

## Once-daily feeding associated with better outcomes 1

1 **Title:** Once-daily feeding is associated with better health in companion dogs: Results from the  
2 Dog Aging Project

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### Abstract

43 A variety of diets have been studied for possible anti-aging effects. In particular, studies  
44 of intermittent fasting and time-restricted feeding in laboratory rodents have found evidence of  
45 beneficial health outcomes. Companion dogs represent a unique opportunity to study diet in a  
46 large mammal that shares human environments. The Dog Aging Project has been collecting  
47 data on thousands of companion dogs of all different ages, sizes, and breeds since 2019. We  
48 leveraged this diverse cross-sectional dataset to investigate associations between feeding  
49 frequency and cognitive function ( $n = 10,474$ ) as well as nine broad categories of health  
50 conditions ( $n = 24,238$ ). Controlling for sex, age, breed, and other potential confounders, we  
51 found that dogs fed once daily rather than more frequently had lower mean scores on a  
52 cognitive dysfunction scale, and lower odds of having gastrointestinal, dental, orthopedic,  
53 kidney/urinary, and liver/pancreas disorders. Therefore, we find that once-daily feeding is  
54 associated with better health in multiple domains. Future research with longitudinal data can  
55 provide stronger evidence for a possible causal effect of feeding frequency on health in  
56 companion dogs.

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### Keywords:

58 Canine, Canine Cognitive Dysfunction, Feeding frequency, Healthy aging, Time-restricted  
59 feeding

60

### Introduction

61 For nearly a century, caloric restriction has been known to extend lifespan and delay age-  
62 associated pathology in laboratory animals [1–5]. More recently, in both animals and humans, a  
63 variety of alternative “anti-aging” diet modalities have been described which are providing new  
64 mechanistic insights and potential clinical applications [6]. These diets include intermittent  
65 fasting [7, 8], fasting mimicking diets [9, 10], ketogenic diets [11–15], protein or essential amino  
66 acid restriction [16, 17], and time-restricted feeding [18–20].

67 These diets have been most extensively studied in rodents in controlled laboratory settings, due  
68 to ease of administering diets on a specific schedule and an enhanced ability to tease apart the  
69 mechanisms through which they act. Time-restricted feeding studies in rodents suggest  
70 improvements in several metabolic parameters, including glucose and insulin homeostasis,  
71 energy expenditure, hepatic pathology, resistance to different obesogenic diets, and improved  
72 circadian rhythm maintenance during aging [21–23]. In one study, mice who experienced time-  
73 restricted feeding demonstrated an 11% extension in lifespan [18]. Additionally, several studies  
74 demonstrate that caloric restriction and intermittent fasting play a protective role in maintaining  
75 and enhancing cognitive function, including memory and spatial learning [24–28]. It remains  
76 unclear, however, whether these benefits in laboratory animals are generally due to reduced  
77 caloric intake or meal frequency or both [6].

78 Despite mainstream popularization in humans of several of these diets, the beneficial health  
79 effects of time-restricted feeding outside of a laboratory setting are less clear. In some human

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80 studies, only mild improvements in body composition and cardiovascular risk factors were  
81 detected [29], even when subjects also reduced their daily caloric intake [30]. In other studies,  
82 detrimental effects on glucose homeostasis were observed with time-restricted feeding [31].  
83 Finally, while some studies have found potential cognitive benefits, especially for memory in  
84 older adults [32, 33], other studies have shown no effect of fasting on cognition [34, 35].

85 Companion dogs provide a potentially powerful animal model to elucidate the relationship  
86 between diet and age-related health outcomes [36]. Having co-evolved alongside people for  
87 thousands of years [37], companion dogs share human environments, experience similar  
88 diseases, and receive similar medical care. Once-daily feeding in dogs serves as a natural  
89 model for the intermittent fasting/time-restricted feeding protocols currently being studied both in  
90 preclinical rodent models and in human trials [38].

91 The Dog Aging Project is a large-scale research initiative following thousands of companion  
92 dogs over their lifetimes to better understand how biology, lifestyle, and environment impact  
93 healthy aging [39]. Participating owners report annually on a variety of aspects related to their  
94 dog's diet, primary and secondary activities, social and physical environments, medications, and  
95 health conditions. In the current study, we used cross-sectional data collected in the first year of  
96 the Dog Aging Project to ask if feeding frequency is associated with cognitive function and  
97 health conditions. Specifically, we hypothesized that dogs fed once-a-day would display lower  
98 rates of physical health issues and better cognitive scores compared to dogs fed more  
99 frequently.

