

1 ***APOE<sup>ε4</sup> and exercise interact to influence systemic***  
2 ***and cerebral risk factors for dementia***

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27 **Abstract**

28 INTRODUCTION: *APOE*<sup>ε4</sup> is the strongest genetic risk factor for Alzheimer's disease and  
29 related dementias (ADRDs) affecting many different pathways that lead to cognitive decline.  
30 Exercise is one of the most widely proposed prevention, and intervention strategies to mitigate  
31 risk and symptomology of ADRDs. Importantly, exercise and *APOE*<sup>ε4</sup> affect similar processes on  
32 the body and brain. While both *APOE*<sup>ε4</sup>, and exercise have been studied extensively, their  
33 interactive effects are not well understood.

34 METHODS: To address this, male and female *APOE*<sup>ε3/ε3</sup>, *APOE*<sup>ε3/ε4</sup> and *APOE*<sup>ε4/ε4</sup> mice ran  
35 voluntarily from wean (1mo) to midlife (12mo). Longitudinal and cross-sectional phenotyping  
36 was performed on the periphery and the brain, on markers of risk for dementia such as weight,  
37 body composition, circulating cholesterol composition, activities of daily living, energy  
38 expenditure, and cortical and hippocampal transcriptional profiling.

39 RESULTS: Data revealed chronic running decreased age-dependent weight gain, lean and fat  
40 mass, and serum LDL concentration dependent on *APOE* genotype. Additionally, murine  
41 activities of daily living and energy expenditure were significantly influenced by an interaction  
42 between *APOE* genotype and running in both sexes. Transcriptional profiling of the cortex and  
43 hippocampus predicted that *APOE* genotype and running interact to affect numerous biological  
44 processes including vascular integrity, synaptic/neuronal health, cell motility, and mitochondrial  
45 metabolism, in a sex-specific manner.

46 DISCUSSION: These data provide compelling evidence that *APOE* genotype should be  
47 considered for population-based strategies that incorporate exercise to prevent ADRDs.

48

## 49 1. Background

50 Aging and *APOE*<sup>ε4</sup> are the strongest risk factors for Alzheimer's disease and related  
51 dementias (ADRDs)[1]. With *APOE*<sup>ε4</sup> implicated in unfavorable systemic changes such as high  
52 BMI, dysregulated cholesterol concentrations, and aberrant metabolism, as well as deficits in  
53 cerebral health such as changes in cerebral metabolism, cerebrovasculature, and neuronal  
54 health, the *APOE*<sup>ε4</sup> allele has been targeted to help reverse these risks[2-7]. The cerebral  
55 changes caused by *APOE*<sup>ε4</sup> emerge in humans at early ages and can worsen with advancing  
56 age[8-12]. Further, the impact of *APOE*<sup>ε4</sup> dosage (such as in the *APOE*<sup>ε3/ε4</sup> versus *APOE*<sup>ε4/ε4</sup>  
57 genotype) on peripheral and brain health during aging is understudied. Targeting *APOE*<sup>ε4</sup>  
58 through pharmacological interventions has resulted in both beneficial and damaging outcomes  
59 meaning therapies targeting APOE-dependent pathways will likely need to be tailored to specific  
60 mechanisms[13-16].

61 While pharmacological interventions are still being investigated, others have turned to  
62 non-pharmacological interventions to reduce risk for ADRDs, such as exercise[13, 17]. Studies  
63 in mice show benefits of exercise to peripheral health, as well as improvements to cognitive  
64 function[18-27]. Though the cognitive changes due to exercise have been controversial, with  
65 human studies showing either no change or improvements with exercise, it is widely accepted  
66 that exercise affects the body in a generally positive manner (i.e., decreasing weight/fat mass,  
67 improving metabolism and circulation, and elevating mood)[19, 28-33]. While understanding the  
68 effect of exercise on neuronal health is critical, other compartments of the brain are largely  
69 neglected. It is essential to understand how exercise affects all mechanisms that pertain to  
70 ADRD risk, such as metabolism and vascular health.

71 It is unknown if the detrimental effects associated with *APOE*<sup>ε4</sup> can be mitigated by  
72 exercise, or conversely, whether the effects of exercise are impacted by *APOE*<sup>ε4</sup> genotype.  
73 Studies in humans are performed later in life after symptom onset, typically measuring  
74 improvements to activities of daily living and quality of life. While important, it is necessary to

75 understand whether running can influence risk factors for dementia before symptomology. We  
76 evaluated the systemic and cerebral effects of running across  $APOE^{\epsilon 3/\epsilon 3}$ ,  $APOE^{\epsilon 3/\epsilon 4}$  and  
77  $APOE^{\epsilon 4/\epsilon 4}$  litter-matched mice during early aging. We show that chronic running affects multiple  
78 ADRD-relevant phenotypes in both the periphery and the brain but these effects are both  $APOE$   
79 genotype- and sex-specific.

80

## 81 **2. Methods**

82

### 83 **2.1 Mouse Husbandry**

84 Novel  $APOE$  mouse strains were created on C57BL/6J (B6) and maintained at The Jackson  
85 Laboratory as previously described prior[34]. Mice were kept in a 12/12-hour light/dark cycle  
86 (06:00 – 18:00 light) and fed ad libitum 6% kcal fat standard mouse chow. Experimental cohorts  
87 were generated by intercrossing male and female  $APOE^{\epsilon 3/\epsilon 4}$  mice to create  $APOE^{\epsilon 3/\epsilon 3}$ ,  $APOE^{\epsilon 3/\epsilon 4}$   
88 and  $APOE^{\epsilon 4/\epsilon 4}$  male and female littermate controls. Animals were divided as evenly as possible  
89 per litter into running and sedentary cohorts. All experiments were approved by the Institutional  
90 Animal Care and Use Committee (IACUC) at The Jackson Laboratory.

91

### 92 **2.2 Exercise by Voluntary Running**

93 Mice were group-housed into two or three per pen and given 24-hour access to an unlocked  
94 (running) or locked (sedentary) running wheel (Innovive Inc). At 5 months (5mo), mice were  
95 singly housed for the duration of the experiment. At 6 and 11mo, running mice were tracked for  
96 number of rotations per minute for five to seven nights during the dark cycle when they are most  
97 active using trackable running wheels (Med Associates Inc.). Any nights that had fewer than 700  
98 minutes tracked were excluded from analysis. For each mouse, sum of rotations per night was  
99 calculated and then averaged across all nights.

100

### 101 **2.3 Harvesting, Tissue Preparation, Plasma Collection**

102 All mice were euthanized by intraperitoneal injection of a lethal dose of Ketamine  
103 (100mg/ml)/Xylazine(20mg/ml). Mice were perfused intracardially with 1XPBS. Brains were  
104 carefully dissected, hemisected sagittally, and one half was then snap frozen on solid CO<sub>2</sub> for  
105 later dissection and RNA-sequencing. At timepoints throughout the experiment, blood plasma  
106 was collected via cheek bleed. Blood was carefully collected in K2 EDTA (1.0mg) microtainer  
107 tubes (BD), allowed to sit at room temperature for at least 30 minutes, and then centrifuged at  
108 21°C for 10 minutes at 5000rpm. Plasma was carefully collected and stored at -20°C. At the  
109 harvest timepoint (12mo), blood was collected in K2 EDTA (1.0mg) microtainer tubes (BD)  
110 through cardiac puncture. Plasma total cholesterol (mg/dL), direct LDL (mg/dL), and HDL  
111 (mg/dL) concentrations were characterized on the Beckman Coulter AU680 chemistry analyzer.  
112 All samples were profiled at the same time at the end of the experiment to avoid batch effects.

113

### 114 **2.4 Nuclear Magnetic Resonance Imaging (NMR)**

115 Each cohort was subjected to NMR imaging at 6 and 11mo. NMR was performed as previously  
116 described[35]. Briefly, weight was measured, and mice were briefly placed into a Plexiglas tube  
117 2.5 in. by 8 in. which was then subjected to NMR (EchoMRI, Houston, TX). Magnetic field was  
118 measured by a 5-gauss magnet. Measurements included weight, lean muscle mass, and fat  
119 mass, as well as fat percentage ((fat/body weight) × 100).

120

### 121 **2.6 Activities of daily living and Indirect Calorimetry**

122 After NMR measurements, groups of 16 mice were measured at a time for energy balance  
123 through indirect calorimetry measurement cages (Sable Promethion). Briefly, these specialized  
124 cages continuously measure food and water intake, general activity (pedometers), wheel  
125 running behavior, energy expenditure (kcal/hr), and respiratory quotient (RQ). Measurements  
126 are collected for five days in five-minute interval bins. The respiratory quotient (RQ) is a ratio of

127 the volume of carbon dioxide (CO<sub>2</sub>) released over the volume of oxygen (O<sub>2</sub>) absorbed. RQ has  
128 been widely used in humans and mice as a tool to determine the starting substrate for energy  
129 metabolism (carbohydrate RQ ~1, protein RQ ~0.8, fat RQ ~0.7, anaerobic respiration RQ ~0,  
130 and multiple energy sources RQ ~0.8) [36-41].

