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1 **Title: Mineral status in canine medial coronoid process disease: A cohort study using analysis of**
2 **hair by mass-spectrometry**

3

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14

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17 **Key words:** medial coronoid process disease; minerals; copper; zinc; sulphur; hair analysis

18

19 **Abstract**

20

21 **Background:** In several species developmental skeletal diseases involving abnormal endochondral
22 ossification have been associated with imbalanced mineral intake. Hair analysis reflects long-term
23 mineral status.

24

25 **Aim:** To determine the mineral content of hair from dogs with or without medial coronoid process
26 disease (MCPD). Hypothesis: dogs with MCPD have a different profile of minerals known to influence
27 metalloenzymes involved in endochondral ossification.

28

29 **Methods:** After cleansing, chelation and acid-digestion of hair samples (n=79 in total: Control dogs,
30 n=70 vs. MCPD, n=9) mineral profile (7 major and 25 trace elements) was determined by inductively
31 coupled plasma-mass spectrometry (ICP-MS). Dogs were of similar age (Control, 4.05 [1.85 – 7.70]
32 vs. MCPD, 4.30 [3.25 – 6.53] median (IQR) years; P=0.78) and gender (Control, n=43/27 vs. MCPD,
33 n=4/5 males/females). 28/70 (40%) of control and 8/9 (88%) of MCPD dogs were neutered,
34 respectively.

35

36 **Results:** Hair from dogs with MCPD contained significantly lower amounts ($\mu\text{g/g DM}^{-1}$) of copper,
37 sulphur and zinc (all at $P < 0.001$). Age, sex and neutered status had no effect on hair mineral status.

38

39 **Conclusions:** Based on hair analysis, a role for mineral imbalance including copper, sulphur and zinc
40 in the aetiopathogenesis of canine MCPD is suggested. Hair mineral analysis may prove useful as a
41 biomarker for susceptible puppies.

42

43 **Introduction**

44

45 In young mammals, linear skeletal growth occurs at the cartilaginous growth plate or physis with
46 subsequent endochondral ossification (Mackie and others 2008) and structural remodelling in
47 response to biomechanical forces under 'Wolffs Law' (Frost 1994). Canine developmental skeletal
48 disease (DSD) is an over-arching term used to describe several conditions including hip dysplasia;
49 premature closure of growth plates; ununited anconeal process (UAP); fragmentation of the
50 coronoid process (medial coronoid process disease - MCPD) and osteochondrosis (OCD). DSD in the
51 elbow is called 'elbow dysplasia' (Lavrijsen and others 2014; Scott 1998).

52

53 Excessive forces applied to insertions on bone usually result in an avulsion fracture. In MCPD, the
54 coronoid process onto which the collateral ligament and brachialis muscle tendon insert develops
55 fissures and disintegrates into multiple fragments (Fitzpatrick and others 2009); (Lau and others
56 2013b) endochondral ossification is delayed (Lau and others 2013a) and affected processes are
57 significantly less mineral dense than processes from unaffected dogs (Burton and others 2010).
58 Occurrence of MCPD, together with other DSDs (Meyer-Lindenberg and others 2006), suggests
59 either a common aetiopathogenesis or a degree of linked heritability.

60

61 Observational studies across several species have associated DSDs with dietary mineral imbalance.
62 This may involve high or low intake of a specific trace mineral or competitive interactions amongst
63 divalent transition metals in the gastrointestinal tract, which reduces mineral bioavailability. For
64 example, high phosphorus (Savage and others 1993a, b) or zinc intake (Gunson and others 1982)
65 have been implicated in the occurrence of OCD in foals. Zinc directly inhibits osteoclast activity
66 (Moonga and Dempster 1995) and high intake reduces the bioavailability of copper resulting in frank

67 deficiency, as has been reported in pigs (Hill and others 1983). Dietary supplementation with copper
68 (Knight and others 1990) or magnesium (Counotte and others 2014) has been shown to reduce the
69 occurrence rate of OCD in foals, highlighting the essentiality of good mineral balance during growth
70 for development of the skeletal system. High calcium intake has been associated with DSDs in dogs
71 (Schoenmakers and others 2000).

72

73 Precisely how imbalanced mineral nutrition causes DSD has not been determined, although it has
74 been suggested that altered activity of endochondral-located metalloenzymes (e.g. zinc-dependent,
75 matrix metalloproteinases (MMPs)) are involved (Page-McCaw and others 2007; Takaishi and others
76 2008). MMPs are important for normal bone development and bone remodelling after repair and
77 maintenance; experimental deletion in mice, particularly of MMP-9 & MMP-13, leads to disrupted
78 bone development (Page-McCaw and others 2007; Takaishi and others 2008). In dogs, MMPs have
79 been proposed to underpin the development of OCD (Kuroki and others 2005). Hence,
80 endochondral metalloenzyme activity may be impaired by inappropriate mineral status thereby
81 increasing the likelihood of DSD.