## 100 **Methods**

### 101 *Subjects*

102 All dogs had been recruited to join the Dog Aging Project (DAP) via mainstream media, social  
103 media, or word of mouth. Their owners then completed the relevant online surveys between  
104 December 26, 2019 and December 31, 2020 [40]. Study data were collected and managed  
105 using REDCap electronic data capture tools hosted through the DAP [41, 42]. REDCap  
106 (Research Electronic Data Capture) is a secure, web-based software platform designed to  
107 support data capture for research studies, providing 1) an intuitive interface for validated data  
108 capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated  
109 export procedures for seamless data downloads to common statistical packages; and 4)  
110 procedures for data integration and interoperability with external sources.

### 111 *Instruments*

112 The first survey that participants completed was the Health and Life Experience Survey (HLES),  
113 which collects information regarding dog demographics, physical activity, environment, dog  
114 behavior, diet, medications and preventives, health status, and owner demographics. In the  
115 current investigation we were principally interested in feeding frequency and health status, and  
116 we identified *a priori* health conditions that could plausibly be affected by feeding frequency.

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117 After completing HLES, all participants were offered the opportunity to complete the Canine  
118 Social and Learned Behavior Survey (CSLB), which measures cognitive function. The CSLB,  
119 renamed by the DAP, is the same as the validated Canine Cognitive Dysfunction Rating Scale  
120 (CCDR) [43], with only a handful of minor wording changes. The Canine Cognitive Dysfunction  
121 Rating Scale was presented to participants as the Canine Social and Learned Behavior Survey  
122 to avoid the negative connotations of the phrase 'cognitive dysfunction. This instrument asks  
123 owners to indicate the frequency with which their dogs exhibit behaviors indicative of dementia  
124 (i.e., disengagement from social activity; difficulty in navigation, searching, and recognition).  
125 Based on owner responses, dogs receive a score that ranges from 16 to 80, where higher  
126 scores are indicative of worse cognitive function.

127 During the study time period, 27,541 DAP participants completed HLES, and 20,096 DAP  
128 participants completed CSLB.

### 129 *Ethical Note*

130 The University of Washington IRB deemed that recruitment of dog owners for the Dog Aging  
131 Project, and the administration and content of the DAP Health and Life Experience Survey  
132 (HLES), are human subjects research that qualifies for Category 2 exempt status (IRB ID no.  
133 5988, effective 10/30/2018). No interactions between researchers and privately owned dogs  
134 occurred; therefore, IACUC oversight was not required.

### 135 *Inclusion/Exclusion Criteria*

136 Given that meal frequency is adjusted as puppies mature, we specified age inclusion as  $1 \leq \text{age}$   
137  $< 18$  years for all health outcomes. For the CSLB outcome, we specified age inclusion as  $6 \leq$   
138  $\text{age} < 18$  years, as 6 years is the youngest age indicated in the literature where signs of  
139 cognitive decline can start to appear in dogs [44–46]. Less than 5% of dogs in the DAP are  
140 intact and these dogs were excluded, as well as dogs (<1%) whose owners reported that their  
141 diet was "not at all consistent." Thus, in our final sample, all dogs were spayed or neutered due  
142 to exclusion criteria, and slightly less than half of dogs were male. About one-fifth of dogs  
143 received daily or more frequent omega-3 or other fatty acid supplementation in their diet.

144 We studied health conditions that were reported in the nine broad categories on HLES that  
145 could plausibly be affected by feeding frequency: dental or oral disease, skin disorders,  
146 orthopedic disorders, gastrointestinal disorders, cancer or tumors, kidney or urinary disorders,  
147 cardiac disorders, neurological disorders, and liver or pancreas disorders. The other broad  
148 categories of health conditions reported in HLES were not analyzed because they were either  
149 based on temporary situational and/or environmental factors and thus unlikely to be associated  
150 with feeding frequency (e.g., trauma, ingesting toxic substances, infectious or parasitic  
151 disorders); were infrequently reported and thus had a very small sample size (less than 3.5% of  
152 the total sample; e.g., respiratory disorders, endocrine disorders, reproductive system disorders,  
153 immune-mediated disorders, and hematopoietic disorders); or there was no compelling rationale  
154 as to why feeding frequency would affect them (e.g., ear, nose, and throat disorders, eye  
155 disorders).

