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RESEARCH ARTICLE

APOE ε4 and exercise interact in a sex-specific manner to modulate dementia risk factors

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Abstract

Introduction: Apolipoprotein E (*APOE*) ε4 is the strongest genetic risk factor for Alzheimer's disease and related dementias (ADRDs), affecting many different pathways that lead to cognitive decline. Exercise is one of the most widely proposed prevention and intervention strategies to mitigate risk and symptomology of ADRDs. Importantly, exercise and *APOE* ε4 affect similar processes in the body and brain. While both *APOE* ε4 and exercise have been studied extensively, their interactive effects are not well understood.

Methods: To address this, male and female *APOE* ε3/ε3, *APOE* ε3/ε4, and *APOE* ε4/ε4 mice ran voluntarily from wean (1 month) to midlife (12 months). Longitudinal and cross-sectional phenotyping were performed on the periphery and the brain, assessing markers of risk for dementia such as weight, body composition, circulating cholesterol composition, murine daily activities, energy expenditure, and cortical and hippocampal transcriptional profiling.

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

Discussion: These data in humanized mouse models provide compelling evidence that *APOE* genotype should be considered for population-based strategies that incorporate exercise to prevent ADRDs and other *APOE*-relevant diseases.

KEYWORDS
Alzheimer's disease, apolipoprotein E, dementia, exercise, running

1 | BACKGROUND

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

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

It is unknown if the detrimental effects associated with *APOE* ε4 can be mitigated by exercise, or conversely, whether the effects of exercise are impacted by *APOE* ε4 genotype. Studies in previous models of *APOE* mice are usually completed in one sex, with some, but not all, studies showing age-related cognitive deficits. Other mouse studies show worsened ADRD pathology in *APOE* ε4 mice, begging the question whether *APOE* ε4's effects on ADRD can be influenced by exercise. Studies on exercise in humans are performed later in life after symptom onset, typically measuring improvements to activities of daily living and quality of life. While important, it is necessary to understand whether running can influence risk factors for dementia before symptomology. We evaluated the systemic and cerebral effects of running across *APOE* ε3/ε3, *APOE* ε3/ε4, and *APOE* ε4/ε4 litter-matched mice during early aging. We show that chronic running affects multiple ADRD-relevant phenotypes in both the periphery and the brain, but these effects are both *APOE* genotype- and sex-specific.

RESEARCH IN CONTEXT

1. **Systematic Review**: The authors have surveyed literature through traditional methods, conference presentations, and other online platforms (Alzforum.org). While exercise is often studied after dementia onset in humans, studies of exercise in dementia mouse models have had significant limitations.
2. **Interpretation**: Our findings support that apolipoprotein E (*APOE*) ε4 dosage affects the body and brain and interacts with running in a unique pattern that differs between males and females. These results should be considered during the development of strategies to prevent or reduce risk of human dementia through exercise.
3. **Future Directions**: This work highlights the interactions between *APOE* ε4 and running in male and female mice; however, interrogation of specific mechanisms is still necessary. While here we studied early aging (birth to midlife), future studies can determine the effects of advanced aging, *APOE* ε4 dosage, and running. Finally, human trials would be useful to validate these *APOE* ε4 genotype effects on the body and brain.

2 | METHODS

2.1 | Mouse husbandry

Novel *APOE* mouse strains were created on C57BL/6J (B6) and maintained at The Jackson Laboratory as previously described.[34] Mice were kept in a 12/12-hour light/dark cycle (06:00–18:00 light) and fed ad libitum 6% kcal fat standard mouse chow. Experimental cohorts were generated by intercrossing male and female *APOE* ε3/ε4 mice to create *APOE* ε3/ε3, *APOE* ε3/ε4, and *APOE* ε4/ε4 male and female littermate controls. As with previous studies, *APOE* ε3/ε3 mice served as the control genotype in these studies to standardize any human *APOE* insertion differences.[3,35–37] Animals were divided as evenly as possible per litter into running and sedentary cohorts. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at The Jackson Laboratory.

2.2 | Exercise by voluntary running

Mice were group-housed into two or three per cage and given 24-hour access to an unlocked (running) or locked (sedentary) running wheel (Innovive). At 5 months, mice were singly housed for the remaining duration of the experiment to enable data to be collected on individual mice. Mice were not returned to group housing to prevent fighting. At 6 and 11 months, running mice were tracked for number of rotations per minute during the dark cycle when they are most active using trackable running wheels (Med Associates, Inc.). Nights during which fewer than 700 minutes of data were tracked were considered incomplete and excluded from analysis. For each mouse, sum of rotations per night ($n = 7$ + nights tracked) was calculated and then averaged across all nights.

