

## Once-daily feeding associated with better outcomes 1

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

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## Once-daily feeding associated with better outcomes 2

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### Abstract

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

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### Keywords:

56 Canine, Canine Cognitive Dysfunction, Feeding frequency, Healthy aging, Time-restricted  
57 feeding

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### Introduction

59 For nearly a century, caloric restriction has been known to extend lifespan and delay age-  
60 associated pathology in laboratory animals [1–3]. More recently, in both animals and humans, a  
61 variety of alternative “anti-aging” diet modalities have been described which are providing new  
62 mechanistic insights and potential clinical applications. These diets include intermittent fasting  
63 [4, 5], fasting mimicking diets [6, 7], ketogenic diets [8–12], protein or essential amino acid  
64 restriction [13, 14], and time-restricted feeding [15–17].

65 These diets have been most extensively studied in rodents in controlled laboratory settings, due  
66 to ease of administering diets on a specific schedule and an enhanced ability to tease apart the  
67 mechanisms through which they act. Isocaloric time-restricted feeding studies in rodents  
68 suggest improvements in several metabolic parameters, including glucose and insulin  
69 homeostasis, energy expenditure, hepatic pathology, resistance to different obesogenic diets,  
70 and improved circadian rhythm maintenance during aging [18–20]. In one study, mice who  
71 experienced time-restricted feeding demonstrated an 11% extension in lifespan [15].  
72 Additionally, several studies demonstrate that caloric restriction and intermittent fasting play a  
73 protective role in maintaining and enhancing cognitive function, including memory and spatial  
74 learning [21–25].

75 Despite mainstream popularization in humans of several of these diets, the beneficial health  
76 effects of time-restricted feeding outside of a laboratory setting are less clear. In some human  
77 studies, only mild improvements in body composition and cardiovascular risk factors were  
78 detected [26], even when subjects also reduced their daily caloric intake [27]. In other studies,  
79 detrimental effects on glucose homeostasis were observed with time-restricted feeding [28].

## Once-daily feeding associated with better outcomes 3

80 Finally, while some studies have found potential cognitive benefits, especially for memory in  
81 older adults [29, 30], other studies have shown no effect of fasting on cognition [31, 32].

82 Companion dogs provide a potentially powerful animal model in which to better understand the  
83 relationship between diet and age-related health outcomes [33]. Having co-evolved alongside  
84 people for thousands of years [34], companion dogs share human environments, experience  
85 similar diseases, and receive similar medical care. Once-daily feeding in dogs serves as a  
86 natural model for the intermittent fasting/time-restricted feeding protocols currently being studied  
87 both in preclinical rodent models and in human trials [35].

88 The Dog Aging Project is a large-scale research initiative following thousands of companion  
89 dogs over their lifetimes to better understand how biology, lifestyle, and environment impact  
90 healthy aging [36]. Participating owners report annually on a variety of aspects related to their  
91 dog's diet, primary and secondary activities, social and physical environments, medications, and  
92 health conditions. In the current study, we used cross-sectional data collected in the first year of  
93 the Dog Aging Project to ask if feeding frequency is associated with cognitive function and  
94 health conditions. Specifically, we hypothesized that dogs fed once-a-day would display lower  
95 rates of physical health issues and better cognitive scores compared to dogs fed more  
96 frequently.

## 97 **Methods**

### 98 *Subjects*

99 All participants were members of the Dog Aging Project (DAP) and had filled out the relevant  
100 online surveys between December 26, 2019 and December 31, 2020 [37]. Study data were  
101 collected and managed using REDCap electronic data capture tools hosted through the DAP  
102 [38, 39]. REDCap (Research Electronic Data Capture) is a secure, web-based software platform  
103 designed to support data capture for research studies, providing 1) an intuitive interface for  
104 validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3)  
105 automated export procedures for seamless data downloads to common statistical packages;  
106 and 4) procedures for data integration and interoperability with external sources.

### 107 *Instruments*

108 The first survey that participants completed was the Health and Life Experience Survey (HLES),  
109 which collects information regarding dog demographics, physical activity, environment, dog  
110 behavior, diet, medications and preventives, health status, and owner demographics. For the  
111 purpose of the current study, we were principally interested in frequency of feeding and health  
112 status. In terms of health outcomes, we focused on conditions that could plausibly be affected  
113 by feeding frequency.