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156 For the health categories examined in this investigation, all participants were assigned a binary  
157 score (affected/unaffected). Dogs were considered ‘affected’ if their owner reported them to  
158 have at least one relevant condition within a given category. However, we did not consider any  
159 congenital health outcomes as ‘affected’: since animals were born with these conditions, their  
160 feeding regimen was by definition instituted after onset and could therefore not have affected  
161 the development of the condition. Similarly, disorders linked to transient situational factors,  
162 including infectious diseases and trauma, were not considered as ‘affected,’ as the  
163 circumstantial nature of these instances made them unlikely to be affected by feeding  
164 frequency.

165 See Supplementary Information 1 for details of all inclusion/exclusion criteria, as well as the  
166 specific conditions that qualified a dog as affected within the dental or oral disease, skin  
167 disorders, orthopedic disorders, gastrointestinal disorders, cancer or tumors, kidney or urinary  
168 disorders, cardiac disorders, neurological disorders, and liver or pancreas disorders categories.

169 After applying exclusion criteria, the final sample consisted of responses from 24,238 HLES  
170 surveys and 10,474 CSLB surveys. The CSLB was always completed at least one week after  
171 completion of HLES. Most participants in the final sample (88%) completed CSLB within 3  
172 months of completing HLES and always within a year (range: 7 to 352 days, average: 47 days).

### 173 *Explanatory variables*

174 We analyzed feeding frequency as a binary exposure, comparing dogs fed once-daily to dogs  
175 fed more frequently. Specifically, owners were asked “How many times per day is your dog  
176 fed?” The dogs of owners who answered “Once” were sorted into the once-daily category,  
177 whereas the dogs of owners who answered “Twice”, “Three or more”, or “Free fed (filling up  
178 bowl when empty or always having food available)” were sorted into the fed-more-frequently  
179 category. In all analyses, 8% of the total sample were fed once daily (Table 1a and Table 1b).

180 In our analyses, we adjusted for sex (spayed female or neutered male), age, breed for purebred  
181 dogs and body size (as captured by weight) for mixed breed dogs. We also adjusted for whether  
182 the owner reported daily omega-3 (or other fatty acid) diet supplementation for all analyses  
183 except for dental/oral disorders and liver/pancreas, as there is evidence in the literature that  
184 fatty acids can have beneficial effects on cognitive function, skin, cardiac, gastrointestinal, renal,  
185 orthopedic, and neoplastic outcomes [47–55]. For analysis of CSLB, we additionally adjusted for  
186 two factors that are thought to affect cognitive function: physical activity level [56] and whether  
187 the dog has a history of training (according to the dog’s primary or secondary activity indicated  
188 by the owner; e.g., show dogs, service dogs, and dogs trained for field trials vs. pets/companion  
189 dogs; see Supplementary Information 2 for full details) [57].

190 We adjusted for the breed of purebred dogs as a categorical variable. After inspecting the  
191 distribution of weight by breed, we subdivided Standard poodles into two breeds for the  
192 analysis, large poodles (weight  $\geq$  13.6 kg (30 lb)) and small poodles (weight  $<$  13.6 kg (30 lb)).  
193 Although there are over 200 breeds represented in DAP data, for each analysis we only  
194 included breeds that had at least one exposed and one unexposed dog because breeds without

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195 variance in the exposure cannot inform the exposure-outcome association. We also restricted  
196 our analyses to breeds with at least 10 dogs meeting inclusion criteria. These restrictions  
197 reduced the number of breeds to 76 breeds for the CSLB analysis and 100 breeds for the  
198 analyses of health outcomes.

### 199 *Statistical Methods*

200 All statistical analyses were carried out in R v.4.0.3 [58]. Age was flexibly modeled using natural  
201 splines with interior knots at 7, 10, and 14 years for CSLB analysis and interior knots at 2, 7,  
202 and 13 years for health outcomes [59]. Weight was similarly modeled using natural splines with  
203 interior knots at 14, 48, and 79 lbs. In each instance, interior knots are at approximately the 10<sup>th</sup>,  
204 50<sup>th</sup>, and 90<sup>th</sup> percentile of each variable. We explored more elaborate adjustment models (e.g.,  
205 4 or 5 interior knots), but these were not supported by metrics such as AIC and examination of  
206 some results suggested overfitting.