2.3 | Harvesting, tissue preparation, plasma collection

All mice were euthanized by intraperitoneal injection of a lethal dose of ketamine (100 mg/ml)/xylazine (20 mg/ml). Mice were perfused intracardially with 1X phosphate buffered saline. Brains were carefully dissected, hemisected sagittally, and one half was then snap frozen on solid CO_2 for later dissection and RNA sequencing. At timepoints throughout the experiment, blood plasma was collected via cheek bleed. Blood was carefully collected in K2 EDTA (1.0 mg) microtainer tubes (BD Biosciences), allowed to sit at room temperature for at least 30 minutes, and then centrifuged at 21°C for 10 minutes at 5031 *g*. Plasma was carefully collected and stored at −20°C. At the harvest timepoint (12 months), blood was collected in K2 EDTA (1.0 mg) microtainer tubes (BD Biosciences) through cardiac puncture. Plasma total cholesterol (mg/dL), direct low-density lipoprotein (LDL; mg/dL), and high-density lipoprotein (HDL; mg/dL) concentrations were characterized on the Beckman Coulter AU680 chemistry analyzer. All samples were profiled at the same time at the end of the experiment to avoid batch effects.

2.4 | Nuclear magnetic resonance imaging

Each cohort was subjected to nuclear magnetic resonance (NMR) imaging at 6 and 11 months (female: $n = 9–15$), male: $n = 11–15$). NMR was performed as previously described.[38] Weight was measured, and mice were briefly placed into a Plexiglas tube 2.5 in. by 8 in., which was then subjected to NMR (EchoMRI). Magnetic field was measured by a 5-gauss magnet. Measurements included weight, lean muscle mass, and fat mass, as well as fat percentage ([fat/body weight] × 100).

2.5 | Murine daily activities and indirect calorimetry

After NMR measurements (female: $n = 9–11$ per genotype/activity, male: $n = 9–12$ per genotype/activity), mice were measured for energy balance through indirect calorimetry measurement cages (Sable Promethion). Briefly, these specialized cages continuously measure food and water intake, general activity (pedometers), wheel running behavior, energy expenditure (kcal/hr), and respiratory quotient (RQ). Measurements are collected for 5 days in 5-minute interval bins. The RQ is a ratio of the volume of carbon dioxide (CO_2) released over the volume of oxygen (O_2) absorbed. RQ has been widely used in humans and mice as a tool to determine the starting substrate for energy metabolism (carbohydrate RQ ≈1, protein RQ ≈0.8, fat RQ ≈0.7, anaerobic respiration RQ ≈0, and multiple energy sources RQ ≈0.8).[39–44]

2.6 | RNA sequencing, linear modeling, and GSEA

We performed RNA sequencing on six brains per group (sex/genotype/activity) at 12 months. RNA extraction, library construction, RNA sequencing, and seq quality control were performed as described previously.[34,38] Genes were then filtered by (1) removing all genes that did not vary in expression (gene count change across all samples was 0) and (2) removing all genes that did not have at least five reads in 50% of the samples. Remaining genes (20,641) were normalized using DEseq2.[45] Principal component analysis (PCA) on the variance stabilized data (vst) identified outliers. To allow for the evaluation of *APOE* ε4 allele dosage, each linear model included two genotype comparisons: (1) *APOE* ε3/ε4 to *APOE* ε3/ε3 and (2) *APOE* ε4/ε4 to *APOE* ε3/ε3. Linear models were run separately for (1) cortex – female, (2) cortex – male, (3) hippocampus – female, and (4) hippocampus – male. *β*-estimates were obtained for all four linear models that evaluated the main effects of *APOE* genotype (*APOE* ε3/ε4, *APOE* ε4/ε4, ref: *APOE* ε3/ε3) and running (run, ref: sed), as well as the interaction between *APOE* genotype and running (*APOE* ε3/ε4:Run, *APOE* ε4/ε4:Run). For each linear model, gseGO from the clusterProfiler package was run on genes significant for each factor. Gene set enrichment analysis (GSEA) was used to determine Gene Ontology (GO) terms for the genes significant for the main (running, *APOE* ε3/ε4, *APOE* ε4/ε4) and interacting factors (*APOE* ε3/ε4:Run and *APOE* ε4/ε4:Run). Normalized enrichment scores (NES) from GSEA were used to identify terms that were positively or negatively associated with each factor. GO terms were ordered based on the NES. Terms with a positive NES had more genes higher on the ranked list (i.e., more positive *β* values) and the terms with a negative NES containing more genes lower on the ranked list (i.e., more negative *β* values). Enriched GO terms had overlapping biological functions that we termed "vascular integrity," "cellular motility," "immune system response," "mitochondrial metabolism," and "synaptic/neuronal health." The top 20 most positive and negative GO terms were visualized for the cortex and hippocampus for both females and males (Figures S10–S24 in supporting information).

2.7 | Statistical analysis

For running comparisons within sex and age, a one-way analysis of variance (ANOVA) with Tukey's multiple comparison was performed. To determine age-related decline a two-sided paired *t*-test was performed.

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

3 | RESULTS

3.1 | *APOE* genotype did not affect voluntary running from young to midlife

To determine the effects of one *APOE* ε4 allele to two *APOE* ε4 alleles, we compared the *APOE* ε3/ε4 or *APOE* ε4/ε4 genotypes to the

control, *APOE* ε3/ε3 genotype (Figure 1A). Previous studies have shown that females run more than males; therefore, we assessed the sexes separately.[38] There was no difference in voluntary running during the dark cycle across *APOE* genotypes; however, there was expected variation between individual mice within the *APOE* genotypes (Figure 1B,C; Figures S1–S2 in supporting information). There was an age-dependent decrease in voluntary running from 6 to 11 months; however, there was no difference between *APOE* genotypes (Figure 1D–G). These findings show that running is not a variable between the *APOE* genotypes, and therefore not a confound in subsequent analyses.

