1 **Long-term improvement in hippocampal-dependent learning ability in healthy, aged**

2 **individuals following high intensity interval training.**

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29 Running title: Exercise improves cognition in the elderly.

Abstract

Physical exercise may reduce dementia risk in ageing, but varying reports on its effectiveness make it challenging to ascribe what level of exercise will have significant longer-term effects on important functions such as hippocampal-based learning and memory. This study compared the effect of three different 6-month exercise regimens on hippocampal-dependent cognition in healthy, elderly individuals. Participants, aged 65-85 with no cognitive deficits, were randomly assigned to one of three exercise interventions (low (LIT), medium (MIT), and High intensity training (HIIT), respectively). Each participant attended 72 supervised exercise sessions over a 6-month period. A total of 151 participants completed all sessions. Cognitive testing for hippocampal performance occurred monthly, as did blood collection, and continued for up to 5 years following initiation of the study. Multimodal 7 Tesla MRI scans were taken at commencement, 6 and 12 months. After 6 months, only the HIIT group displayed significant improvement in hippocampal function, as measured by paired associative learning (PAL). MRI from the HIIT group showed abrogation of the age-dependent volumetric decrease within several cortical regions including the hippocampus and improved functional connectivity between multiple neural networks not seen in the other groups. HIIT-mediated changes in the circulating levels of brain-derived neurotrophic factor (BDNF) and cortisol correlated to improved hippocampal-dependent cognitive ability. These findings demonstrate that HIIT significantly improves and prolongs the hippocampal-dependent cognitive health of aged individuals. Importantly, improvement was retained for at least 5 years following initiation of HIIT, suggesting that the changes seen in hippocampal volume and connectivity underpin this long-term maintenance. Sustained improvement in hippocampal function to this extent confirms that such exercise-based interventions can provide significant protection against hippocampal cognitive decline in the aged population. The changes in specific blood factor levels also may provide useful biomarkers for choosing the optimal exercise regimen to promote cognitive improvement.

Key words: exercise, hippocampal-dependent, memory, biomarkers

INTRODUCTION

Aging dementia, including Alzheimer's disease (AD), is a significant health concern, with more than 130 million people worldwide predicted to suffer from the condition by 2050 [1]. Delaying the onset of dementia by five years would result in a decreased prevalence of 41% by 2050 [2]. Therefore, it is critical to identify approaches that delay, slow or even reverse age-associated cognitive decline. Modifiable lifestyle factors such as physical activity [3] have been proposed to be effective at altering the trajectory of ageing dementia.

A key feature of ageing dementia is the decline in specific domains of cognitive function, especially those related to spatial learning and memory. The hippocampus is a critical region of the brain that is responsible for the consolidation of spatial information into memories and is particularly susceptible to age [4], with reports of age-dependent decreased hippocampal volume [5] and connectivity [6]. Physical exercise is a promising non-invasive approach to ameliorate age-associated hippocampal cognitive deficits. Rodent studies have clearly demonstrated that physical exercise improves hippocampal-dependent spatial learning [7, 8], through several key exercise-mediated mechanisms including increased neural stem cell (NSC) activation [9, 10], hippocampal neurogenesis [11], and improved hippocampal connectivity [12]. Some of these processes, including NSC activation and neurogenesis, have also been linked to specific circulating biomarkers [8, 13], and the duration of exercise has been shown to be critical for these effects [8, 12].

Despite the work conducted in adult and aged animal models demonstrating exercise-mediated improvement in hippocampal-dependent cognition, there is a lack of studies examining the extent to which physical exercise affects hippocampal function in aged

82   humans. Indeed, there is only one study that expressly examined hippocampal-dependent

83   spatial learning in the healthy elderly following MIT exercise [14]. While the authors found a

84   marked increase in hippocampal volume with 12 months of MIT exercise, no improvement in

85   hippocampal-dependent spatial memory above that of the control group was observed [14].

86   The same study also measured the change in concentration of BDNF but found no correlation

87   to hippocampal cognitive ability. However, most studies that have examined exercise-

88   mediated changes in circulating analytes and cognitive function have been limited to one

89   factor [15] or, at most, a few analytes, as recently reviewed [16]. Such an approach limits the

90   ability to accurately identify circulating biomarkers that may be involved in exercise-mediated

91   changes in cognitive ability. Further, in a recent umbrella review of meta-analyses examining

92   the link between physical exercise and various domains of cognition in healthy populations,

93   only small effect sizes were found, cautioning against claims of exercise-mediated effects on

94   cognition [17]. A lack of accounting for key moderators such as active controls and baseline

95   differences between groups were identified as major limitations when reporting the effect of

96   exercise on cognition [17]. These, coupled with a heterogenous population, a propensity to

97   rely on self-reported measures including exercise intensity [14, 18], and the duration of

98   exercise intervention ranging from weeks [19] to years [20], make the large variation in

99   reported outcomes unsurprising. There is evidence that the age-associated volumetric

100   atrophy in the hippocampus [21] and decrease in key functional networks [22] such as the

101   default mode network (DMN), can be ameliorated following exercise [23, 24]. However, as

102   with cognitive studies, the amount of exercise required to achieve optimal outcomes in

103   structural and functional connectivity remains unclear.

104

105   Therefore, to address these shortcomings, we conducted a randomized control study to

106   investigate the effect of exercise in the healthy elderly. Our primary objective was to measure

107   changes in cognitive function following exercise. This was examined using a longitudinal

108   approach where we took advantage of electronic cognitive testing as described below. The

109   secondary outcomes we also examined included: 1) if exercise could improve hippocampal-

110   dependent spatial learning in healthy, aged individuals, 2) if there was a dose-dependent

111   component, 3) if hippocampal cognitive improvements could be predicted by longitudinal

112   changes in biochemical and/or physiological parameters and 4) if any exercise-mediated long-

113   term improvement in hippocampal learning was observed. To do this, we performed a

114   multimodal, randomized 6-month exercise intervention with increasing doses of exercise

115   comprising an active control group (low intensity training- LIT), a MIT group and a high

116   intensity interval training (HIIT) group. Participants exercised three times a week under

117   constant supervision by qualified exercise physiologists to ensure that personalized target

118   heart rates (HR) were reached and maintained during the exercise sessions. Numerous

119   parameters were recorded monthly to examine each participant before, during and following

120   the exercise intervention. These included cognitive function, as assessed electronically

121   through multiple CANTAB® tests, including the hippocampal-dependent paired associated

122   learning (PAL) test. Other cognitive domains including working memory, visual working

123   memory, and emotional recognition were also examined. Monthly blood samples were also

124   collected immediately before and after exercise to examine changes in several circulating

125   biochemical markers. Training diaries recorded HR, and treadmill parameters for MIT and HIIT

126   participants multiple times each session. Multimodal, high resolution brain scans using ultra-

127   high field magnetic resonance imaging (MRI) were also obtained for a subset of participants

128   at the initiation and at completion of the intervention, as well as 6 months later.

129

130   We found that:

- Hippocampal-dependent cognition improved significantly in the HIIT group after 6 months of exercise and that the improvement was maintained for up to 5 years from the initiation of the exercise intervention.

- In the LIT and MIT groups, hippocampal-dependent cognition was maintained during the exercise intervention and showed no age-related decline compared with baseline performance at the later time points.

- HIIT abrogated the age-associated decreases in brain volume observed in the LIT and MIT groups.

- Significant increases in functional connectivity occurred between multiple key resting-state network circuits following 6 months of HIIT.

- An increase in initial exercise-mediated cortisol and BDNF levels predicted cognitive improvement in HIIT participants.