82

83 Mineral status may be assessed in a number of ways. Blood and urine samples provide only a short-
84 term assessment of mineral status (i.e. hours to days) which would not cover the growth period.
85 Tissue biopsy can be definitive for identifying micronutrient deficiencies (e.g. in liver), but is invasive
86 and traumatic and therefore unacceptable as a routine diagnostic or screening tool. We consider
87 that mineral composition of hair is a better indicator of long-term mineral nutrition status. Hair
88 concentrations of some minerals are 10-15 times the levels in blood or urine (Ahmad and others
89 2013), sampling is non-invasive (e.g. by grooming or clipping) and reflects mineral accumulation
90 over a long-period (months to years). Many factors are involved in mineral incorporation into the

91 structure of hair: at the time of synthesis of each hair in the follicle, through absorption from
92 sebaceous or other secretions, and through environmental contamination. Studies using radioactive
93 isotopes have shown that mineral uptake into hair can occur within hours of dietary intake, but that
94 uptake rates vary from one mineral to another; for example, zinc incorporation into hair takes longer
95 than other minerals (Strain and others 1971). Similar to other keratinous structures, like horse
96 hooves or human nails, hair does not accurately reflect diet, but dietary fluctuations can influence
97 hair composition. In cattle, variation in dietary magnesium (low vs. high intake; (Anke 1966; Fisher
98 and others 1985)), or dietary zinc and selenium (Perry and others 1976) were reflected as high or
99 low content in hair. Whilst accurate data are not available for all dog breeds and variable rates of
100 growth or periods between shedding may affect hair content, it is generally accepted that dog hairs
101 remain in the inactive, 'resting' telogen phase for a long period (e.g. months to years) and for some
102 breeds such as Nordic dogs, hair may only be shed after many years.

103

104 Based on an accumulation of evidence, we posit that imbalanced mineral nutrition or impaired
105 bioavailability, is an underlying cause of canine MCPD. We hypothesise that the mineral composition
106 of hair from dogs with MCPD will be significantly different, especially in zinc and copper content, to
107 the mineral composition of hair from dogs that are free from MCPD.

108

109 This study was approved by the Ethics Committee of the School of Veterinary Medicine and Science,
110 University of Nottingham

111 **Materials and methods**

112

113 ***Collection and preparation of hair for elemental analysis:*** A total of 79 samples (10-200g) of hair
114 were collected from 32 different breeds of dogs without DSD during routine grooming from a UK
115 Veterinary Hospital and from 9 dogs with confirmed elbow dysplasia and fragmentation of the
116 coronoid process (MCPD) attending several UK practices during September and October 2015.
117 Breeds with MCPD included Labradors (n=6 of 9; 66%), two crossbred dogs (22%) and one
118 Staffordshire bull terrier. Hair was placed into sealed plastic bags and labelled with the dog's name,
119 age, breed, sex and neutered status. Of the 79 hair samples, n=70 (males, n=43; females, n=27) were
120 healthy dogs free from any clinical signs of developmental skeletal disease based on their clinical
121 records (Control). The remaining n=9 dogs (males, n=4; females, n=5), were confirmed to have
122 MCPD by experienced radiologists assessing CT scans. 28 of the healthy dogs and one of the dogs
123 with MCPD were not neutered. The age range of each population of dogs was similar: Control, 4.05
124 [1.85 – 7.70] vs. MCPD, 4.30 [3.25 – 6.53] median (IQR) years; P=0.78).

125

126 During preparation of hair, gloves were worn at all times to avoid trace ion contamination of the
127 samples. In order to remove external contaminants (dust, dirt, skin cells, cosmetic and cleaning
128 treatments) a sample of hair (10-50g) was washed in a series of steps, as described previously (Forte
129 and others 2005). In brief, the sample was immersed in a mixture of 3:1 (v/v) di-ethyl ether-acetone
130 (Sigma, UK) and stirred at room temperature for 10 min to remove any sebaceous film covering the
131 hair. At this time, samples were re-immersed and stirred for 1 h in 5% sodium ethylenediamine
132 tetracetic acid (EDTA) (Sigma, UK) to chelate free chemical elements present on the surface of the
133 hair. Finally, samples were repeatedly rinsed for 10 mins (x3) in ultrapure milli-Q water (18.2 MΩ
134 cm) (Fisher Scientific UK Ltd, Loughborough, UK). After cleansing, a known quantity of hair was