207 To adjust for physical activity, we performed principal component analysis on three HLES-  
208 reported activity variables: lifestyle activity level (reported as not active, moderately active, or  
209 very active over the past year), average activity intensity level (reported as low: walking,  
210 medium: jogging, or vigorous: sprinting, such as playing fetch or frisbee), and average daily time  
211 spent physically active (reported in hours and minutes). Parallel analysis recommended  
212 retaining one principal component. This principal component captured 52% of the variance, and  
213 we used the loadings onto the first principal component as a physical activity score (PA-score).  
214 We adjusted for PA-score using natural splines with interior knots at approximately the 10<sup>th</sup>, 50<sup>th</sup>,  
215 and 90<sup>th</sup> percentiles.

216 Mixed breed dogs were included as a separate category of breed, and we adjusted for body  
217 size, as measured by weight, for mixed breed dogs only by constructing a variable *weight\*MB*  
218 where *weight* is the dog's weight and *MB* = 1 for mixed breed dogs and *MB* = 0 for purebred  
219 dogs. This is analogous to grouping mixed breed dogs by weight and including each group as a  
220 breed, except that our approach uses continuous weight information.

221 We used linear regression for analysis of CSLB and logistic regression for analysis of all health  
222 outcomes. For linear regression, the large number of parameters in the model due to 76 breeds  
223 does not cause statistical issues. However, large models are problematic for logistic regression  
224 when using conventional maximum likelihood model-fitting [60]. Therefore, we fit the logistic  
225 models using a conditional likelihood, where the conditioning was on the breed categories. This  
226 approach allowed breed to be in the model without necessitating the estimation of 100 breed  
227 parameters. Due to the large size of the data set, maximizing the exact conditional likelihood  
228 was not computationally feasible, and we used the Efron approximation. We investigated the  
229 fidelity of the approximation with follow-up analyses (reported in Supplementary Information 3)  
230 on mixed breed dogs plus dogs from the 10 most common breeds and fitting the model with  
231 ordinary logistic regression. The 10 most common breeds were Australian shepherd, beagle,  
232 border collie, chihuahua, dachshund, German shepherd, golden retriever, Labrador retriever,  
233 poodles (large), and pugs (Table 1b). We also treated these analyses as secondary analyses to  
234 assess the robustness of our findings. We used robust standard error estimates for all



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235 regression analyses. All hypothesis tests were two-sided and we did not adjust for multiple  
236 comparisons.

### 237 **Results**

238 In the CSLB analysis ( $n = 10,474$  dogs), 56% of dogs were mixed breed with the remaining  
239 dogs belonging to 76 breeds (Table 1a and Supplementary Information 4). Dogs fed once per  
240 day had, on average, a 0.62 point lower CSLB score than dogs fed more than once per day  
241 (95%: 0.27, 0.97;  $p < 0.001$ ), adjusting for age, sex, weight (for mixed breed dogs), breed (for  
242 purebred dogs), cognitive activity, physical activity level, and fatty acid supplementation (Figure  
243 1; see Supplementary Information 3 for the full model). This effect size of 0.62 points is roughly  
244 the same difference in mean CSLB score between 11- and 7-year-old dogs.

245 In analyses of health conditions ( $n = 24,238$  dogs), 55% of dogs were of mixed breed and the  
246 remaining dogs belonged to 100 breeds (Table 1b and Supplementary Information 5). For five of  
247 nine health conditions analyzed, we found evidence that being fed once per day vs. more often  
248 is associated with lower odds of having the health condition (Figure 2; Table 2). Adjusted odds  
249 ratios were less than one and statistically significant for gastrointestinal, dental/oral, orthopedic,  
250 kidney/urinary, and liver/pancreas health conditions. Adjusted odds ratios were also less than  
251 one for the remaining four categories of health conditions (cardiac, skin, neurological, cancer),  
252 but not statistically significant (see Supplementary Information 3 for detailed model results).  
253 Results were similar in secondary analyses including only mixed breed dogs and dogs from the  
254 ten most common breeds (see Supplementary Information 3 for full report).

### 255 **Discussion**

256 Using observational data from the Dog Aging Project, this is the largest study to date of feeding  
257 frequency conducted in companion dogs. We found that adult dogs fed once daily have better  
258 average cognitive scores and are less likely to have gastrointestinal, dental/oral, orthopedic,  
259 kidney/urinary, and liver/pancreas health conditions than dogs fed more frequently. While it is  
260 important to note that this study does not demonstrate causality, our observations are consistent  
261 with prior work in laboratory mice and observational studies in humans [61] suggesting that diets  
262 that restrict the timing of feeding are associated with better cognitive function and physical  
263 health.