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

Weight, body composition (e.g., lean mass, fat mass, and fat percentage) and cholesterol levels are commonly used as a general proxy for health in humans.[46–48] These biometrics are typically measured at routine physicals and are considered indicative of general health status, and markers for obesity, cardiovascular disruption, and lipid dysregulation.[49–52] We examined whether running affected weight, body composition, and cholesterol across *APOE* genotypes. Monthly weights (from 1 to 12 months) revealed an expected age-dependent weight gain in sedentary mice that was significantly attenuated by running (Figure 2A–F; Figure S3A–D in supporting information). In females, but not males, the *APOE* ε4/ε4 genotype caused a greater running-based attenuation in weight gain compared to *APOE* ε3/ε3 and *APOE* ε3/ε4. These results suggest that the beneficial effects of running on weight loss are *APOE* genotype-dependent in females only.

Overall, running mice had a lower fat composition compared to sedentary mice for both sexes at 6 and 11 months (Figures S4,5 in supporting information). In females, only *APOE* ε4/ε4 mice showed a significant attenuation of fat mass and fat percentage in running compared to sedentary mice (Figure 2G–I; Figure S3E–G). There were no *APOE* genotype differences in male mice; however, there was an effect of running on lean and fat mass. This effect was most pronounced in *APOE* ε3/ε4 male mice, with running attenuating lean and fat mass (Figure 2J–L; Figure S3H–J). Running attenuated the age-related increase in lean and fat mass across all genotypes and sexes. However, there was a pronounced reduction of age-related fat mass accumulation in female *APOE* ε4/ε4 running mice. Also, male *APOE* ε3/ε4 running mice showed the greatest reduction in age-related lean and fat mass accumulation.

No effect of running or *APOE* genotype was determined for total cholesterol or HDL concentration at 12 months (Figure S6 in supporting information). There was a significant sex-specific effect of *APOE* genotype on LDL concentration in the plasma. In running females, LDL concentrations decreased in an *APOE* ε4 dose-dependent manner (Figure S6H). Conversely, in running males, LDL concentrations were significantly lower than sedentary mice (Figure S6K). Cholesterol composition in running mice did not correlate with running distance for both sexes (Figure S6C,F,I,L,O,R).

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

3.3 | Running affects murine daily activities in an *APOE* genotype- and sex-specific manner

In humans, prior to more severe cognitive decline in ADRDs, activities of daily living (i.e., sleep, general movement, feeding) are often disrupted.[53–55] To evaluate murine daily activities in mice, we measured feeding and walking behavior (pedometers) across four dark cycles (active/awake period), and three light cycles (inactive/sleep period) at 11 months. Feeding behavior revealed significant changes in the dark cycle, but not in the light cycle for both sexes. In females, running mice consumed more food than sedentary mice during the dark cycle across all *APOE* genotypes (Figure 3A–C; Figure S7 in supporting information). However, in males, there was an interaction between *APOE* genotype and running during the dark cycle (Figure 3D–F). In sedentary mice, male *APOE* ε3/ε4 ate more than *APOE* ε3/ε3 and *APOE* ε4/ε4. This pattern was not apparent in running mice, suggesting running mitigates the *APOE* genotype differences observed in sedentary mice (Figure 3D; Figure S7).

We next determined whether movement in the home cage was affected by *APOE* genotype and/or running by measuring walking (Ped meters; see Methods). Female sedentary mice showed an *APOE* ε4 dose-dependent increase in Ped meters that was attenuated by running (Figure 3G,H; Figure S7). During the light cycle only *APOE* ε4/ε4 females showed a significant reduction in Ped meters compared to their sedentary counterparts (Figure 3I; Figure S7). In male mice, both *APOE* genotype and running interacted to alter Ped meters during both the dark and light cycle; however, running more strikingly increased cumulative Ped meters of *APOE* ε4/ε4 mice compared to the other *APOE* genotypes (Figure 3K,L; Figure S7).

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

3.4 | *APOE* genotype affects running-dependent increase in energy expenditure during the dark cycle

Previous studies in humans demonstrated *APOE* genotype affects metabolism on a cellular, regional, and organismal level.[37,56] To determine whether running and *APOE* genotype affect metabolic processes, energy expenditure (kcal/hr) was measured at 11 months. In female sedentary mice, energy expenditure showed an *APOE* ε4 dose-dependent increase during the dark cycle. In general, running resulted in significantly higher energy expenditure in the dark cycle in male and female mice. However, this effect was not observed in male *APOE* ε3/ε4 mice (Figure 4A–C; Figure S8 in supporting information). This suggests the *APOE* ε3/ε4 genotype attenuates the effects of