135 placed into a 250ml solvent-resistant container (Sarstedt, UK) and contents freeze dried. After
136 drying, moisture content was calculated by difference (<5% for all samples) and a known weight of
137 dry hair (100-200 mg) acid-digested using standard techniques for inductively-coupled plasma mass-
138 spectrometry (ICP-MS). Briefly, each sample was microwave-digested in 3.0 mL of 70% Trace
139 Analysis Grade (TAG) HNO₃, 2.0 mL H₂O₂ and 3.0 mL milli-Q water (18.2 MΩ cm) (Fisher Scientific
140 UK Ltd, Loughborough, UK). Hair samples were prepared and run in batches. To control for sources
141 of contamination (e.g. in acids or water) two blank tubes were run with each batch (containing all
142 liquids but no sample). Additionally, a certified reference material (CRM) was run in duplicate for
143 each batch. For hair digests, the CRM was powdered human hair (ERM-DB001; Sigma-Aldrich, UK)
144 certified (all as µg/g DM⁻¹) for As (0.044 ± 0.006), Cd (0.125 ± 0.007), Cu (33 ± 4), Se (3.24 ± 0.24)
145 and Zn (209 ± 12).

146

147 **Elemental analysis by ICP-MS:** Elemental analysis was by inductively coupled plasma-mass
148 spectrometry (ICP-MS; iCAPTM Q, Thermo Fisher Scientific Inc., Waltham, MA, USA) using a He
149 collision cell with 'kinetic energy discrimination' to reduce polyatomic interference in the analysis
150 of Ag, Al, As, B, Ba, Cd, Ca, Co, Cr, Cs, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb, Rb, S, Sr, Tl, U, V and Zn.
151 Lithium, Be and P were determined in standard (vacuum) mode and Se in 'hydrogen-cell' mode, with
152 'in-sample switching'. Internal standards were Ge, Rh and Ir. Final hair elemental composition is
153 presented after correction for blanks and batch variation (using the CRM as reference) as µg/g of
154 dry matter [DM; 1 µg/g = 1ppm]. For major and trace elements, recovery was >95% (e.g. Ca, 97%;
155 Zn, 106%; Cu, 100%) with <5% coefficient of variation for each (n=11 separate analyses). Operational
156 blanks (n = 10) were run to determine the operational Limit of Detection (LOD; 3*SD) and Limit of
157 Quantification (LOQ, 10*SD). Intra-assay variability for all elements was <2%.

158

159 **Statistical Analysis**

160 Continuous data (e.g. age of the dogs, elemental composition) was analysed by one-way analysis of
161 variance (ANOVA; Healthy Controls vs. Developmental Skeletal Disease). Appropriateness of each
162 statistical comparison was assessed by visualizing histograms of residuals and further residual (on
163 y-axis) plots of 1) fitted-values and 2) expected normal quantiles. Non-parametric data (i.e. those
164 with skewed residual errors, common for elemental analysis) were statistically analysed after \log_{10}
165 transformation. Data are reported as means \pm 1 standard deviation (S.D.), unless otherwise
166 indicated in the text. 95% confidence intervals around the mean may be approximated from the
167 data as $\times 2$ S.D. We considered $P < 0.05$ as indicating statistical significance, but adjusted P-values to
168 account for the number of comparisons ($0.05/17$ minerals present above LOQ). Multivariate, linear
169 discriminant analyses were used to identify patterns in complex (i.e. multiple variates), non-
170 independent data (e.g. all elemental data for hair) using principal component analysis (PCA) and
171 orthogonal partial least squares discriminant analysis (OPLS-DA) using SIMCA-P v12 (Umetrics,
172 Umea, Sweden). The method is an objective means to effectively demonstrate statistically
173 significant patterns in complex, non-independent datasets. All data were otherwise analyzed using
174 Genstat v17 (VSNi, Rothampsted, UK).

175

176 **Power and sample size:** We estimated that over the period of study (June 2015 – November 2015)
177 we would collect hair from approximately five:one healthy dogs (i.e. no clinical signs of
178 developmental skeletal disease) vs. dogs with developmental skeletal disease. In a previous study
179 (Forte and others 2005), the response within each subject group was normally distributed with a
180 standard deviation of 3.41 and 47 (for copper and zinc, respectively). If the true difference in our
181 control vs. disease means is 4 (Cu) and 56 (Zn) $\mu\text{g/g DM}^{-1}$ (>25% mean difference) we will need to
182 recruit at least 33 control subjects and 7 experimental subjects to be able to reject the null

183 hypothesis that the population means of the experimental and control groups (for Cu and Zn) are
184 equal with probability (power) 0.80. The Type I error probability associated with this test of the null
185 hypothesis is 0.05.