143

METHODS

Study design and participants. We conducted a multidomain, randomized controlled study over a period of 6 years at a single site located in a purpose-built clinic at The University of Queensland (UQ). The participants who were recruited ranged from 65-85 years of age. Detailed recruitment, screening, inclusion, exclusion, randomization, and withdrawal of participants are described in S1 Appendix. Inclusion criteria included the ability to provide written, informed consent to participant in the study, be between 65-85 years of age, have to ability to communicate in English, no history of stroke, heart or brain surgery or brain trauma, stratified as "not high risk of experiencing a cardiac event during exercise' according to pre-exercise screening, no presently diagnosed mental illness or cognitive impairment, as per baseline Addenbrooke's cognitive examination-revised (ACE-R) scores, not be medicated for dementia or psychiatric illnesses, have a healthy body mass index; and be able and willing to commit to the duration of the exercise program.

Exclusion criteria included 1) being younger than 65 or older than 85 years of age; 2) illness or disability that precluded exercise or hindered completion of the study; 3) poorly controlled hypertension, cardiomyopathy, unstable angina, heart failure or severe arrhythmia, cancer, or chronic communicable infectious diseases; 4) current use of antipsychotics and/or antidepressants; 5) current or planned use of dehydroepiandrosterone, testosterone, transdermal oestrogens or other medications known to affect the growth hormone releasing hormone/growth hormone/insulin-like growth factor-1 axis; 6) currently diagnosed significant psychiatric illness, such as depression or schizophrenia, or dementia; 7) any contraindication for 7 Tesla MRI investigation, including recent joint replacements, stents, brain or heart surgery, bullet or shrapnel wounds and metal in the eye; 8) tobacco use,

168   excessive alcohol intake (more than four alcoholic drinks per day), excessive caffeine intake

169   (more than four cups of coffee per day); 9) excessive exercise involvement (more than twice

170   the weekly recommendations for adults or >600 min moderate to vigorous exercise per

171   week); 10) test results that indicated study participation was unsafe or; 11) participation in

172   conflicting studies.

173

174   Randomization of participants: We randomly assigned participants to one of three arms using

175   a centrally controlled computer-generated randomization list, generated by an independent

176   staff member. The treatment was assigned using sealed envelopes based on order of

177   recruitment and stratified for sex only. All investigators were blinded to the allocation order;

178   however, due to practicality, outcome assessors and exercise trainers were not blinded to the

179   allocation treatment arm.

180

181   After screening, 194 participants were recruited into the study, randomised into one of three

182   groups in a 1:1:1 ratio with 151 completing the 6-month exercise intervention (see Fig. 1 for

183   cohort diagram). Baseline demographic and descriptive data compared between all three

184   exercise groups showed no significant differences (Table. 1).

185

186   **Procedures.** Participants completed three, 36-45 min exercise sessions per week over a 6-

187   month period for a total of 72 exercise sessions; all training sessions were supervised by

188   qualified exercise physiologists and were conducted in a small group setting. Exercise

189   intensity was individually prescribed and regularly monitored via HR. Visual HR cues were

190   provided on an individualized poster and HR was recorded at regular intervals during each

191   session in a digital training diary. Peak aerobic capacity (VO_{2peak}) and peak HR (HR_{peak}) were

192 determined via a maximal graded exercise test to volitional fatigue using automated indirect

193 calorimetry and 12-lead ECG cardiac monitoring (COSMED). This was conducted at baseline

194 and at the 3- and 6-month time points and served as the basis for the calculation of HR

195 intensity zones for each participant during each exercise session.

196

197 All groups completed a 10 min warm up, followed by an exercise session at their appointed

198 intensity with a 5 min cool-down period at the end of each session. The LIT control group

199 conducted 30 min exercise sessions that included 5-8 stretching, balance, range of motion

200 and relaxation tasks where HR was maintained at 45-55% HR_{peak} to ensure a negligible

201 cardiometabolic effect. The MIT exercise sessions comprised of continuous treadmill walking

202 at 60-75% HR_{peak} that lasted 30 min. Participants were required to reach their target HR at

203 the end of the warm-up and were instructed to maintain the HR for the duration of the

204 session. Each exercise session for the HIIT group totalled 25 min and comprised of four, 4 min

205 working periods at 85-95% of HR_{peak} interspersed by three, 3 min active recovery intervals at

206 60-70% HR_{peak}. Participants were required to reach their target HR within the first 2 min of

207 the interval and maintain this HR until the end of the 4 min interval. In the MIT and HIIT

208 groups, participants were free to alter the treadmill speed and/or treadmill incline to

209 maintain the required HR (see S2 Appendix for detailed description for physiological

210 associated methods and safety parameters). The total period for each session was therefore:

211 LIT- 45 min, MIT – 45 min and HIIT- 40 min.

212

213 Cognitive testing was conducted electronically each month during the exercise intervention

214 and then every 6 months during the follow-up period which extended up to 60 months.

215 Testing was carried out using the Cambridge Automated Neuropsychological Test Battery

216 (CANTAB; Cambridge Cognition Ltd., 2015). These tests were self-administered using an iPad

217 touch screen interface while in the clinic. Outcomes were automatically recorded in the

218 application for later retrieval (see S3 Appendix).

219

220 MRI was conducted on a subset of participants prior to exercise (0 months), immediately

221 following completion of the exercise intervention (6 months) and 6 months after completion

222 of the exercise intervention (12 months). Scans were conducted at the Centre for Advanced

223 Imaging at UQ using an ultra-high field 7T MRI, Siemens scanner (see S4 Appendix for detailed

224 description of MRI-associated methods).

225

226 Blood biochemical properties were obtained from monthly blood samples that were collected

227 immediately pre- and post-exercise. ELISAs were conducted as per the manufacturer's

228 recommendations for a total of 17 analytes (see S5 Appendix for detailed description of

229 biochemical associated methods).

230

231 Physiological and functional fitness measurements were collected during in-person visits. At-

232 home questionnaires were also completed by participants during the intervention and

233 beyond (see S6 Appendix). Habitual physical activity was measured for 7 consecutive days

234 using tri-axial accelerometry (Actigraph®) at 0, 3, 6, 12 and 42 months. This was done in the

235 absence of the prescribed exercise sessions.

236

237 **Safety.** An event was defined as any untoward medical occurrence in a participant undergoing

238 an intervention. The reporting period for an adverse event was defined as the period from

239 initiation of the study treatment to the end of the 12-month follow-up. All adverse events

240   were recorded using authorized incident report forms and were reviewed by Occupational

241   Health and Safety staff, project staff and the heads of organizational units. The principal

242   investigator registered all adverse events with the ethical review committee within 24 h of

243   the incident. No adverse events resulted in hospitalization. During the study there were a

244   total of 2 adverse events. One participant from the LIT group fell into the moderate adverse

245   event range (cardiac discomfort that occurred at home). One participant from the HIIT group

246   fell into the severe adverse event range (injured knee requiring physiotherapy).

247

248   **Data storage, access and sharing.** In accordance with the Australian Privacy Principles 2014,

249   the Australian Code of the Responsible Conduct of Research and NHMRC National Guidelines

250   for the Ethical Conduct of Human Research (2007), data were de-identified by referencing

251   through a six-character participant code. DICOM images from the brain imaging sessions were

252   anonymized by only using the participant code. Digital data including DICOM images and

253   scanned paperwork in pdf format were stored on an access-controlled dedicated shared

254   network drive with high integrity permanent backup capability, with access limited to

255   authorized research and IT staff (see S7 Appendix for detailed description of data storage and

256   access associated methods).