114 After completing HLES, all participants were offered the opportunity to complete the Canine  
115 Social and Learned Behavior Survey (CSLB), which measures cognitive function. The CSLB,  
116 renamed by the DAP, is the same as the validated Canine Cognitive Dysfunction Rating Scale  
117 (CCDR) [40], with only a handful of minor wording changes. The Canine Cognitive Dysfunction

## Once-daily feeding associated with better outcomes 4

118 Rating Scale was presented to participants as the Canine Social and Learned Behavior Survey  
119 to avoid the negative connotations of the phrase 'cognitive dysfunction. This instrument asks  
120 owners to indicate the frequency with which their dogs exhibit behaviors indicative of dementia  
121 (i.e., disengagement from social activity; difficulty in navigation, searching, and recognition).  
122 Based on owner responses, dogs receive a score that ranges from 16 to 80, where higher  
123 scores are indicative of worse cognitive function.

124 During the study period, 27,541 DAP participants completed HLES, and 20,096 DAP  
125 participants completed CSLB.

### 126 *Inclusion/Exclusion Criteria*

127 Given that meal frequency is adjusted as puppies mature, we specified age inclusion as  $1 \leq \text{age}$   
128  $< 18$  years for all health outcomes. For the CSLB outcome, we specified age inclusion as  $6 \leq$   
129  $\text{age} < 18$  years, as 6 years is the youngest age indicated in the literature where signs of  
130 cognitive decline can start to appear in dogs [41–43]. Less than 5% of dogs in the DAP are  
131 intact and these dogs were excluded, as well as dogs (<1%) whose owners reported that their  
132 diet was "not at all consistent." Thus, in our final sample, all dogs were spayed or neutered due  
133 to exclusion criteria, and slightly less than half of dogs were male. About one-fifth of dogs  
134 received daily or more frequent omega-3 or other fatty acid supplementation in their diet.

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

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

## Once-daily feeding associated with better outcomes 5

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

160 After applying exclusion criteria, the final sample consisted of responses from 24,238 HLES  
161 surveys and 10,474 CSLB surveys. The CSLB was always completed at least one week after  
162 completion of HLES. The exact amount of time elapsed between the two surveys varied across  
163 participants, but time until CSLB completion was no longer than a year (range: 7 to 352 days,  
164 average: 46.61 days), and the majority of participants (88%) completed CSLB within 3 months  
165 of completing HLES.

### 166 *Explanatory variables*

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

173 In our analyses, we adjusted for sex (spayed female or neutered male), age, breed for purebred  
174 dogs and body size (as captured by weight) for mixed breed dogs. We also adjusted for whether  
175 the owner reported daily omega-3 (or other fatty acid) diet supplementation for all analyses  
176 except for dental/oral disorders and liver/pancreas, as there is evidence in the literature that  
177 fatty acids can have beneficial effects on cognitive function, skin, cardiac, gastrointestinal, renal,  
178 orthopedic, and neoplastic outcomes [44–52]. For analysis of CSLB, we additionally adjusted for  
179 two factors that are thought to affect cognitive function: physical activity level [53] and whether  
180 the dog has a history of training (according to the dog’s primary or secondary activity indicated  
181 by the owner; e.g., show dogs, service dogs, and dogs trained for field trials vs. pets/companion  
182 dogs; see Supplementary Information 2 for full details) [54].

183 We adjusted for the breed of purebred dogs as a categorical variable. After inspecting the  
184 distribution of weight by breed, we subdivided Standard poodles into two breeds for the  
185 analysis, large poodles (weight  $\geq$  13.6 kg (30 lb)) and small poodles (weight  $<$  13.6 kg (30 lb)).  
186 Although there are over 200 breeds represented in DAP data, for each analysis we only  
187 included breeds that had at least one exposed and one unexposed dog because breeds without  
188 variance in the exposure cannot inform the exposure-outcome association. We also restricted  
189 our analyses to breeds with at least 10 dogs meeting inclusion criteria. These restrictions  
190 reduced the number of breeds to 76 breeds for the CSLB analysis and 100 breeds for the  
191 analyses of health outcomes.

### 192 *Statistical Methods*

## Once-daily feeding associated with better outcomes 6

193 Age was flexibly modeled using restricted cubic splines with knots at 7, 10, and 14 years for  
194 CSLB analysis and knots at 2, 7, and 13 years for health outcomes [55]. Weight was similarly  
195 modeled using restricted cubic splines with knots at 14, 48, and 79 lbs. In each instance, knots  
196 are at approximately the 10<sup>th</sup>, 50<sup>th</sup>, and 90<sup>th</sup> percentile of each variable. We explored more  
197 elaborate adjustment models (e.g., 4 or 5 knots), but these were not supported by metrics such  
198 as AIC and examination of some results suggested overfitting.