131

## 132 **2.7 RNA-sequencing, linear modeling and GSEA**

133 RNA extraction, library construction, RNA sequencing and seq quality control was performed as  
134 described previously[34, 35]. Genes were then filtered by 1) removing all genes that did not vary  
135 in expression (gene count change across all samples was 0) and 2) removing all genes that did  
136 not have at least five reads in 50% of the samples. Remaining genes (20,641) were normalized  
137 using DEseq2[42]. Principal component analysis (PCA) on the variance stabilized data (vst)  
138 identified outliers. To allow for the evaluation of *APOE*<sup>ε4</sup> allele dosage, each linear model  
139 included two genotype comparisons: 1) *APOE*<sup>ε3/ε4</sup> to *APOE*<sup>ε3/ε3</sup> and 2) *APOE*<sup>ε4/ε4</sup> to *APOE*<sup>ε3/ε3</sup>.  
140 Linear models were run separately for 1) cortex - female, 2) cortex – male, 3) hippocampus –  
141 female, and 4) hippocampus – male. β-estimates were obtained for all four linear models that  
142 evaluated the main effects of *APOE* genotype (*APOE*<sup>ε3/ε4</sup>, *APOE*<sup>ε4/ε4</sup>, ref: *APOE*<sup>ε3/ε3</sup>) and running  
143 (run, ref: sed), as well as the interaction between *APOE* genotype and running (*APOE*<sup>ε3/ε4</sup>:Run,  
144 *APOE*<sup>ε4/ε4</sup>:Run). For each linear model, gseGO from the clusterProfiler package was run on  
145 genes significant for each factor. Gene Set Enrichment Analysis (GSEA) was used to determine  
146 GO terms for the genes significant for the main (running, *APOE*<sup>ε3/ε4</sup>, *APOE*<sup>ε4/ε4</sup>) and interacting  
147 factors (*APOE*<sup>ε3/ε4</sup>:Run and *APOE*<sup>ε4/ε4</sup>:Run). Normalized Enrichment Scores (NES) from GSEA  
148 were used to identify terms that were positively or negatively associated with each factor. GO  
149 terms were ordered based on the NES. Terms with a positive NES had more genes higher on  
150 the ranked list (ie. more positive β values) and the terms with a negative NES containing more  
151 genes lower on the ranked list (ie., more negative β values). Enriched GO terms had

152 overlapping biological functions that we termed ‘vascular integrity’, ‘cellular motility’, ‘immune  
153 system response’, ‘mitochondrial metabolism’, and ‘synaptic/neuronal health’. The top 20 most  
154 positive and negative GO terms were visualized for the cortex and hippocampus for both  
155 females and males (**Supp Figs 10-24**).

156

## 157 **2.10 Statistical Analysis**

158 For all weights and body composition analysis, a two-way ANOVA for *APOE* genotype, activity,  
159 and the interaction between *APOE* genotype and activity was calculated. Bonferroni post hoc  
160 corrections were calculated and significance within genotype (the effect of running per  
161 genotype) was visualized.

162

## 163 **3. Results**

### 164 ***APOE* genotype did not affect voluntary running from young to midlife**

165 To determine the effects of one *APOE*<sup>ε4</sup> allele to two *APOE*<sup>ε4</sup> alleles, we compared the  
166 *APOE*<sup>ε3/ε4</sup> and *APOE*<sup>ε4/ε4</sup> genotypes to the control, *APOE*<sup>ε3/ε3</sup> genotype (**Fig 1A**). Previous studies  
167 have shown that females run more than males, therefore we assessed the sexes separately[35].  
168 There was no difference in voluntary running during the dark cycle across *APOE* genotypes,  
169 however there was expected variation between individual mice within the *APOE* genotypes (**Fig**  
170 **1B-C, Supp Fig 1-2**). There was an age-dependent decrease in voluntary running from 6-11mo,  
171 however there was no difference between *APOE* genotypes (**Fig 1D-G**). These findings show  
172 that running is not a variable between the *APOE* genotypes, and therefore not a confound in  
173 subsequent analyses.

174

175 ***APOE* genotype and running interact in a sex-specific manner to modulate general**  
176 **markers of healthy aging**

177 Weight, body composition (e.g., lean mass, fat mass, and fat percentage) and  
178 cholesterol levels are commonly used as a general proxy for health in humans[43-45]. These  
179 biometrics are typically measured at routine physicals and are considered indicative of general  
180 health status, and markers for obesity, cardiovascular disruption, and lipid dysregulation[46-49].  
181 We examined whether running affected weight, body composition and cholesterol across *APOE*  
182 genotypes. Monthly weights (from 1-12mos) revealed an expected age-dependent weight gain  
183 in sedentary mice that was significantly attenuated by running (**Fig 2AF, Supp Fig 3A-D**). In  
184 females, but not males, the *APOE*<sup>ε4/ε4</sup> genotype caused a greater running-based attenuation in  
185 weight gain compared to *APOE*<sup>ε3/ε3</sup> and *APOE*<sup>ε3/ε4</sup>. These results suggest that the beneficial  
186 effects of running on weight loss are *APOE* genotype-dependent in females only.

187 Overall, running mice had a lower fat composition compared to sedentary mice for both  
188 sexes at 6 and 11mo (**Supp Fig 4,5**). In females, only *APOE*<sup>ε4/ε4</sup> mice showed a significant  
189 attenuation of fat mass and fat percentage in running compared to sedentary mice (**Fig 2G-I,**  
190 **Supp Fig 3E-G**). There were no *APOE* genotype differences in male mice, however there was  
191 an effect of running on lean and fat mass. This effect was most pronounced in *APOE*<sup>ε3/ε4</sup> male  
192 mice, with running attenuating lean and fat mass (**Fig 2J-L, Supp Fig 3H-J**). Running  
193 attenuated the age-related increase in lean and fat mass across all genotypes and sexes.  
194 However, there was a pronounced reduction of age-related fat mass accumulation in female  
195 *APOE*<sup>ε4/ε4</sup> running mice. Also, male *APOE*<sup>ε3/ε4</sup> running mice showed the greatest reduction in  
196 age-related lean and fat mass accumulation.

197 No effect of running or *APOE* genotype was determined for total cholesterol or HDL  
198 concentration at 12mo (**Supp Fig 6**). There was a significant sex-specific effect of *APOE*  
199 genotype on LDL concentration in the plasma. In running females, LDL concentrations  
200 decreased in an *APOE*<sup>ε4</sup> dose-dependent manner (**Supp Fig 6H**). Conversely, in running males,  
201 LDL concentrations were significantly lower than sedentary mice (**Supp Fig 6K**). Cholesterol



202 composition in running mice did not correlate with running distance for both sexes (**Supp Fig**  
203 **6C,F,I,L,O,R**).

204 Collectively, these data demonstrate that weight, body composition and cholesterol  
205 levels, commonly used markers of healthy aging, are significantly affected by voluntary running,  
206 but the effects are dependent upon both sex and *APOE* genotype.

207

### 208 **Running affects activities of daily living in an *APOE* genotype- and sex-specific manner**

209 In humans, prior to more severe cognitive decline in ADRDs, activities of daily living (i.e.,  
210 sleep, general movement, feeding) are often disrupted[50-52]. To evaluate activities of daily  
211 living in mice, we measured feeding and walking behavior (pedometers) across four dark cycles  
212 (active/awake period), and three light cycles (inactive/sleep period) at 11 mos. Feeding behavior  
213 revealed significant changes in the dark cycle, but not in the light cycle for both sexes. In  
214 females, running mice consumed more food than sedentary mice during the dark cycle across  
215 all *APOE* genotypes (**Fig 3A-B, Supp Fig 7**). However, in males, there was an interaction  
216 between *APOE* genotype and running during the dark cycle. In sedentary mice, *APOE*<sup>ε3/ε4</sup> ate  
217 more than *APOE*<sup>ε3/ε3</sup> and *APOE*<sup>ε4/ε4</sup>. This pattern was not apparent in running mice, suggesting  
218 running mitigates the *APOE* genotype differences observed in sedentary mice (**Fig 3D, Supp**  
219 **Fig 7**).

220 We next determined whether movement in the home cage was affected by *APOE*  
221 genotype and/or running by measuring walking (Ped meters) (**see Methods**). Female sedentary  
222 mice showed an *APOE*<sup>ε4</sup> dose-dependent increase in Ped meters that was attenuated by  
223 running (**Fig 3G-H, Supp Fig 7**). During the light cycle only *APOE*<sup>ε4/ε4</sup> females showed a  
224 significant reduction in Ped meters compared to their sedentary counterparts (**Fig 3I, Supp Fig**  
225 **7**). In male mice, both *APOE* genotype and running interacted to alter Ped meters during both  
226 the dark and light cycle, however running more strikingly increased cumulative Ped meters of  
227 *APOE*<sup>ε4/ε4</sup> mice compared to the other *APOE* genotypes (**Fig 3K-L, Supp Fig 7**).