257

258   **Primary and secondary outcomes.** The primary outcome - changes in cognitive function using

259   CANTAB following exercise - was assessed at the completion of the 6-month exercise study

260   using the detailed methodology provided in Appendix 3. The secondary outcomes examined

261   in this manuscript include changes in blood biomarkers at month intervals for the duration of

262   the intervention, changes in hippocampal volume, and changes in resting state functional

263   activity. These outcomes were also assessed at the completion of the exercise intervention

264 study and were measured using the methods described above and in the relevant

265 supplementary appendices.

266

267 **Statistical analysis.** Statistical description was provided as means and standard deviation

268 (SD), mean and standard error (SEM) or median and inter-quartile range (IQR) for continuous

269 data and frequency and percentage for categorical data as appropriate. Normality of the

270 distribution of continuous variables was tested using the Shapiro-Wilk test with an alpha level

271 <0.05. Data were log-transformed when appropriate and re-checked for normality of

272 distribution. Associations between continuous variables was assessed using Pearson's

273 correlation coefficient for data normally distributed or Spearman's correlation coefficient

274 when normality assumption was not met. The comparison in continuous outcomes between

275 groups at baseline was performed using one-way analysis of variance (ANOVA) where

276 appropriate. Analysis of continuous outcomes over time and between groups was performed

277 using either two-way repeated measures (RM) ANOVA or linear mixed-effects models (LMM)

278 considering time, group and group by time as main effect and participants as random effect.

279 Post-hoc tests with Bonferroni adjustments were performed where appropriate. Potential

280 confounders effect such as age, gender, BMI were considered and tested in the model as fixed

281 effects but were not found significant and therefore not included in the final models

282 presented. Variables showing a significant difference at baseline were included in the

283 regression analyses for the cognitive function outcome. Cohen's d= "($\mu1$-$\mu2$)/ SD pooled" was

284 used to calculate effect sizes where appropriate. Comparison of the functional connectivity

285 between groups was assessed and reported using q-values, using an optimised false discovery

286 rate (FDR) approach. Analyses were performed using the R statistical software (version 4.2),

287   SPSS (version 22.0, SPSS, Inc.) and GraphPad Prism (version 10.0.3). P-values were two-tailed,

288   with $p< 0.05$ considered statistically significant.

289

290   **Ethics and Trial Registration.** The ethics approval number was 2016-01-038-A-5 (Bellberry

291   Ltd- https://bellberry.com.au). The Universal Trial Number (UTN) is: U1111-1185-5102. The

292   trial application approval number from ANZCTR is ACTRN12618000700235.

293

294 **RESULTS**

295 **HIIT exercise significantly improves hippocampal-dependent spatial learning following 6**

296 **months of exercise.** Longitudinal changes in PALTEA performance during the intervention

297 period showed that only the HIIT group demonstrated a significant improvement in ΔPALTEA

298 (decreased errors) during each month of the exercise intervention, with the largest

299 improvement being reached after 6 months of exercise. In contrast, both the LIT and MIT

300 groups remained stable at each time point during the 6-month exercise intervention (Fig. 2A).

301 By the completion of the exercise intervention, the PALTEA performance for the HIIT group

302 was significantly better than that of both the LIT and MIT groups (p= 0.004 and p= 0.008

303 respectively).

304

305 Prior to, and during the intervention, participants underwent a comprehensive battery of

306 physiological tests. We found no significant differences either between groups or within

307 groups for physiological measures such as weight, BMI, grip strength or blood pressure at

308 either baseline (Table 1) or immediately following 6 months of exercise (Supp. Table 1). A

309 graded balance test was also performed with both the LIT and HIIT group demonstrating a

310 significant increase in balance at the conclusion of the exercise intervention (Supplementary

311 Fig. 1A). Cardiorespiratory tests were also conducted immediately prior to, at the 3-month,

312 6-month and 12-month periods (Supplementary Fig. 1B). There was a main effect of exercise

313 intensity during the testing period when we examined VO_2/kg (2-way RM-ANOVA, exercise

314 intensity: $F_{(2,149)}$= 4.675, p =0.011) with post hoc analysis showing a significant difference

315 between LIT and HIIT groups at the 3-month test period. While both MIT and HIIT groups

316 showed an increase in cardiorespiratory fitness during the exercise intervention period, this

317 did not reach significance. Further, comparing the change in VO_2/kg to ΔPALTEA showed no

318 correlation between these two metrics.

319

320 **Hippocampal-dependent cognitive improvements are maintained for up to 5 years**

321 **following initiation of the HIIT intervention**. Participants were re-tested 6 months after the

322 completion of the exercise intervention (the 12-month timepoint) and then regularly for the

323 following 4 years. The results showed that the HIIT group maintained the PALTEA

324 improvement (decreased errors) not only at the 12-month time point but continued to do so

325 for up to 5 years following the initiation of the exercise trial (Fig. 2B). PALTEA performance

326 for the LIT and MIT groups was not significantly different from baseline (Fig. 2B). At each

327 testing period following the conclusion of the exercise trial, the HIIT group performed

328 significantly better than both the LIT and MIT groups. These improvements were not due to

329 differences in lifestyle or physical activity following the 6-months exercise period as measured

330 by both the Godin Leisure Time Physical Activity Questionnaire and accelerometry data

331 (Supplementary Fig. 1). Rather, the increase in, and retention of improved cognitive function

332 in the HIIT group appeared to be a direct result of the 6-month HIIT exercise intervention.

333

334 **Participants who initially perform poorly on PALTEA show marked improvement with HIIT.**

335 As there was a large variation in PALTEA performance at screening, we examined whether the

336 group with the highest errors (poor performers), which were +1SD above mean at screening,

337 responded to the HIIT. We found that this group showed the largest increase in performance

338 which again was maintained for 5 years. Interestingly, the poor performers in the MIT group

339 also showed some improvement, although the magnitude of the effect was much smaller

340 than that seen with the HIIT group (Fig. 2C, and Supplementary Fig. 2).

341

342 In contrast to PALTEA, we found little evidence that exercise affected other cognitive domains

343 including working memory (WM), visual working memory and emotional recognition

344 (Supplementary Fig. 3). The LIT control group showed comparable improvement to MIT and

345 HIIT groups during WM testing for all parameters including total errors (ΔWMTE), between

346 trial errors (ΔWMBE) and strategy to complete the task (Supplementary Fig. 3A-C), suggesting

347 that these measures were not sensitive to exercise intensity. Visual working memory, as

348 tested by the delayed match to sample (DMS) task, showed no significant difference between

349 groups, and did not show significant improvement during the intervention (Supplementary

350 Fig. 3D-I). In addition, we found no difference in the time required to correctly identify

351 emotional facial cues using the emotional recognition task (ERT) between groups or with time

352 (Supplementary Fig. 3J-O). Taken together, these results show that the HIIT intervention was

353 specific to the hippocampal-dependent spatial learning task.

354

355 **Age-related volumetric decreases within specific brain regions are eliminated in response**

356 **to HIIT.** To quantitatively compare, in an unbiased fashion, changes in whole brain volumes

357 that occurred between and within exercise groups, we modified an established deformation-

358 based framework pipeline [25]. We found that, by the end of the 6-month exercise

359 intervention, the right-hand side (RHS) hippocampal volume remained stable in the HIIT

360 group but decreased significantly in both the LIT and MIT groups (Fig. 3A and Supplementary

361 Fig. 4A-C, $p= 0.0011$ and $p= 0.0002$ respectively), whereas all groups showed a significant

362 decrease in volume for the left-hand side hippocampus (Supplementary Fig. 4D-F).