264 In addition to being able to observe the animals in a naturalistic versus laboratory setting, one of  
265 the major strengths of our investigation is the large sample size of dogs included (CSLB  
266 assessment: 10,474 dogs; all other health conditions: 24,238 dogs). Furthermore, our statistical  
267 methods used flexible adjustment of continuous covariates (age, weight, and physical activity),  
268 reducing the possibility of residual confounding by these factors.

269 The key limitation of this work is that it is a cross-sectional analysis. Thus, we cannot rule out  
270 the possibility that dog owners shifted to more frequent feeding in response to health conditions,  
271 and observed associations are due in whole or part to reverse causality. This is a particular  
272 concern for gastrointestinal conditions and liver conditions, which are the two health conditions

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273 with the strongest observed associations. Such a shift would not be captured in the current  
274 dataset as owners reported on their dog's current feeding frequency but did not provide  
275 information on feeding frequency history. In the future, we will gather this information through  
276 annual 'snapshots' since participants complete HLES each year. As the Dog Aging Project  
277 accrues longitudinal data over the next several years, investigations can compare dogs with  
278 different feeding frequencies who do not have a health condition and prospectively examine  
279 incidence of the condition. Such analyses can provide stronger evidence for a causal effect of  
280 feeding frequency on health.

281 It is plausible that once-daily feeding tends to result in lower overall caloric intake compared to  
282 more frequent feeding. Since we do not have data on caloric intake, we cannot analyze whether  
283 observed associations are mediated by caloric intake or reflect a possible effect of feeding  
284 frequency through other pathways. Caloric restriction has been previously reported to extend  
285 lifespan and improve health in Labrador retrievers maintained in a laboratory setting [5],  
286 although it has not yet been studied in other breeds of dog or in companion dogs.

287 This study has other limitations. All data are owner-reported and thus subject to error in recall  
288 and interpretation. However, while a given owner's responses on their dog's cognitive function,  
289 physical activity, and other health conditions might reflect individual differences in interpretation  
290 and reporting errors, it is unlikely that these would generate the specific associations we  
291 observed. We were also unable to account for dogs reported as fed once-daily but who received  
292 snacks and treats throughout the day. Although HLES gathers data on frequency of treats, we  
293 did not use these data because the caloric content of treats was unknown. Finally, due to the  
294 rarity of intact dogs in our sample, analyses included only spayed and neutered dogs. While age  
295 at spay or neuter might be an important factor for some health outcomes [62, 63], this  
296 information was not incorporated into our analyses because data on the timing of gonadectomy  
297 were not available with sufficient detail or completeness.

298 Studies of obesity, including possible associations with feeding frequency [23], will be an  
299 important area of future research. This investigation did not consider obesity because  
300 information on dogs' body condition scores was not available. We anticipate that these data will  
301 be available in the future when owners share their dogs' veterinary electronic medical records  
302 (VEMR) with the Dog Aging Project.

303 Given the limitations of this cross-sectional, observational study, the results of this investigation  
304 should not be used to make decisions about the feeding or clinical care of companion dogs.  
305 However, if supported by future studies, it may be prudent to revisit the currently predominant  
306 recommendation that adult dogs be fed twice daily. The rationale for twice-daily feeding in dogs  
307 is obscure (although see [64]), and our study suggests that more frequent feeding may, in fact,  
308 be suboptimal for several age-related health outcomes.

309 We view these results as an exciting first step of an ongoing exploration of the impact of diet on  
310 companion dogs living in human environments. Given the intense interest in, and popularization  
311 of, "longevity diets" such as intermittent fasting and time-restricted feeding, these types of



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312 studies in dogs are both timely and important. We believe these studies will ultimately offer  
313 insights into factors that promote health and longevity for both dogs and humans.

### 314 **Author Contributions**

315 All authors contributed to writing – review & editing. E.B.: conceptualization, methodology, data  
316 curation, writing – original draft, and project administration. Z.Z.: conceptualization,  
317 methodology, formal analysis, and visualization. K.T.: conceptualization and data curation. B.M.:  
318 data curation. DAP consortium: resources. M.K.: conceptualization, writing – original draft, and  
319 funding acquisition. K.K.: conceptualization, methodology, formal analysis, data curation, writing  
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### 333 **Conflicts of interest/Competing interests**

334 The authors declare no competing interests.

### 335 **Data availability statement**

336 These data are housed on the Terra platform at the Broad Institute of MIT and Harvard.

### 337 **Code availability statement**

338 This study did not use custom code or mathematical algorithms.

### 339 **Supplementary Information captions**

340 Supplementary Information 1: Summary of the inclusion and exclusion criteria for subjects  
341 across all analyses, including guidelines for how all relevant variables were coded.