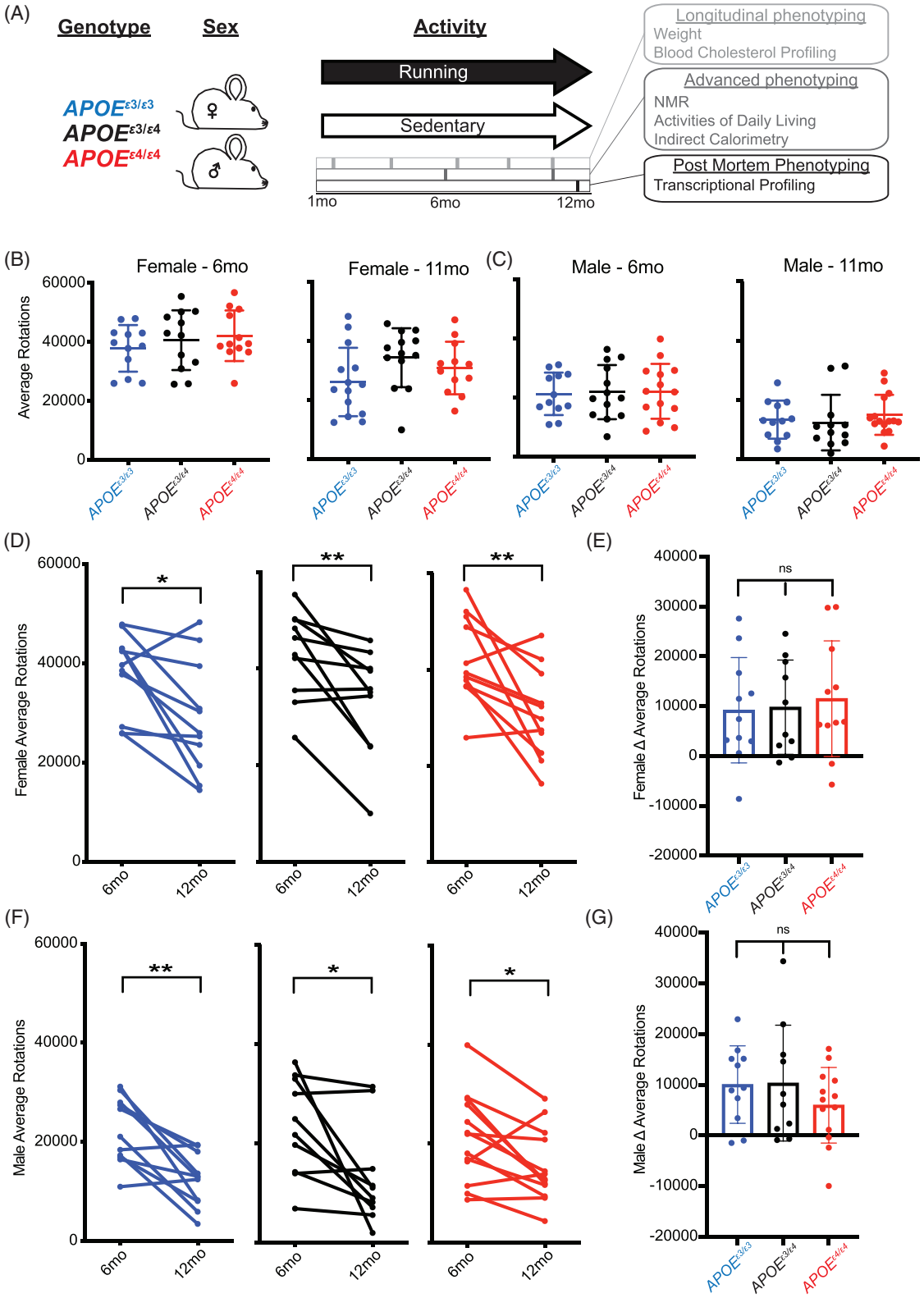

FIGURE 1 Voluntary chronic running to midlife is not different between apolipoprotein E (*APOE*) genotypes. A, Schematic of the voluntary running paradigm in which *APOE* ε3/ε3, *APOE* ε3/ε4, and *APOE* ε4/ε4 male and female mice were introduced to a locked (control – sedentary) or unlocked (treatment – running) running wheel at 1 month until 12 months (midlife). Longitudinal, advanced, and *post mortem* phenotyping is indicated. B-C, No difference in running (average rotations across multiple consecutive nights) between *APOE* genotypes at both 6 and 11 months in female (B) or male (C) mice ($n = 10–15$). D-G, Average rotations per mouse at 6 and 12 months showed an age-dependent decrease for both females (D) and males (F); however, the change over time was not significantly different between genotypes (E,G) ($n = 10–15$). Data presented as mean ± standard deviation, one-way analysis of variance with Tukey's multiple comparison performed for B,C,E,G. Two-sided paired *t*-test performed for D,F. $^{*}P < .05$, $^{**}P < .01$