363

364 We also found significant decreases in the volume of the corticospinal tract (CST) (Fig. 3B) and

365 the arcuate fasciculus (Fig. 3C) in the LIT group (paired t-tests, *p*= 0.011 and *p*= 0.006

366 respectively), whereas the volume in both the MIT and HIIT groups remained stable

367 (Supplementary Fig. 4G-I). When examining the longitudinal change for each participant as a

368 change in percentage volume from baseline, we found an exercise intensity-dependent

369 difference in CST volume, with the volume in both the MIT and HIIT groups being significantly

370 larger than that in the LIT group at the completion of the exercise intervention (Fig. 3B; *p*=

371 0.014 and *p*< 0.0001 respectively). Exercise intensity-dependent, post-exercise changes were

372 also observed for the arcuate fasciculus, with significant differences between the 6-month v

373 baseline difference of LIT compared to MIT and HIIT groups (Fig. 3C and Supplementary Fig.

374 4J-L; *p*= 0.012 and *p*< 0.0001 respectively), with the LIT group having a significantly higher

375 volume decrease going from the baseline to the 6-month timepoint. Voxel-wise analysis

376 showed significant differences in white matter between the LIT and HIIT group. These areas

377 were shown to be connected to the premotor cortex and the motor cortex (Fig. 3D).

378

379 The final MRI scan, at 12 months showed significantly less RHS hippocampal volumetric loss

380 in the HIIT group when compared to both the MIT and LIT groups (Fig. 3E). Similarly, in the

381 HIIT group, the volume of both the CST and arcuate fasciculus remained stable at the 12-

382 month time point whereas significant volumetric loss was observed in the LIT group (Fig. 3F

383 and G respectively).

384

385 **Resting-state functional connectivity significantly increases following HIIT exercise**. We next

386 examined whether the 6-month exercise intervention affected functional connectivity (FC)

387 during a resting-state scan. When comparing the within-group change in FC strength, we

found that only the HIIT group exhibited significant increases between several inter-network pairs including the motor-visual (MOT-VIS), default mode-attention (DMN-ATTN) and DMN-frontal (DMN-FRNT) network following exercise compared to baseline (Fig. 4A). Between group effect changes in FC network pairs were also examined following exercise, with the HIIT group showing increased connectivity between networks in comparison with the LIT (Fig. 4B) and MIT groups (Fig. 4C). In both cases, the HIIT group showed significant increases in the following inter-network FC pairs: MOT-VIS and DMN-MOT (two-way RM-ANOVA; FDR corrected at Q <0.1). Examining the Group x Time effects between exercise groups revealed that the HIIT group only exhibited significant increases in FC between numerous network pairs compared to the LIT control group. These included the DMN-MOT, DMN-VIS, DMN-FRNT, DMN-ATTN, VIS-MOT and VIS-FRNT (Fig. 4D; two-way RM-ANOVA; FDR corrected at Q <0.1). The X, Y, Z planes for each independent components (ICs) that showed significant FC interactions are shown in Fig. 4E (all ICs generated are included in Supplementary Appendix 9). Of note, at the 12-month time point, we found no significant differences in FC for any network pairs of the exercise groups relative to baseline (data not shown).

The longitudinal changes to functional connectivity (ΔFC) for these regions of interest were then compared to exercise-mediated changes in PALTEA performance (ΔPALTEA). The LIT group showed both positive (ATTN06-MOT28) and negative FC interaction (DMN04-VIS23 and FRNT38-ATTN13) changes (Supplementary Fig. 5A) whereas the MIT group demonstrated a single positive interaction (DMN04-VIS23; Supplementary Fig. 5B). The HIIT group, however, had three positive interactions between ΔFC and ΔPALTEA (FRNT14-ATTN06, DMN04-VIS23 and ATTN06-MOT28; Supplementary Fig. 5C).

Circulating biomarkers differentially respond to exercise intensity. To examine any biochemical changes accompanied the different intensities of exercise and may be associated with the observed hippocampal-dependent cognitive improvement, we next analysed several circulating factors, some of which have previously been reported to be modulated by exercise in humans and/or animal models. Of the 17 analytes initially examined (see Supplementary Table 1), only those exhibiting exercise-mediated changes were analysed further (Supplementary Fig. 6). Both growth hormone (GH) and β-hydroxybutyrate (BHB) showed consistent post-exercise increases relative to pre-exercise levels for all groups (Supplementary Fig. 6A-B respectively) while cortisol, prolactin and BDNF showed post-exercise responses that were dependent on exercise intensity (Supplementary Fig. 6C-E respectively).

We also calculated the cumulative pre-post exercise delta value (Δ) at each month for each biomarker (Fig. 5). This approach provides insight into the exercise-mediated longitudinal changes in circulating biomarkers at both the individual and group level. We found that cumulative ΔGH increased for all groups during the exercise intervention, with the largest increase occurring in the HIIT group. This value was significantly higher than that of either the LIT or MIT group at the completion of the intervention (Fig. 5A). The longitudinal change in cumulative ΔBHB, however, displayed an exercise intensity-dependent increase (Fig. 5B): with a negligible change observed in the LIT group, a significant increase in the MIT group, and exhibiting the highest cumulative increase in ΔBHB in the HIIT group. In the case of BDNF, which has been extensively examined for its association with exercise-mediated changes and cognition, we found that MIT and HIIT produced a similar increase in ΔBDNF whereas the ΔBDNF levels remained stable in the LIT group (Fig. 5C). Examination of the changes in cortisol

436   levels revealed that most LIT and MIT participants showed a decrease in Δcortisol levels

437   whereas HIIT participants were evenly split between increased and decreased cortisol levels

438   and so remained stable as a group overall (Fig. 5D). An increase in cumulative Δprolactin

439   levels was restricted to the HIIT group whereas the levels in both the LIT and MIT groups

440   remained stable during the exercise intervention (Fig. 5E). Inflammation markers including IL-

441   1β, IL-6, IL-10, IL-12 and IFNγ were also examined (Supplementary Fig. 7) and showed no

442   significant differences between groups over the duration of the exercise intervention.

443

444   **Exercise-mediated changes in cortisol predict PAL ability and correlate to endpoint**

445   **hippocampal cognitive function in HIIT participants.** We next determined if correlations

446   between circulating analytes and hippocampal cognitive function were present. Only those

447   that showed significance are presented here. Restricted to the HIIT group, we found a

448   significant correlation between initial pre-post exercise delta value of cortisol (Δcortisol) and

449   improved PALTEA performance at the end of the exercise intervention (Fig. 6A). When the

450   delta between pre/post-exercise cortisol levels across the entire period of the exercise

451   intervention was accumulated, we found a significant positive correlation with improved

452   PALTEA learning for the HIIT group (Fig. 6B). We also found that the 6-month pre/post-

453   exercise delta value for cortisol positively correlated to the endpoint PALTEA performance for

454   the HIIT group (Fig. 6C).

455

456   **The initial change in BDNF predicts hippocampal cognitive improvement for HIIT**

457   **participants.** In the HIIT group, we found no relationship between the absolute levels of

458   circulating BDNF and PALTEA performance for either pre- or post-exercise samples

459   (Supplementary Fig. 8A-E) until the 6-month timepoint where a higher post-exercise BNDF

concentration correlated to better PALTEA ability (S8F Fig). Surprisingly, when we compared

the exercise-mediated difference between pre-and post-exercise BDNF (ΔBDNF), we found

that higher initial ΔBDNF concentrations significantly correlated with improved PALTEA

learning (ΔPALTEA) at the end of the trial (Fig. 6D). This correlation remained significant after

1 month of exercise (Supplementary Fig. 9A) and there was a strong trend for an association

between higher cumulative ΔBDNF concentration and improved ΔPALTEA learning which was

maintained until the end of the exercise intervention (Supplementary Fig. 9). No correlations

between BDNF and PALTEA were observed for the LIT or MIT groups (data not shown).

