

1 **Title:** Associations between physical activity and cognitive dysfunction in older companion
2 dogs: Results from the Dog Aging Project

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Abstract

42 Canine Cognitive Dysfunction (CCD) is a form of dementia that shares many similarities
43 with Alzheimer's disease. Given that physical activity is believed to reduce risk of Alzheimer's
44 disease in humans, we explored the association between physical activity and cognitive health in
45 a cohort of companion dogs, aged 6-18 years. We hypothesized that higher levels of physical
46 activity would be associated with lower (i.e., better) scores on a cognitive dysfunction rating
47 instrument and lower prevalence of dementia, and that this association would be robust when
48 controlling for age, comorbidities, and other potential confounders. Our sample included 11,574
49 companion dogs enrolled through the Dog Aging Project, of whom 287 had scores over the
50 clinical threshold for CCD. In this observational, cross-sectional study, we used owner-reported
51 questionnaire data to quantify dog cognitive health (via a validated scale), physical activity
52 levels, health conditions, training history, and dietary supplements. We fit regression models
53 with measures of cognitive health as the outcome, and physical activity—with several important
54 covariates—as predictors. We found a significant negative relationship between physical activity
55 and current severity of cognitive dysfunction symptoms (estimate = -0.10, 95% CI: -0.11 to -
56 0.08, $p < 0.001$), extent of symptom worsening over a 6-month interval (estimate = -0.07, 95%
57 CI: -0.09 to -0.05, $p < 0.001$), and whether a dog reached a clinical level of CCD (odds ratio =
58 0.53, 95% CI: 0.45 to 0.63, $p < 0.001$). Physical activity was robustly associated with better
59 cognitive outcomes in dogs. Our findings illustrate the value of companion dogs as a model for
60 investigating relationships between physical activity and cognitive aging, including aspects of
61 dementia that may have translational potential for Alzheimer's disease. While the current study
62 represents an important first step in identifying a relationship between physical activity and
63 cognitive function, it cannot determine causality. Future studies are needed to rule out reverse
64 causation by following the same dogs prospectively over time, and to evaluate causality by
65 administering physical-activity interventions.

66

Keywords

67 Canine, Canine Cognitive Dysfunction, Healthy aging, Physical activity

68

Introduction

69 Alzheimer's disease is a devastating, age-related progressive neurodegenerative brain disorder
70 that leads to cognitive decline and dementia. It is therefore a high priority for researchers to
71 identify early, modifiable risk factors that can be targeted as interventions (Raichlen &
72 Alexander, 2017; Yu et al., 2020). Over the past few decades, physical activity has emerged as
73 one such factor that may play an important role in reducing the risk of Alzheimer's disease.
74 There is evidence in humans that engaging in physical activity can have protective effects on
75 cognitive function (Ahlskog, Geda, Graff-Radford, & Petersen, 2011; Santos-Lozano et al.,
76 2016). In one large interventional study of adults with memory impairment, participating in a
77 physical activity program for six months led to measurable increases in cognitive performance

78 over the next year and a half (Lautenschlager et al., 2008). In a different intervention, researchers
79 documented an increase in hippocampal volume linked to aerobic exercise training (Erickson et
80 al., 2011). A meta-analysis across 12 cohorts including thousands of participants also concluded
81 that physical activity significantly protected against cognitive decline, even at low to moderate
82 levels (Sofi et al., 2011). A recent study found that late-life physical activity was associated with
83 higher presynaptic protein levels, known to positively affect cognition (Casaletto et al., 2021).
84 Indeed, recent meta-analyses of randomized controlled trials using physical activity interventions
85 reveal notable protective effects for dementia risk (Beckett, Ardern, & Rotondi, 2015; Xu et al.,
86 2017).

87
88 Several nonhuman species have been used as animal models for the cognitive impairments
89 associated with Alzheimer's disease (Cotman & Berchtold, 2007). Similarly to the human
90 studies, there is preliminary evidence from work in rodents (Berchtold, Castello, & Cotman,
91 2010; Jahangiri, Gholamnezhad, & Hosseini, 2019; Van Praag, Shubert, Zhao, & Gage, 2005)
92 and primates (Rhyu et al., 2010) that exercise enhances cognitive function and leads to
93 neurogenesis, potentially protecting against the development of dementia. However, current
94 model systems have limited translational potential due to reliance on genetically homogenous
95 populations studied in artificial environments. To date, most comparative studies have been
96 conducted using transgenic mouse models that attempt to mimic specific aspects of Alzheimer's
97 disease neuropathology, including the pathological deposition of amyloid- β (A β) plaques and
98 neurofibrillary tangles with hyperphosphorylated tau (Jankowsky & Zheng, 2017). However,
99 these models have typically focused on the least prevalent form in humans (Webster, Bachstetter,
100 Nelson, Schmitt, & Van Eldik, 2014). No mouse model exhibits the full progression of
101 Alzheimer's disease, and the supraphysiological overexpression of amyloid precursor protein
102 transgenes may alter brain development in ways that limit translational potential (Elder, Gama
103 Sosa, & De Gasperi, 2010). In addition, studies with laboratory mice have limited ability to
104 model the complex gene \times environment interactions believed to underlie the heterogeneity
105 observed in the development and progression of Alzheimer's disease (Chouliaras et al., 2010).

106
107 Companion dogs have been proposed as a model for aging research with high translational
108 potential (Creevy, Akey, Kaeberlein, & Promislow, 2022; Kaeberlein, Creevy, & Promislow,
109 2016). Unlike laboratory populations, companion dogs are genetically heterogeneous, and share
110 many important features with humans, including the same living environments, disease risks and
111 burdens, patterns of actuarial aging, and access to a sophisticated health care system (Hoffman,
112 Creevy, Franks, O'Neill, & Promislow, 2018). Dogs have also been suggested as a valuable
113 natural complementary model for the age-related dementia of Alzheimer's disease. With
114 advanced age, many dogs spontaneously develop a range of cognitive and behavioral
115 impairments that resemble those associated with brain aging and Alzheimer's dementia. Dozens
116 of studies have shown that signs of age-related neurodegeneration in dogs are often accompanied
117 by cognitive dysfunction in learning and memory analogous to impairments often seen in aging

118 and Alzheimer's disease (Head, 2011, 2013; Milgram et al., 2004; Packer et al., 2018; Ruehl et
119 al., 1995). Although the full complement of Alzheimer's disease neuropathology has yet to be
120 consistently observed in any naturally occurring non-human animal model, Alzheimer-like
121 pathology, e.g., A β 1-42, increases with age in companion dogs (Urfer et al., 2021) and has been
122 described in the context of diffuse plaque deposition that has been related to cognitive
123 decrements in older dogs (Cotman and Head, 2008). There is also preliminary evidence for
124 tauopathy, another feature of Alzheimer-like pathology, in the brains of dogs diagnosed with
125 canine cognitive dysfunction (Abey et al., 2021).