228           These results show that *APOE* genotype modulates the effects of running on natural  
229 home cage behaviors such as feeding and general movement, considered equivalent to  
230 activities of daily living in humans.

231  
232 ***APOE* genotype affects running-dependent increase in energy expenditure during the**  
233 **dark cycle**

234           Previous studies in humans demonstrated *APOE* genotype affects metabolism on a  
235 cellular, regional, and organismal level[53, 54]. To determine whether running and *APOE*  
236 genotype affect metabolic processes, energy expenditure (kcal/hr) was measured at 11mo. In  
237 female sedentary mice, energy expenditure showed an *APOE*<sup>ε4</sup> dose-dependent increase  
238 during the dark cycle. In general, running resulted in significantly higher energy expenditure in  
239 the dark cycle in male and female mice. However, this effect was not observed in male  
240 *APOE*<sup>ε3/ε4</sup> mice (**Fig 4A-C, Supp Fig 8**). This suggests the *APOE*<sup>ε3/ε4</sup> genotype attenuates the  
241 effects of running in a sexually dimorphic manner. During the light cycle, all genotypes showed  
242 decreased energy expenditure with running; except for female *APOE*<sup>ε3/ε3</sup> mice that showed an  
243 increase (**Fig 4D-F, Supp Fig 8**). Subtle but significant changes in substrate usage (based on  
244 respiratory quotient, RQ, **see methods**) were also determined across groups in both the light  
245 and dark cycle (**Fig 4G-L, Supp Fig 8**). Significant changes were small, however may worsen  
246 with more advancing age (**Fig 4H-L, Supp Fig 8**). These results highlight that *APOE* genotype  
247 and running affect energy expenditure, however changes in starting energy substrate usage  
248 were minute (**Fig 4A-F**).

249  
250 ***APOE* genotype causes subtle sex-specific changes to the effects of running on the**  
251 **aging brain**

252           Unbiased transcriptional profiling was utilized to capture molecular effects across *APOE*  
253 genotype and activities (12 groups per brain region, **Fig. 5A, See Methods**). Principal

254 component analysis (PCA) revealed brain region (PC1, 65%) and sex (PC2, 20%) were the  
255 primary drivers of variance, suggesting *APOE* genotype and running are not exerting strong  
256 effects (**Fig 5B**). Therefore, to determine subtle effects of *APOE* genotype and running, linear  
257 modeling was used for male or female cortex or hippocampus samples separately (4 linear  
258 models in total) (**Fig 5C**). Supporting the PCA data, linear modeling revealed fewer than 200  
259 significant genes in females for the cortex and hippocampus, and fewer than 800 genes in  
260 males. These numbers align with published data from human studies but are somewhat fewer  
261 than previous mouse studies (**Supp Fig 12**). Several significant genes including *Ephx1* (main  
262 effect:  $APOE^{\epsilon 3/\epsilon 4}$ ), *Ctsf* (main effect:  $APOE^{\epsilon 4/\epsilon 4}$ ), *C3* (interaction  $APOE^{\epsilon 3/\epsilon 4}$ :Run) and *Cav3*  
263 (interaction  $APOE^{\epsilon 4/\epsilon 4}$ :Run) are known to function in lipid homeostasis, neuroinflammation and  
264 membrane integrity, key processes implicated in ADRD risk (**Fig 5H**).

265 For the female cortex, GO terms showed positive NES for the main effects (running,  
266  $APOE^{\epsilon 3/\epsilon 4}$ , and  $APOE^{\epsilon 4/\epsilon 4}$ ), but negative NES for the interactive terms ( $APOE^{\epsilon 3/\epsilon 4}$ :Run,  
267  $APOE^{\epsilon 4/\epsilon 4}$ :Run) for vascular and synaptic/neuronal functions (**Fig 6B-C**). Also, interestingly, in  
268 males, there were few significantly enriched GO terms for  $APOE^{\epsilon 3/\epsilon 4}$ , suggesting in males, but  
269 not females, the  $APOE^{\epsilon 3/\epsilon 4}$  genotype exerts little to no effect compared to the  $APOE^{\epsilon 3/\epsilon 3}$   
270 genotype on genes associated with vascular integrity-related processes (**Fig 6D**).

271 Transcriptional profiling supplemented our peripheral findings, determining that running and  
272 *APOE* genotype interact in sex-specific ways to influence mechanisms involved in dementia-  
273 relevant biological processes.

274

#### 275 4. Discussion

276 Exercise is generally considered to have beneficial effects, but our results show *APOE*  
277 genotype impacts the effects of running. Significant interactions between *APOE* genotype and  
278 running were observed across body weight, body composition, activities of daily living, systemic  
279 metabolism, and cortical and hippocampal gene expression. Male and female mice were

280 evaluated separately as ADRD risk varies between the sexes, with higher risk in women  
281 compared to men[55, 56]. Sex is typically used as a covariate in human studies, but our data  
282 show that *APOE* genotype and sex interact across multiple domains. Additionally, there is a lack  
283 of consideration that odds ratios are sex-specific when assessing clinical trials, obfuscating the  
284 effects of sex. Our data suggest *APOE* genotype for each sex should be considered for studies  
285 assessing exercise interventions to reduce risk for dementia.

286 While the brain has been shown to be plastic throughout adulthood, environmental  
287 influences can exert a greater effect on a younger brain compared to an older brain[19, 23, 35,  
288 57-60], prompting us to study the effects of *APOE* genotype and running from early age to  
289 midlife. We assessed 12mo mice to understand the effect of *APOE* and running up until midlife,  
290 likening our findings to prodromal studies in humans[61, 62]. *APOE* genotype-specific effects  
291 may also be apparent at older ages so studying later timepoints in the mouse, even beginning  
292 running at midlife would be informative. This would relate more closely with human clinical trials  
293 that conduct studies on older, affected human populations (i.e., nursing home/hospice  
294 patients)[63-65].

295 Transcriptomic approaches have revolutionized our understanding of ADRDs and has  
296 therefore become a hypothesis generating tool for identifying the molecular pathways impacted  
297 by genetic and environmental risk factors. Therefore, we used transcriptional profiling to identify  
298 interactions between *APOE* genotype and running. Our data revealed a reversal of NES  
299 direction from the main effects and the interaction of *APOE* genotype and running. This was  
300 unexpected, as we saw similar patterns of positive (or negative) enrichment for 1) running  
301 compared to sedentary, 2)  $APOE^{\epsilon 3/\epsilon 4}$  compared to  $APOE^{\epsilon 3/\epsilon 3}$  and 3)  $APOE^{\epsilon 4/\epsilon 4}$  compared to  
302  $APOE^{\epsilon 3/\epsilon 3}$ . These results contradict the assumption that running would have the opposite effect  
303 on the brain as  $APOE^{\epsilon 4}$ , particularly when considering each of these terms collectively (vascular,  
304 immune, mitochondrial, neuronal/synaptic). We propose that there is a possibility for  
305 overcompensation for the  $APOE^{\epsilon 4}$  allele. While evidence shows  $APOE^{\epsilon 4}$  causes gains and

306 losses of APOE function across many processes, it is unknown whether there is a preemptive  
307 response that has not been considered. Further, the  $APOE^{\epsilon 3/\epsilon 4}$  genotype may be responding to  
308 early aging phenotypes different than  $APOE^{\epsilon 4/\epsilon 4}$  genotype. Precise experimentation on this  
309 phenomenon is needed in both mice and humans to better understand which  $APOE^{\epsilon 4}$ - specific  
310 pathways are mitigated by running. Lastly, while these models are key for interpretation of  
311 APOE biology, other important pathological interactions (e.g., amyloid or TAU) are not present  
312 in this study. Future studies are necessary to interrogate the interaction between APOE,  
313 exercise, and hallmark ADRD pathologies in order to provide further translatable outcomes.

314         Advancements in RNA-sequencing have made it cheaper and faster to sequence the  
315 brains of ADRD patients (ROSMAP, MAYO, ADSP). Recently, research programs have  
316 explored whether *APOE* influences the human cerebral transcriptome. In three largescale AMP-  
317 AD studies, reports included few to no changes in multiple brain regions in  $APOE^{\epsilon 4}$ + cases  
318 (ROSMAP: syn8456629, MAYO: syn8466812, MSBB: syn8484987)[66-68] (**Supp Fig 12**). The  
319 ROSMAP dataset analysis showed no differences due to  $APOE^{\epsilon 4}$  status across the dorsolateral  
320 prefrontal cortex region[66]. The MAYO dataset showed a significant differential expression  
321 (DE) of only 173 genes between  $APOE^{\epsilon 3/\epsilon 4}$  and  $APOE^{\epsilon 3/\epsilon 3}$ , and a significant DE of only 88 genes  
322 between  $APOE^{\epsilon 4/\epsilon 4}$  and  $APOE^{\epsilon 3/\epsilon 3}$  in the temporal cortex[67]. The Mount Sinai Brain Bank  
323 (MSBB) reported fewer than 5 genes DE between all *APOE* genotype comparisons in the frontal  
324 pole region, parahippocampal gyrus region, frontal superior temporal gyrus region, and inferior  
325 frontal gyrus region[68]. Our mouse data aligns more closely with these human studies, possibly  
326 due to litter-matched mice, and further analyses using GSEA showed subtle changes that  
327 escaped detection through traditional DE analysis. Moving forward, our data show the  
328 importance of including heterozygous genotypes (e.g.,  $APOE^{\epsilon 3/\epsilon 4}$ ) and varying degrees of  
329 chronic voluntary exercise (e.g., low, medium, high) in mouse studies to improve the alignment  
330 to ADRD in human studies.

