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Effects of a ketogenic diet on ADHD-like behaviour in dogs with idiopathic epilepsy

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ABSTRACT

Objectives: Epilepsy in humans and rodent models of epilepsy can be associated with behavioural comorbidities including an increased prevalence of attention-deficit/hyperactivity disorder (ADHD). ADHD symptoms and seizure frequency have been successfully reduced in humans and rodents using a ketogenic diet (KD). The aims of this study were (i) to describe the behavioural profile of dogs with idiopathic epilepsy (IE) while on a standardised non-ketogenic placebo diet, to determine whether ADHD-like behaviours are present, and (ii) to examine the effect of a ketogenic medium chain triglyceride diet (MCTD) on the behavioural profile of dogs with idiopathic epilepsy (IE) compared to the standardised placebo control diet, including ADHD-like behaviours.

Methods: A 6-month prospective, randomised, double blinded, placebo controlled, crossover dietary trial comparing the effects of the MCTD to a standardised placebo diet on canine behaviour was carried out. Dogs diagnosed with IE, with a seizure frequency of at least 3 seizures in the past 3 months (n=21), were fed the MCTD or placebo diet for 3 months, then were switched to the alternative diet for 3 months. Owners completed a validated behavioural questionnaire to measure 11 defined behavioural factors at the end of each diet period to report their dogs' behaviour, with three hypothesised to be related to ADHD: excitability, chasability and trainability.

Results: The highest scoring behavioural factors in the placebo and MCTD period were excitability (mean ± SE: 1.910±0.127), and chasing (1.824±0.210). A markedly lower trainability score (0.437±0.125) than previously studied canine populations was observed. The MCTD resulted in a significant improvement in the ADHD-related behavioural factor chasing, and a reduction in stranger-directed fear ($p<0.05$) compared to the placebo diet. The latter effect may be attributed to previously described anxiolytic effects of a KD.

Conclusions: This data supports the supposition that dogs with IE may exhibit behaviours that resemble ADHD symptoms seen in humans and rodent models of epilepsy, and that a MCTD may be able to improve some of these behaviours, along with potentially anxiolytic effects.

Key words: canine; attention-deficit/hyperactivity disorder; ketogenic; medium chain triglyceride; comorbidity; anxiolytic

35

List of abbreviations

37

38   ADHD: Attention Deficit Hyperactive Disorder

39   AED: Anti-Epileptic Drug

40   AKC: American Kennel Club

41   C-BARQ: Canine Behavioural Assessment and Research Questionnaire

42   CPRS-R: Conners' Parent Rating Scale

43   IE: Idiopathic Epilepsy

44   KD: Ketogenic Diet

45   MCTD: Medium Chain Triglyceride Diet

46   MRI: Magnetic Resonance Imaging

47   QoL: Quality of Life

48

1. Background

1.1 ADHD and Epilepsy

Psychiatric disorders are common in human patients with epilepsy, with attention-deficit/hyperactivity disorder (ADHD) being one of the most common co-occurring disorders alongside depression and anxiety. Up to one third of epilepsy patients are diagnosed with ADHD [1]. In a recent large-scale community-based survey, ADHD symptoms were reported in nearly one of five adults with self-reported epilepsy, which was associated with increased psychosocial morbidity and lowered quality of life (QoL) [2]. The hypothesised association between epilepsy and ADHD is not recent; a 'hyperkinetic syndrome' was described in child epilepsy patients which resembles current definitions of ADHD nearly 60 years ago [3]. Attention/associative deficits combined with impulsivity and hyperactivity are the defining features of ADHD [4].

Hyperactivity is 5.7 times more prevalent in children with epilepsy than control children [5]. ADHD affects children and adolescents, with symptoms often persisting into adulthood [6]. Significant ADHD symptoms are present in many patients before the onset of the first seizure. Of children newly diagnosed with epilepsy, 31% showed symptoms of ADHD [7], with 82% of these children with epilepsy and ADHD showing ADHD symptoms prior to seizure onset [7]. A bidirectional association between epilepsy and ADHD has been demonstrated, with epilepsy patients at an increased risk of ADHD, and ADHD patients at an increased risk of epilepsy. For example, in a population-based cohort study of Taiwanese children <19 years old, the possibility of developing ADHD in epilepsy patients was significantly higher (adjusted hazard ratio 2.54), and the possibility of developing epilepsy in ADHD patients was also significantly higher (adjusted hazard ratio 3.94) [8].

1.2 Animal models of epilepsy and ADHD

The link between seizure activity and ADHD-like behaviours is not limited to human epilepsy. Hallmarks of ADHD (e.g. easy distraction and slow learning) have been demonstrated in a strain of epilepsy-prone laboratory rats using various behavioural paradigms with a disinhibited or impulsive behavioural style [9]. This has been thought to establish the disorders as truly comorbid [10]. In a rat model of temporal lobe epilepsy, a fast-kindling selectively bred strain

('*Fast*' rats) exhibit different behavioural features from slow-kindling rats ('*Slow*' rats). *Fast* rats show signs such as hyperactivity, impulsivity and easy distraction compared to *Slow* rats [11]. *Fast* rats are comparatively hyperactive in an open field exploration task [12], and when restrained, struggle far longer and with more ferocity than *Slow* rats, indicating a higher level of hyperactivity/impulsivity [13]. High levels of impulsivity and distractibility may result in learning deficits. In a delayed alternation test *Fast* rats displayed a high degree of impulsivity and learning deficits [14]. In addition, in several variants of a Morris water maze, *Fast* rats were more likely to be distracted by irrelevant cues during acquisition [9]. *Fast* rats appear to retain more juvenile like features [15], with impulsivity, distractibility and reduced fear than typically shown in juvenile mammals [16]. In addition, *Fast* rats also exhibit age-inappropriate juvenile and aggressive play behaviours that are not seen in *Slow* rats [15].