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

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

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

## 228 **Results**

229 In the CSLB analysis ( $n = 10,474$  dogs), 56% of dogs were mixed breed with the remaining  
230 dogs belonging to 76 breeds (Table 1a and Supplementary Information 4). Dogs fed once per  
231 day had, on average, a 0.63 point lower CSLB score than dogs fed more than once per day  
232 (95%: 0.28, 0.98;  $p < 0.001$ ), adjusting for age, sex, weight (for mixed breed dogs), breed (for

## Once-daily feeding associated with better outcomes 7

233 purebred dogs), cognitive activity, physical activity level, and fatty acid supplementation (Figure  
234 1; see Supplementary Information 3 for the full model). This effect size of 0.63 points is roughly  
235 the same difference in mean CSLB score between 11- and 7-year-old dogs, and should be  
236 interpreted in the context of large variances in CLSB scores across all ages (Table 1a).

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

### 247 **Discussion**

248 Using observational data from the Dog Aging Project, this is the largest study to date of feeding  
249 frequency conducted in companion dogs. We found that adult dogs fed once daily have better  
250 average cognitive scores and are less likely to have gastrointestinal, dental/oral, orthopedic,  
251 kidney/urinary, and liver/pancreas health conditions than dogs fed more frequently. While it is  
252 important to note that this study does not demonstrate causality, our observations are consistent  
253 with prior work in laboratory mice and observational studies in humans [56] suggesting that diets  
254 that restrict the timing of feeding are associated with better cognitive function and physical  
255 health.

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

261 A limitation of this work is that it is a cross-sectional analysis. Thus, we cannot rule out the  
262 possibility that dog owners shifted to more frequent feeding in response to health conditions,  
263 and observed associations are due in whole or part to reverse causality. This is a particular  
264 concern for gastrointestinal conditions and liver conditions, which are the two health conditions  
265 with the strongest observed associations. As the Dog Aging Project accrues longitudinal data  
266 over the next several years, incident analyses have the potential to provide stronger evidence of  
267 a possibly causal link between feeding frequency and health.

268 This study has other limitations. All data are owner-reported and thus subject to error in recall  
269 and interpretation. We were also unable to account for dogs reported as fed once-a-day but who  
270 received snacks and treats throughout the day. Although HLES gathers data on frequency of  
271 treats, we did not use these data because the caloric content of treats was unknown. Finally,

## Once-daily feeding associated with better outcomes 8

272 due to the rarity of intact dogs in our sample, analyses included only spayed and neutered dogs.  
273 While age at spay or neuter might be an important factor for some health outcomes [57, 58], this  
274 information was not incorporated into our analyses because data on the timing of gonadectomy  
275 were not available with sufficient detail or completeness.

276 Studies of obesity, including possible associations with feeding frequency [20], will be an  
277 important area of future research. This investigation did not consider obesity because  
278 information on dogs' body condition scores was not available. We anticipate that these data will  
279 be available in the future when owners share their dogs' veterinary electronic medical records  
280 (VEMR) with the Dog Aging Project.

281 Given the limitations of this cross-sectional, observational study, the results of this investigation  
282 should not be used to make decisions about the feeding or clinical care of companion dogs.  
283 However, if supported by future studies, it may be prudent to revisit the currently predominant  
284 recommendation that adult dogs be fed twice daily. The rationale for twice-daily feeding in dogs  
285 is obscure, and our study suggests that more frequent feeding may, in fact, be suboptimal for  
286 several age-related health outcomes.

287 We view these results as an exciting first step of an ongoing exploration of the impact of diet on  
288 companion dogs living in human environments. Given the intense interest in, and popularization  
289 of, "longevity diets" such as intermittent fasting and time-restricted feeding, these types of  
290 studies in dogs are both timely and important. We believe these studies will ultimately offer  
291 insights into factors that promote health and longevity for both dogs and humans.

### 292 **Author Contributions**

293 All authors contributed to writing – review & editing. E.B.: conceptualization, methodology, data  
294 curation, writing – original draft, and project administration. Z.Z.: conceptualization,  
295 methodology, formal analysis, and visualization. K.T.: conceptualization and data curation. B.M.:  
296 data curation. DAP consortium: resources. M.K.: conceptualization, writing – original draft, and  
297 funding acquisition. K.K.: conceptualization, methodology, formal analysis, data curation, writing  
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## Once-daily feeding associated with better outcomes 9

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### 311 **Conflicts of interest/Competing interests**

312 The authors declare no competing interests.

### 313 **Data availability statement**

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

### 315 **Supplementary Information captions**

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

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

320 Supplementary Information 3: Regression outputs from the CSLB analysis, as well as the  
321 regression outputs from the health outcome analyses (both primary and secondary).