331 The *APOE<sup>ε4</sup>* allele emerged as our early hominin ancestors adapted to changes in  
332 habitat and food availability to include more aerobic exercise such as running[69]. The *APOE<sup>ε4</sup>*  
333 allele was beneficial for storage of fats, increasing cholesterol. While the *APOE<sup>ε4</sup>* conferred  
334 longer lifespan 200,000 years ago, the diet and exercise of an individual was drastically  
335 different[69]. Currently, western culture sees some of the highest rates of ADRD, due to the  
336 interaction between *APOE<sup>ε4</sup>* and our environment, and as we show, running. This work supports  
337 that *APOE<sup>ε4</sup>* interacts with running in a genotype- and sex- specific manner, influencing ADRD  
338 risks in the periphery and brain.

339

#### 340 **Keywords**

341 APOE

342 Exercise

343 Alzheimer's disease

344 Dementia

345 Running

346

#### 347 **Author contributions**

348 KEF and GRH conceived and designed this project. KEF, CAD, AAH performed mouse  
349 experiments. KEF performed IF, experimental analysis, and bioinformatic analysis. KEF and  
350 GRH consulted for statistical approach and analysis. KEF and GRH wrote and prepared this  
351 manuscript. All authors read and approved the final manuscript.

352

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360

### 361 **Declarations**

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364

### 365 **Ethics Approval and Consent to Participate**

366 No human subjects or data was used in this study. All experiments involving mice were  
367 approved by the Animal Care and Use Committee at The Jackson Laboratory in  
368 accordance with guidelines set out in The Eighth Edition of the Guide for the Care and  
369 Use of Laboratory Animals. All euthanasia used methods were approved by the  
370 American Veterinary Medical Association.

371

### 372 **Availability of Data and Materials**

373 All data is being uploaded to the AD Knowledge Portal. ID numbers will be provided  
374 once the process is complete.

375

### 376 **Competing interests and Disclosures**

377 The authors declare they have no competing interests or disclosures.

378

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571

572 **Figure 1: Voluntary chronic running to midlife is not different between *APOE* genotypes**

573 **(A)** Schematic of the voluntary running paradigm where *APOE*<sup>ε3/ε3</sup>, *APOE*<sup>ε3/ε4</sup> and *APOE*<sup>ε4/ε4</sup>  
574 male and female mice were introduced to a locked (control – sedentary) or unlocked (treatment  
575 - running) running wheel at 1 month (mo) until 12mo (midlife). Longitudinal, advanced, and post-  
576 mortem phenotyping is indicated. **(B-C)** No difference in running (average rotations across  
577 multiple consecutive nights) between *APOE* genotypes at both six and eleven months in female  
578 (B) or male (C) mice. **(D-G)** Average rotations per mouse at 6mo and 12mo showed an age-  
579 dependent decrease for both females (D) and males (F) however the change over time was not  
580 significantly different between genotypes **(E,G)**. Data presented as mean ± SD, one way  
581 ANOVA with Tukey's multiple comparison performed for **B,C,E,G**. Two-sided paired t-test  
582 performed for **D,F**. \*P < 0.05, \*\*P < 0.01.

583

584 **Figure 2: Running attenuated age-dependent weight gain and fat accumulation across**  
585 ***APOE* genotypes**

586 **(A-B)** Expected age-dependent weight gain from one to twelve months in females (A) and  
587 males (B). **(C-D)** Running mice weighed significantly less at 12mo in both females (C) and  
588 males (D). **(E-F)** Running significantly attenuated age-dependent weight gain (the difference in  
589 body weight from 1 to 12mo) in both females (E) and males (F). **(G-I)** Significant effect of  
590 running on the change in lean mass (G), fat mass (H), and fat percentage (I) between six and  
591 eleven months, with an overall reduction in running mice compared to sedentary mice across all  
592 *APOE* genotypes in females. **(J-L)** Running had a significant reduction on the change in lean  
593 mass (J) and fat mass (K) between six and eleven months, but no change in fat percentage (L)  
594 in male mice. Data presented as mean ± SEM, two-way ANOVA performed for *APOE* genotype  
595 (significant marked above 'Sed' column, indicating an effect of *APOE* genotype), Running  
596 (significance marked above 'Run' column, indicating an effect of running), and the interaction

597 between *APOE* genotype:Running (significance marked to the right of the graph). Bonferroni's  
598 multiple comparisons performed for within genotype running effects (significance marked in  
599 smaller stars directly to the right of the run column, within graph limits, in the color of the  
600 genotype). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

601

### 602 **Figure 3: Activities of daily living are influenced by *APOE* genotype and running**

603 **(A,D)** Cumulative food consumed per gram for female (A) and male (D) mice across four dark  
604 cycles and three light cycles. **(B-C)** Running significantly increased food consumed during the  
605 dark cycle (B), but not the light cycle (C) in female mice. **(E-F)** *APOE* genotype and *APOE*  
606 genotype:running interaction affected food consumption in males during the dark cycle (E), but  
607 no effect was seen during the light cycle (F). **(G,J)** General movement (cumulative ped meters)  
608 for female (G) and male (J) mice across four dark cycles and three light cycles. **(H-I)** *APOE*  
609 genotype, Running, and *APOE* genotype:Running interaction all significantly affected ped  
610 meters during the dark cycle (H) with running decreasing ped meters differently across the  
611 genotypes. Only *APOE* genotype was significant during light cycle (I) in female mice. **(K-L)**  
612 *APOE* genotype:Running interaction significantly affected ped meters in males, with *APOE*<sup>ε4/ε4</sup>  
613 showing increased ped meters during the dark cycle (K), as well as the light cycle (L). There  
614 was also an *APOE* genotype effect (L). Data presented as mean ± SEM, two-way ANOVA  
615 performed for *APOE* genotype (significance marked above 'Sed' column, indicating an effect of  
616 *APOE* genotype), Running (significance marked above 'Run' column, indicating an effect of  
617 running), and *APOE* genotype:Running interaction (significance marked in to the right of the  
618 graph). Bonferroni's multiple comparisons performed for within genotype running effects  
619 (significance marked in smaller stars directly to the right of the run column, within graph limits, in  
620 the color of the genotype). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

621

622

623 **Figure 4: Running influences energy expenditure differently between females and male**

624 ***APOE* mice**

625 **(A-C)** Energy expenditure (kcal/hr) across four dark cycles and three light cycles for female  
626 mice. Energy expenditure (kcal/hr) significantly affected by *APOE* genotype:Running, and  
627 Running during the dark cycle (B), with an increase in energy expenditure in running mice.  
628 *APOE* genotype:Running, *APOE* genotype, and Running all influenced light cycle energy  
629 expenditure (C), with *Apoε3/3* increasing with running while *APOE<sup>ε3/ε3</sup>* and *APOE<sup>ε4/ε4</sup>*  
630 decreased energy expenditure with running. **(D-F)** Energy expenditure (kcal/hr) across four dark  
631 cycles and three light cycles for male mice. (E) *APOE* genotype:Running, *APOE* genotype, and  
632 Running all affected dark cycle energy expenditure in male mice. (F) *APOE* genotype:Running  
633 and Running showed an overall decrease in energy expenditure in running male mice. **(G-I)**  
634 Respiratory Quotient (RQ) across four dark cycles and three light cycles for female mice (G).  
635 *APOE* genotype:Running significantly affected RQ during the dark cycle for female mice (H).  
636 *APOE* genotype and Running significantly affected RQ during the light cycle for female mice (I).  
637 **(J-L)** Respiratory Quotient (RQ) across four dark cycles and three light cycles for male mice (J).  
638 *APOE* genotype:Running, *APOE* genotype, and Running all significantly affected RQ during the  
639 dark cycle in male mice (K). *APOE* genotype:Running significantly affected RQ during the light  
640 cycle (L). Data presented as mean ± SEM, two-way ANOVA performed for *APOE* genotype  
641 (significant marked above 'Sed' column, indicating an effect of *APOE* genotype), Running  
642 (significance marked above 'Run' column, indicating an effect of running), and the interaction  
643 between *APOE* genotype:Running (significance marked to the right of the graph). Bonferroni's  
644 multiple comparisons performed for within genotype running effects (significance marked in  
645 smaller stars directly to the right of the run column, within graph limits, in the color of the  
646 genotype). \*P < 0.05, \*\*P < 0.01, \*\*\*P < 0.001, \*\*\*\*P < 0.0001.