342 Supplementary Information 2: Criteria for determining whether or not a dog had a history of  
343 training (coded as a binary variable).

- 344 Supplementary Information 3: Regression outputs from the CSLB analysis, as well as the  
345 regression outputs from the health outcome analyses (both primary and secondary).
- 346 Supplementary Information 4: Complete list of purebred dogs ( $n = 76$ ) included in the CSLB  
347 analysis, with sample sizes.
- 348 Supplementary Information 5: Complete list of purebred dogs ( $n = 100$ ) included in analysis of  
349 health conditions, with sample sizes.

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521 *Table 1a. Characteristics of 10,474 Dogs in CSLB Analysis (76 pure breeds included).*

<b>Explanatory variable</b>	<b>n (%) or mean (SD)</b>
<b>Age</b>	
6-9.9 years	4956 (47%)
10-18 (not including 18) years	5518 (53%)
<b>Sex</b>	
Male, castrated	5042 (48%)
Female, spayed	5432 (52%)
<b>Body Size &amp; Weight</b>	
Small (<10 kg)	2673 (26%)
Middle (10-29.9 kg)	5064 (48%)
Large (30-44.9 kg)	2389 (23%)
Giant (≥45 kg)	348 (3%)
<b>Breed</b>	
Mixed-breed dog	5885 (56%)
Labrador Retriever	616 (6%)
Golden Retriever	463 (4%)
German Shepherd	192 (2%)
Dachshund	159 (2%)
Australian Shepherd	149 (1%)
Poodle (Standard) (≥13.6 kg)	130 (1%)
Border Collie	124 (1%)
Chihuahua	107 (1%)
Beagle	105 (1%)
Shih Tzu	93 (1%)

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<b>Explanatory variable</b>	<b>n (%) or mean (SD)</b>
Other purebred dogs <sup>1</sup>	2451 (23%)
<b>Feeding Frequency</b>	
Fed once per day	860 (8%)
<b>Fatty Acid Supplement</b>	
Given daily or more often	2252 (22%)
<b>Cognitive Activity</b>	
Primary or Secondary Activity requires training	1759 (17%)
<b>CSLB</b>	
Score	36.4 (5.3)

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<sup>1</sup> See Supplementary Information 4 for full list and numbers of purebred dogs included in the CSLB analysis.

Once-daily feeding associated with better outcomes 17

536 *Table 1b. Characteristics of 24,238 Dogs in Analysis of Health Outcomes (100 pure breeds*  
537 *included).*

<b>Explanatory variable</b>	<b>n (%)</b>
<b>Age</b>	
1-1.9 years	1672 (7%)
2-5.9 years	7721 (32%)
6-9.9 years	7082 (29%)
10-18 (not including 18) years	7763 (32%)
<b>Sex</b>	
Male, castrated	11853 (49%)
Female, spayed	12385 (51%)
<b>Body Size &amp; Weight</b>	
Small (<10 kg)	5510 (23%)
Middle (10-29.9 kg)	12267 (51%)
Large (30-44.9 kg)	5504 (23%)
Giant ( $\geq$ 45 kg)	957 (4%)
<b>Breed</b>	
Mixed-breed dog	13308 (55%)
Labrador retriever	1467 (6%)
Golden retriever	1199 (5%)
German Shepherd	502 (2%)
Australian Shepherd	373 (2%)
Poodle (Standard) ( $\geq$ 13.6 kg)	306 (1%)
Dachshund	305 (1%)
Border Collie	257 (1%)

## Once-daily feeding associated with better outcomes 18

<b>Explanatory variable</b>	<b>n (%)</b>
Chihuahua	205 (1%)
Beagle	187 (1%)
Pug	180 (1%)
Other purebred dogs <sup>2</sup>	5949 (25%)
<b>Feeding Frequency</b>	
Fed once per day	1884 (8%)
<b>Fatty Acid Supplement</b>	
Given daily or more often	4383 (18%)
<b>Health Outcomes</b>	
Dental/Oral	6414 (26%)
Skin	5619 (23%)
Orthopedic	4270 (18%)
Gastrointestinal	2429 (10%)
Cancer	1906 (8%)
Kidney/Urinary	1740 (7%)
Cardiac	1243 (5%)
Neurological	972 (4%)
Liver/Pancreas	673 (3%)

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<sup>2</sup> See Supplementary Information 5 for full list and numbers of purebred dogs included in the analysis of health outcomes.