1.3 Epilepsy and canine behaviour

Epilepsy is a common chronic neurological disorder in dogs as well as humans, with an estimated prevalence in dogs of 0.6 in the first opinion practice population [17]. The dog also shows some similar aspects of human behaviour, possibly owing to the similarity of evolutionary processes that have shaped their behaviour [18]. Parallels have been drawn between behavioural disorders in humans and canines, such as separation anxiety and obsessive-compulsive disorder [19]. Although parallels between childhood ADHD and canine activity and attention-related behavioural problems have been considered [20], the behavioural profiles of dogs with epilepsy has been little studied thus far, despite being considered as a naturally occurring model of human epilepsy [21, 22]. In a recent single-breed study of Lagotto Romagnolo dogs with or without a history of Benign Familial Juvenile Epilepsy (BFJE; where dogs often experience spontaneous seizure remission before 13 weeks of age), dogs with BFJE (n=25) showed significantly higher scores on the behavioural factors 'Inattention' and 'Excitability/Impulsivity' than did the control group without BFJE [23]. The authors considered these behaviours to be comparable with ADHD in humans. These behavioural changes were observed after at least four years following the last observed seizure, which demonstrates that behavioural comorbidities can be present in the absence of seizure activity. As this study was limited to one breed with one specific type of epilepsy, whether these results are more widely applicable to the canine IE population is unknown, as different epilepsy syndromes may pose different risks for behavioural development problems.

1.4 The influence of diet on behaviour

External factors associated with diet and the dog's lifestyle may also have an impact upon the seizure activity and behaviour. Diet induced behavioural modifications in dogs have been reported in peer-reviewed literature and anecdotal notes [24]. For example, a low protein diet has been shown to reduce certain types of aggression in dogs [25, 26], and supplementation of casozepine or the proportion of protein in a given diet may reduce anxiety-related behaviour [27]. The ketogenic diet (KD), which is a high fat, low protein, and low carbohydrate diet used in the treatment of intractable human epilepsy, also appears to improve symptoms of ADHD in individuals with both disorders in humans. For example, the KD can decrease seizure activity or lead to seizure freedom in children refractory to anti-epileptic drug (AED) therapy allowing reduction or cessation of medication [28, 29]. The KD has also been found to decrease ADHD symptoms in both adults and children [30-33]. This improvement in ADHD symptoms appears to be independent of seizure control, with behaviour found to improve even if seizure control is not obtained [30, 31]. Similar effects on behaviour have also been investigated in laboratory rodents, where reversible reductions in activity are observed [34].

Around 20–30% of dogs with IE will remain poorly controlled (<50% reduction of seizure frequency) despite adequate treatment with common first and second line AEDs phenobarbitone (PB) and/or potassium bromide (KBr) [35-37]. Consequently, there is a need for further treatment options, particularly for pharmacoresistant patients. A novel diet with relatively low MCT levels (MCTD) was recently developed for canine cognitive function and shown to be ketogenic [38]. To date, this diet has been found to have a cognition-enhancing effect in aged dogs [38], and show antiepileptic properties [39].

The aims of this study were twofold:

1. To describe the behavioural profile of dogs with IE while on a standardised diet, to determine whether ADHD-like behaviours are present in this population
2. To examine the effect of a ketogenic MCTD on the behavioural profile of dogs with IE compared to the standardised placebo control diet, including ADHD-like behaviours

2. Methods

2.1 Study design

150 The present study comprised of a 6-month prospective, randomised, double blinded, placebo

151 controlled, crossover dietary trial comparing the effects of the MCTD to a standardised placebo

152 diet on behaviour in canine epilepsy. Dogs were fed either the MCTD or placebo diet for 3

153 months (Day 1 to Day 90 ±2 days) followed directly by a subsequent respective switch of diet

154 for a further 3 months (Day 90 to Day 180 ±2 days).

155

156 *2.2 Recruitment of cases*

157

158 Owners of dogs with IE were recruited by contacting primary care veterinary practices to

159 identify cases and through social media e.g. canine epilepsy support groups. These dogs were

160 recruited for a study investigating the efficacy of a diet on seizure reduction, with the dual aim

161 of examining their behavioural profile during the study. As such, specific inclusion and

162 exclusion criteria were employed. Dogs were deemed suitable for inclusion in this study if they

163 were of mixed or pure breed status and met the following requirements:

164   (i)   Had IE: unremarkable former magnetic resonance imaging (MRI) scan and

165   cerebrospinal fluid (CSF) analysis; no clinically significant findings on

166   haematology, biochemistry or bile acid results; unremarkable interictal neurological

167   examinations for dogs on antiepileptic treatment;

168   (ii)   Were between 6 months and 12 years of age;

169   (iii) Weighed between 4kg and 65kg;