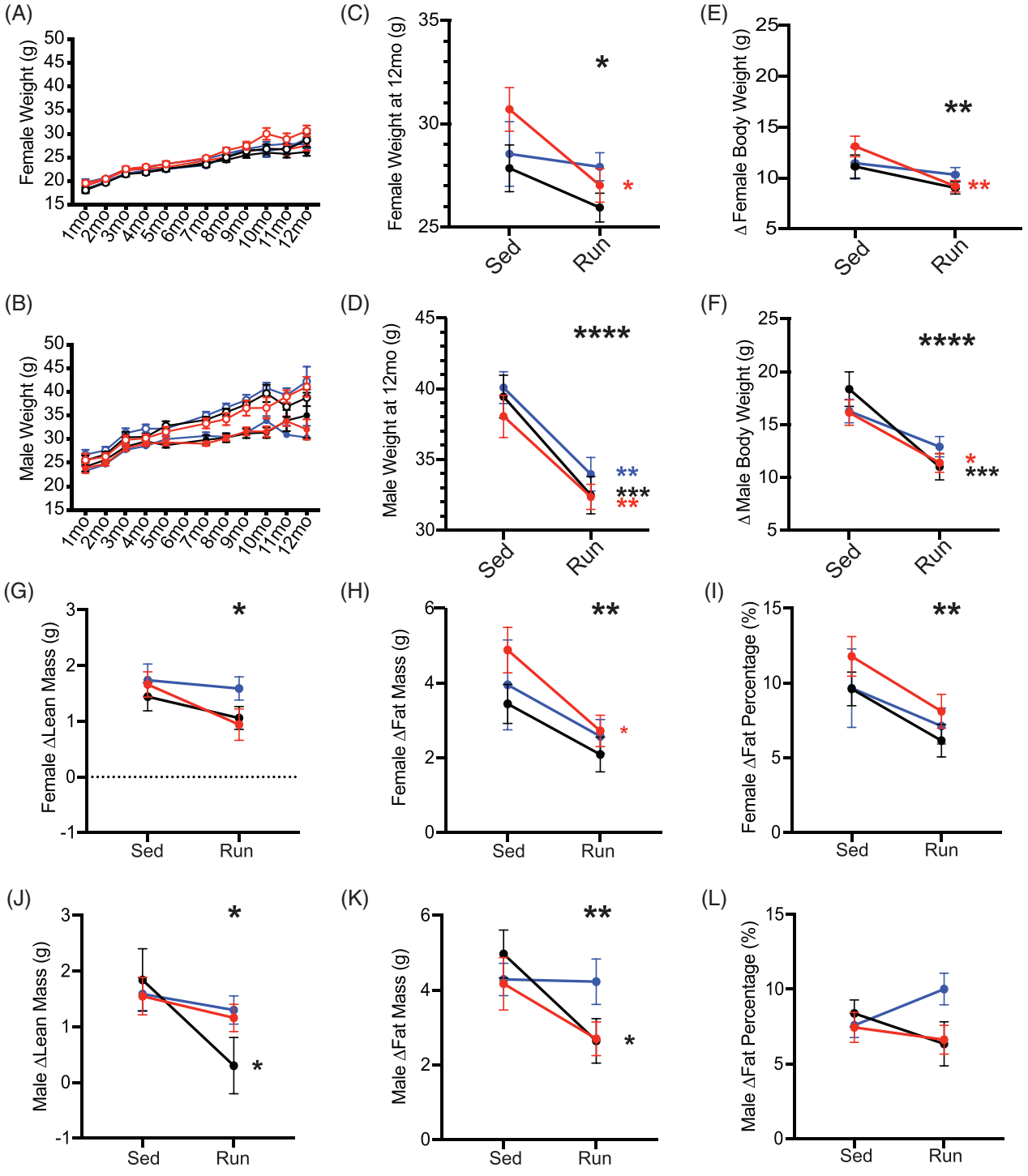

FIGURE 2 Running attenuated age-dependent weight gain and fat accumulation across apolipoprotein E (*APOE*) genotypes. A-B, Expected age-dependent weight gain from 1 to 12 months in females (A, $n = 9–15$) and males (B, $n = 11–15$). C-D, Running mice weighed significantly less at 12 months in both females (C) and males (D). E-F, Running significantly attenuated age-dependent weight gain (the difference in body weight from 1 to 12 months) in both females (E) and males (F). G-I, Significant effect of running on the change in lean mass (G), fat mass (H), and fat percentage (I) between 6 and 11 months, with an overall reduction in running mice compared to sedentary mice across all *APOE* genotypes in females. J-L, Running had a significant reduction on the change in lean mass (J) and fat mass (K) between 6 and 11 months, but no change in fat percentage (L) in male mice. Data presented as mean ± standard error of the mean, two-way analysis of variance performed for *APOE* genotype (significant marked above "Sed" column, indicating an effect of *APOE* genotype), Running (significance marked above "Run" column, indicating an effect of running), and the interaction between *APOE* genotype:Running (significance marked to the right of the graph). Bonferroni's multiple comparisons performed for within-genotype running effects (significance marked in smaller stars directly to the right of the run column, within graph limits, in the color of the genotype). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$