**Decreased prolactin levels in the MIT group correlate to improved hippocampal cognitive
function.** For the MIT group alone, we found that exercise-mediated changes in cumulative

Δprolactin levels correlated to improved PALTEA performance (Fig. 7A). This relationship

emerged at 2 months, was strongest at 4 months and was maintained throughout the

remainder of the exercise intervention (Supplementary Fig. 10A-F). We then conducted a

subgroup analysis for the MIT group using a change of ±1SD PALTEA performance to divide

the MIT group into improvers, stable participants and deteriorators. This showed that those

participants with improved hippocampal cognition had significantly lower prolactin levels

than those that deteriorated (Supplementary Fig. 10G).

Higher workload levels correlate with improved PAL performance. Controlling for exercise

dose is typically accomplished by reaching and maintaining specified HR ranges, an approach

we also used. However, controlling for target HR necessarily results in different mechanical

work for each participant. As multiple parameters, including HR, treadmill speed and treadmill

angle, were recorded every session, we were able to accurately calculate the exercise

484 workload throughout the trial. Calculation of the workload in this manner clearly revealed the

485 difference in the total exercise workload that each participant completed during the

486 intervention for both the HIIT (Supplementary Fig. 10H) and MIT groups (Fig. 7B). Although,

487 we did not find a correlation between HIIT exercise workload and improved cognitive function

488 (data not shown), the MIT group exhibited a strong correlation between higher workload

489 levels and improved PALTEA performance (Fig. 7C).

490

491 **DISCUSSION**

492 This is the first study, to our knowledge, to reveal that a 6-month HIIT intervention

493 significantly improves hippocampal-dependent spatial learning, as measured by PAL, in

494 healthy, aged human individuals. At the completion of the exercise intervention PAL in the

495 HIIT group had improved significantly from baseline as well as when compared to both the

496 LIT and MIT groups. Remarkably, the hippocampal-dependent PAL improvement in the HIIT

497 group was retained for up to 5 years. The non-hippocampal-dependent WM cognitive tests

498 failed to show significant differences between exercise groups whereas visual working

499 memory and ERT revealed no improvement during the testing period. High-resolution MRI

500 showed that HIIT abrogated age-dependent volumetric decreases in specific brain regions,

501 including the hippocampus, which is especially sensitive to ageing. Resting-state MRI

502 indicated that the HIIT-mediated hippocampal-dependent cognitive improvement was

503 associated with enhanced functional connectivity between key networks, including the DMN

504 and those associated with attention, motor function and vision. Biomarker analysis revealed

505 a variety of exercise-mediated changes; however, only increased BDNF and cortisol levels

506 correlated to improved hippocampal-dependent cognitive function in the HIIT group,

507   whereas a decrease in prolactin correlated to improved hippocampal-dependent cognition in

508   the MIT group.

509

510   Our primary goal was to determine the effect of 6 months of exercise on hippocampal-

511   dependent cognitive function in the healthy elderly. An age-dependent decrease in

512   hippocampal function has been clearly established in both humans [4, 26] and rodents [7, 8]

513   and we chose PAL to assess the potential for exercise to ameliorate this loss because PAL is a

514   hippocampal-dependent cognitive paradigm [27] that is sufficiently sensitive to identify those

515   at risk of developing dementia [28, 29]. Importantly, the test-re-test reliability of the

516   computerized and automated PAL test we used is robust and falls into the acceptable to high

517   range [30, 31]. At the conclusion of the 6-month exercise intervention, only the HIIT group

518   showed significantly improved PAL performance, whereas both the MIT and LIT groups

519   remained stable. Previous studies examining hippocampal-dependent cognitive changes

520   following MIT also reported no improvement when using neuropsychological tests including

521   the mini-mental state examine (MMSE)[32, 33] or Montreal cognitive assessment (MoCA)[34,

522   35], which are unlikely to possess the required sensitivity to discriminate subtle changes [27],

523   especially within cognitively healthy individuals. Similarly, an electronic match/ non-match

524   spatial learning task following MIT exercise did not detect significant improvements above

525   controls [14]. Initial PAL scores allowed us to examine the effect of exercise on those who

526   may be most at risk of developing cognitive impairments, namely those that began the

527   intervention more than 1SD above the median performance. Encouragingly, we found that

528   they showed the greatest improvement in PAL performance with HIIT; moreover, they also

529   responded to MIT, although to a much lesser degree, suggesting that even moderate exercise

530   may be beneficial to this at-risk group.

531

532 Surprisingly, the improvements in hippocampal performance following HIIT exercise were not

533 observed with other cognitive domains such as WM, visual working memory or emotional

534 recognition, where we found minimal changes and no significant differences between the

535 groups at the conclusion of the 6-month exercise period. The lack of an exercise intensity-

536 mediated effect on working memory was of interest given that it has been the focus of several

537 studies and meta-analyses with some concluding no effect [36] while others found only small

538 effect sizes [37]. A lack of sensitivity in WM cognitive tests may have also contributed to this

539 result as a recent study found that PAL was superior to WM at successfully discriminating

540 cognitive abilities in high functioning elderly individuals [38]. More studies are therefore

541 required to determine whether exercise can indeed mediate WM or if more sensitive and

542 challenging cognitive tests are required for determining subtle differences over time.

543 Regardless, our results highlight the specificity of HIIT in significantly improving hippocampal-

544 dependent spatial learning in the healthy elderly.

545

546 The maintenance of hippocampal function in the active control LIT group for up to 5 years

547 was an intriguing observation, especially as this contrasts to previous reports that multiple

548 cognitive domains significantly decline within a 4 year re-test period during normal ageing

549 [39]. A possible explanation for this may be associated with the improvement in balance that

550 occurred during the intervention. Indeed, it has been demonstrated previously that a 12-

551 week balance training paradigm was sufficient to improve spatial learning, even in the

552 absence of altered cardiorespiratory fitness [40]. Thus our 6-month, LIT motor function and

553 balance regimen may be a viable approach to maintain cognitive function for participants that

554 have reduced physical ability and movement limitations.

555

556 Another major aim we addressed with our study is the long-term effectiveness of an exercise

557 intervention [41], which has significant ramifications for the trajectory of cognitive decline

558 [42]. As exercise at high intensity becomes increasingly difficult in older individuals,

559 intervention at a younger age that has a significant impact on long-term performance

560 becomes increasingly important. Our results demonstrate that a relatively acute, 6-month

561 HIIT intervention can maintain the improvement in hippocampal function for at least 4.5 years

562 with no diminution of performance. Importantly, this was observed even though there was

563 no discernable change to the original physical or lifestyle practice of the participants once the

564 exercise intervention was complete. Nor was there a significant change in cardiorespiratory

565 fitness during the intervention, indicating that this did not play a major role in improving

566 hippocampal function.

567

568 It is well established that there is an age-associated decrease in whole brain volume [43, 44]

569 and the hippocampus is especially susceptible to volumetric decrease [5, 43], with an annual

570 hippocampal volumetric loss between 1.4-2.81% being reported [21, 45] which increases

571 beyond 65 years of age [5]. A concomitant decrease in specific domains of cognitive function

572 such as executive function and spatial learning is also typically reported [21, 46]. Using high-

573 resolution 7T MRI we found that HIIT maintained hippocampal volume at 6 and 12 months

574 whereas both the LIT and MIT groups exhibited significant volumetric decreases at the

575 completion of the exercise intervention. This effect was most profound in the right

576 hippocampus which has been identified as having a role in spatial learning [47]. In support of

577 this, while one study previously reported a significant increase in hippocampal volume in

578 healthy aged individuals [14] following 12 months of MIT exercise, our findings are more in

579 line with the meta-analytic examination of the literature which shows retention of volume

580 rather than an overall increase [48] is more likely.