170   (iv) Had at least 3 seizures in the 3 months prior to start of study;

171   (v)   Were being treated chronically with at least one antiepileptic treatment;

172 Dogs were excluded from the study if they were:

173   (i)   Were receiving drugs that could influence the metabolism of PB and KBr;

174   (ii)   Were intended for breeding less than two weeks from start of study, or were females

175   known or suspected to be pregnant or lactating;

176   (iii) Had a known cause of epilepsy such as brain neoplasm, brain trauma, encephalitis

177   and meningitis;

178   (iv) Were affected by chronic or acute renal, hepatic or cardiac failure;

179   (v)   Had an acute or surgical condition at the time of enrolment

180

181 Only one dog per household was allowed in the study to maintain independence. A unique

182 Study Case Number (SCN), consisting of a two-digit number ascending in a chronological

183   order of enrolment, was allocated and used to identify each dog on all documents and samples
184   throughout the study.

185

186   *2.3 Diet*

187

188   Full details of each diet are available in Law et al [40]. The experimental placebo and test
189   formulas were dry extruded kibble (Nestle Purina PetCare, St. Louis, Missouri, USA)
190   formulated to meet or exceed nutritional guidelines established by the Association of American
191   Feed Control Officials. Both formulas were of the same nutrient composition, and formulated
192   to contain less than 10% moisture, at least 28% crude protein (as fed basis), at least 15% crude
193   fat (as fed basis), less than 6% ash (as fed basis), and less than 2% as crude fiber (as fed basis).
194   The one composition exception is that zero MCTs were added to the placebo formula, and lard
195   was used as fat substitute to ensure that the formulas were isocaloric (373 kcal/100 g), whereas
196   the test formula contained 5.5% MCTs. MCT content was about 10% of total formula calories
197   (based on fat as 8.5 kcal/g and MCT as 6.8 kcal/g). All dogs were housed indoors and the
198   majority were fed once/day, with no restrictions on water consumption. The owners were
199   educated to keep diet consistent throughout the study period. Amount of food given per day
200   was calculated according to the weight for each dog to provide sufficient nutritional needs. A
201   deviation of ±10% food consumption (kg) was allowed to account for the individual needs of
202   each dog taking into consideration differences in activity level and physical condition. Dogs
203   were restricted to consumption of only study food, hence treats or snacks were replaced by the
204   respective placebo or MCTD food.

205

206   *2.4 Behavioural data*

207

208   At the end of each three month period (placebo or MCTD), owners were asked to complete a
209   previously validated behavioural questionnaire to report on their dog's behaviour during that
210   period, the C-BARQ [41] (Supplementary Table 1). The C-BARQ was chosen to quantify the
211   behavioural profiles of dogs during both diet periods as it has been validated [41], studied in
212   international canine populations, and covers a broad range of behaviours. Although aim (1) of
213   this study focuses on ADHD-like behaviours, quantifying changes in other behaviours is
214   important when testing a novel diet, to detect any unexpected effects.

215

216 The questionnaire comprises 68 questions, owners are asked to score their dogs on either a 5-
217 point frequency scale (i.e. 0= never - 4=always) for particular responses e.g. attention-seeking
218 behaviour, or a 5-point qualitative rating scale (i.e. 0=no signs of behaviour – 4=severe signs
219 of the behaviour) for intensity of behaviours e.g. excitability. If owners were unable to answer,
220 an 'N/A' option was included, which was treated as missing data in the analyses. Behaviours
221 were previously grouped into 11 broad categories using factor analysis: 'stranger-directed'
222 aggression; 'owner-directed' aggression; 'stranger-directed' fear; non-social fear; 'dog-
223 directed' fear; 'separation-related' behaviour; attachment or attention seeking behaviour;
224 trainability; chasing behaviour; excitability; and touch sensitivity. The loading factors for each
225 question established in the validation study were used for the analysis of each category.

227 Children diagnosed with ADHD frequently have unusually high activity and emotional
228 reactivity, and may be more distractible and impulsive [42]. As such, from the C-BARQ 11
229 behavioural factors, we would expect to observe relatively high levels of the following factors
230 compared to other factors, and compared to 'normal' dogs:
231 (i) Excitability: dogs with high scores for this factor have strong reactions to exciting events
232   and have difficulty calming down after events [41] which may resemble high emotional
233   reactivity and excitability seen in ADHD. The revised Conners' Parent Rating Scale
234   (CPRS-R) [43] is a popular research and clinical tool for obtaining parental reports of
235   childhood behaviour problems. It includes 'excitable' as a component of the
236   hyperactivity-impulsivity score. Another component measured in the CPRS-R
237   hyperactivity-impulsivity score is 'difficulty waiting' which may resemble components
238   of the Excitability factor, namely a dog being 'excitable just before being taken for a
239   walk' and 'excitable just before being taken on a car trip'.
240 (ii) Chasing: dogs with high scores for this factor have a tendency to chase cats, birds,
241   squirrels and/or other small animals [41] which may indicate a degree of hyperactivity
242   seen in ADHD. The CPRS-R includes the components 'runs excessively', 'restless' and
243   'always on the go' in the hyperactivity-impulsivity score [43] which the Chasing factor
244   may resemble.
245 Conversely, we would expect to observe relatively low levels of:
246 (i) Trainability: dogs with low scores are not attentive to their owners or willing to obey
247   basic commands, are easily distracted, do not tend to be fast learners, and do not tend
248   to retrieve thrown objects/toys [41], which may resemble impaired attention resulting
249   in learning deficits.

