
329 significantly over three years of treatment at the higher ALN dose, decreases in toughness are
330 probably not dependent on damage accumulation.

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341

342 FIGURE LEGENDS

343 Figure 1. Microdamage parameters in vertebral trabecular bone following three years of daily
344 vehicle (VEH) or alendronate (ALN) treatment (0.20 or 1.00 mg/kg/day). (A) Crack density, the
345 number of microcracks normalized to bone area, was significantly higher ($p = 0.032$) in animals
346 treated with the higher dose of alendronate (ALN1.0). (B) Mean crack length was significantly
347 lower ($p = 0.013$) with both doses of ALN. (C) Crack surface density, the product of crack
348 density and crack length, was not significantly different among groups ($p = 0.149$). There was no
349 significant difference between doses of ALN for any microdamage parameter. * $p < 0.05$ versus
350 VEH.

351

352 Figure 2. Strength-density (A) and energy absorption-density (B) relationships of vertebral bone
353 from beagles treated for 3 years with vehicle (VEH) or alendronate (ALN). Areal bone mineral
354 density (aBMD) was assessed by densitometry while strength and energy absorption were
355 assessed by monotonic compression biomechanical tests. The strength-density relationship was
356 similar for vehicle (\circ , $y = 20397x - 2065$) and alendronate-treated animals (\bullet , $y = 23385x - 3033$).
357 The slope of the energy absorption-density relationship was similar yet the intercepts differed
358 significantly between vehicle (\circ , $y = 9912x - 1306$) and alendronate-treated animals (pooled (\bullet),
359 $y = 11489x - 2228$). After adjusting for aBMD, the energy absorption capacity was significantly
360 lower (-20%) in ALN-treated specimens compared to VEH. ALN-treated groups were combined
361 as there was no difference between the two doses for either relationship.

362

363 Figure 3. Differences in activation frequency (A) crack density (B) and toughness (C) between
364 animals treated for 1 and 3 years with vehicle (VEH) or alendronate (ALN0.2 and ALN1.0). (A)
365 Activation frequency was significantly lower in both VEH and ALN0.2 after 3 years of treatment
366 compared to animals at 1 year. (B) Crack density, the number of microcracks normalized to
367 bone area, was not significantly different for either dose of ALN but was significantly higher in
368 VEH-treated animals after 3 years compared to animals treated for 1 year. (C) Toughness, the
369 material-level energy absorption capacity, was significantly lower in animals treated with the

370 higher dose of alendronate (ALN1.0) after 3 years compared to values at 1 year. * $p < 0.05$
371 versus 1 year animals within treatment.

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Table 1. Dynamic histomorphometry of the second lumbar vertebrae

	Vehicle 1 ml/kg/day	Alendronate 0.20 mg/kg/day	Alendronate 1.00 mg/kg/day	p value
MAR, $\mu\text{m}/\text{day}$	1.36 ± 0.08	$1.13 \pm 0.06^*$	$1.11 \pm 0.07^*$	0.028
MS/BS, %	7.63 ± 0.99	$4.15 \pm 0.51^*$	$3.30 \pm 0.92^*$	0.002
BFR/BS, $\mu\text{m}^3/\mu\text{m}^2/\text{year}$	36.75 ± 0.10	$16.70 \pm 0.04^*$	$14.31 \pm 0.09^*$	0.0004
Ac.f, #/year	0.793 ± 0.097	$0.328 \pm 0.037^*$	$0.319 \pm 0.094^*$	0.0002

N= 12 animals per treatment group. MAR, mineral apposition rate; MS/BS, mineralizing surface per unit bone surface; BFR/BS, bone formation rate normalized to bone surface; Ac.f, activation frequency. * $p < 0.05$ vs vehicle.

Table 2. Compressive biomechanical properties of the fourth lumbar vertebrae

	Vehicle 1 ml/kg/day	Alendronate 0.20 mg/kg/day	Alendronate 1.00 mg/kg/day	p value
Ultimate Load, N	4656 ± 234	4966 ± 254	4847 ± 304	0.710
Stiffness, N/mm	11889 ± 1153	14241 ± 1146	13622 ± 956	0.299
Energy to Ultimate Load, Nmm	1961 ± 198	1764 ± 140	1581 ± 136	0.260
Ultimate Stress/(BVTv)	1.78 ± 0.11	1.64 ± 0.14	1.51 ± 0.07	0.229
Modulus/(BVTv)	67.3 ± 6.9	74.1 ± 9.1	62.9 ± 3.2	0.518
Toughness/(BVTv)	0.049 ± 0.004	0.036 ± 0.003 *	0.033 ± 0.002 *	0.004

N= 12 animals per treatment group. BV/TV, bone volume normalized to tissue volume. * p < 0.05 vs vehicle.