126
127 In addition, similarly to humans, physical activity as part of enrichment programs in dogs has
128 been associated with reductions in A β Alzheimer-like pathology and improved cognitive
129 performance (Cotman & Berchtold, 2007). Despite the strong potential for dog models of
130 Alzheimer's disease, most studies to date have used small laboratory samples that do not
131 capitalize on the many potential benefits of a companion dog model (e.g., large heterogeneous
132 populations living in the same environments as humans).

133
134 Previous exploratory work has looked broadly for associations between a wide range of
135 characteristics and Canine Cognitive Dysfunction, finding that age as well as a single rating of
136 physical activity were associated with Canine Cognitive Dysfunction (Yarborough, 2021).
137 Building upon these findings, in the current observational study we focused our investigation on
138 the relationship between physical activity and age-related impairments in cognitive function in
139 companion dogs, using questionnaire data generated by The Dog Aging Project. Specifically,
140 owners were asked to report the dog's lifestyle (not active to active) as well as the typical
141 duration and intensity of their dog's physical activity. This dataset was analyzed alongside the
142 owners' responses to a validated instrument (Salvin, McGreevy, Sachdev, & Valenzuela, 2011)
143 assessing behaviors indicative of cognitive dysfunction and dementia (i.e., changes in social
144 activity; challenges in navigation, searching, and recognition). We hypothesized that higher
145 levels of physical activity would be associated with lower (i.e., better) scores on a cognitive
146 dysfunction rating instrument, and decreased risk of dementia, and that this association would be
147 robust when controlling for age, comorbidities, and potential confounders (e.g., joint
148 supplements, motor impairments, exercise intolerance). Additionally, given that we know little
149 about potential risk factors and protective effects for canine dementia, we also examined
150 associations between several lifestyle factors (i.e., use of neuroprotective supplements and
151 engagement in formal dog training activities) and categories of health conditions (i.e., neurologic
152 conditions, sensory deficits, periodontal disease, and liver failure) with dementia outcomes.

153 **Methods**

154 *Subjects*

155 All dogs were members of the Dog Aging Project (DAP), a nationwide research study of
156 companion dogs that aims to better understand the biological and environmental factors that
157 impact health span and lifespan (Creedy et al., 2022; Kaeberlein et al., 2016). While the DAP is
158 an ongoing longitudinal study, the data in the current study were cross-sectional, drawing on
159 initial responses from owners whose dogs are enrolled in the first cohort. Owners completed the
160 requested online surveys between December 26, 2019 and December 31, 2020 (Dog Aging
161 Project, 2021). Study data were collected and managed using REDCap electronic data capture
162 tools hosted through the DAP (Harris et al., 2019; Harris et al., 2009). These data are publicly
163 available and housed on the Terra platform at the Broad Institute of MIT and Harvard.

164 *Instruments*

165 Upon enrollment in the DAP, owners completed the Health and Life Experience Survey (HLES).
166 In addition to collecting dog and owner demographics, this detailed questionnaire also asked
167 owners to report on their dog's physical activity, environment, behavior, diet, medications and
168 preventatives, and health status. For the current study, we were mainly interested in the data
169 reflecting physical activity and health status.

170 After completing HLES, all participants were asked to participate in a second survey: the Canine
171 Social and Learned Behavior Survey (CSLB). The intent of this survey was to measure owner-
172 report of cognitive function. The CSLB, renamed by the DAP, is based on the Canine Cognitive
173 Dysfunction Rating Scale (CCDR) (Salvin et al., 2011), with minor wording modifications to
174 select items. The CCDR was presented to participants as the Canine Social and Learned
175 Behavior Survey to avoid the negative connotations of the phrase 'cognitive dysfunction'. This
176 instrument asks owners to indicate the frequency with which their dogs exhibit behaviors
177 indicative of cognitive dysfunction and dementia (i.e., disengagement from social activity;
178 difficulty in navigation, searching, and recognition). Based on owner responses, dogs receive a
179 score that ranges from 16 to 80, where higher scores are indicative of worse cognitive function.
180 This instrument was previously validated in a sample of dogs 8 years and older as a way of
181 distinguishing dogs with CCD from those without (Salvin et al., 2011). In the current manuscript,
182 we also explored its utility as a continuous measure.

183 During the study period, we received HLES responses from 27,541 unique DAP participants, of
184 which 20,096 went on to also complete a CSLB.

185 *Ethical Note*

186 The University of Washington IRB deemed that recruitment of dog owners for the DAP, and the
187 administration and content of the DAP HLES, are human subjects research that qualifies for
188 Category 2 exempt status (IRB ID no. 5988, effective 10/30/2018). No interactions between
189 researchers and privately owned dogs occurred; therefore, IACUC oversight was not required.

190 *Inclusion/Exclusion Criteria*

191 Given that cognitive decline is not typically observed in dogs until at least six years of age
192 (Harvey, 2021; Packer et al., 2018; Studzinski et al., 2006), we specified age of inclusion as $6 \leq$
193 age < 18 years at the time of CSLB completion.

194 After applying this exclusion criterion, the final sample consisted of 11,574 dogs whose owners
195 completed both the HLES and CSLB surveys. CSLB was always completed at least one week
196 after completion of HLES. Most participants in the final sample (87.8%) completed CSLB
197 within 3 months of completing HLES and always within one year (range: 7 to 352 days, mean:
198 47.14 days).

199 *Outcome variable*

200 Our outcome of interest was the owner-reported symptoms of cognitive dysfunction of each dog,
201 which we measured via three scores derived from CSLB responses. We first performed principal
202 component analysis (PCA) on the 13 response items (see SI 1, Appendix A for survey
203 questions). Parallel analysis recommended retaining two principal components. We used an
204 oblimin rotation to allow correlation between the two PCs (see Table S1 in SI 1 for loadings).
205 The first PC, which we called ‘change’, was loaded highly by questions regarding reported
206 changes in cognitive dysfunction symptoms over the prior 6 months. The second PC, which we
207 called ‘severity’, was loaded highly by items measuring reported current symptom severity.
208 Finally, we analyzed Canine Cognitive Dysfunction (CCD) status as a binary exposure, wherein
209 dogs who scored 50 or above were deemed to be above the diagnostic clinical threshold for
210 CCD, and dogs below this score were not (Salvin et al., 2011).