Once-daily feeding associated with better outcomes 19

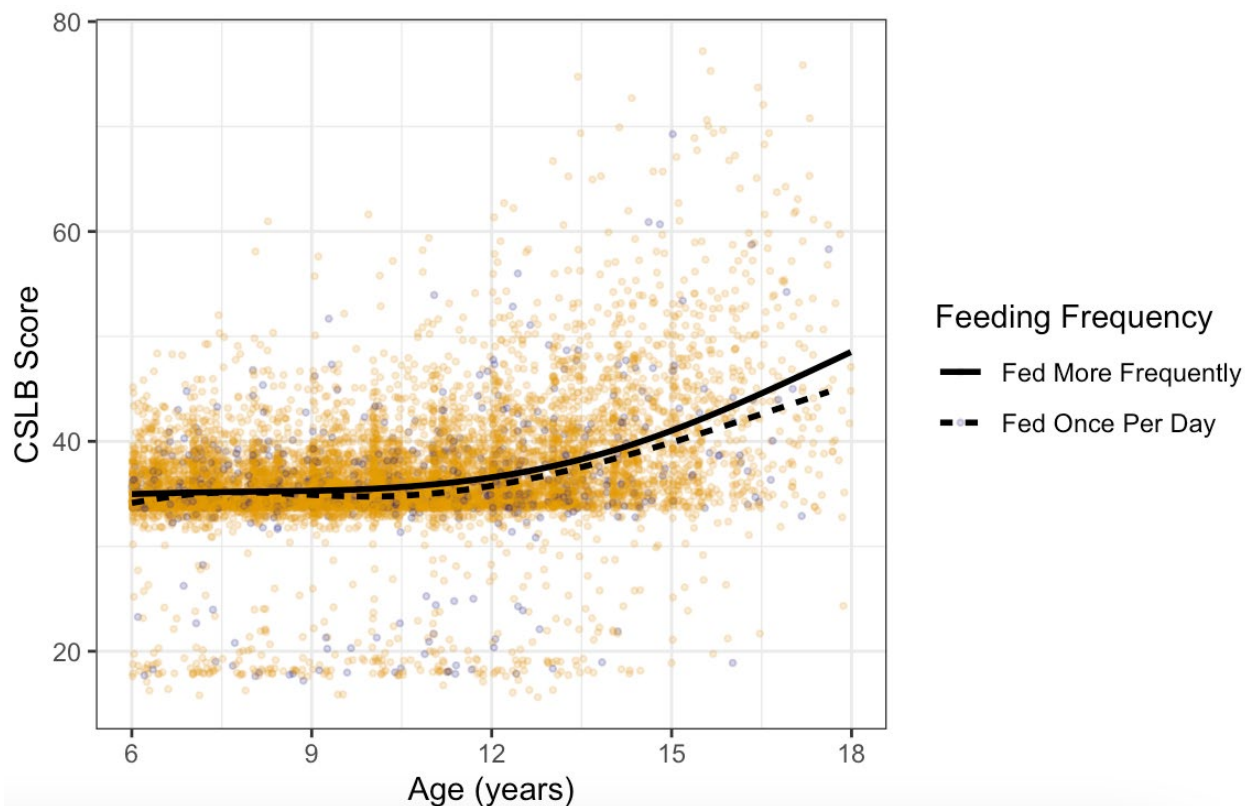
542 *Table 2. Estimated odds ratios of specific health condition for dogs fed once per day compared*  
543 *to more frequently, adjusted for sex, age, breed for purebred dogs, and body size (as captured*  
544 *by weight) for mixed breed dogs. All analyses except Liver/Pancreas and Dental/Oral are also*  
545 *adjusted for omega-3 (or other fatty acid) supplementation.*