Table 3. Lumbar vertebrae bone mineral density, geometry, and bone volume

	Vehicle 1 ml/kg/day	Alendronate 0.20 mg/kg/day	Alendronate 1.00 mg/kg/day	p value
Whole aBMD, g/cm ²	0.330 ± 0.010	0.343 ± 0.011	0.337 ± 0.009	0.644
Total vBMD, mg/cm ³	554 ± 14	591 ± 13	597 ± 12	0.056
Trabecular vBMD, mg/cm ³	329 ± 7	349 ± 7	341 ± 6	0.111
Cortical vBMD, mg/cm ³	1020 ± 10	1019 ± 7	1027 ± 6	0.746
CSA, mm ²	136 ± 4.5	131 ± 5.3	127 ± 5.1	0.461
Trabecular BV/TV, %	19.9 ± 1.3	24.5 ± 1.7 *	25.8 ± 1.7 *	0.029

N= 12 animals per treatment group. aBMD, areal bone mineral density; vBMD, volumetric bone mineral density; CSA, cross sectional area; BV/TV, bone volume normalized to tissue volume.

* p < 0.05 vs vehicle.

Table 4. Percent difference between animals treated for 1 and 3 years within treatment

	Vehicle 1 ml/kg/day	Alendronate 0.20 mg/kg/day	Alendronate 1.00 mg/kg/day
aBMD, g/cm ²	-1	-5	-4
CSA, mm ²	+17	+15	+15
Ultimate Load, N	+24	+21	+14
Ultimate Stress/(BVTV)	+20	+18	-1
Stiffness, N/mm	+68	+55	+42
Modulus/(BV/TV)	+62	+65	+30
Energy to Ultimate Load, Nmm	+13	+7	-3

Comparisons between parameters of lumbar vertebrae after 1 and 3 years of treatment (1 year data from Allen et al, Bone 2006). Values represent percent difference between 1 and 3 year animals. **Bold** denotes significance ($p < 0.05$). N= 12 animals per treatment group per time point. aBMD, areal bone mineral density; CSA, cross sectional area; BV/TV, bone volume normalized to tissue volume.

Figure 1.

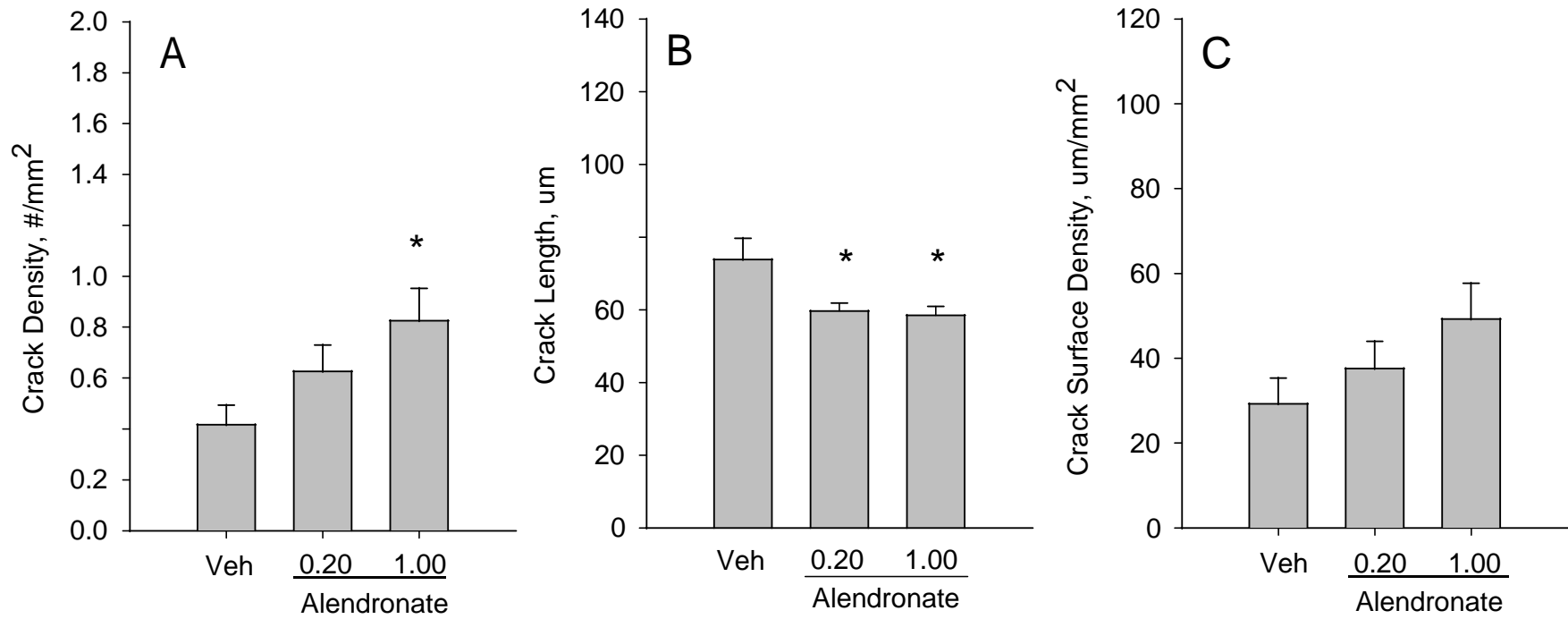


Figure 2.