186

187

188 **Results**

189 The elemental composition of dog hair is presented in Table 1. A number of elements were
190 measureable, but without high confidence i.e. above limits of quantification (LOQ) and whilst we
191 provide pooled averages below, we do not report further on these elements. For information,
192 elements at low levels in hair samples (median [IQR] $\mu\text{g/g DM}^{-1}$ for pooled estimates) were: Arsenic
193 (As, 0.040 [0.01-0.17]), Beryllium (Be, 0.001 [0.000-0.001]), Cadmium (Cd, 0.009 [0.004-0.033]),
194 Cobalt (Co, 0.014 [0.010-0.031]), Caesium (Cs, 0.002 [0.001-0.005]), Lead (Pb, 0.18 [0.10-0.37]),
195 Lithium, (Li, 0.040 [0.025-0.080]), Molybdenum (Mo, 0.071 [0.041-0.130]), Rubidium (Rb, 0.044
196 [0.025-0.094]), Silver (Ag, 0.047 [0.028-0.076]), Titanium (Ti, 0.60 [0.32-1.00]), Thallium (Tl, 0.001
197 [0.000-0.002]), Uranium (U, 0.002 [0.001-0.004]) and Vanadium (Va, 0.080 [0.045-0.150]).
198 Nevertheless, it is of interest that for a few isolated cases some values were well above LOQ in hair.
199 For example, three cases had hair arsenic of 3.85, 4.60 and 27.6 $\mu\text{g/g DM}^{-1}$.

200

201 Comparison of 'Other Breeds' (i.e. not including Labrador) indicated that hair from dogs with MCPD
202 contained significantly lower amounts ($\mu\text{g/g DM}^{-1}$) of copper (27% lower, $P=0.03$) and zinc (21%,
203 $P=0.01$) (Table 1 & Figure 1). Comparison of Labrador retrievers only (Healthy Labradors vs.
204 Labradors with MCPD) also indicated that Labradors with MCPD had significantly lower hair ($\mu\text{g/g}$
205 DM^{-1}) copper (17% lower, $P<0.001$), zinc (23%; $P=0.01$) and also sulphur (18%, $P=0.009$). Hence, hair
206 zinc and copper composition may biomark MCPD regardless of breed, whereas hair sulphur content
207 may only biomark MCPD in Labradors, suggesting a breed pre-disposition to altered sulphur
208 metabolism and consequent incorporation into hair. Correction for age, sex and neutered status
209 had no effect on the significance of these results.

210

211 Multivariate analysis of the whole elemental dataset (normalised to account for marked variation
212 in elemental composition) according to 1) disease status (MCPD Disease = 'Yes') and 2) sex of dog
213 (Male/Female) clearly discriminates disease status as the largest contributor (linear discriminant
214 vector 1, 68.1%) to variation in the dataset, with gender also being important (25.6% of the
215 variation) but not with respect to disease status (Figure 2A). Whilst affected animals (blue dots in
216 Figure 2C) retained significant variation in overall hair mineral composition, in that the group are
217 not distinct from unaffected animals, the loadings attributable to individually elements in those dogs
218 with vs. those without disease clearly distinguish reduced hair zinc >>copper>sulphur (Figure 2D).
219

220 **Discussion**

221 In this study, we report for the first time a significant difference in accumulated mineral
222 concentrations in hair from dogs with MCPD compared to dogs without MCPD, suggesting that
223 mineral status – as reflected in the long-term through hair analysis – could be used as a biomarker
224 for this disorder.

225

226 It was to be expected that Labradors would be overrepresented in the MCPD group, since Labradors
227 are currently the most popular breed in the UK (www.thekennelclub.org.uk) and they are a breed
228 predisposed to develop MCPD (Lewis and others 2013). Lower hair concentrations of copper,
229 sulphur and zinc may be explained by one of several mechanisms including malnutrition,
230 malabsorption or high mineral presence competing for bioavailability or utilisation in the
231 gastrointestinal tract. In a previous study, 61% of dogs with skeletal abnormalities had consumed
232 unnecessarily high calcium (Dobenecker and others 1998). High calcium intake may chelate other
233 divalent minerals in the gastrointestinal tract, reducing their bioavailability (Schoenmakers and
234 others 2000).

235

236 Metalloenzymes (matrix metalloproteinases or MMPs) are vital for normal endochondral
237 ossification (Page-McCaw and others 2007). Both zinc and copper, two minerals found to be at lower
238 levels in hair from dogs with MCPD, are fundamental to proper functioning of matrix
239 metalloenzymes (Page-McCaw and others 2007). It is therefore possible that diet-induced
240 interference with metalloenzyme activity, engendered by dietary mineral imbalance during
241 endochondral ossification may underpin MCPD in dogs. Indeed, in a separate study, we have
242 recently reported that over 90% of UK ‘complete’, wet, pet foods examined did not comply with EU
243 guidelines for mineral content (Davies, Davis and Gardner - unpublished observations). Many diets

244 contained either less than the nutritional minimum or exceeded the nutritional or legal maximums.
245 Furthermore, many had gross mineral imbalance such as an inappropriately low (1:10) or high (10:1)
246 Ca:P ratio, the optimum being 1:1. Such an imbalance, together with other specific deficiencies –
247 copper for example – suggests that nutrition of companion animals may be an important
248 contributory factor to variable mineral status and development of MCPD.