250

2.5 Clinical data

251

252

253 In addition, seizure frequency, seizure days, body weight and measurements of serum PB

254 and/or KBr concentration as appropriate were recorded for each dog. Visual analogue score

255 (VAS) for ataxia, sedation and quality of life (QoL) were recorded by the owner, using a line

256 ranging from 0mm to 100mm. The owner was asked to draw a secondary intersecting line,

257 perpendicular to the line of measurement that best represented the subjective severity. A

258 perpendicular line at 0mm represented either asymptomatic/normal and at 100mm represented

259 either 'ataxia so severe dog is unable to walk', 'sedation to the extent dog only sleeps' and

260 extremely poor 'QoL, respectively.

261

2.6 Ethics statement

262

263

264 This study was conducted in accordance with the guidelines laid down in the International

265 Cooperation of Harmonization of Technical Requirements for Registration of Veterinary

266 Products (VICH) GL9 Good Clinical Practices (GCP) and the European Agency for the

267 evaluation of Medical Products (EMEA). The study protocol was approved by the Royal

268 Veterinary College's Ethics and Welfare Group (EWG) (URN 2011 1132). The owners of the

269 dogs gave consent for their animals to be used in this study.

270

2.7 Statistical analysis

271

272

273 Weighted scores for the 11 behavioural factors were calculated for each dog using the loading

274 factors for each question established in the validation study, with descriptive statistics

275 calculated for each factor. Wilcoxon Signed Rank tests were used to compare the differences

276 in behavioural factors between diet groups. Spearman's rank test was used to test for

277 correlations between the behavioural factors and seizure frequency, PB/KBr levels, and VAS

278 scores for ataxia, sedation and QoL. The Kruskall-Wallis test was used to test for associations

279 between behaviour and breed, and the Mann-Whitney U test used to test for associations

280 between behaviour and gender, neuter status and the presence of cluster seizures. All tests were

281 two-sided and $P < 0.05$ was considered to be significant. Data are presented as mean $\pm$ standard

282 deviation (SD), or median (25th-75th quartile), where appropriate.

283

3 Results

3.1 Population demographics

Twenty-one dogs of 17 breeds and three cross breeds were included in the study. Ten dogs were neutered male, five entire male, four neutered female and two entire female. The mean age was 4.59 ±1.73 years of age and mean weight 29.79 ±14.73 kg. All 21 dogs received PB, and the majority (n=18) were also treated with KBr. The mean serum concentrations of PB was 31.05ug/ml±6.36, and mean potassium bromide was 1.05mg/ml±0.71. Several dogs were chronically treated with a third AED, imepitoin (n=1) or levetiracetam (n=8). 3 dogs were treated with 1 AED, 11 with 2 AEDs, and 7 with 3 AEDs. To avoid confounding influences, concomitant AED medication and dosages were unchanged throughout the study. Twelve owners had been prescribed rectal diazepam or levetiracetam for pulse therapy by their first opinion vet for at home treatment of cluster seizure episodes. The mean age at first seizure event was 27.85 months (SD: 23.16). Three dogs were outside of the '>6 month to <6 year age bracket at seizure onset. recognized to be indicative of idiopathic epilepsy in dogs [44]. As is recommended in this circumstance, all dogs underwent MRIs that were found to be clear of structural abnormalities and thus were diagnosed with idiopathic epilepsy. Nine dogs experienced cluster seizures and twelve experienced only single seizures. Full details of demographics are available in the original study of the effect of the MCTD on seizure frequency [39].

3.1 Baseline behaviour: Placebo diet

The highest scoring behavioural factors in the placebo period were excitability (mean ± SE: 1.910±0.127) and chasing (1.824±0.210). Regarding the individual questions that constitute each factor, for excitability, over half of dogs (52.38%) were reported to act in an 'extremely excitable' way (score 4 on a scale of 0-4) when a member of the household returns after a brief absence, when playing with a member of the household and just before being taken for a walk. A further 47.62% act in an 'extremely excitable' way before being taken on a car trip, 42.86% when visitors arrive at the home, and 33.33% when the doorbell rings. For chasing, over half of dogs (52.38%) were reported to 'always' chase squirrels and other small animals if given the chance, nearly half 'always' (47.62%) chase cats, and a third (33.33%) 'always' chase birds or act aggressively to cats, squirrels, and other animals entering their garden.

11

In contrast, the 'trainability' behavioural factor was relatively low (0.437±0.125). Two thirds of owners (66.67%) reported that their dog is 'always' easily distracted by interesting sights, sounds and smells. In contrast, less than one quarter (23.81%) dogs would 'always' obey a sit command, and less than a tenth (9.52%) obey a stay command immediately.

The baseline median seizure frequency per month during the placebo diet phase was 2.67 seizures/month (1.78-4.91), and the median seizure days per month was 1.69 days/month (1.16-3.30). The mean VAS score for ataxia was 42.38 ± 16.48, sedation 38.19 ± 4.37, and QoL 31.14 ± 18.24. No associations were found between the ADHD-related behavioural factors (excitability,chasing and trainability) and clinical variables (e.g. seizure frequency, experience of cluster seizures, VAS scores for ataxia and sedation).