322 Supplementary Information 4: Complete list of purebred dogs ( $n = 76$ ) included in the CSLB  
323 analysis, with sample sizes.

324 Supplementary Information 5: Complete list of purebred dogs ( $n = 100$ ) included in analysis of  
325 health conditions, with sample sizes.

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482 *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%)

## Once-daily feeding associated with better outcomes 15

<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 16

504 *Table 1b. Characteristics of 24,238 Dogs in Analysis of Health Outcomes (100 pure breeds*  
505 *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 17

<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.

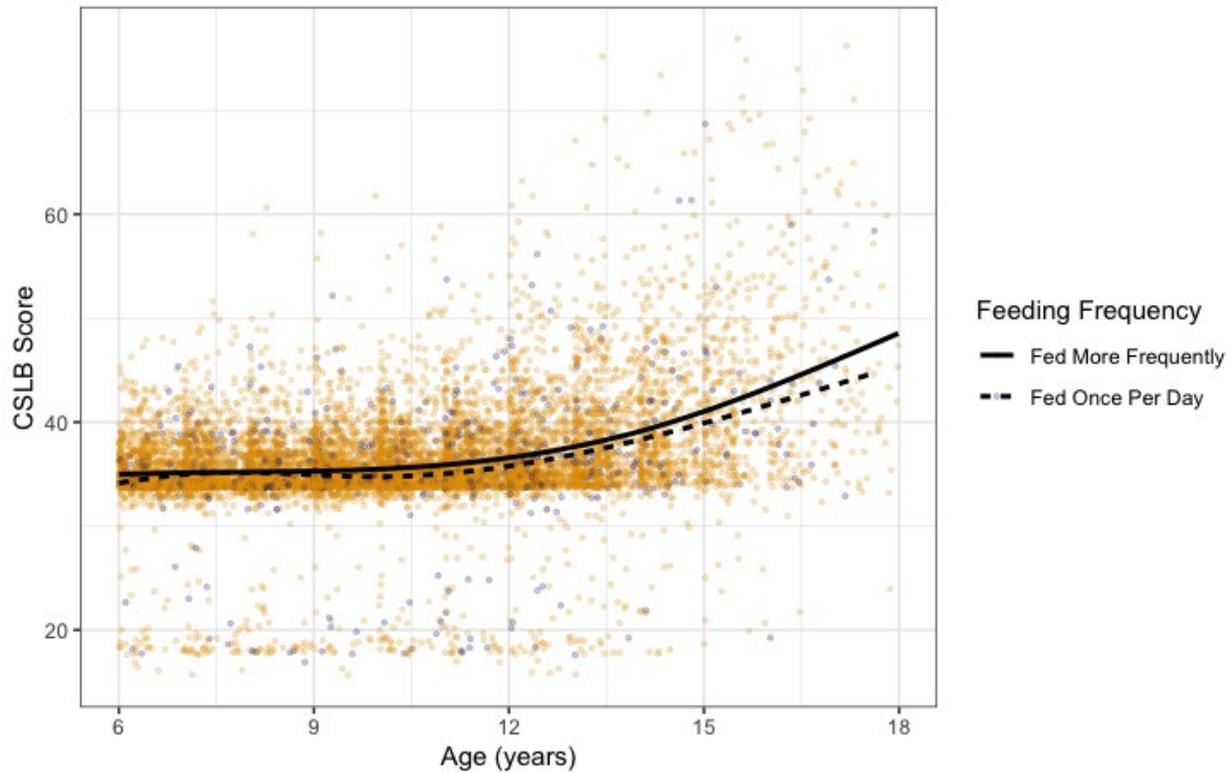
Once-daily feeding associated with better outcomes 18

510 *Table 2. Estimated odds ratios of specific health condition for dogs fed once per day compared*  
511 *to more frequently, adjusted for sex, age, breed for purebred dogs, and body size (as captured*  
512 *by weight) for mixed breed dogs. All analyses except Liver/Pancreas and Dental/Oral are also*  
513 *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 - 0.78	<0.001
Kidney/Urinary	0.72	0.58 - 0.88	0.0012
Orthopedic	0.78	0.69 - 0.88	<0.001
Dental/Oral	0.84	0.76 - 0.92	<0.001
Cardiac	0.87	0.70 - 1.08	0.20
Cancer	0.90	0.76 - 1.08	0.25
Neurological	0.90	0.71 - 1.16	0.42
Skin	0.94	0.85 - 1.04	0.24