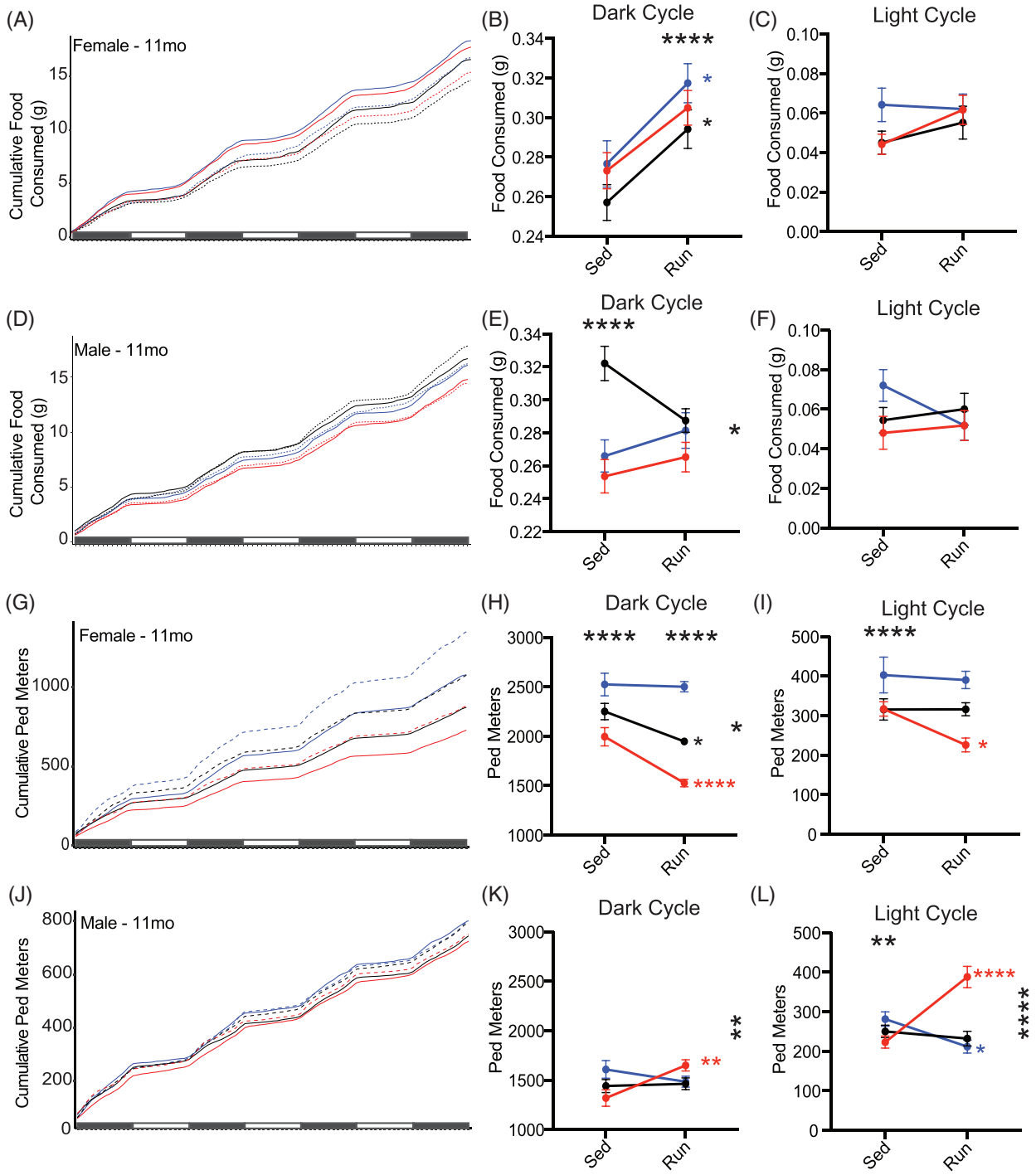

FIGURE 3 Murine daily activities are influenced by apolipoprotein E (*APOE*) genotype and running. A,D, Cumulative food consumed per gram for female (A, $n = 9–11$) and male (D, $n = 9–12$) mice across four dark cycles and three light cycles. B-C, Running significantly increased food consumed during the dark cycle (B), but not the light cycle (C) in female mice. E-F, *APOE* genotype and *APOE* genotype:running interaction affected food consumption in males during the dark cycle (E), but no effect was seen during the light cycle (F). G,J, General movement (cumulative ped meters) for female (G) and male (J) mice across four dark cycles and three light cycles. H-I, *APOE* genotype, running, and *APOE* genotype:running interaction all significantly affected ped meters during the dark cycle (H) with running decreasing ped meters differently across the genotypes. Only *APOE* genotype was significant during light cycle (I) in female mice. K-L, *APOE* genotye:Running interaction significantly affected ped meters in males, with *APOE* ε4/ε4 showing increased ped meters during the dark cycle (K), as well as the light cycle (L). There was also an *APOE* genotype effect (L). Solid lines indicate Run mice, dashed lines indicate Sed mice (A,D,G,J). Data presented as mean ± standard error of the mean, two-way analysisof variance performed for *APOE* genotype (significant marked above "Sed" column, indicating an effect of APOE genotype), running (significance marked above "Run" column, indicating an effect of running), and *APOE* genotype:running interaction (significance marked in to the right of the graph). Bonferroni's multiple comparisons performed for within-genotype running effects (significance marked in smaller stars directly to the right of the run column, within graph limits, in the color of the genotype). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$