249

250 One particular target of interest for further study is the zinc-containing matrix metalloproteinase
251 MMP-13, as it is expressed in the skeleton when restructuring of the collagen matrix is required for
252 bone mineralization (Inada and others 2004; Takaishi and others 2008). MMP-13 null mice show
253 profound developmental delays in formation, ossification and vascularization of the primary
254 endochondral ossification centres (Inada and others 2004). Suppressed MMP-13 activity, as may
255 occur with inadequate zinc bioavailability, could explain the histopathological features of OCD.
256 Furthermore, a form of human chondrodysplasia – the Missouri variant of spondyloepimetaphyseal
257 dysplasia – is caused by a mutation in the MMP-13 gene (Patel and others 1993). In a study involving
258 50 explants from dogs with OCD, no MMP-13 staining was present in control samples but in dogs
259 with OCD 42% (21 of 50 specimens) were positive for MMP-13 (Kuroki and others 2005). MMP-13
260 therefore appears integral to non-physiologic, extra-cellular matrix turnover resulting in, or from,
261 abnormal endochondral ossification.

262

263 The scientific basis of using hair as a biomarker of mineral or disease status (e.g. accuracy, reliability,
264 repeatability) has met with controversy (Barrett 1985; Shamberger 2002; Zlotkin 1985). Few
265 scientific studies have evaluated the concept of hair as a marker of underlying disease status. In one,
266 Forte *et al* found little association between mineral composition of hair and progression of
267 Parkinsons Disease (Forte and others 2005). To our knowledge, no study has analysed canine hair

268 samples and directly linked mineral composition to a disease such as MCPD. Many factors may affect
269 canine hair growth and shedding which could confound interpretation of hair mineral analysis
270 (Shamberger 2002; Zlotkin 1985). Most concern has centred over variability in methodologies used
271 between different laboratories such that results from the same individual analysed at different
272 laboratories give different outcomes (Seidel and others 2001). In a recent pilot study, two samples
273 of hair taken from one healthy volunteer were submitted to three different commercial hair mineral
274 analysis laboratories (Namkoong and others 2013); results were consistent for hair mineral
275 composition, with variability only introduced by laboratory-specific differences in reference ranges.
276 As reported in the current manuscript, use of a standardised and appropriate (e.g. hair) Certified
277 Reference Material (CRM) obviates this inherent study-study and batch variability. Further, for a
278 technique as sensitive as ICP-MS it is equally important to include within-run controls to account for
279 machine drift and suitable operational blanks to address potential sources of contamination during
280 acid-digestion. In summary, hair is an accessible biomarker of long-term mineral status but has
281 received a bad press, largely through poor experimental practice in order to maximise its
282 commercial potential. Indeed, in 1979, the U.S. Environmental Protection Agency conducted a
283 meta-analysis of over 400 studies reporting hair as a biomarker of a given disease state and
284 concluded that, *“if hair samples are properly collected and cleaned, and analyzed by the best analytic
285 methods, using standards and blanks as required, in a clean and reliable laboratory by experienced
286 personnel, the data are reliable”*.

287

288 A limitation of this study is that some dogs in the control group may have had underlying,
289 asymptomatic skeletal disease without any mention of such in their clinical records. Age-matched
290 controls were not specifically selected and examined. Furthermore, the precise timing of hair-shaft

291 formation to shedding is not known for many different breeds and ages, although the available
292 evidence would suggest it to likely be months or even years, as oppose to weeks.

293

294 **Conclusions**

295 This study demonstrates for the first time that dogs with MCPD have lower levels of copper, sulphur
296 and zinc in their hair compared to dogs without MCPD. Sulphur is a major constitutive element of
297 hair and an important constituent of cartilage. Zinc and copper are important dietary elements with
298 known roles in endochondral metalloenzyme expression and function. Dietary mineral imbalance
299 may therefore contribute toward MCPD. We propose mineral analysis of hair as an informative,
300 relatively cheap, non-invasive adjunctive tool of diagnostic value for MCPD. Further prospective
301 studies are warranted to determine a causal pathway from variation in the mineral content of diet
302 to mineral content of hair and development of MCPD, or other DSDs. A number of factors are likely
303 to influence hair mineral composition; dietary intake, mineral interactions in the foods, or factors
304 involved in the digestion, absorption, assimilation and distribution of minerals. Further studies in
305 this area are warranted. Hair analysis may offer a mechanism for early screening of puppies to
306 perhaps identify those at risk of developing MCPD or other DSDs. Preventive dietary intervention
307 may then be possible.