581

582 As with our hippocampal findings, only HIIT mitigated the age-associated decrease observed

583 in the white matter tracts for the CST and arcuate fasciculus. The CST, which connects the

584 motor cortex to the spinal cord to enable voluntary control of the distal extremities, decreases

585 during age [49]. This is the first report of the effect of exercise on CST volume in the elderly.

586 We also found that the arcuate fasciculus was maintained following HIIT exercise as opposed

587 to decreases observed in the LIT and MIT groups. The arcuate fasciculus is a bundle of axons

588 that connects the temporal and inferior parietal cortices to the frontal temporal lobe and is

589 involved in producing and understanding language. Interestingly, it has been reported that

590 healthier middle aged people exhibit higher fasciculus integrity compared to age-matched

591 sedentary individuals [50], although a 6-month MIT intervention failed to improve the

592 integrity of these tracts in aged individuals [51]. These results indicate that HIIT exercise

593 ameliorates the age-dependent decrease in volume observed in multiple regions including

594 the hippocampus and arcuate fasciculus, both of which have been associated with learning

595 [21] and memory [52].

596

597 It has been suggested that changes in the functional organization of the brain may precede

598 both structural differences and clinical symptoms, especially in relation to disorders such as

599 Alzheimer's disease [53]. Synchronization of blood oxygenation level dependent (BOLD)

600 signals in the absence of explicit stimuli between regions is referred to as resting-state MRI

601 [6], which provides a measure of FC both within and between networks. In aged mice, we

602 recently demonstrated significant decreases in FC within the hippocampus which could be

603 reversed via an exercise-mediated increase in hippocampal neurogenesis [12]. We therefore

604 examined if FC was also altered during exercise in our cohort of elderly participants. We found

605 increases in FC between the DMN-ATTN and DMN-FRNT networks in the HIIT group,

606 suggesting that these changes contributed to the observed improvement in PAL performance.

607 This is supported by our recent finding that exercise in aged mice increases FC between the

608 entorhinal cortex [12], which is part of the ATTN network [54], and the hippocampus (included

609 in the DMN), resulting in improved performance in hippocampal-dependent spatial learning

610 [12]. Further, in young adults, an increase in FC within the ATTN network correlates to

611 improved spatial cognitive tasks [55]. Taken together, these findings demonstrate that HIIT

612 exercise increases the FC between networks that have been identified to be either altered

613 during ageing or play a role in cognitive function [6, 55, 56]. Considering the similarities in

614 brain structure and function between rodents and humans, our studies in aged mice following

615 exercise showing increased hippocampal neurogenesis [8, 12], provide crucial mechanistic

616 insight as to how exercise in humans may abrogate the age-associated decrease in

617 hippocampal cognitive function by reducing the trajectory of hippocampal volume decrease

618 and improve FC between key networks.

619

620 To gain insight into putative humoral mechanisms which may be associated with, or mediate

621 the improved hippocampal cognitive function, multiple studies have explored various

622 circulating biomarkers in humans and animals [14, 15, 57]. Foremost amongst these analytes

623 is the neurotrophin BDNF. In animal models, circulating levels of BDNF have been reported to

624 directly correlate to those in the hippocampus [58]. This, combined with the established role

625 of BDNF in neurogenesis and synaptic plasticity, makes circulating BDNF an attractive

626 potential biomarker of human cognitive health [14, 19]. Considering that humans [15] and

627 animals [8] both show exercise-mediated increases in circulating BDNF, similar mechanisms

628 may be involved in mediating cognitive improvement. For example, animal studies have

629 shown that an exercise-mediated increase in BDNF, specifically in hippocampal tissue, directly

630 correlate to improved hippocampal-dependent spatial learning performance [59] as well as

631 increased neurogenesis [60], which we and others have shown to be critical for hippocampal-

632 dependent spatial learning [8, 12, 61].

633

634 Our approach of calculating the acute exercise-mediated changes in BDNF (ΔBDNF) revealed

635 a similar increase in BDNF for both the MIT and HIIT groups at the completion of the exercise

636 intervention. A similar study found that a single session of MIT or HIIT exercise in middle aged

637 and elderly participants resulted in BDNF increasing to the same degree; however, no

638 correlation between pre- or post-exercise BDNF and cognitive function was observed [62].

639 Interestingly, we found that in the HIIT, but not the MIT or LIT groups, it was the difference

640 between pre- and post-exercise BDNF levels (ΔBDNF) that predicted improved hippocampal-

641 dependent learning ability in the HIIT group at the end of the exercise program rather than

642 the absolute concentration of either the pre- or post-exercise circulating BDNF levels. As we

643 observed this correlation from the start of the exercise program, it implies an intrinsic

644 capacity of the individuals to respond to HIIT. This response is unlikely to be associated with

645 a previous history in exercise as we found no evidence of a relationship between initial

646 concentrations of BDNF and initial hippocampal cognitive ability. Indeed, preliminary data

647 suggest that the observed correlations between higher initial ΔBDNF and Δcortisol levels with

648 improved PALTEA performance may be due to primed epigenetic factors that allow for

649 immediate responses to external stimuli, a prospect we are currently investigating.

650

651   As with our recent study in aged mice [8], we found no change in circulating inflammation

652   markers during exercise in all groups and did not observe any correlations between

653   inflammation markers and hippocampal cognitive function. Low level chronic inflammation

654   has often been associated with advanced age [63]; however the effect of exercise on

655   circulating cytokines remains to be fully elucidated, as highlighted in recent meta-analyses

656   [63-65]. While this remains an area for further examination, it suggests that modulation of

657   inflammatory cytokines may not play a crucial role in the observed effects of exercise.

658

659   Chronically elevated cortisol levels have been associated with deficits in cognition,

660   particularly in the aged population [66]; however, acute elevations in cortisol induced by

661   physiological stimuli such as exercise have also been reported to be beneficial [57]. We found

662   that the HIIT intervention maintained total Δcortisol levels whereas a decrease was observed

663   in both the LIT and MIT groups. One study examined the effect of vigorous physical activity

664   on cortisol and quality of life (QoL) outcomes and found that a moderate, non-pathological

665   increase in cortisol was associated with a better perception of QoL [57]. Focusing on the

666   exercise-mediated change in cortisol, we found that a higher initial Δcortisol level predicted

667   improved PAL performance and a higher total Δcortisol correlated with end-point cognitive

668   ability for the HIIT group. Taken together, these results suggest that initial exercise-mediated

669   changes in both BDNF and cortisol appear to be good candidates to predict the effectiveness

670   of HIIT in improving cognitive function in the healthy elderly. How the exercise-dependent

671   increase in cortisol mediates hippocampal-dependent cognitive improvement, however,

672   remains to be elucidated. There is some suggestion that cortisol acts indirectly by modulating

673   BDNF gene expression [67], whereas others have shown that acute cortisol exposure

674   enhances neurogenesis and hippocampal-dependent learning in rodent models [68].

675

676   The divergence in hippocampal learning response, coupled with the exercise intensity-

677   dependent differences of multiple circulating factors between ostensibly similar exercise

678   interventions (MIT and HIIT) suggests that some component of the HIIT intervention is

679   intrinsically different from more traditional steady-state exercise. A lack of significant

680   differences in cardiorespiratory fitness between MIT and HIIT during the intervention

681   suggests mechanisms other than those related to cardiorespiratory fitness are involved. One

682   major difference between the interventions is the exercise workload experienced, especially

683   by the leg skeletal muscles. Importantly, myokines which are factors released following

684   skeletal muscle contractions [69] in an exercise intensity-dependent manner, have been

685   associated with improvements in hippocampal function [70]. Indeed, BDNF is one of the most

686   extensively studied myokines in relation to brain function [71].