211 *Predictor Variables*

212 Our main predictor of interest was physical activity. To calculate this variable for each dog, we
213 performed PCA on three HLES-reported activity variables: lifestyle activity level (reported as
214 not active, moderately active, or very active over the past year), average activity intensity level
215 (reported as low: walking, medium: jogging, or vigorous: sprinting, such as playing fetch or
216 frisbee), and average daily time spent physically active (reported in hours and minutes). Parallel
217 analysis recommended retaining one principal component from these measures. This principal
218 component explained 52% of the variance and was loaded positively by all three questions
219 regarding physical activity. We used the scores from this component as our measure of physical
220 activity (PA-score). Initial exploratory analyses suggested substantial and linear declines in
221 physical activity with age (Fig S1 in SI 1).

222 We used information reported in HLES about diverse medical conditions with potential to
223 influence cognitive function or physical activity level as covariates. Specifically, based on past
224 literature, we expected the following health-related factors to be associated with risk of cognitive
225 impairment in dogs: neurologic conditions, such as epilepsy (Hobbs et al., 2020; Watson, Packer,
226 Rusbridge, & Volk, 2020; Winter, Packer, & Volk, 2018), sensory deficits in the visual and

227 auditory domains (Fischer et al., 2016; Ford et al., 2018; Szabó, Miklósi, & Kubinyi, 2018),
228 periodontal disease (Dewey & Rishniw, 2021; Harding, Gonder, Robinson, Crean, & Singhrao,
229 2017; Singhrao, Harding, Poole, Kesavalu, & Crean, 2015), and liver failure (Butterworth, 2016;
230 Felipo, 2013).

231 We also created covariates for orthopedic conditions and exercise intolerance, which we
232 expected to be negatively associated with physical activity levels. In the exercise intolerance
233 category, we accounted for cardiac and respiratory conditions that negatively affect a dog's
234 ability to exercise—either by rendering the dogs unable to exert themselves physically, or
235 because the prevailing veterinary advice for the diagnosis is restricted activity.

236 Lastly, to control for other factors potentially influencing general health, we created variables for
237 whether dogs had been diagnosed with certain systemic disorders, including cancer and those
238 affecting the kidneys and the endocrine system.

239 For each of the health condition categories described above, all participants were assigned a
240 binary score (affected/unaffected). Dogs were considered 'affected' if their owner reported them
241 to have one or more relevant conditions within a given category. We only included chronic
242 conditions that were likely to affect the relevant systems, and thus excluded temporary
243 conditions that, given standard recommended medical care, would only temporarily affect the
244 relevant systems. For example, in the orthopedic category, we scored hip dysplasia as an
245 'affected' condition, as it is a long-term issue that affects mobility, whereas fractured bones were
246 not included because the most likely prognosis is complete recovery and therefore the impact on
247 physical activity is temporary. For cataracts and ligament ruptures, we only included dogs as
248 affected (in the sensory impairment and orthopedic categories, respectively) if the diagnosis was
249 *not* followed by surgery. Our curated list of health conditions included in each covariate category
250 can be found in SI 2, and the full list of health conditions that owners were asked about is listed
251 in SI 3.

252 Additionally, we created covariates for lifestyle factors that preliminary evidence suggests might
253 have ameliorating or protective effects for physical activity and/or cognition. If dogs received
254 glucosamine and/or other joint supplements daily, they were considered 'affected' in the joint
255 supplement category (McCarthy et al., 2007). If dogs received omega 3, vitamins, probiotics,
256 antioxidants, taurine, carnitine, and/or coenzyme Q10 daily, they were considered 'affected' in
257 the neuroprotective supplement category (Heath, Barabas, & Craze, 2007; Mad'ari, Farbakova,
258 & Žilka, 2017; Milgram et al., 2004; Pan, Kennedy, Jönsson, & Milgram, 2018). Finally, we also
259 created a variable accounting for whether a dog had a history of training (Bray et al., 2022),
260 given intriguing preliminary evidence that this sort of enrichment is linked to delay in cognitive
261 decline (Bray et al., 2022; Milgram, Siwak-Tapp, Araujo, & Head, 2006; Szabó et al., 2018).
262 Training history was determined according to what the owner reported as the dog's primary or

263 secondary activity (e.g., service dogs, agility dogs, and dogs trained for field trials vs.
264 pets/companion; see SI 1, Appendix B for full details).

265 A summary of the demographic variables, incidence of health conditions, physical activity
266 levels, training history, and dietary supplement use within our sample is reported in Table 1,
267 broken down by participants who met the diagnostic score for CCD ($n = 287$) and those who did
268 not ($n = 11,287$).

269 *Statistical Methods*

270 All statistical analyses were carried out in R v.4.0.3 (R Development Core Team, 2016).

271 We fit three tiers of models for each of our outcome variables. In our first tier of analysis, we
272 built a base model that included only key predictor variables (physical activity and age) and a
273 minimal set of covariates. The effect of age was modelled using a second-order polynomial term
274 because preliminary exploratory analyses revealed a non-linear relationship between age and the
275 cognitive outcomes (see Fig S2 in SI 1). The other covariates included in our base models
276 included dog sex (female, intact; female, spayed; male, intact; male, castrated), dog size (lbs),
277 and owner age (18-24, 25-34, 35-44, 45-54, 55-64, 65-74, 75+). For models using the categorical
278 measure of dementia status as the outcome, the owner age variable was collapsed to two levels
279 (18-54, 55+) and dog sex was collapsed to two levels (male, female) to avoid small cell sizes.

280 In our second tier of analysis, we built a model that included all the variables from our base
281 model as well as hypothesis-driven confounders and risk or protective factors. The additional
282 variables for these models included whether a given dog exhibited sensory impairments (e.g.,
283 visual and/or auditory), motor impairments (e.g., orthopedic challenges), exercise intolerance
284 (e.g., cardiac and/or respiratory challenges), neurological conditions other than dementia (i.e.,
285 dogs with a reported diagnosis of dementia or senility—and no other neurological conditions—
286 were considered ‘unaffected’ in this category), periodontal disease, liver disease, as well as
287 whether they were currently receiving joint and/or neuroprotective supplements, and whether
288 they had a history of training. For models using the categorical measure of dementia as the
289 outcome, liver disease was removed as a covariate due to small cell sizes when stratifying on this
290 covariate.