647

648 **Figure 5: Transcriptional profiling reveals subtle changes due to *APOE* genotype and**  
649 **running in the cortex and hippocampus**

650 **(A)** Diagram of the cortical and hippocampal regions of the brain taken for transcriptional  
651 profiling. **(B)** PCA revealed clear separations between brain regions (cortex and hippocampus,  
652 65% variance explained), as well as by sex (female and male, 20% of variance explained). **(C)**  
653 Schematic of the computational analysis approach; first RNA-seq was separated by brain  
654 region, next separated again by sex. Four linear models were run to examine gene expression  
655 as it varies with Running, *APOE* genotype, and the interaction between *APOE*  
656 genotype:Running.  $\beta$ -value is the association of the gene with the factor tested – positive  $\beta$ -  
657 value indicates a positive correlation, negative  $\beta$ -value indicates a negative correlation. **(D-G)**  
658 Number of significant genes (FDR corrected) for female cortex (D), female hippocampus (E),  
659 male cortex (F), and male hippocampus (G). **(H)** Example of a significant gene for each of the  
660 main effects and interactive effects: *Meox1* (Hippocampus, Male), *Ephx1* (Hippocampus, Male),  
661 *Ctsf* (Hippocampus, Female), *C3* (Hippocampus, Male), *Cav3* (Cortex, Male) .

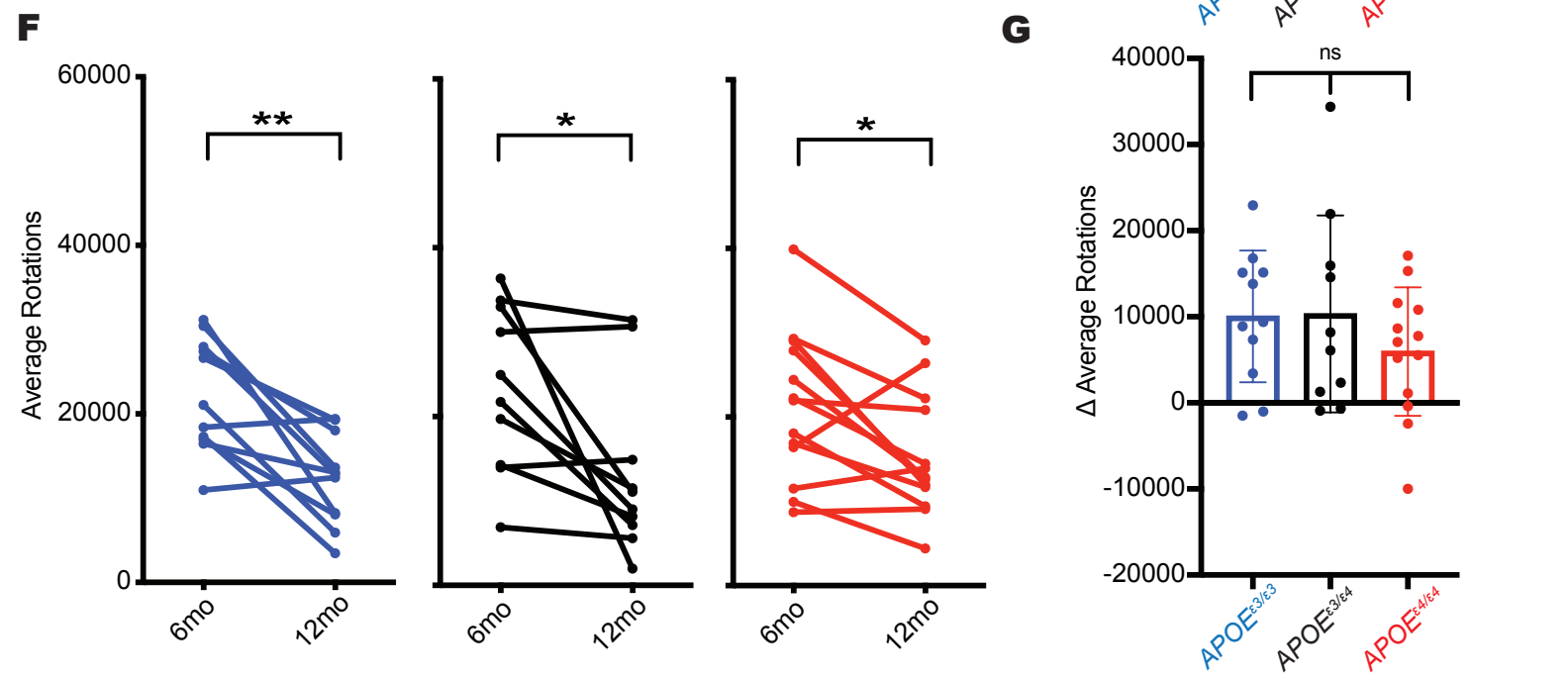
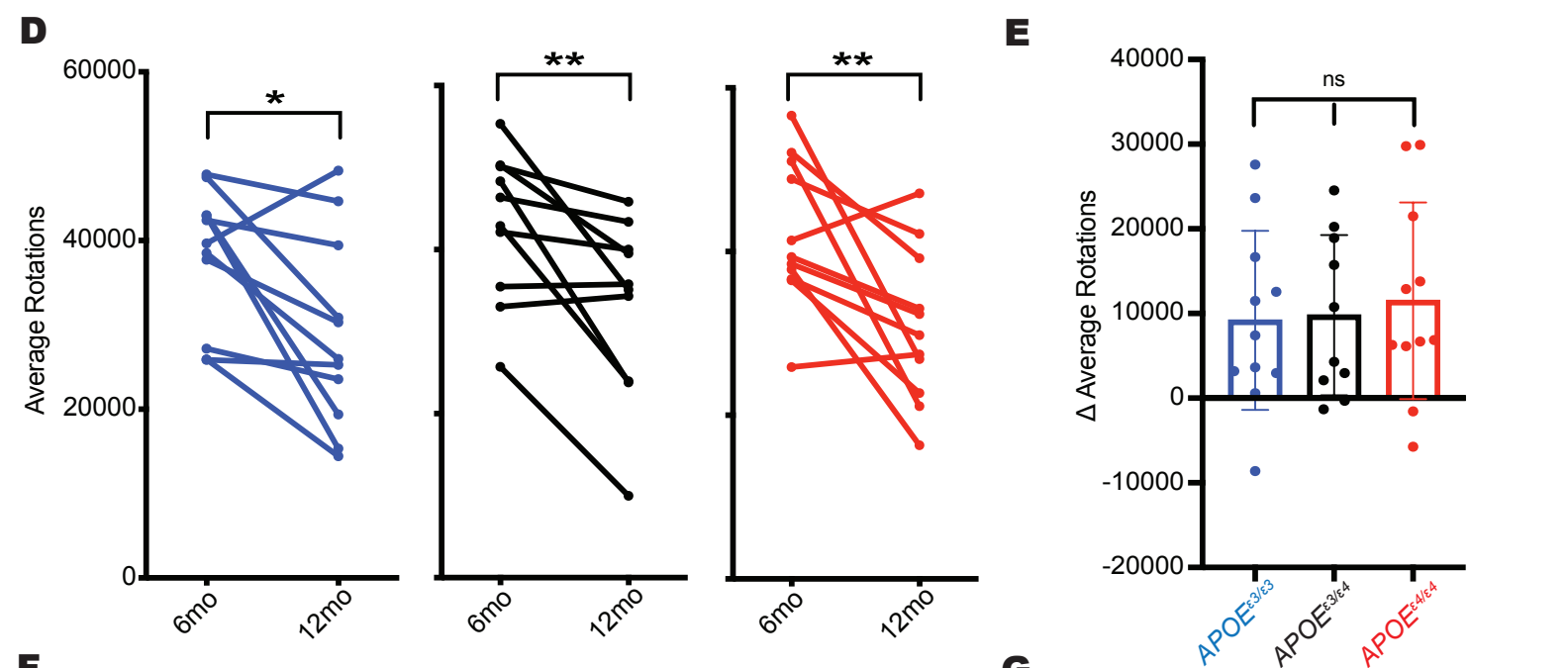
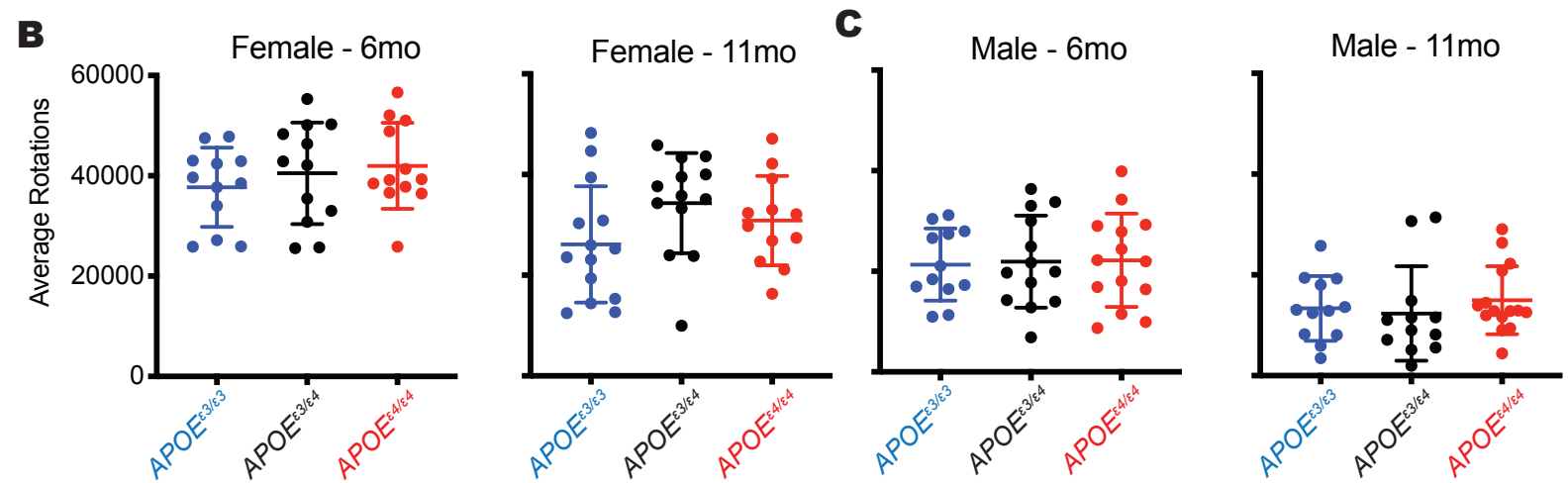
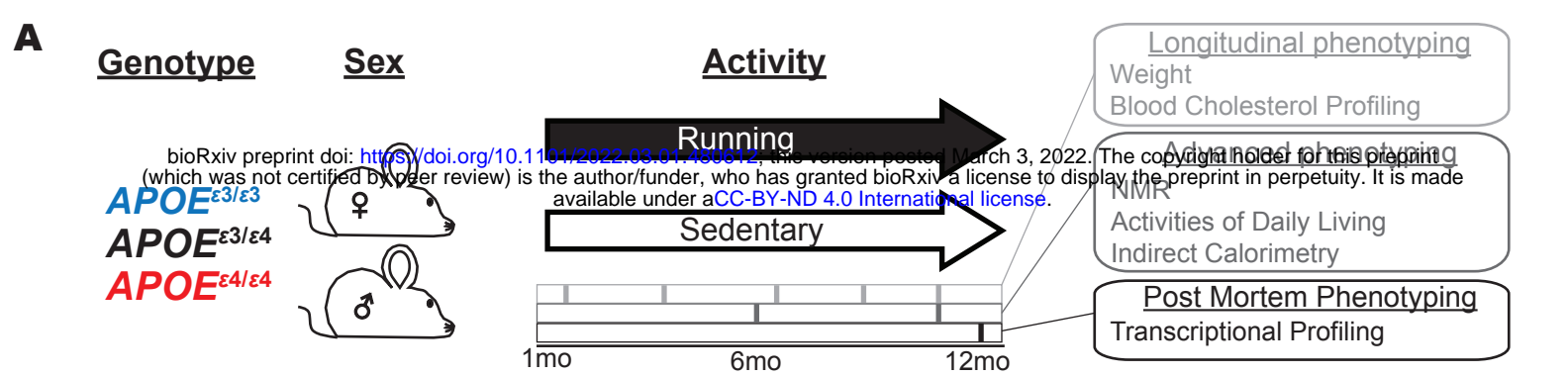
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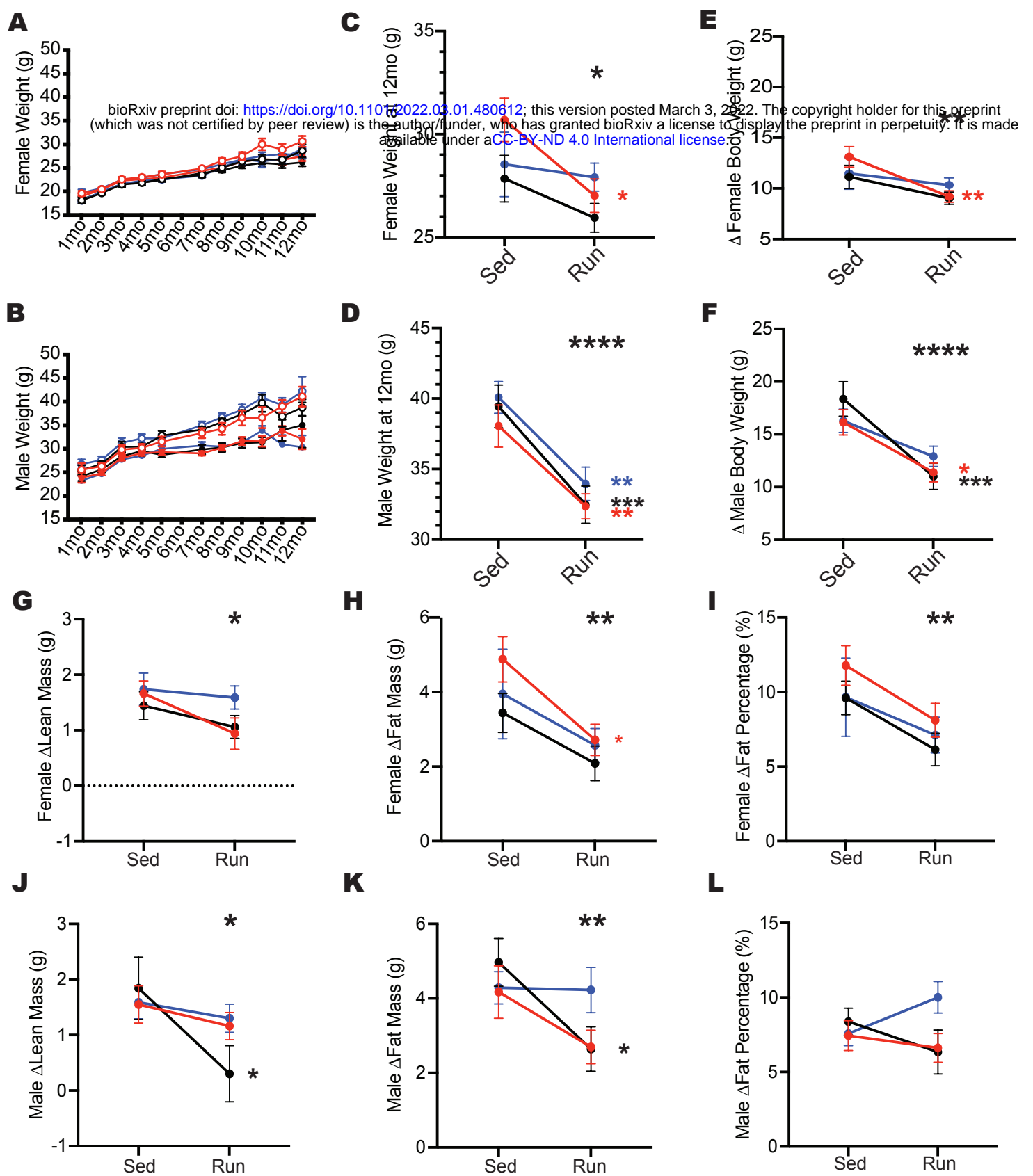
663 **Figure 6: GSEA predicts *APOE* genotype and running interact to mitigate main effects**