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## Once-daily feeding associated with better outcomes 19



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517 **Figure 1: Scatterplot of CSLB Scores vs. Age with Superimposed Trend Lines.** Darker  
518 points represent dogs fed once daily and other points represent dogs fed more frequently. Trend  
519 lines were constructed separately for the two groups using restricted cubic splines. Dogs fed  
520 once daily have slightly lower mean CSLB score at all ages ( $6 \leq \text{age} < 18$  years).  
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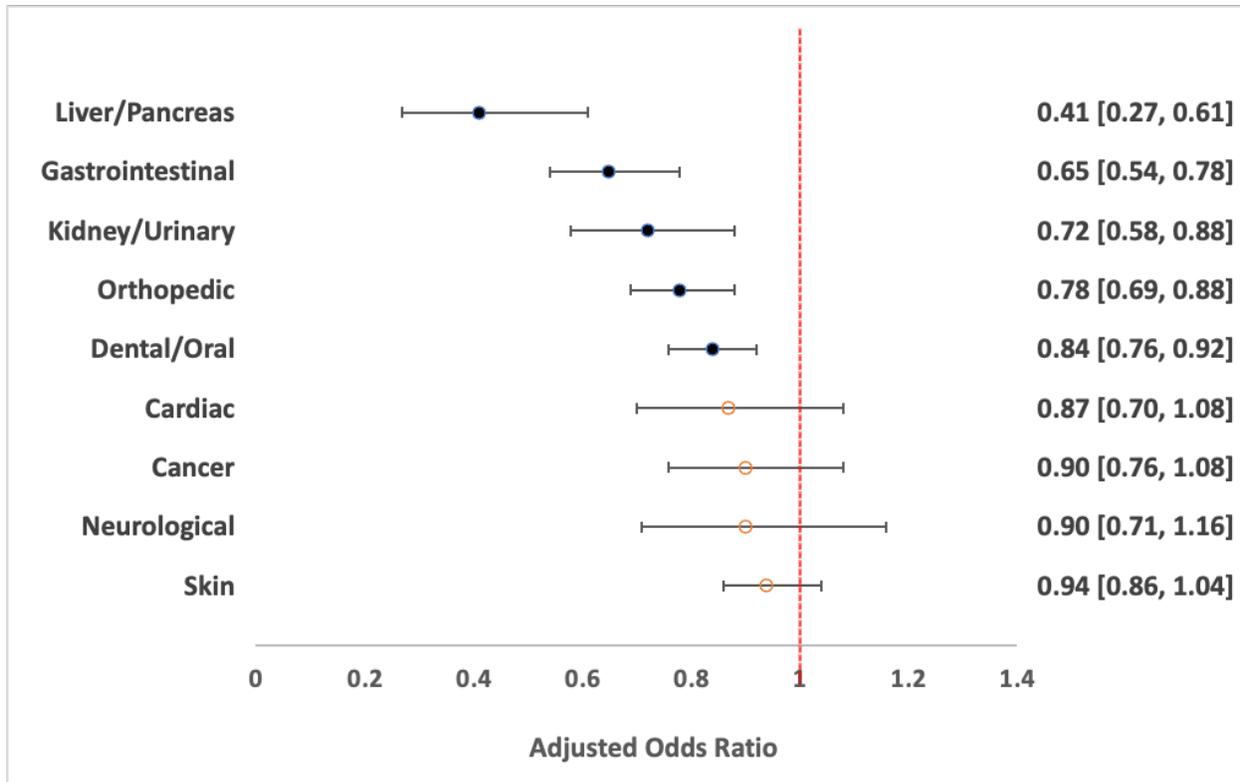
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## Once-daily feeding associated with better outcomes 20



532 **Figure 2: Summary of Results for Analysis of Health Outcomes.** Circles represent point  
533 estimates of adjusted odds ratios, with filled circles indicating statistically significant results.  
534 Bars represent 95% confidence intervals. Odds ratios less than 1 indicate lower odds of the  
535 outcome among dogs fed once daily; odds ratios greater than 1 indicate higher odds of the  
536 outcome among dogs fed once daily.