308

309 **Clinical relevance**

310 The underlying aetiopathogenesis of canine medial coronoid process disease (MCPD) has not been
311 fully elucidated. This study suggests that mineral status during development of the skeletal system
312 in dogs may be a contributory factor for MCPD. Our study reinforces the need for owners, and for
313 the manufacturers of diets, to ensure that the ration provided to growing puppies contains the
314 correct balance of minerals. Mineral profiling of hair might provide a useful, non-invasive screening

315 tool to identify individual puppies at risk of developing MCPD or other DSDs early in life, so that
316 appropriate dietary intervention can be made to help prevent onset of the disease.

317

318

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322

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326 disclose.

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417 **Table 1: Hair elemental composition of Healthy dogs or dogs with Medial Coronoid Process Disease.**

Major element ($\mu\text{g g DM}^{-1}$)	Control Healthy Dogs (n=70)		Medial Coronoid Process Disease (n=9)		P-value Control vs. MCPD		
	Other Breeds (n=60)	Labrador (n=10)	Other Breeds (n=3)	Labrador (n=6)	Other Breed	Lab	AB × Lab
Sulphur (mgs)	53.7 (49.1,55.9)	54.3 (51.8,57.7)	50.1 (49.6,50.3)	45.5 (43.2,49.7)	0.34	0.009	0.26
Calcium	520 (229,1242)	1360 (757,1618)	627 (600,1242)	516 (362,994)	0.97	0.29	0.50
Phosphorus	248 (195,305)	305 (267,329)	310 (278,369)	235 (200,272)	0.43	0.37	0.07
Sodium	80.9 (45.8,160)	260 (205,678)	131 (119,338)	107 (68.2,382)	0.46	0.22	0.05
Magnesium	68.2 (35.6,160)	254 (134,301)	116 (107,299)	121 (61.7,264)	0.41	0.31	0.09
Potassium	10.3 (3.43,18.8)	37.0 (27.7,89.6)	16.6 (12.8,37.4)	11.0 (8.80,12.9)	0.65	0.04	0.03
Boron	0.27 (0.20,0.76)	0.79 (0.43,1.10)	0.51 (0.46,0.51)	0.29 (0.16,0.50)	0.75	0.20	0.35
Trace element (all $\mu\text{g g DM}^{-1}$)							
Zinc	199 (179,210)	167 (155,173)	160 (141,161)	123 (94,157)	0.01	0.01	0.64
Nickel	99.4 (0.42,245)	0.14 (0.07,0.39)	76.3 (19.1,103)	83.6 (38.0,274)	0.42	0.16	0.22
Iron	28.4 (17.1,42.7)	27.7 (22.6,59.4)	31.8 (24.3,35.2)	35.6 (19.8,75.7)	0.54	0.26	0.19
Aluminum	23.1 (13.0,44.1)	6.91 (3.70,50.4)	34.0 (19.8,34.4)	21.6 (13.0,63.8)	0.65	0.284	0.11
Copper	13.3 (11.1,15.1)	9.27 (8.14,10.4)	10.0 (8.9,10.2)	6.93 (6.58,9.14)	0.03	<.001	0.60
Manganese	0.99 (0.44,2.82)	0.97 (0.48,1.56)	0.75 (0.62,0.86)	2.58 (1.05,3.61)	0.38	0.29	0.37
Strontium	0.68 (0.32,1.70)	0.71 (0.58,1.05)	0.71 (0.58,1.05)	1.10 (0.86,2.26)	0.57	0.78	0.54
Selenium	0.69 (0.60,0.85)	0.69 (0.67,0.97)	0.69 (0.67,0.97)	0.66 (0.58,0.88)	0.67	0.85	0.97
Barium	0.56 (0.31,1.13)	0.55 (0.36,0.92)	0.26 (0.23,0.78)	1.54 (0.50,3.81)	0.49	0.10	0.22
Chromium	0.20 (0.12,0.33)	0.53 (0.19,0.65)	0.16 (0.13,0.32)	0.22 (0.18,0.33)	0.59	0.50	0.34