3.2 MCTD

The median seizure frequency per month in the MCTD period was 2.31 seizures/month (1.00-4.46), and the median seizure days per month was 1.63 days/month (0.67-2.32). As previously reported [39], seizure frequency was significantly lower when dogs were fed the MCTD in comparison to placebo diet (p<0.05).

The highest scoring behavioural factors in the MCTD period were again excitability (mean ± SD: 1.863±0.136) and chasing (1.516±0.200). Two behavioural factors differed significantly between the placebo diet and MCTD phases (Table 1). Significant reductions in one of the ADHD-related factors, chasing behaviour (p=0.037) was observed when dogs were on the MCTD in comparison to the placebo diet, but not excitability (Table 1, Figure 1). A reduction in stranger-directed fear was also observed during the MCTD in comparison to the placebo diet (p=0.046). There were no significant changes in stranger-directed aggression, owner-directed aggression, dog-directed fear, separation-related behaviour, non-social fear, attachment or attention seeking behaviour, trainability, excitability, and touch sensitivity behavioural factors between diet groups.

The mean VAS score for ataxia was 41.05 ± 20.21, sedation 38.62 ± 23.03, and QoL 28.29 ± 18.78. No associations were again found between the ADHD-related behavioural factors (excitability,chasing and trainability) and clinical variables (e.g. seizure frequency, experience

352 of cluster seizures, VAS scores for ataxia and sedation). As such, the reductions in chasing
353 were not thought to be due to an increase in sedation or ataxia inhibiting these behaviours.
354 There was no effect of the number of AEDs a dog was treated with on any of the behavioural
355 factors in either diet period, or changes in seizure frequency.

356

357 **4. Discussion**

358

359 The data presented show that (i) behaviours observed in dogs with IE resemble those seen in
360 humans and rodent models of epilepsy, with relatively high levels of excitability and chasing
361 behaviour, and relatively low levels of trainability and (ii) the MCTD significantly reduces one
362 of these behavioural factors 'chasing', and reduced stranger directed fear. This study adds
363 further evidence to the notion that there may be common neurobiological mechanisms present
364 in epilepsy and ADHD. Although direct comparisons between specific behaviours are not
365 possible between humans, rats and dogs, the profile of excitability, a propensity towards active
366 chasing behaviour, and a reduced ability to learn because of distraction resembles the
367 behaviours described in individuals of these species with epilepsy-related ADHD.

368

369 The trainability of this population when compared to average trainability values of healthy dogs
370 are markedly lower, with the average score ~2.5 in the 30 most popular American Kennel Club
371 (AKC) registered breeds [45], but just 0.437 in this study. The MCTD diet was associated with
372 an increase in trainability, from 0.437 to 0.600; however, this difference was not significant.
373 Even within the MCTD period, trainability did not increase to 'normal levels' seen in the AKC
374 population study [45]. No change was observed in the behavioural factor excitability,
375 hypothesised to be ADHD-related. As such, further research may be required to investigate
376 why the MCTD differentially affects these potential components of an ADHD–like behavioural
377 profile, and whether further interventions such as behavioural therapy or obedience training are
378 required to further increase trainability and reduce excitability.

379

380 *4.1 Effect of diet*

381

382 Studies in rat models of epilepsy have previously demonstrated positive effects of diet on
383 ADHD symptoms [34, 46]. To date there have been no studies investigating the relationship
384 between diet, seizure activity and behaviour in dogs. The present study is the first to present
385 significant results showing dietary induced behavioural modifications in dogs with epilepsy.

386 KDs have been shown to not only control seizure activity in human patients with epilepsy, but

387 also to improve behaviour in general [47]. Symptoms of ADHD have also been reported to

388 decrease in both adults and children on a KD irrespective of level of seizure control [48].

389 Although the exact mechanisms involved with behavioural improvements and KDs are

390 unknown it has been suggested that alterations of energy metabolism in the brain may

391 contribute to behavioural changes [48].

392

393 Increases in fear/anxiety have previously been documented in drug-naïve dogs with idiopathic

394 epilepsy [49]. The reduction in stranger directed fear was unexpected in this study, and may

395 indicate an anxiolytic effect of the MCTD. KDs such as the MCTD in this study have recently

396 been shown to have anxiolytic effects. In a mouse model of Alzheimer's disease, mice who

397 were supplemented with a ketone ester, a precursor of the physiological forms of ketone bodies

398 that increase during a KD, showed reduced anxiety in an elevated plus maze and open field

399 testing [50]. Pilot study results have also shown reductions in some of the behavioural, social

400 communication and cognitive deficits seen in children with autism on a KD[51]. The ketogenic

401 MCTD utilised in this study has previously been demonstrated to improve the cognitive

402 function of aged dogs, to be due to the diet providing the brain with an alternative energy source

403 [38]. The significant behavioural improvements in stranger directed fear and chasing behaviour

404 seen in this study gives credence to dietary modifications of behaviour and provides motivation

405 for further investigations on the causal link between KDs and behavioural change.