<b>Health condition</b>	<b>Adjusted odds ratio</b>	<b>95% CI</b>	<b>p</b>
Liver/Pancreas	0.41	0.27 - 0.61	<0.001
Gastrointestinal	0.65	0.54 - 0.77	<0.001
Kidney/Urinary	0.71	0.58 - 0.88	0.0012
Orthopedic	0.78	0.69 - 0.88	<0.001
Dental/Oral	0.84	0.77 - 0.92	<0.001
Cardiac	0.86	0.70 - 1.07	0.18
Cancer	0.90	0.75 - 1.07	0.24
Neurological	0.90	0.71 - 1.16	0.42
Skin	0.94	0.85 - 1.04	0.22

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## Once-daily feeding associated with better outcomes 20

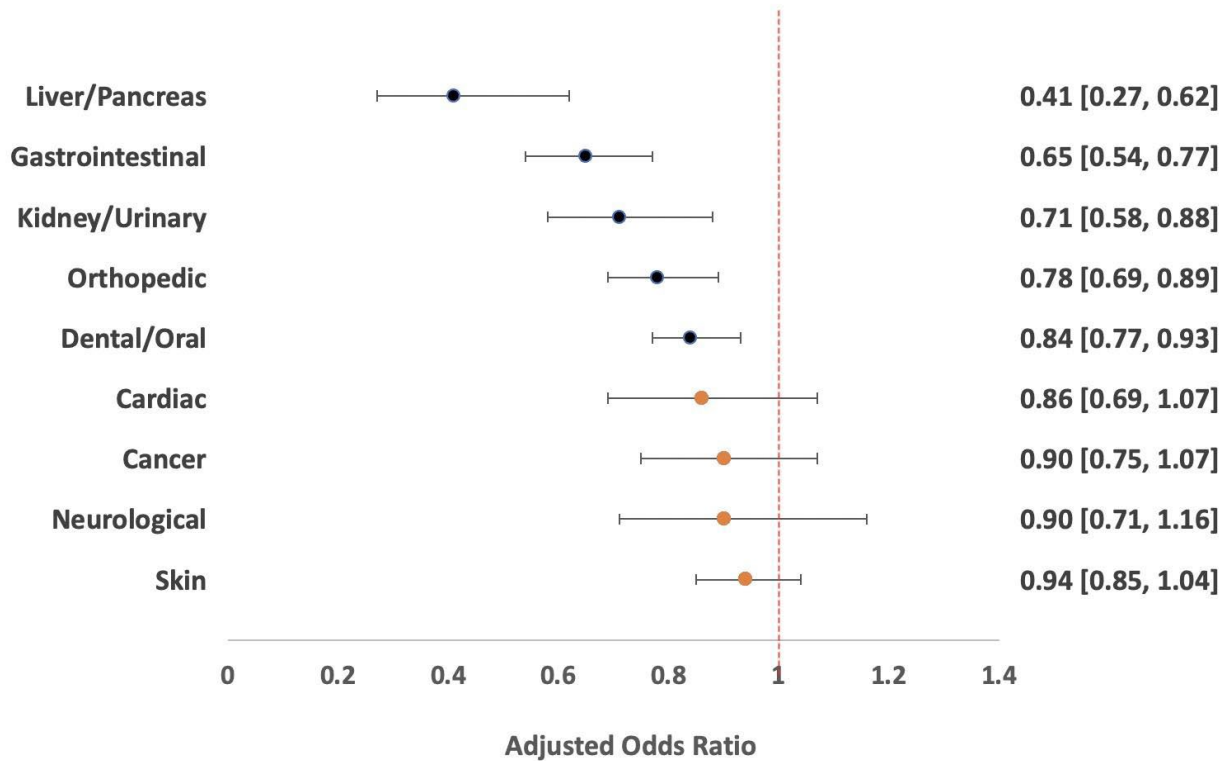


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550 **Figure 1: Scatterplot of CSLB Scores vs. Age with Superimposed Trend Lines.** Darker  
551 points represent dogs fed once daily and other points represent dogs fed more frequently. Trend  
552 lines were constructed separately for the two groups using natural splines. Dogs fed once daily  
553 have slightly lower mean CSLB score at all ages ( $6 \leq \text{age} < 18$  years).  
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## Once-daily feeding associated with better outcomes 21



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566 **Figure 2: Summary of Results for Analysis of Health Conditions.** Circles represent point

567 estimates of adjusted odds ratios, with filled circles indicating statistically significant results.

568 Bars represent 95% confidence intervals. Odds ratios less than 1 indicate lower odds of the

569 outcome among dogs fed once daily; odds ratios greater than 1 indicate higher odds of the

570 outcome among dogs fed once daily.

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