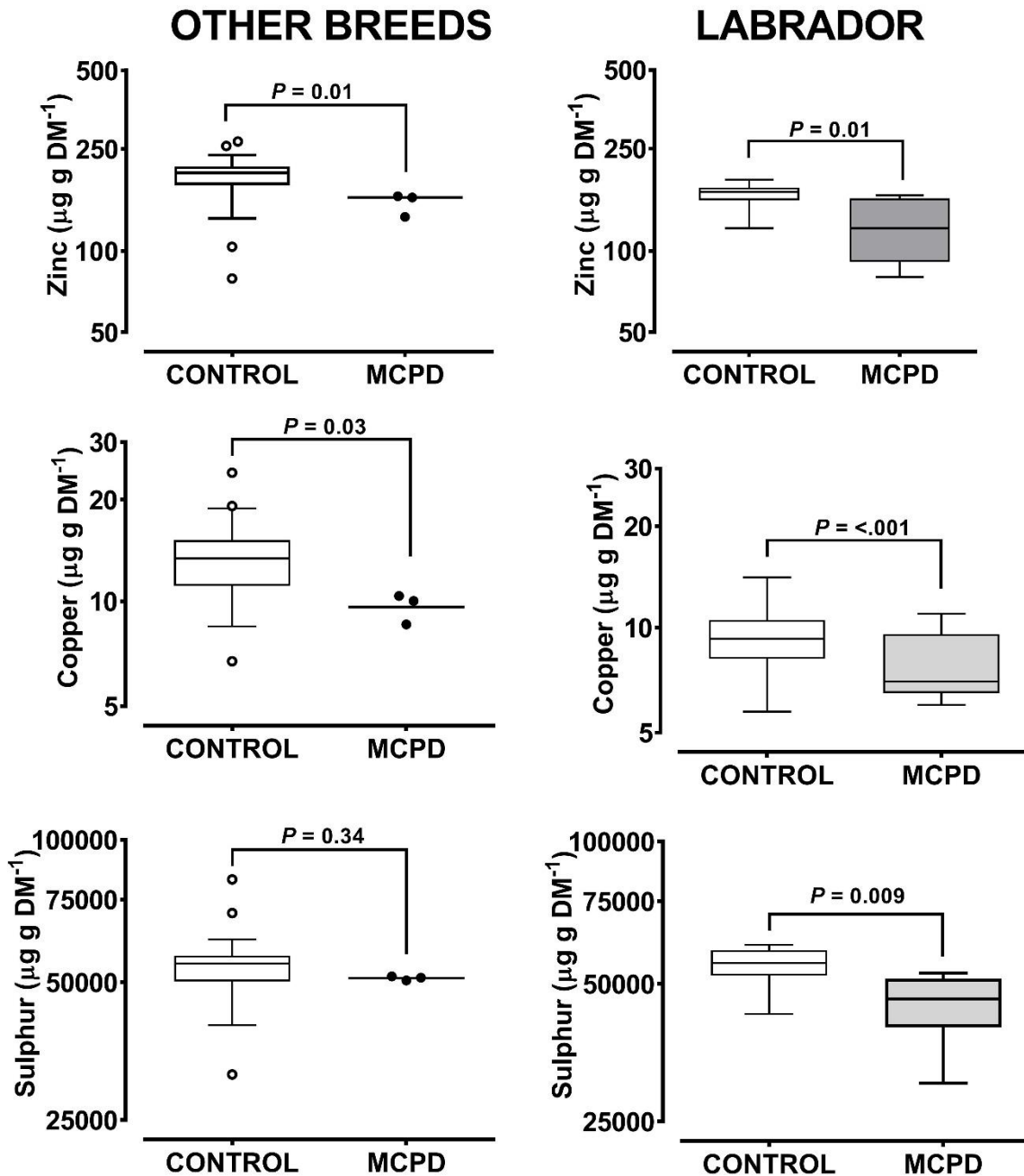
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419 **Table 1.** Major and trace elements were measured in dried hair samples by inductively-coupled plasma mass
420 spectrometry (ICP-MS). Data are presented as medians (1st to 3rd interquartile range). ‘Other breeds’ includes
421 all breeds of dog other than Labrador. Due to a breed pre-disposition to DSD in Labradors, we analysed data
422 as a 2 (Control vs MCPD Disease) × 2 (Other Breeds vs Labrador) factorial ANOVA design together with a pre-
423 specified main effects interaction in order to determine if being a Labrador *per se* alleviated or exacerbated
424 select mineral deficiencies in hair. Significance was accepted at a P-value of ≤ 0.01 (to adjust for multiple
425 comparisons).