406

407 *4.2 Identifying and quantifying ADHD-like behaviours*

408

409 These results are an early indicator of ADHD-like behavioural profiles in dogs with epilepsy,

410 and thus must be taken as a preliminary descriptive finding. Further studies are required to

411 compare dogs with epilepsy with healthy dogs to quantify the degree of behavioural

412 abnormality in IE cases. Although one previous study identified ADHD-like behaviours in dogs

413 with epilepsy when compared to healthy controls, this was limited to one breed with a specific

414 type of epilepsy [23], and thus it's broader applicability was unknown. Despite this limitation,

415 the similar findings between that study and the present study indicate that this behavioural co-

416 morbidity may well be present in the dog. Both of these studies relied on pet-owner

417 questionnaires of canine behaviour. To complement and strengthen the evidence of this

418 comorbidity, direct objective behavioural observations should be employed to establish a

419 relationship between ADHD and canine epilepsy, to avoid potential biases of owner-reporting.

420 The Activity-Impulsivity Behavioural Scale (AIBS) [52] is a four part behavioural test that is

421 significantly correlated with the Dog ADHD rating scale (ADHD-RS), a questionnaire adapted

422 from human psychology and developed in healthy dogs [20]. Levels of activity-impulsivity as

423 quantified by the AIBS scale have been associated with the TH intron 4 polymorphism [52].

424 In addition to direct behavioural observation and tests, technology such as activity monitors

425 [53] could also be used to quantify the degree of movement (and if present, hyperactivity) in

426 these dogs compared to healthy controls of the same breed.

427

428 *4.3 AED side effects*

429

430 It is possible that some of the behaviours observed in this population were related to the AEDs

431 the dogs were receiving, with the majority receiving both phenobarbital and potassium

432 bromide. Both of these drugs can result in sedation and lethargy [54]; however, the mean score

433 for sedation in this study was 38.19/100 (with 100 representing sedation to the extent dog only

434 sleeps) and thus did not appear a major problem for these animals, with no correlation between

435 VAS scores for sedation and QoL. It would be expected that sedation would reduce the ADHD-

436 like behavioural signs in these dogs, and thus this side effect would not explain the behavioural

437 profile observed. There have been reports of restlessness and hyperactivity as side effects of

438 the AED phenobarbital [55, 56]. In humans, AEDs associated with ADHD-like side effects

439 include phenobarbital, gabapentin, vigbatrin and topiramate [57]. Canine literature in this area

440 is sparse and whether these behaviours originate from the disease or as AED side effects has

441 not yet been determined. If related to AEDs it would be expected that dogs would exhibit

442 different behaviour profiles before and after AED treatment. It has further been noted that

443 ADHD-like behaviours due to AEDs would be expected to change when medication

444 type/dosage is altered, and as such clinical signs may be transient in contrast to true ADHD

445 symptoms which are likely to be present and persistent from an early age [58]. Longitudinal

446 studies are required to ascertain the temporal pattern of potential behaviour changes in these

447 dogs.

448

449 The dogs in this study were of a particularly severe epilepsy phenotype, with a median seizure

450 frequency of 2.67 seizures/ month during the placebo phase despite treatment with PB and/or

451 KBr, with over half experiencing cluster seizures. As such, whether the same behavioural

452 profile would be seen in dogs that were more responsive to AED treatment is unknown, as only

453 20–30% of dogs with IE remain poorly controlled despite adequate treatment these AEDs [35-
454 37].

455

456 *4.4 Influences on behaviour*

457

458 The behaviour of companion dogs is complex, with many potential internal and external factors
459 impacting upon the behaviours observed by the owner. Previous studies have found that young
460 dogs exhibit higher levels of attention-seeking behaviour and were more likely to have control
461 problems than older dogs [59]. This is due to higher cortical centres in the brain developing
462 with age and experience, resulting in increasingly inhibited pathways from these centres that
463 inhibit the immediate behavioural response to emotions [60]. This study found no effect of age
464 on the levels of excitability and chasing. There were also no effects of factors such as sex,
465 neuter status, age, breed or reported level of AED side effects (sedation and ataxia) on these
466 behavioural factors. Previous larger scale studies have demonstrated differences in C-BARQ
467 factors by breed for example [61], and due to the relatively low sample size of this study, a
468 larger sample may be required to allow the detection of such effects. The lack of effect of
469 seizure frequency on ADHD-related behavioural factors in both the placebo and MCTD phases
470 supports previous research in this area demonstrating that seizure activity is not essential for
471 the presence comorbid ADHD-like behaviours. Dogs with BFJE demonstrated ADHD-like
472 behaviours more than four years after their last observed seizure, which demonstrates that
473 behavioural comorbidities can be present in the absence of seizure activity.

474

475 Data from cross-fostering studies have demonstrated that the behavioural profiles of *Fast* and
476 *Slow* rats are likely genetic or prenatal, and not likely to be due to the postnatal environment,
477 with behaviour phenotypes in-line with their strain exhibited even if reared by a mother of the
478 opposite strain, which show distinct maternal behaviours [62]. Genetic studies and longitudinal
479 studies of behaviour are required to determine whether this also applies to dogs with epilepsy.
480 External influences, for example the degree and type of training method used by the owner [59]
481 may influence these behavioural factors associated with ADHD, as owners may perceive these
482 behaviours as undesirable and aim to improve them through behavioural modification
483 techniques. This is an inherent limitation of working with owned companion dogs rather than
484 kennel-housed dogs living in a controlled setting, reflecting a real world situation. Larger scale
485 future studies should attempt to quantify the degree of training that a dog has had to determine
486 its influence on behaviour.