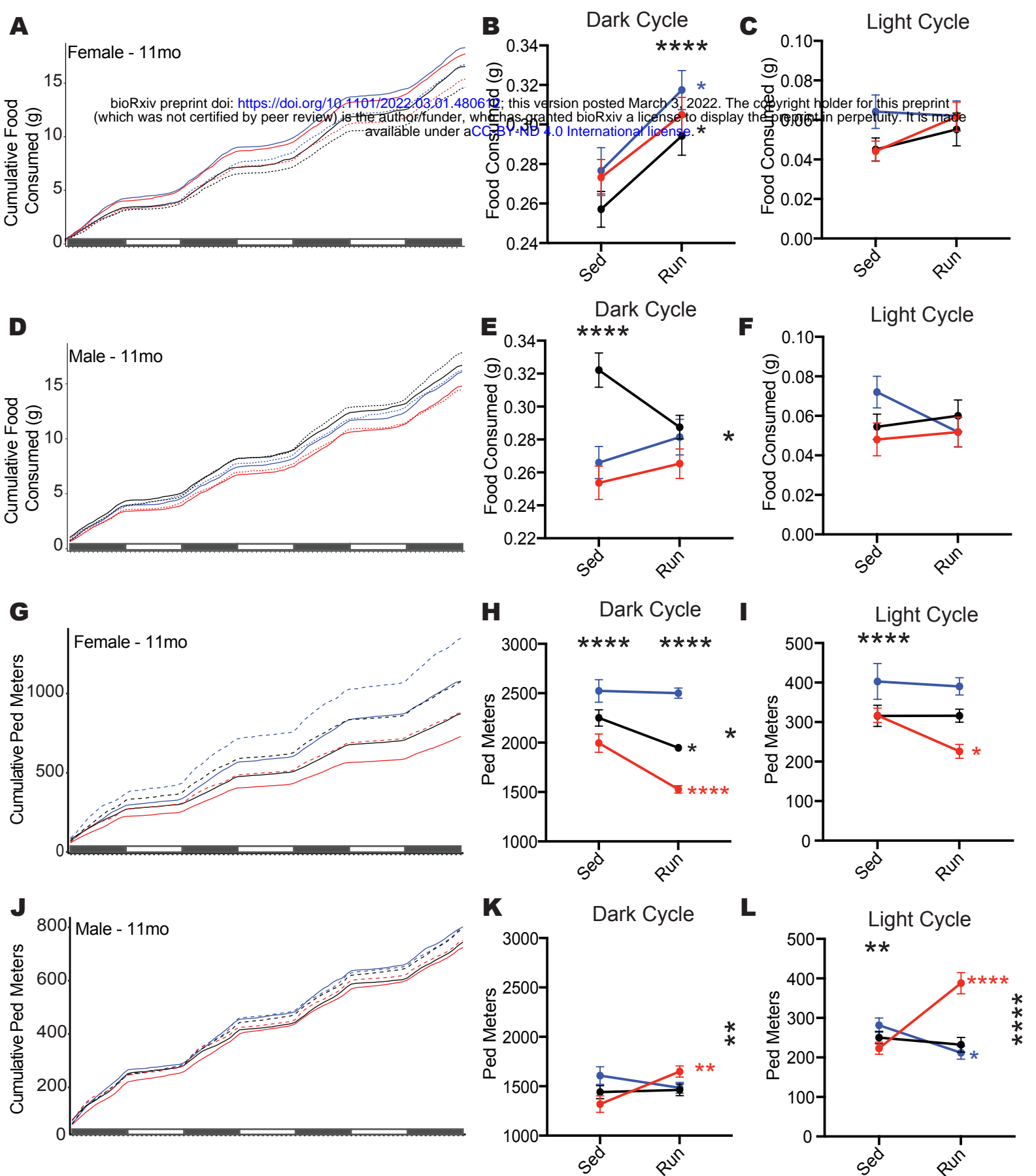
664 **(A)** Schematic of computational approach;  $\beta$ -values from linear models were passed through  
665 GSEA for gene ranking, GSEA plots were used to visualize results and main effects of running,  
666 *APOE* genotype, and *APOE* genotype:Running were interpreted per GO term. **(B)** GSEA plots  
667 for ‘Collagen Fibril Organization’ in the female cortex. Main effects of Running and  $APOE^{\epsilon 3/\epsilon 4}$ ,  
668 and  $APOE^{\epsilon 4/\epsilon 4}$  all show positive Enrichment scores, while the interactions,  $APOE^{\epsilon 3/\epsilon 4}$ :Run and  
669  $APOE^{\epsilon 4/\epsilon 4}$ :Run reveal negative Enrichment Scores. **(C)** In the female cortex data GO terms that  
670 fit the pattern shown in (B), colored by Normalized Enrichment Score (NES), are represented  
671 specifically vascular integrity, mitochondrial metabolism, and synaptic/neuronal health. **(D)** The  
672 pattern observed in male cortex was different to that seen in female cortex (B,C) with  $APOE^{\epsilon 3/\epsilon 4}$