291 Finally, in the third tier of analysis, we added the remaining, non-hypothesis driven covariates,
292 for health condition categories including endocrine disease, kidney disease, and cancer.

293 We applied our three-tier modeling approach to the three different outcome variables, using
294 linear regressions for symptom severity and recent symptom change, and a logistic regression for
295 CCD status. Continuous outcomes (severity and change) were subjected to an inverse rank
296 normal transformation to better meet the assumptions of linear modeling, and then standardized
297 to have a mean of 0 and standard deviation of 1, to facilitate interpretation. We fit a total of nine

298 statistical models (three for each dependent measure). To identify the best model for each
299 outcome, we compared the Akaike information criterion scores across models.

300 We also performed some sensitivity analyses. To determine if any observed associations would
301 still hold in a cognitively healthy population, we re-ran our original analyses but removed all
302 dogs above the CCD threshold ($n = 11,287$, Tables S2 and S3 in SI 1). Given that over half of
303 our sample was comprised of mixed breed dogs ($n = 6,027$ (52%)), a highly heterogenous group,
304 we did not control for breed in our main analyses. Thus, in a follow-up set of sensitivity
305 analyses, we first repeated all models but eliminated all purebred dogs from the sample ($n =$
306 $6,027$, Table S4-S6 in SI 1). Additionally, we then repeated all models but only included
307 purebred dogs—using breeds with at least 10 dogs in the dataset ($n = 5,167$ dogs from 92 breeds;
308 Table S7 in SI 1), and, for the CCD model, at least one member of the breed above the CCD
309 threshold ($n = 3,945$ dogs from 53 breeds; Table S8 in SI 1)—and added breed as a covariate
310 (Table S4-S6 in SI 1). Finally, based on the possibility that CSLB scores below 20 may be
311 implausible, we re-ran the models from our main analyses, excluding the subset of dogs with a
312 score of 19 and lower ($n = 11,368$; see SI 1 for details).

313 **Results**

314 For all outcomes, results from each of the three tiers of analysis displayed the same pattern but
315 the fully adjusted model fit the best in all cases, as assessed by the lowest Akaike information
316 criterion (Tables 2-4). Therefore, the results reported below are derived from the models
317 including all candidate covariates.

318 As expected, all three cognitive outcomes were negatively impacted by age, with effect of age
319 increasing at older ages (Fig 1). In all models, there was also a significant relationship between
320 physical activity and cognitive outcomes (Fig 2).

321 In the severity model, we found a significant negative association between physical activity and
322 severity of cognitive symptoms, whereby high levels of activity were linked to lower (i.e., better)
323 scores on the CSLB (Fig 2; Table 2). We also identified associations between two other
324 hypothesized protective factors (training history and neuroprotective supplements), in which
325 both a history of training and daily consumption of neuroprotective supplements were associated
326 with better cognitive outcomes. For the final hypothesized protective factor (joint supplements),
327 the beta coefficient was negative but not statistically significant. We also observed that poor
328 health in certain domains was a risk factor for symptom severity. For our medical covariates,
329 beta coefficients were positive and statistically significant for six categories of conditions
330 (sensory impairment, endocrine, orthopedic, neurological, cancer, and periodontal) and positive
331 but not statistically significant for the final three categories of conditions (kidney, liver, and
332 exercise intolerance; Fig 2; Table 2). Results were similar in the analysis that excluded dogs
333 above the CCD threshold (Table S2 in SI 1), suggesting that these relationships hold below the
334 clinical cutoff for a diagnosis of dementia. Results were also similar in secondary analyses

335 including only mixed breed dogs and dogs from the most common breeds (see Table S4 in SI 1).
336 Across all three models, the negative association between symptom severity and our main
337 exposure of interest (physical activity) remained significant, as did the negative associations with
338 training history and neuroprotective supplements and the positive associations with two
339 categories of medical conditions (sensory impairment and orthopedic). Finally, removing dogs
340 with reported CSLB scores less than 20 did not change our findings (Table S9 in SI 1).

341 In the symptom change model, we again found a significant negative relationship between
342 physical activity and reported change in cognitive symptoms as recalled by owners over the prior
343 6-month period, whereby higher levels of activity were linked to less owner-reported cognitive
344 decline across the preceding six months (Fig 2; Table 3). We also identified a negative
345 association with one of our other hypothesized protective factors (training history), in which
346 dogs with an extensive training history exhibited less cognitive decline in the preceding six
347 months. For the two other hypothesized protective factors (neuroprotective and joint
348 supplements), the beta coefficients were near zero and not statistically significant. We also found
349 evidence that poor health in certain domains was a risk factor for symptoms worsening over a 6-
350 month period. For our medical covariates, beta coefficients were positive and statistically
351 significant for five categories of medical conditions (sensory impairment, orthopedic,
352 neurological, cancer, and periodontal), and not statistically significant for four categories of
353 conditions (kidney, endocrine, exercise intolerance, and liver). Results were similar when
354 performing our original analyses but removing all dogs above the CCD threshold (Table S3 in SI
355 1), suggesting that these relationships hold below the clinical cutoff for a diagnosis of dementia.
356 Results were also similar in secondary analyses including only mixed breed dogs and dogs from
357 the most common breeds (see Table S5 in SI 1): across all three models, the negative association
358 between symptom change and physical activity remained significant, as did the positive
359 associations with three categories of medical conditions (sensory impairment, orthopedic, and
360 periodontal). Finally, removing dogs with reported CSLB scores less than 20 did not change our
361 findings (Table S10 in SI 1).

362 In the CCD status model, we found that higher levels of physical activity were associated with
363 lower odds of being over the diagnostic threshold for CCD (Fig 2; Table 4). The adjusted odds
364 ratio was 0.53 (95% CI: 0.45 to 0.63) and statistically significant for physical activity, but there
365 were no significant associations with the other hypothesized protective factors (training history,
366 neuroprotective supplements, and joint supplements). We also found evidence that poor health in
367 certain domains was associated with CCD, whereby individuals with CCD were also likely to
368 have other owner-reported health issues. For our medical covariates, we observed OR > 1.0 and
369 statistically significant for three categories of medical conditions (sensory impairment, kidney,
370 and endocrine) with none of the other six categories of conditions (orthopedic, neurological,
371 cancer, liver, exercise intolerance, and periodontal) reaching statistical significance. Results were
372 similar in secondary analyses including only mixed breed dogs and dogs from the most common
373 breeds (see Table S6 in SI 1 for full report): across all three models, the negative association

374 between being over the diagnostic threshold for CCD and physical activity remained significant,
375 as did the positive association with sensory impairment. Removing dogs with reported CSLB
376 scores less than 20 did not change our findings (Table S11 in SI 1).