5. Conclusions

Our data suggest that dogs with IE may exhibit similar signs of ADHD found in children with epilepsy, and rat models of epilepsy. These results corroborate previous findings of ADHD-like behaviours observed in the Lagotto Romagnolo, but in a variety of breeds. The use of the MCTD reduced one of these ADHD-related behaviours, chasing, and reduced stranger related fear, and thus may have anxiolytic properties. Further studies are required to corroborate the relationship between epilepsy and ADHD in dogs, using ADHD specific rating scales (e.g. [20]) or objective behavioural testing to confirm these results in a larger mixed-breed sample of dogs. Further investigations using the similarities between human and dogs may increase our understanding of epilepsy and its comorbidities, benefitting both species.

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663

Table 1 Comparison of behavioural scores for C-BARQ behavioural factors between a placebo and MCTD diet. Significant reductions were observed in the behavioural factors chasing and stranger directed fear (p<0.05).

Behaviour Factor	Diet (Mean ± SE)		Wilcoxon results
	Placebo	**MCTD**	
Stranger directed aggression	0.305 ± 0.145	0.210 ± 0.131	W=25.5, P=0.290
Owner directed aggression	0.119 ± 0.080	0.030 ± 0.019	W=13.5, P=0.104
Stranger directed fear	**0.498 ± 0.203**	**0.313 ± 0.142**	**W=15.0, P=0.042**
Non-social fear	0.296 ± 0.082	0.323 ± 0.082	W=25.5, p=0.051
Dog directed fear or aggression	0.392 ± 0.137	0.292 ± 0.110	W=29.0, p=0.123
Separation related behaviour	0.321 ± 0.100	0.300 ± 0.097	W=53.0, p=0.600
Attachment/attention-seeking behaviour	1.320 ± 0.131	1.30 ± 0.139	W=98.0, p=0.904
Trainability	0.437 ± 0.125	0.600 ± 0.130	W=79.0, p=0.205
Chasing	**1.824 ± 0.210**	**1.516 ± 0.200**	**W=115.5, p=0.037**
Excitability	1.91 ± 0.127	1.863 ±0.136	W=112.0, p=0.794
Pain sensitivity	0.458 ± 0.144	0.246 ± 0.101	W=35.0, p=0.444

Figure 1 Comparison of behavioural scores for C-BARQ behavioural factors between a placebo and MCTD diet. Significant reductions were observed in the behavioural factors chasing and stranger directed fear ($p<0.05$).
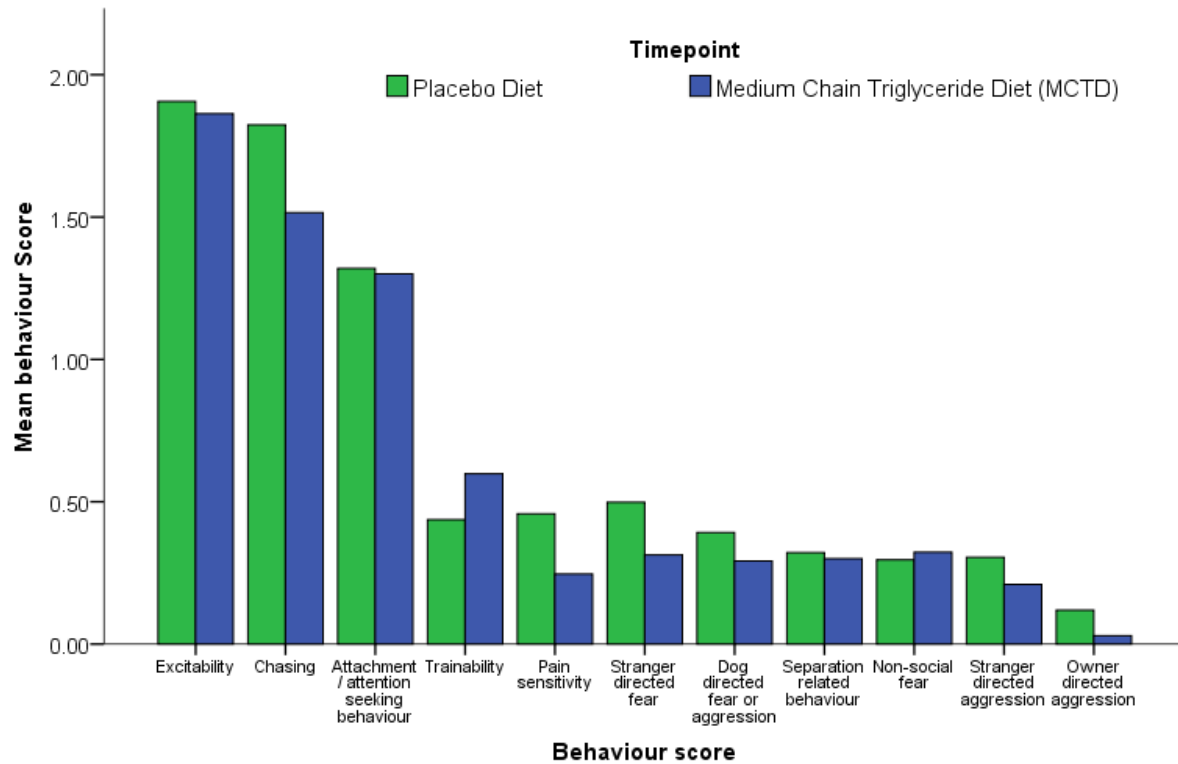
Supplementary Table C-BARQ behavioural questionnaire used to quantify eleven behavioural factors during the MCTD and placebo diet