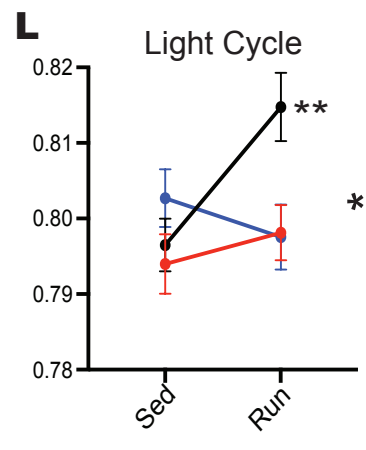
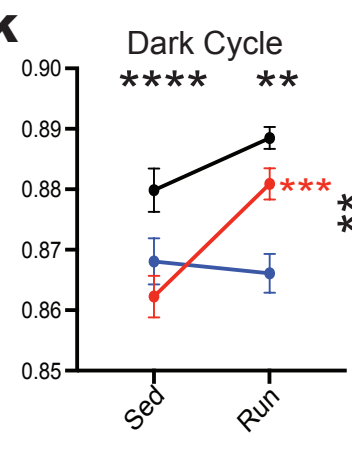
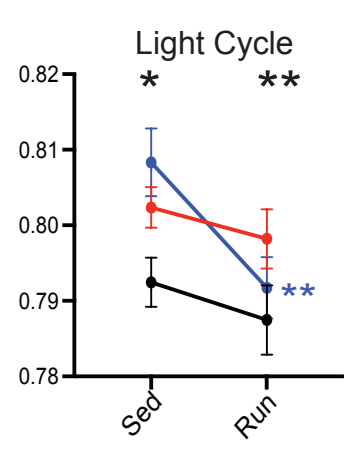
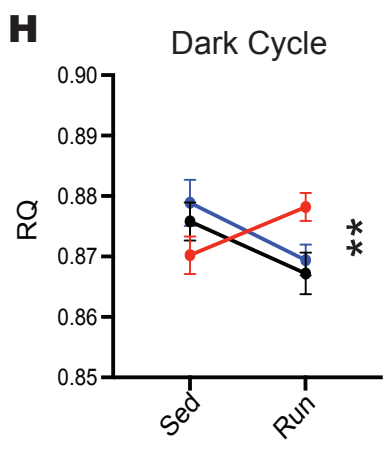
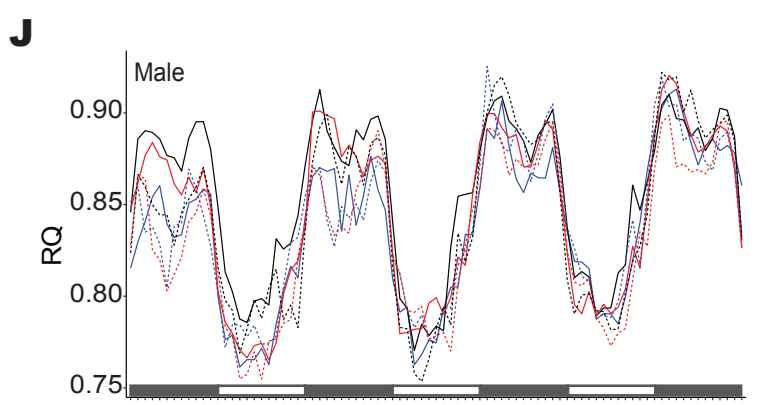
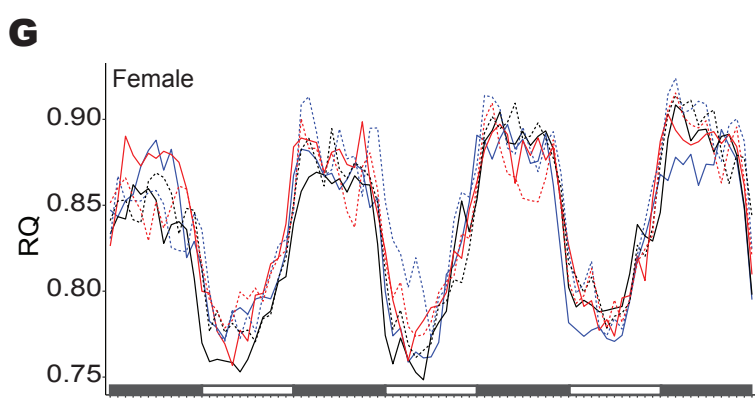
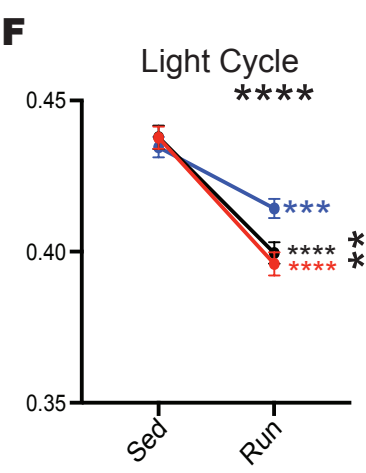
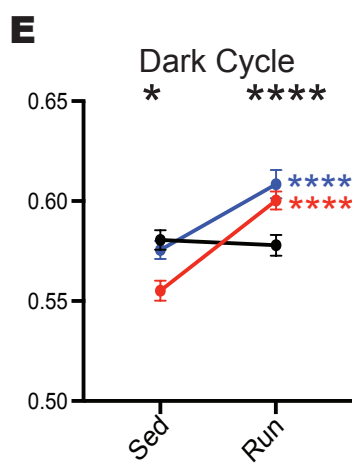
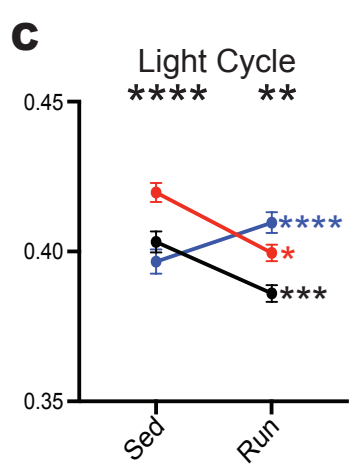
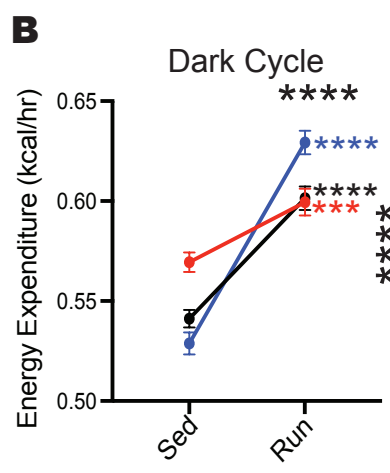
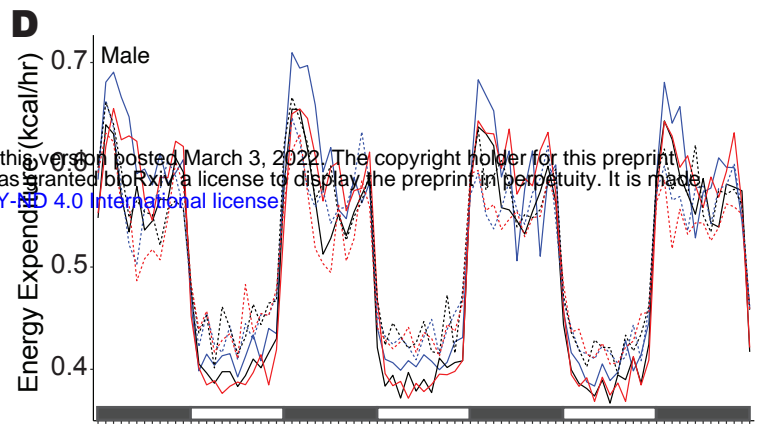
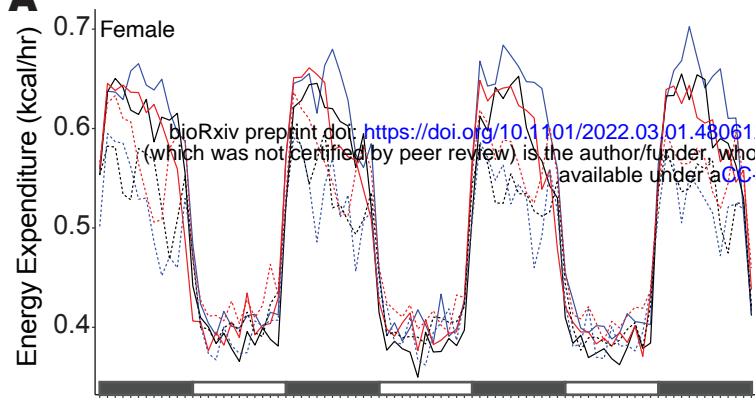