377 **Discussion**

378 We investigated the relationship between physical activity and cognitive health in a sample of
379 over 10,000 companion dogs. By exploring this relationship in a large population living in an
380 environment shared with humans, we aimed to gain insight regarding factors associated with
381 healthy cognitive aging and to identify potential modifiable risk factors that may prevent
382 cognitive dysfunction and dementia (Deckers et al., 2015).

383 Across all models, we observed robust associations between physical activity and cognitive
384 health. Physical activity was significantly negatively associated with three metrics of cognitive
385 dysfunction: current symptom severity, extent of worsening over a 6-month interval, and whether
386 a dog had reached a clinical threshold for CCD. These results held when controlling for basic
387 demographic factors (weight, sex, and age of the dog, as well as age of the owner), hypothesis-
388 driven confounders and risk factors related to lifestyle (joint-enhancing supplements,
389 neuroprotective supplements, and training history) and health (sensory impairments, exercise
390 intolerance, orthopedic conditions, neurological conditions other than dementia, periodontal
391 disease, liver conditions), and other general health conditions (endocrine conditions, kidney
392 failure, and cancer).

393 Furthermore, sensitivity analyses indicated that the association between physical activity and
394 cognitive function held even when dogs who met the CCD threshold were removed from the
395 sample. Thus, even in non-clinical cohorts physical activity may be associated with measurable
396 cognitive benefits in older dogs, and/or declines in cognitive function may be associated with
397 declines in owner-reported physical activity.

398 In addition to the association between physical activity and cognition, our analyses revealed
399 relationships between cognitive health and several other health and lifestyle variables. For
400 example, one of the strongest observed associations was between CSLB scores indicating worse
401 cognitive health and sensory impairment, in line with the findings of a similar questionnaire-
402 based study of 1,300 companion dogs (Szabó et al., 2018). While it may be that sensory
403 impairment is a confounder (i.e., owners may mistakenly attribute a change in behavior to
404 cognitive dysfunction when really it is the result of failing vision and/or audition), there is also
405 evidence in the human literature that such impairments are potential risk factors for dementia
406 (Hwang et al., 2020; Luo et al., 2018; Maharani, Dawes, Nazroo, Tampubolon, & Pendleton,
407 2020).

408 We also found a positive association between taking daily neuroprotective supplements (e.g.,
409 fish oil) and cognitive symptom severity. This finding is consistent with some clinical studies in

410 dogs (Pan, Kennedy, et al., 2018; Pan, Landsberg, et al., 2018) and humans (Fotuhi, Mohassel, &
411 Yaffe, 2009; Nolan, Mulcahy, Power, Moran, & Howard, 2018), although other studies in the
412 human literature have found no effect (Danthiir et al., 2018; van de Rest et al., 2008). A potential
413 limitation of this finding is that owners who are motivated to provide potentially neuroprotective
414 supplements may be biased in their evaluation of their pet's dementia symptoms. However, these
415 supplements (e.g., fish oil) are also recommended by veterinarians for numerous other perceived
416 benefits (e.g., heart health, coat shine, allergy relief, and pain management), so we do not know
417 what expectations owners have regarding their potential effects on cognition.

418 Finally, we identified an association between two of our cognitive outcomes—symptom severity
419 and cognitive change over the last 6 months—and training, whereby dogs who had a history of
420 training were less likely to exhibit signs of cognitive decline. This finding is consistent with the
421 idea that both physical exercise *and* mental exercise can have a beneficial impact on the brain
422 (Marx, 2005; Raichlen & Alexander, 2017; Raichlen et al., 2020). Furthermore, this measure
423 accounted for previous activity (i.e., history of training versus current training regimen) and so,
424 given the timeline, cannot be readily explained by reverse causality. While the literature in
425 humans (Kramer, Bherer, Colcombe, Dong, & Greenough, 2004) and laboratory animals (Birch
426 & Kelly, 2019), including beagles (Milgram et al., 2005; Milgram et al., 2006), supports the idea
427 that enrichment can lead to better cognitive functioning in old age, only one other study has
428 demonstrated this relationship in companion dogs (Szabó et al., 2018). Nonetheless, this
429 relationship has interesting potential parallels to associations between cognitive training and
430 educational attainment in the context of dementia and Alzheimer's disease risk in humans (Xu et
431 al., 2016).

432 Our study has several notable limitations. First, despite the large sample size and wide range of
433 covariates able to be accounted for, we cannot rule out unmeasured confounding. Second, all
434 data were owner-reported and thus subject to potential pitfalls associated with self-report.
435 Despite this limitation, the survey used in our analyses is known to have excellent diagnostic
436 accuracy and test-retest reliability (Salvin et al., 2011). Third, we categorized dogs as either
437 'affected' or 'not affected' on each health covariate based on owner-reported diagnoses when
438 filling out the HLES survey. However, HLES does not capture information about a condition's
439 severity. While all dogs were included in each category if they had a relevant diagnosis, in reality
440 that condition might not have had a measurable impact. For example, we included all dogs with
441 heart disease in our 'exercise intolerance' category; in moderate to severe cases, this condition
442 will inevitably impact a dog's ability to exercise (and likely lead to a veterinary recommendation
443 of exercise restriction). However, in mild cases, this condition may have minimal impact on a
444 dog's ability to exercise.

445 The most important limitation of our study is that we cannot determine causality given the
446 observational, cross-sectional nature of the design. Given existing knowledge about the
447 relationships between physical activity and cognitive function, it is plausible that higher rates of

448 physical activity play a causal role in reducing risk of later-life cognitive impairment in dogs.
449 However, the observed association between physical activity and cognitive outcomes could also
450 indicate that as dogs decline cognitively, it causes them to become less active. Finally, there is a
451 third possibility of unmeasured confounding, whereby neither physical activity nor cognitive
452 decline have causal effects on one another. The fact that our sensitivity analyses revealed an
453 association between CSLB scores and physical activity even in clinically ‘normal’ dogs suggests
454 that the first explanation is more likely; however, future research incorporating additional study
455 designs, including interventions and the analysis of longitudinal data, will be critical for causal
456 inferences in this domain.