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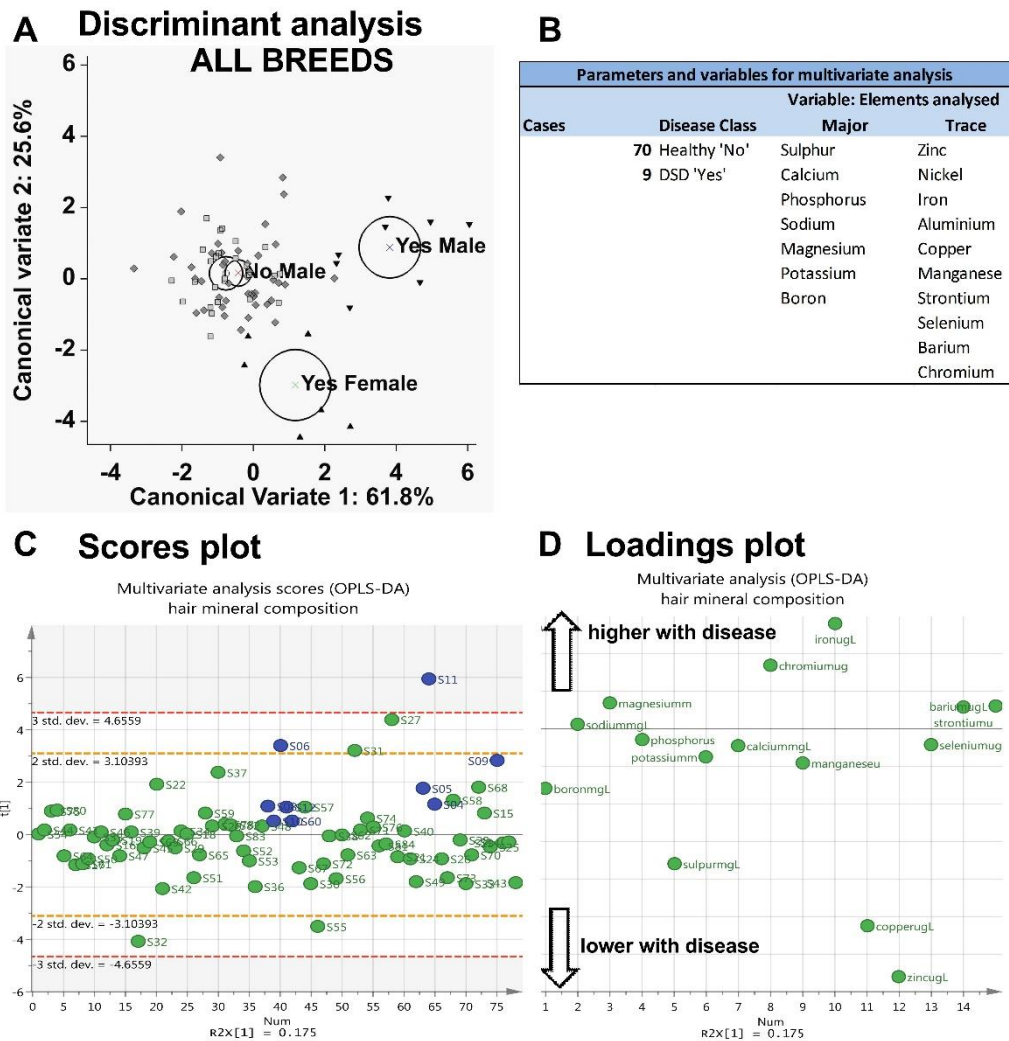


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431 **Figure 1.** Selected minerals were measured in freeze-dried hair samples by inductively-coupled plasma mass
 432 spectrometry (ICP-MS). Data are presented as box or scatter-plots. For boxes; line is at median, box
 433 represents 1st to 3rd interquartile range and whiskers are 5th to 95th percentile. 'Other Breeds' includes all
 434 breeds of dog other than Labrador. Data was first analysed as a 2 (Breed; Other vs Labrador) × 2 (Disease,
 435 Healthy vs MCPD) factorial ANOVA. Significance was accepted at a P-value of ≤ 0.002 (to adjust for multiple
 436 comparisons) and if indicated, further post-hoc comparison was made using Mann-Whitney U-test ('Other
 437 breeds') or one-way ANOVA.

438

439 **Figure 2: Multivariate analysis of hair mineral composition in dogs with or without medial coronoid**
 440 **process disease**



441

442 **Figure 2. Multivariate analysis of hair mineral composition in dogs with or without medial coronoid**
 443 **process disease. A)** linear discriminant plot after multivariate analysis of all cases, according to
 444 disease class (MCPD = 'Yes', Control = 'No') and including all variables as described in (B). Each point
 445 is an independent sample with circles representing 95% confidence interval around group means.
 446 Groups are healthy dogs (i.e. No Disease, 'No') or dogs with MCPD ('Yes'). Gender of the individuals
 447 is indicated. 61.8% of the variation in the whole dataset may be attributed to the first principle
 448 component, disease status (i.e. along x-axis). A further 25.6% may be attributed to the second
 449 principle component, gender (i.e. along y-axis). C. In SIMCA, with all parameters and variables
 450 included according to (B) indicates that the mineral composition of hair is broadly similar amongst
 451 all dogs; dogs with MCPD ('Yes') are indicated in blue, healthy dogs in green ('No'). D). In SIMCA,
 452 loading scores from orthogonal partial least squares – discriminant analysis (OPLS-DA) indicate
 453 elements contributing toward most variability in the mineral composition of hair. Scores at 0 do not
 454 contribute toward variability in hair mineral content. Highly positive (e.g. iron) or negative (e.g.
 455 copper and zinc) scores indicate higher or lower incorporation into hair respectively, in animals with
 456 MCPD. Arrows indicate direction of change and are for clarity.