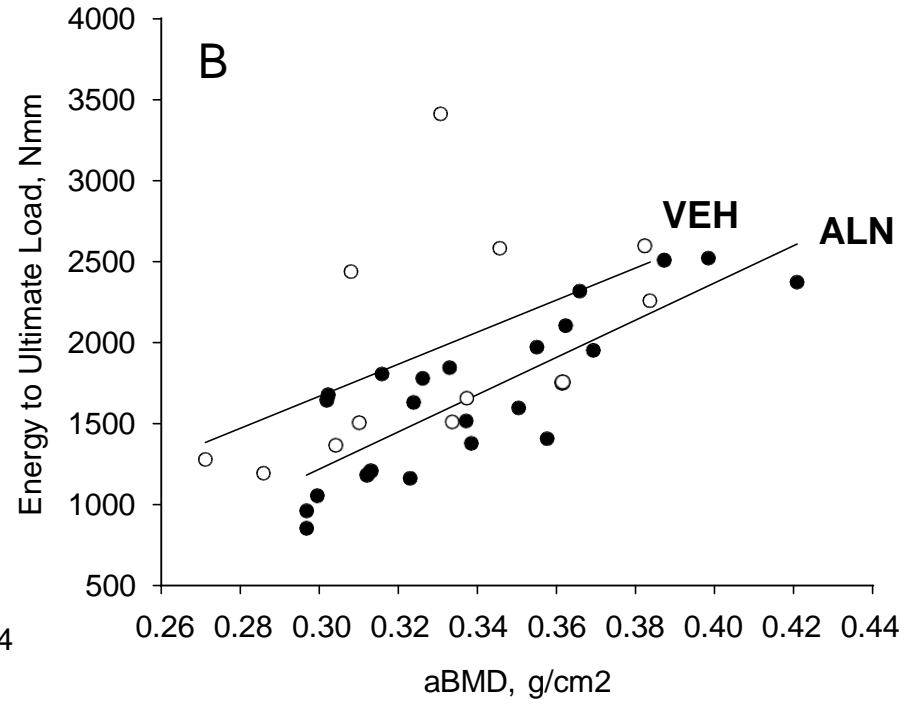
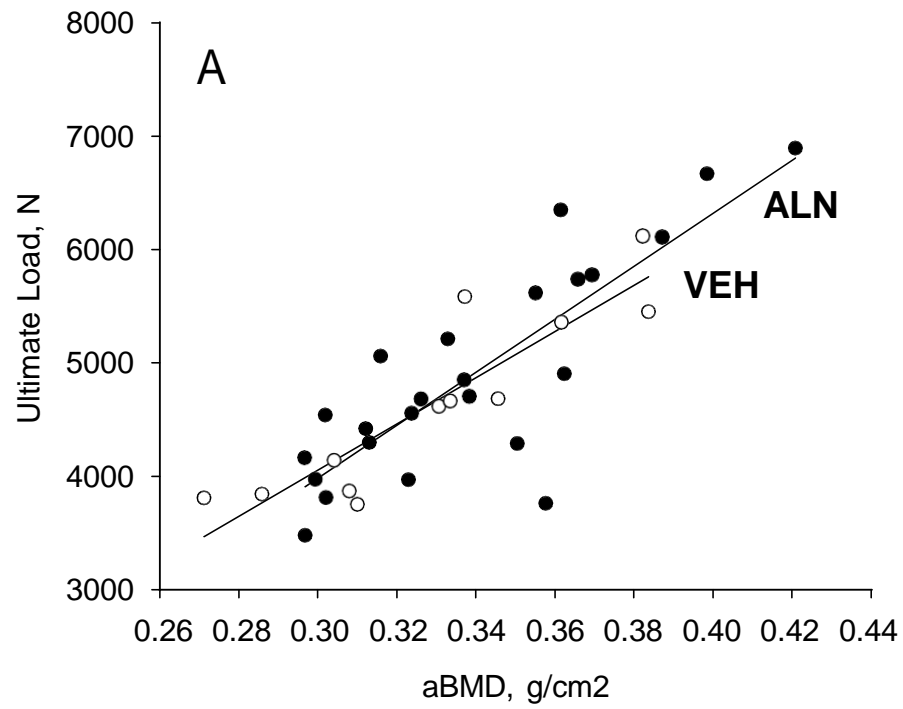


Figure 3.