457 In conclusion, our findings indicate that signs of cognitive decline in dogs, and the likelihood of
458 developing CCD, increase with age. Furthermore, the associations presented here are consistent
459 with the hypothesis that physical activity may partially mitigate these risks, although they are
460 also consistent with the hypothesis that cognitively impaired dogs exercise less, or that
461 unidentified confounding variables influence changes in both physical activity and cognitive
462 function. We also identified several categories of medical conditions that were associated with
463 cognitive dysfunction: sensory deficits showed the strongest associations, and there was also
464 some evidence to suggest associations with endocrine disorders, neurological conditions,
465 orthopedic impairments, periodontal disease, cancer, and kidney disorders. Across a subset of
466 our outcome measures, training history and neuroprotective supplements were associated with
467 reduced cognitive impairment. However, in support of our key hypothesis, physical activity was
468 the only lifestyle factor that was robustly associated with reduced risk of cognitive dysfunction
469 across all three of our outcome measures. These findings establish the value of companion dogs
470 as a model for relationships between physical activity and cognitive aging, and lay a foundation
471 for future longitudinal studies, including randomized controlled trials, with this valuable
472 population.

473 **Author Contributions**

474 All authors contributed to writing – review & editing. E.B.: conceptualization, methodology,
475 formal analysis, data curation, writing – original draft, and supervision. D.R.: conceptualization
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491 **Conflicts of interest/Competing interests**

492 The authors declare no competing interests.

493 **Data availability statement**

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

495 **Code availability statement**

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

497 **Supplementary Information captions**

498 **Supplementary Information 1.** Supplementary tables and appendices.

499 **Supplementary Information 2.** Summary of HLES items that contributed to each of the
500 following covariates in our full model, along with the total number of unique affected dogs from
501 our sample: sensory impairment, orthopedic, exercise intolerance, neurological, periodontal,
502 liver, endocrine, kidney, and cancer.

503 **Supplementary Information 3.** A list of all 288 specific health conditions from HLES; Dog
504 Aging Project owners were asked to report, for each condition, whether their dog had been
505 diagnosed. Each of the broad general categories also had an ‘other’ option where owners could
506 write in an answer.

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Tables

787 **Table 1. Summary statistics of our sample.**

Variable	Canine Cognitive Dysfunction Case (score ≥ 50)			Canine Cognitive Dysfunction Control (score < 50)		
	N	Mean	SD	N	Mean	SD
age	287	14.15	2.32	11287	10.10	2.61
sex	287			11287		
... female intact	3	1%		85	1%	
... female spayed	133	46%		5668	50%	
... male intact	7	2%		304	3%	
... male neutered	144	50%		5230	46%	
dog weight (lbs)	287	33.56	24.73	11287	48.9	28.57
physical activity	287	-0.79	0.83	11287	0.02	1
training history	287	-0.21	0.78	11287	0.01	1
neurological	287	0.18	0.38	11287	0.07	0.25
periodontal	287	0.37	0.48	11287	0.24	0.43
exercise intolerance	287	0.13	0.34	11287	0.07	0.25
orthopedic	287	0.41	0.49	11287	0.21	0.41
sensory impairment	287	0.63	0.48	11287	0.13	0.34
neuroprotective supplement	287	0.37	0.48	11287	0.37	0.48
joint supplement	287	0.45	0.50	11287	0.40	0.49
endocrine	287	0.13	0.34	11287	0.05	0.22
kidney	287	0.09	0.28	11287	0.01	0.12
cancer	287	0.17	0.38	11287	0.09	0.29
liver	287	0.02	0.14	11287	0.01	0.08

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Table 2. Model comparisons between the three tiers of models predicting symptom severity, reporting the beta coefficients and the 95% confidence interval based on robust standard errors in parentheses. Age effects are shown in Fig 1.

Parameter	Symptom Severity					
	Minimally Adjusted		Moderately Adjusted		Fully Adjusted	
	Beta (95% CI) ¹	p-value	Beta (95% CI) ¹	p-value	Beta (95% CI) ¹	p-value
physical activity	-0.116 (-0.134 to -0.098)	<0.001	-0.096 (-0.114 to -0.079)	<0.001	-0.095 (-0.113 to -0.077)	<0.001
dog weight (lbs)	0.000 (-0.001 to 0.000)	0.123	0.000 (-0.001 to 0.001)	0.720	0.000 (-0.001 to 0.001)	0.941
sex						
female intact	—		—		—	
female spayed	0.238 (0.056 to 0.419)	0.010	0.198 (0.020 to 0.376)	0.029	0.195 (0.018 to 0.373)	0.031
male intact	0.208 (0.004 to 0.411)	0.046	0.171 (-0.029 to 0.372)	0.093	0.170 (-0.030 to 0.370)	0.096
male neutered	0.290 (0.108 to 0.471)	0.002	0.244 (0.066 to 0.422)	0.007	0.243 (0.066 to 0.420)	0.007
owner age						
18-24	—		—		—	
25-34	-0.348 (-0.567 to -0.129)	0.002	-0.336 (-0.545 to -0.127)	0.002	-0.334 (-0.542 to -0.125)	0.002
35-44	-0.578 (-0.794 to -0.362)	<0.001	-0.556 (-0.762 to -0.350)	<0.001	-0.554 (-0.759 to -0.348)	<0.001
45-54	-0.725 (-0.939 to -0.510)	<0.001	-0.710 (-0.915 to -0.505)	<0.001	-0.707 (-0.911 to -0.503)	<0.001
55-64	-0.876 (-1.09 to -0.664)	<0.001	-0.861 (-1.06 to -0.658)	<0.001	-0.856 (-1.06 to -0.654)	<0.001
65-74	-0.99 (-1.20 to -0.774)	<0.001	-0.97 (-1.18 to -0.772)	<0.001	-0.97 (-1.17 to -0.767)	<0.001
75 and older	-1.07 (-1.29 to -0.852)	<0.001	-1.05 (-1.26 to -0.844)	<0.001	-1.05 (-1.25 to -0.839)	<0.001
sensory impairment			0.408 (0.351 to 0.464)	<0.001	0.405 (0.349 to 0.461)	<0.001
orthopedic			0.087 (0.044 to 0.130)	<0.001	0.084 (0.041 to 0.127)	<0.001
exercise intolerance			0.045 (-0.020 to 0.111)	0.177	0.043 (-0.023 to 0.108)	0.203
neurological			0.076 (0.008 to 0.143)	0.028	0.073 (0.005 to 0.140)	0.035
periodontal			0.063 (0.024 to 0.101)	0.002	0.060 (0.021 to 0.099)	0.003

liver		0.041 (-0.182 to 0.264)	0.720	0.030 (-0.193 to 0.253)	0.790
joint supplement		-0.032 (-0.074 to 0.009)	0.125	-0.031 (-0.073 to 0.010)	0.139
neuroprotective supplement		-0.078 (-0.119 to -0.038)	<0.001	-0.082 (-0.123 to -0.042)	<0.001
training history		-0.031 (-0.047 to -0.014)	<0.001	-0.031 (-0.047 to -0.014)	<0.001
endocrine				0.085 (0.009 to 0.161)	0.029
kidney				0.112 (-0.025 to 0.248)	0.109
cancer				0.057 (0.001 to 0.113)	0.047
AIC	30,326	30,010		30,003	