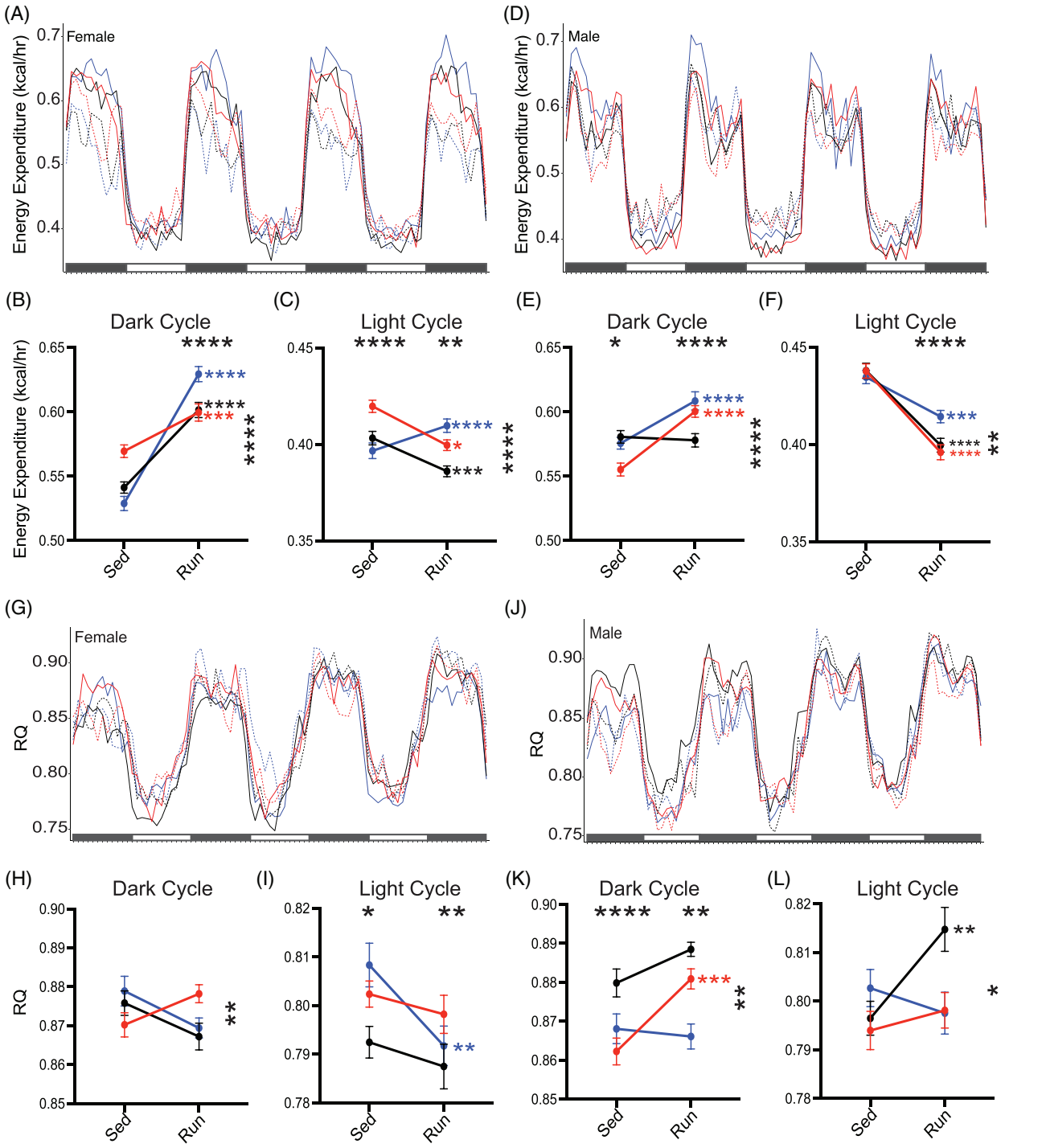

FIGURE 4 Running influences energy expenditure differently between females and male apolipoprotein E (*APOE*) mice. A-C, Energy expenditure (kcal/hr) across four dark cycles and three light cycles for female mice ($n = 9–11$). Energy expenditure (kcal/hr) significantly affected by *APOE* gentoype:running, and running during the dark cycle (B), with an increase in energy expenditure in running mice. *APOE* genotype:running, *APOE* genotype, and running all influenced light cycle energy expenditure (C), with *APOE* ε3/ε3 increasing with running while *APOE* ε3/ε4 and *APOE* ε4/ε4 decreased energy expenditure with running. D-F, Energy expenditure (kcal/hr) across four dark cycles and three light cycles for male mice ($n = 9–12$). E, *APOE* genotype:running, *APOE* genotype, and running all affected dark cycle energy expenditure in male mice. F, *APOE* genotype:running and running showed an overall decrease in energy expenditure in running male mice. G-I, Respiratory quotient (RQ) across four dark cycles and three light cycles for female mice (G). *APOE* genotype:running significantly affected RQ during the dark cycle for female mice (H). *APOE* genotype and running significantly affected RQ during the light cycle for female mice (I). J-L, RQ across four dark cycles and three light cycles for male mice (J). *APOE* genotype:running, *APOE* genotype, and running all significantly affected RQ during the dark cycle in male mice (K). *APOE* genotype:running significantly affected RQ during the light cycle (L). Solid lines indicate Run mice, dashed lines indicate Sed mice (A,D,G,J). Data

running in a sexually dimorphic manner. During the light cycle, all genotypes showed decreased energy expenditure with running; except for female *APOE* ε3/ε3 mice that showed an increase (Figure 4D–F; Figure S8). Subtle but significant changes in substrate usage (based on RQ; see Methods) were also determined across groups in both the light and dark cycle (Figure 4G–L; Figure S8). Significant changes were small; however, they may worsen with more advancing age (Figure 4H–L; Figure S8). These results highlight that *APOE* genotype and running affect energy expenditure; however, changes in starting energy substrate usage were minute (Figure 4A–F).