687

688   More recently, it has been found that the exercise induced secretion of factors extends

689   beyond skeletal muscle to other organs and is now collectively referred to as exerkines [72].

690   In support of a role of exerkines in hippocampal-dependent learning, we recently

691   demonstrated that the exerkine platelet factor 4 was effective at increasing hippocampal

692   neurogenesis and cognitive function in old mice [73]. With multiple organs now shown to be

693   sensitive to exercise, the level of crosstalk and signaling cascades becomes far more complex.

694   For example, β-hydroxybutyrate, which we showed increased in an exercise intensity-

695   dependent manner, was originally described as an alternate energy source secreted from the

696   liver for use by peripheral organs including the brain [74]. More recently however, β-

697   hydroxybutyrate has been shown to have additional downstream effects, including changes

698   to BDNF expression [75]. Similarly, our observed exercise intensity-mediated release of

699 cortisol from adrenal glands, and growth hormone and prolactin from the pituitary gland, fall

700 into this definition of exerkines. Collectively, our findings add to the expanding identification

701 of exercise-mediated metabolite release from multiple organs and underscores the emerging

702 picture of complexity required to positively influence hippocampal function. Additionally, the

703 physiological switch between aerobic and anaerobic exercise and the effect on myokines and

704 exerkines has yet to be thoroughly explored.

705

706 In line with differential responses between aerobic and anaerobic exercise, we noted a

707 difference in response between the MIT and HIIT groups for Prolactin. In this instance, an

708 exercise-mediated decrease in Δprolactin correlated to improved PAL performance, a finding

709 that was restricted to the MIT group. This is likely due to the exercise-intensity mediated

710 switch that has been observed in young adults [76] in which prolactin decreases following

711 moderate intensity exercise but increases when anaerobic, high intensity exercise is

712 undertaken. Our finding suggests that the physiological switch in prolactin secretion patterns

713 due to exercise intensity is retained in the elderly.

714

715 The differential response in prolactin and other biomarkers between the MIT and HIIT groups

716 highlights the existence of exercise intensity-dependent physiological responses. This is likely

717 due to the switch between aerobic and anaerobic exercise. In our study, MIT exercise was

718 purely aerobic whereas HIIT included a combination of both aerobic and anaerobic

719 components. The switch between aerobic and anaerobic exercise is an important distinction

720 that has not received the appropriate level of attention, and future studies should consider

721 this variable when analysing circulating biochemical markers. Taken together, we would

722 argue that the exercise-mediated improvement in hippocampal function requires the

723   complex orchestration of multiple organs and mechanisms, some of which we and others

724   have identified, while others have yet to be discovered and will require further studies to be

725   fully elucidated.

726

727   **Limitations**

728   Our study was highly supervised to ensure that participants conducted the appropriate

729   exercise level. Regardless of this, there were limitations. For example, this study only

730   examined the effect of exercise on aged individuals that did not exhibit cognitive deficits. We

731   also did not include an isolated non-exercise, sedentary control group to determine the

732   effects of social interactions alone may have on performance. Selection of participants was

733   designed to include healthy aged individuals capable of participating in a 6-month exercise

734   intervention. It therefore remains to be seen if this type of exercise intervention would be

735   possible in less physically healthy individuals. Further, it is currently unknown if other

736   modalities of HIIT such as low impact exercise bikes or rowing machines would provide similar

737   hippocampal-dependent cognitive improvements.

738

739   **Conclusions**

740   This is the first study to our knowledge to identify that a HIIT paradigm for the healthy elderly

741   is suitable and effective at significantly improving and retaining long-term hippocampal-

742   dependent learning, for up to 5 years. Our finding of individualized, exercised-mediated

743   responses of biomarkers as predictors for improved hippocampal functional outcomes offers

744   a quantifiable metric to provide an effective exercise regimen. The improvement, and long-

745   term retention of hippocampal learning ability following HIIT exercise provides a new insight

746   into how the elderly could be insulated from cognitive decline even though their exercise

747 capabilities may decline with advanced age. This approach could greatly enhance the capacity

748 of clinicians to tailor personalized exercise paradigms, including those at risk of cognitive

749 decline.

750

751 **Contributors**
752 DGB, MAS, MZ, SR and PFB conceived and designed the trial. SB, MAS, TS, GR, JM, MB and MZ

753 supervised intervention components. EC created the study database. DGB, XVT, IO, MZ, AB

754 did data analysis. DGB, AB, XVT, TLS, FN, SR and PFB interpreted the results and drafted the

755 paper. All authors revised the article for intellectual content and approved the final version.

756 PFB was the principal investigator.

757

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998

999 Table captions

1000 Table 1: **Demographic and descriptive baseline data**. Comparison of basic demographic and descriptive data

1001 prior to the initiation of the exercise intervention. All groups were evenly distributed and showed no significant

1002 differences. All data represent a group mean (±SE). Statistical comparisons were conducted using one-way

1003 ANOVA with Bonferroni post-hoc tests.

1004

1005 Figure captions

1006 Figure 1: **Cohort schematic illustrating recruitment and participation for each group during the 6-month**

1007 **exercise trial and beyond**. The number of participants who were screened and assigned to each group is

1008 provided. The reasons for withdrawal are summarized.

1009

1010 Figure 2: **Learning performance was significantly improved during the exercise intervention period and**

1011 **beyond.** (**A**) Only the HIIT group exhibited significantly improved PALTEA performance (fewer errors) during the

1012 exercise intervention. Neither the LIT nor the MIT group exhibited altered PALTEA performance during the

1013 exercise intervention. At the 6-month timepoint the HIIT group significantly outperformed the LIT group

1014 (mean±SE; two-way RM-ANOVA [time x exercise intensity effect $F_{(10,738)} = 2.272$, $p= 0.012$], with Bonferroni

1015 post-hoc tests, $p= 0.004$ and $p= 0.008$ respectively, effect sizes LIT: HIIT = 0.72, MIT: HIIT = 0.74). (**B**) The

1016 improvement in PALTEA performance for the HIIT group observed following 6 months of exercise was

1017 maintained for up to 5 years following exercise initiation. Conversely, both the LIT and MIT groups remained

1018 stable at each time point measured (mean±SE; two-way RM-ANOVA [time x exercise intensity effect $F_{(6,317)} =$

1019 4.076, *p*= 0.0006], repeated measures with Bonferroni's post-hoc tests, *p*= 0.036 and *p*= 0.017 respectively, 12m

1020 effect sizes; LIT: HIIT = 0.5, MIT: HIIT effect size = 0.62. >48m effect sizes; LIT: HIIT = 0.73, MIT: HIIT = 0.77). (**C**)

1021 Comparing cognitive performance for participants who were +1SD above mean PALTEA performance at the start

1022 of testing showed that the HIIT group significantly improved and outperformed both the LIT and MIT groups

1023 (mean±SD; one-way ANOVA F (2,32) = 10.71, *p*= 0.0003, with Bonferroni's post hoc tests, effect sizes: LT: HIIT=

1024 1.4, MIT: HIIT= 1.7). Within group statistical comparisons are represented by significance indicators of the same

1025 colour whereas black significance indicators represent differences between groups. ns = non-significant, *p<

1026 0.05, **p< 0.01, ***p< 0.001, ****p< 0.0001

1027

1028 Figure 3: **HIIT exercise prevents volumetric loss in specific brain regions.** (**A**) Comparison of the % change in

1029 volume from baseline to the end of the exercise intervention showed that the hippocampal volume from the

1030 right-hand side (RHS) remained stable for the HIIT group. However, both the LIT and MIT groups showed a

1031 significant reduction (mean±SD; mixed effects model with post hoc tests, *p*< 0.001 and *p*< 0.0001 respectively).