¹ CI = Confidence Interval

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799 **Table 3. Model comparisons between the three tiers of models predicting cognitive decline in previous six months, reporting**
 800 **the beta coefficients and the 95% confidence interval based on robust standard errors in parentheses. Age effects are shown in**
 801 **Fig 1.**

Parameter	Symptom Change; Previous 6 Months					
	Minimally Adjusted		Moderately Adjusted		Fully Adjusted	
	Beta (95% CI) ¹	p-value	Beta (95% CI) ¹	p-value	Beta (95% CI) ¹	p-value
physical activity	-0.086 (-0.104 to -0.068)	<0.001	-0.070 (-0.089 to -0.052)	<0.001	-0.069 (-0.087 to -0.051)	<0.001
dog weight (lbs)	0.001 (0.000 to 0.002)	<0.001	0.001 (0.000 to 0.002)	0.001	0.001 (0.000 to 0.002)	0.004
sex						
female intact	—		—		—	
female spayed	0.114 (-0.089 to 0.318)	0.271	0.074 (-0.126 to 0.274)	0.469	0.072 (-0.127 to 0.272)	0.479
male intact	0.044 (-0.184 to 0.272)	0.704	0.008 (-0.217 to 0.232)	0.947	0.008 (-0.215 to 0.232)	0.942
male neutered	0.126 (-0.078 to 0.330)	0.225	0.082 (-0.118 to 0.283)	0.421	0.082 (-0.118 to 0.282)	0.421
owner age						
18-24	—		—		—	
25-34	-0.034 (-0.358 to 0.290)	0.835	-0.037 (-0.357 to 0.283)	0.820	-0.033 (-0.353 to 0.286)	0.839
35-44	-0.067 (-0.389 to 0.255)	0.684	-0.063 (-0.382 to 0.255)	0.696	-0.060 (-0.378 to 0.258)	0.713
45-54	-0.038 (-0.359 to 0.283)	0.815	-0.038 (-0.355 to 0.279)	0.815	-0.033 (-0.349 to 0.284)	0.841
55-64	-0.019 (-0.339 to 0.301)	0.907	-0.018 (-0.334 to 0.298)	0.911	-0.010 (-0.326 to 0.305)	0.949
65-74	-0.081 (-0.401 to 0.239)	0.618	-0.086 (-0.402 to 0.230)	0.595	-0.077 (-0.393 to 0.238)	0.632
75 and older	-0.112 (-0.437 to 0.212)	0.498	-0.103 (-0.424 to 0.218)	0.528	-0.095 (-0.415 to 0.226)	0.562
sensory impairment			0.233 (0.169 to 0.297)	<0.001	0.230 (0.166 to 0.294)	<0.001
orthopedic			0.156 (0.108 to 0.204)	<0.001	0.153 (0.106 to 0.201)	<0.001
exercise intolerance			0.056 (-0.019 to 0.132)	0.146	0.054 (-0.021 to 0.130)	0.161
neurological			0.089 (0.013 to 0.165)	0.021	0.087 (0.011 to 0.163)	0.025
periodontal			0.066 (0.023 to 0.108)	0.003	0.063 (0.020 to 0.106)	0.004

liver			-0.012 (-0.289 to 0.265)	0.930	-0.023 (-0.298 to 0.253)	0.872
joint supplement			0.015 (-0.029 to 0.059)	0.504	0.016 (-0.028 to 0.060)	0.477
neuroprotective supplement			0.001 (-0.042 to 0.044)	0.972	-0.003 (-0.046 to 0.040)	0.888
training history			-0.021 (-0.039 to -0.004)	0.016	-0.021 (-0.039 to -0.004)	0.016
endocrine					0.033 (-0.056 to 0.122)	0.464
kidney					0.089 (-0.091 to 0.268)	0.333
cancer					0.106 (0.043 to 0.170)	0.001
AIC	31,513		31,365		31,356	

¹ CI = Confidence Interval

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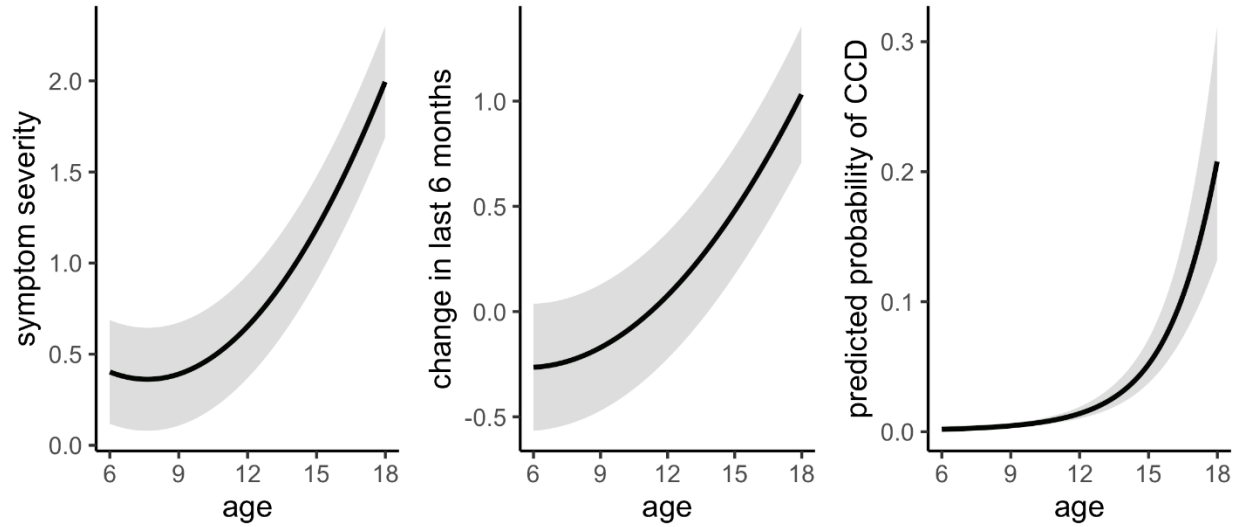
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813 **Table 4. Model comparisons between the three tiers of models predicting CCD status, reporting the odds ratio and the 95%**
 814 **confidence interval in parentheses. Age effects are shown in Fig 1.**