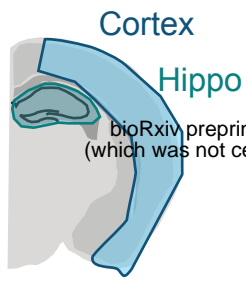
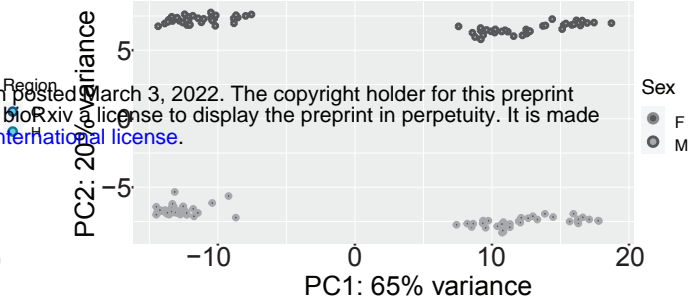
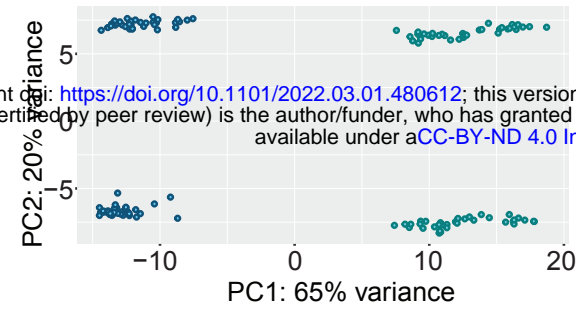
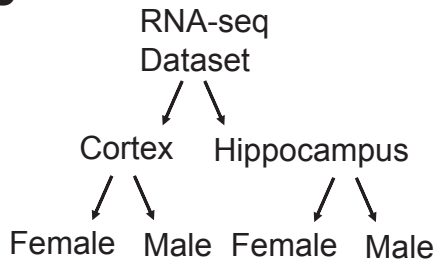
673 appearing more similar to *APOE*<sup>ε3/ε3</sup> (indicated by gray boxes) for enrichment terms grouped as  
674 cell motility, mitochondrial metabolism, vascular integrity, and immune system response.







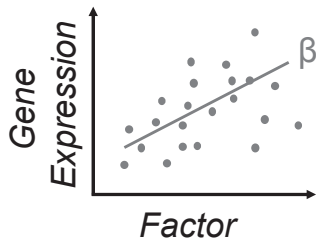
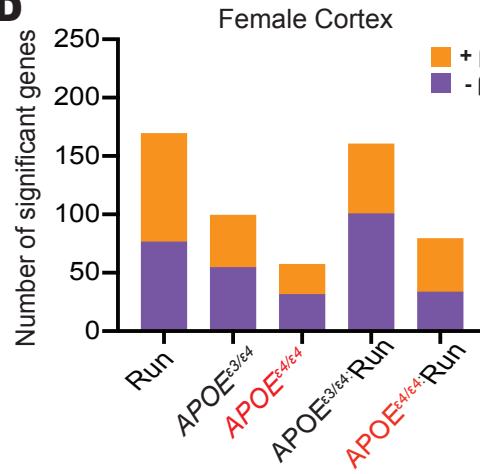
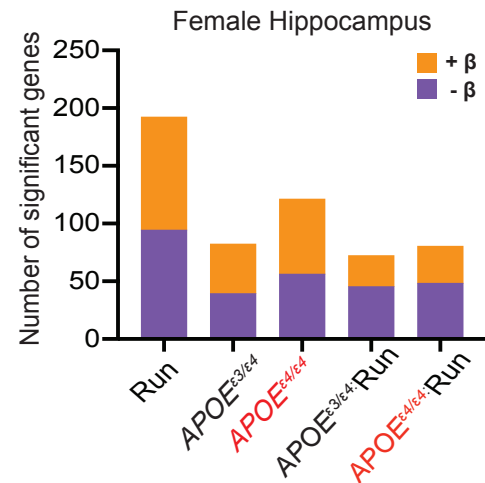
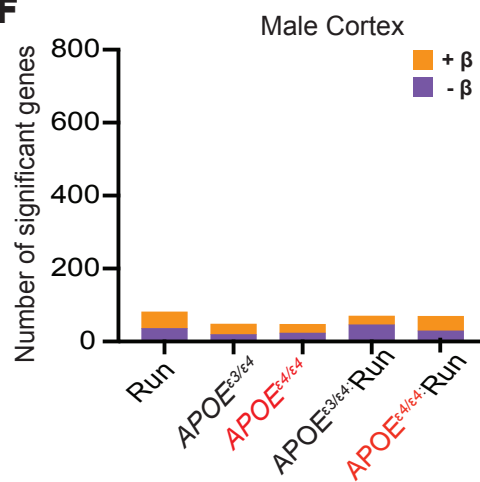
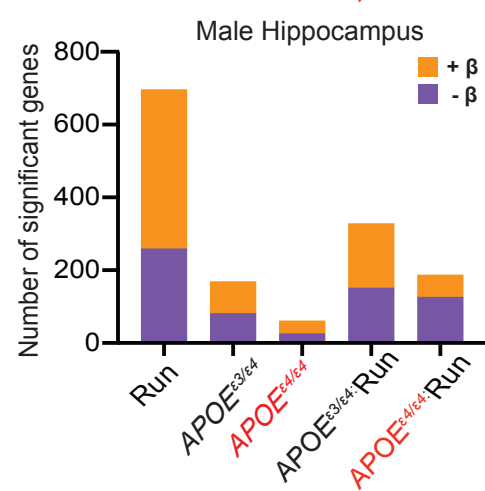


**A****B****C**

Linear Model:

Gene Expr.<sub>[ij]</sub> =

$$\beta(\text{Run}_{[ij]}) + \beta(\text{APOE Genotype}_{[ij]}) + \beta(\text{Run:APOE Genotype}_{[ij]})$$

**D****E****F****G****H**