1032 (**B**) Following the intervention, the cortical spinal tract remained stable for both the HIIT and MIT groups;

1033 however, there was a significant decrease in the LIT group. Differences in % volume change were also observed

1034 between the MIT and LIT groups and the HIIT and LIT groups (mean±SD; mixed effects model with post hoc tests

1035 [time x exercise intensity effect F (2,214) = 4.71, *p*= 0.01], with Bonferroni post-hoc tests). (**C**) Following exercise

1036 there was a significant % difference in the volume of white matter associated with the arcuate fasciculus in the

1037 HIIT group compared to both the MIT and LIT groups (mean±SD; mixed effects model with post hoc tests [time

1038 x exercise intensity effect F (2,214) = 4.718, *p*= 0.001], with post-hoc tests. (**D**) The increased white matter of

1039 both the cortical spinal tract and the arcuate fasciculus, as compared between the HIIT and LIT groups, was

1040 shown to connect to the premotor cortex (purple) and the primary motor cortex (cyan) by voxel-wise analysis.

1041 (**E**) The amount of volumetric loss in the RHS Hippocampus after 12 months was significantly reduced in the HIIT

1042 group compared to both the LIT and MIT groups. (**F**) At the 12-month time point the HIIT group showed an

1043 increase in cortical spinal tract volume whereas the LIT showed a decrease, resulting in a significant difference

1044 between these groups (*p*= 0.004). (**G**) The arcuate fasciculus remained stable in the HIIT and MIT groups whereas

1045 a significant decrease was observed in the LIT group at the 12-month time point (mean±SD; mixed effects model

1046 with post hoc tests). Coloured significance indicators are relative to baseline cognitive performance over time

1047 for each respective group whereas black significance indicators represent significant differences between

1048 groups. *$p< 0.05$, **$p< 0.01$, ***$p< 0.001$, ****$p< 0.0001$

1049

1050 Figure 4: **HIIT exercise improves connectivity between multiple resting-state networks**. (**A**) Comparison of

1051 baseline to post-exercise scans revealed FC in the HIIT group significantly increased between network pairs

1052 including the DMN-FRNT, DMN-ATTN and VIS-MOT networks when FDR was corrected at Q< 0.2. (**B**) Following

1053 exercise, the HIIT group showed increased network FC between the DMN-MOT and VIS-MOT network pairs

1054 compared to the LIT group. (**C**) Comparison of the MIT and HIIT groups following exercise revealed increases in

1055 connectivity in the HIIT group, including in the DMN-FRONT, DMN-MOT, VIS-FRNT, ATTN-FRNT and VIS-MOT

1056 circuits (FDR corrected at Q <0.1). (**D**) Comparison of Group x Time (the change in connectivity between baseline

1057 and following exercise) interactions between the LIT and HIIT groups showed that the HIIT group had significant

1058 FC increases between the DMN-MOT, DMN-VIS, DMN-FRNT, DMN-ATTN, VIS-MOT and VIS-FRNT networks (FDR

1059 corrected at Q< 0.1). (**E**) Representative MRI scans showing the regions of the brain that belong to the

1060 independent components (ICs) used to determine the MOT, VIS, DMN, ATTN and FRNT networks. Hot colours

1061 represent significant increases in functional connectivity (FC) while cool colours represent a decrease in FC. The

1062 X, Y and Z planes are shown for each ICs. Motor (MOT), visual (VIS), default mode (DMN), attention (ATTN) and

1063 frontal (FRNT) networks. The numbers listed for each network group represent different anatomical regions of

1064 the brain. Significance was calculated as a log p value and only those that survived FDR correction were included.

1065

1066 Figure 5: **Cumulative analyte deltas show biochemical and exercise intensity-specific changes.** Tracking

1067 individual participants cumulative pre-post exercise deltas (Δ) revealed large differences both between and

1068 within groups. (**A**) The majority of individuals in each exercise group had a positive cumulative ΔGH value. The

1069 cumulative ΔGH slope was significantly higher for the HIIT group than the LIT group (mean±SE; two-way RM-

1070 ANOVA [time x exercise intensity effect $F_{(12,888)} = 2.802$, $p= 0.0009$], with post hoc tests, effect size LIT: HIIT =

1071 0.43). (**B**) Several of the LIT participants had a negative cumulative ΔBHB value whereas the majority of MIT and

1072 HIIT individuals had a positive value. The cumulative ΔBHB slope was significantly higher for both the HIIT and

1073 MIT groups compared to the LIT group, and the HIIT slope was significantly steeper than that of the MIT group.

1074 Following 6 months of exercise there was a significant difference in ΔBHB between groups (mean±SE; two-way

1075 RM-ANOVA [time x exercise intensity effect $F_{(12,870)} = 20.39$, $p< 0.0001$], with post hoc tests, effect sizes LIT:

HIIT = 1.62, LIT: MIT = 1.13). (**C**) There was a large spread in cumulative ΔBDNF between participants for each exercise intensity. The cumulative ΔBDNF slope for the LIT group was close to zero after 6 months of exercise whereas both the MIT and HIIT groups had positive values (mean±SE; two-way RM-ANOVA [time x exercise intensity effect F (12,883) = 1.814, p= 0.042], with post hoc tests, effect size LIT: HIIT = 0.33). (**D**) The majority of participants in the MIT and LIT groups had a negative cumulative Δcortisol value whereas the HIIT group had a large spread in cumulative values. Both the LIT and MIT groups had significant negative cumulative Δcortisol slopes whereas that of the HIIT group remained steady (mean±SE; two-way RM-ANOVA [time x exercise intensity effect F (12,888) = 16.63, p< 0.0001], with post hoc tests, effect sizes LIT: HIIT = 0.68, MIT: HIIT = 1.18). (**E**) The LIT and MIT participants were evenly distributed with negative and positive cumulative Δprolactin values whereas the majority of HIIT participants had positive Δprolactin levels. Both the LIT and MIT cumulative Δprolactin levels remained stable over 6 months whereas the HIIT group exhibited a significant positive slope (mean±SE, two-way, RM-ANOVA [time x exercise intensity effect F (12,888) = 17.16, p< 0.0001], with post-hoc tests, effect sizes LIT: HIIT = 0.99, MIT: HIIT = 1.07). * p< 0.05, ** p< 0.01, and *** p< 0.001.

Figure 6: **Pre/post exercise delta values for cortisol and BDNF predict improved learning ability for the HIIT group**. (**A**) The HIIT group showed a significant correlation between the initial exercise-mediated cortisol pre/post delta values (Δ) and learning ability (ΔPALTEA), with higher cortisol values correlating to improved learning. (**B**) The total cumulative Δcortisol levels at 6 months showed that higher Δcortisol levels correlated to better learning ability for the HIIT group. (**C**) The 6-month pre/post delta Δcortisol levels correlated to superior endpoint cognitive ability for the HIIT group. (**D**) The HIIT group showed a significant correlation between the initial, pre/post-exercise-mediated ΔBDNF values and PALTEA learning ability. Spearman correlations were used for analysis.

Figure 7: **Lower prolactin levels and higher cumulative exercise workload correlate to improved cognition in MIT participants.** (**A**) Exercise-mediated changes in total cumulative Δprolactin correlated to improved PALTEA performance for the MIT group. (**B**) Individual MIT workload levels subdivided based on gender, with solid lines representing males and dotted lines representing females. (**C**) The total cumulative workload after 6 months of exercise for the MIT group significantly correlated to ΔPALTEA change with higher total workload correlating to better PALTEA improvement. Spearman correlations were used for analysis.