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Parameter	Canine Cognitive Dysfunction (Clinical Cutoff)					
	Minimally Adjusted		Moderately Adjusted		Fully Adjusted	
	OR (95% CI) ¹	p-value	OR (95% CI) ¹	p-value	OR (95% CI) ¹	p-value
physical activity	0.51 (0.43 to 0.60)	<0.001	0.53 (0.45 to 0.62)	<0.001	0.53 (0.45 to 0.63)	<0.001
dog weight (lbs)	0.99 (0.99 to 1.00)	0.003	0.99 (0.99 to 1.00)	0.008	0.99 (0.99 to 1.00)	0.006
dog sex						
male	—		—		—	
female	0.85 (0.66 to 1.09)	0.198	0.85 (0.65 to 1.09)	0.202	0.83 (0.64 to 1.07)	0.152
owner age						
18-54	—		—		—	
55 and older	0.78 (0.61 to 1.01)	0.062	0.75 (0.58 to 0.97)	0.029	0.78 (0.60 to 1.02)	0.070
sensory impairment			3.23 (2.45 to 4.28)	<0.001	3.20 (2.43 to 4.24)	<0.001
orthopedic			1.22 (0.92 to 1.61)	0.160	1.22 (0.92 to 1.62)	0.162
exercise intolerance			0.98 (0.66 to 1.43)	0.928	0.97 (0.65 to 1.42)	0.887
neurological			1.31 (0.91 to 1.86)	0.137	1.29 (0.89 to 1.84)	0.162
periodontal			0.80 (0.60 to 1.05)	0.105	0.78 (0.59 to 1.02)	0.076
joint supplement			0.96 (0.70 to 1.32)	0.822	1.00 (0.72 to 1.37)	0.979
neuroprotective supplement			1.02 (0.74 to 1.40)	0.898	0.97 (0.71 to 1.34)	0.872
training history			0.89 (0.75 to 1.04)	0.174	0.88 (0.74 to 1.03)	0.133
endocrine					1.46 (0.97 to 2.16)	0.062
kidney					1.85 (1.09 to 3.04)	0.017
cancer					1.15 (0.80 to 1.61)	0.437
AIC	1,977		1,911		1,908	

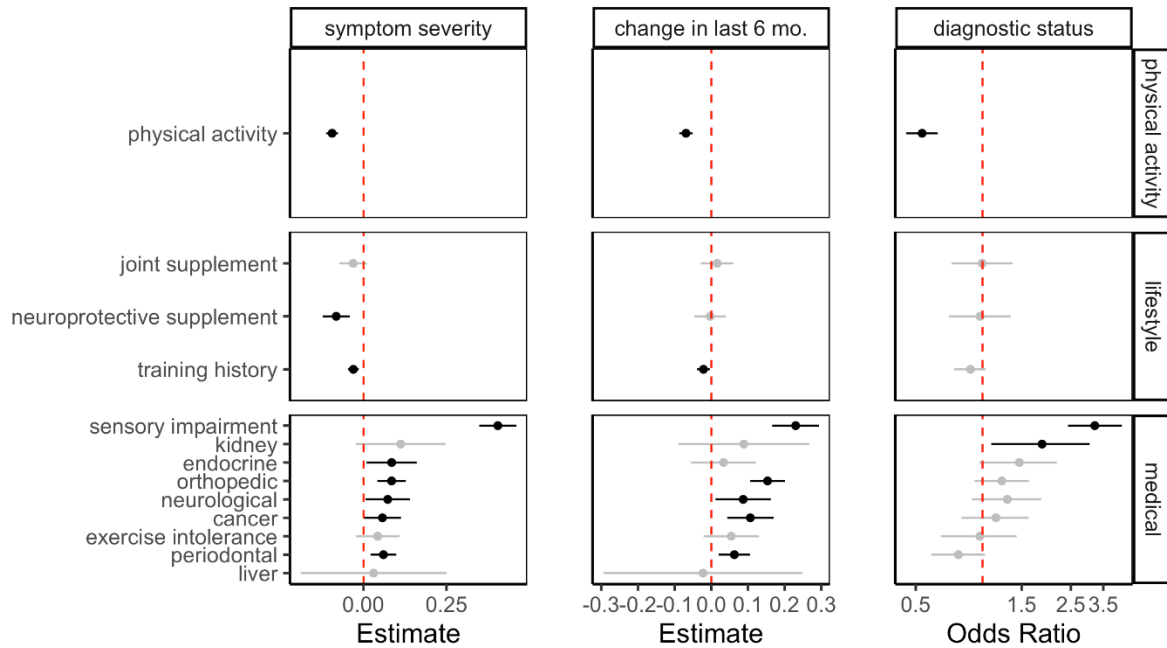
¹OR = Odds Ratio, CI = Confidence Interval



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817 **Fig 1.** The estimated association between age and symptom severity (PCA-derived score),
818 symptom change in last 6 months (PCA-derived score), and probability of a CCD diagnosis,
819 respectively (with 95% confidence intervals indicated in gray). Results are from our fully
820 adjusted models and include both linear and quadratic terms for age.

Physical activity and cognitive dysfunction associations 29



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822 **Fig 2.** The beta coefficients (for the severity and change models) and odds ratios (for the CCD
 823 diagnosis model) of physical activity, as well as the other lifestyle (joint supplement,
 824 neuroprotective supplement, training history) and medical (sensory impairment, kidney,
 825 endocrine, orthopedic, neurological, cancer, liver, exercise intolerance, periodontal) covariates
 826 from the fully adjusted models. The red dotted line indicates the null expectation (i.e., 0 for the
 827 betas and 1 for the odds ratios). Significant findings are presented in black, while nonsignificant
 828 findings are presented in gray. The bars represent the 95% confidence intervals.

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