Does your dog ever react aggressively to the following situations	Factor	Rating
1. When approached directly by an unfamiliar male adult while being walked or exercised on a lead	Stranger directed aggression	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. When approached directly by an unfamiliar female adult while being walked or exercised on a lead		
3. When approached directly by an unfamiliar child while being walked or exercised on a lead		
4. Toward unfamiliar persons approaching the dog while it is in the owner's car		
5. When an unfamiliar person approaches the owner or a member of the owner's family at home		
6. When an unfamiliar person approaches the owner or a member of the owner's family away from home		
7. When mailmen or other delivery workers approach the home		
8. When strangers walk past the home while the dog is in the garden		
9. When joggers, cyclists, roller skaters, or skateboarders pass the home while the dog is in the garden		
10. Toward unfamiliar persons visiting the home		
11. Toward unfamiliar dog visiting the home	Dog directed fear or aggression	
12. When approached directly by an unfamiliar dog of the same or larger size		
13. When approached directly by an unfamiliar dog of a smaller size		
Does your dog ever respond aggressively to the following situations	**Factor**	**Rating**
1. When verbally corrected or punished by a member of the household	Owner directed aggression	Never (0) Seldom (1) Sometimes (2) Usually (3) Always (4)
2. When toys, bones, or other objects are taken away by a member of the household		
3. When bathed or groomed by a member of the household		
4. When approached directly by a member of the household while it is eating		
5. When food is taken away by a member of the household		
6. When stared at directly by a member of the household		
7. When stepped over by a member of the household		
8. When a member of the household retrieves food or objects stolen by the dog		
Please assess the following situations and determine how likely your dog is to respond in a fearful or anxious way	**Factor**	**Rating**
1. When approached directly by an unfamiliar male adult while away from the home	Stranger directed fear	Never (0) Seldom (1)
2. When approached directly by an unfamiliar female adult while away from the home		

		Factor	Rating
3.	When approached directly by an unfamiliar child while away from the home		Sometimes (2)
4.	When unfamiliar persons visit the home		Usually (3)
			Always (4)
5.	In response to sudden or loud noises		
6.	In heavy traffic		
7.	In response to strange or unfamiliar objects on or near the pavement	Non-social fear	
8.	During thunderstorms		
9.	When first exposed to unfamiliar situations		
10.	In response to wind or wind-blown objects		
Does your dog display the following behaviour?		**Factor**	**Rating**
1.	Shaking, shivering, or trembling when left or about to be left on its own		
2.	Excessive salivation when left or about to be left on its own		
3.	Restlessness, agitation, or pacing when left or about to be left on its own		Never (0)
4.	Whining when left or about to be left on its own		Seldom (1)
5.	Barking when left or about to be left on its own	Separation related behaviour	Sometimes (2)
6.	Howling when left or about to be left on its own		Usually (3)
7.	Chewing or scratching at doors, floor, windows, and curtains when left or about to be left on its own		Always (4)
8.	Loss of appetite when left or about to be left on its own		
Which category best describes your dog's behaviour?		**Factor**	**Rating**
1.	Displays a strong attachment for a particular member of the household		
2.	Tends to follow a member of household from room to room about the house		
3.	Tends to sit close to or in contact with a member of the household when that individual is sitting down	Attachment/attention seeking behaviour	
4.	Tends to nudge, nuzzle, or paw a member of the household for attention when that individual is sitting down.		
5.	Becomes agitated when a member of the household shows affection for another person		Never (0)
6.	Becomes agitated when a member of the household shows affection for another dog or animal		Seldom (1)
7.	Returns immediately when called while off leash		Sometimes (2)
8.	Obeys a sit command immediately		Usually (3)
9.	Obeys a stay command immediately	Trainability	Always (4)
10.	Will fetch or attempt to fetch sticks, balls, and other objects		
11.	Seems to attend to or listen closely to everything the owner says or does		

	Factor	Rating
12. Is slow to respond to correction or punishment		
13. Is slow to learn new tricks or tasks		
14. Is easily distracted by interesting sights, sounds, or smells		
15. Acts aggressively toward cats, squirrels, and other animals entering its garden	Chasing	
16. Chases cats if given the chance		
17. Chases birds if given the chance		
18. Chases squirrels and other small animals if given the chance		
19. Tends to nudge, nuzzle, or paw a member of the household for attention when that individual is sitting down.		
Does your dog respond in a highly excitable way to the following situations?	**Factor**	**Rating**
1. When a member of the household returns home after a brief absence		Never (0)
2. When playing with a member of the household		Seldom (1)
3. When the doorbell rings	Excitability	Sometimes (2)
4. Just before being taken for a walk		Usually (3)
5. Just before being taken on a car trip		Always (4)
6. When visitors arrive at its home		
Does your dog react in a fearful or anxious way to the following situations?	**Factor**	**Rating**
1. When examined or treated by a veterinarian		Never (0)
2. When having its claws clipped by a household member	Pain sensitivity	Seldom (1)
3. When groomed or bathed by a household member		Sometimes (2)
		Usually (3)
		Always (4)