3.5 | *APOE* genotype causes subtle sex-specific changes to the effects of running on the aging brain

Unbiased transcriptional profiling was used to capture molecular effects across *APOE* genotype and activities (12 groups per brain region, Figure 5A; see Methods). PCA revealed brain region (PC1, 65%) and sex (PC2, 20%) were the primary drivers of variance, suggesting *APOE* genotype and running are not exerting strong effects (Figure 5B). Therefore, to determine subtle effects of *APOE* genotype and running, linear modeling was used for male or female cortex or hippocampus samples separately (four linear models in total; Figure 5C). Supporting the PCA data, linear modeling revealed fewer than 200 significant genes in females for the cortex and hippocampus, and fewer than 800 genes in males (Figure 5D–G). These numbers align with published data from human studies but are somewhat fewer than previous mouse studies (Figure S12). Several significant genes including *Ephx1* (main effect: *APOE* ε3/ε4), *Ctsf* (main effect: *APOE* ε4/ε4), *C3* (interaction *APOE* ε3/ε4:Run), and *Cav3* (interaction *APOE* ε4/ε4:Run) are known to function in lipid homeostasis, neuroinflammation, and membrane integrity, key processes implicated in ADRD risk (Figure 5H).

Through Gene Set Enrichment Analysis (GSEA), for the female cortex, GO terms showed positive normalized enrichment scores (NES) for the main effects (running, *APOE* ε3/ε4, and *APOE* ε4/ε4), but negative NES for the interactive terms (*APOE* ε3/ε4:Run, *APOE* ε4/ε4:Run) for vascular and synaptic/neuronal functions (Figure 6A–C)). Also, interestingly, in males, there were few significantly enriched GO terms for *APOE* ε3/ε4, suggesting in males, but not females, the *APOE* ε3/ε4 genotype exerts little to no effect compared to the *APOE* ε3/ε3 genotype on genes associated with vascular integrity–related processes (Figure 6D). Transcriptional profiling supplemented our peripheral findings, determining that running and *APOE* genotype interact in sex-specific ways to influence mechanisms involved in dementia-relevant biological processes.

4 | DISCUSSION

Exercise is generally considered to have beneficial effects, but our results show *APOE* genotype impacts the effects of running. Significant interactions between *APOE* genotype and running were observed across body weight, body composition, murine daily activities, systemic metabolism, and cortical and hippocampal gene expression. As with previous studies, to enable genotype comparisons between littermates, *APOE* ε3/ε3 were considered controls in all comparisons.[3,35–37] This was because *APOE* ε3 is considered the neutral allele in human studies, although this design meant comparisons between human *APOE* and mouse *Apoe* could not be made. Male and female mice were evaluated separately as ADRD risk varies between the sexes, with higher risk in women compared to men.[57,58] Sex is typically used as a covariate in human studies, but our data show that *APOE* genotype and sex interact across multiple domains. Additionally, there is a lack of consideration that odds ratios are sex-specific when assessing clinical trials, obfuscating the effects of sex. Our data suggest *APOE* genotype for each sex should be considered for studies assessing exercise interventions to reduce risk for dementia and more broadly any diseases for which APOE genotype has been associated.

While the brain has been shown to be plastic throughout adulthood, environmental influences can exert a greater effect on a younger brain compared to an older brain,[19,23,38,59–62] prompting us to study the effects of *APOE* genotype and running from early age to midlife. We assessed 12-month-old mice to understand the effect of *APOE* and running up until midlife, likening our findings to prodromal studies in humans.[63,64] *APOE* genotype-specific effects may also be apparent at older ages so studying later timepoints in the mouse, even beginning running at midlife, would be informative. This would relate closely to some human clinical trials that conduct studies on older, affected human populations (i.e., nursing home/hospice patients).[65–67] Additionally, it is unknown if exercise affects APOE production in the brain and periphery. While APOE production is assumed to be stable, it is possible that expression is directly or indirectly affected by exercise-related changes, such as BDNF, FNDC5/Irisin, and other systemic factors that promote neuroprotection. However, one human study suggested that exercise paradigms helped preserve cognition in *APOE* ε4 carriers more effectively over *APOE* ε3 controls suggesting *APOE* ε4 carriers to be more responsive to exercise-induced myokines; however, the APOE levels were not tested.[68] It is still necessary to carry out further clinical analyses, and while many ADRD studies include exercise as an intervention, the mechanisms by which *APOE* ε4 and exercise interact to affect amyloid beta deposition, tau tangle accumulation, neuroinflammation, vascular disruption, and other important ADRD pathologies are still to be performed.[69–71]

presented as mean ± standard error of the mean, two-way analysis of variance performed for *APOE* genotype (significant marked above "Sed" column, indicating an effect of *APOE* genotype), Running (significance marked above "Run" column, indicating an effect of running), and the interaction between *APOE* genotype:running (significance marked to the right of the graph). Bonferroni's multiple comparisons performed for within genotype running effects (significance marked in smaller stars directly to the right of the run column, within graph limits, in the color of the genotype). $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{****}P < .0001$

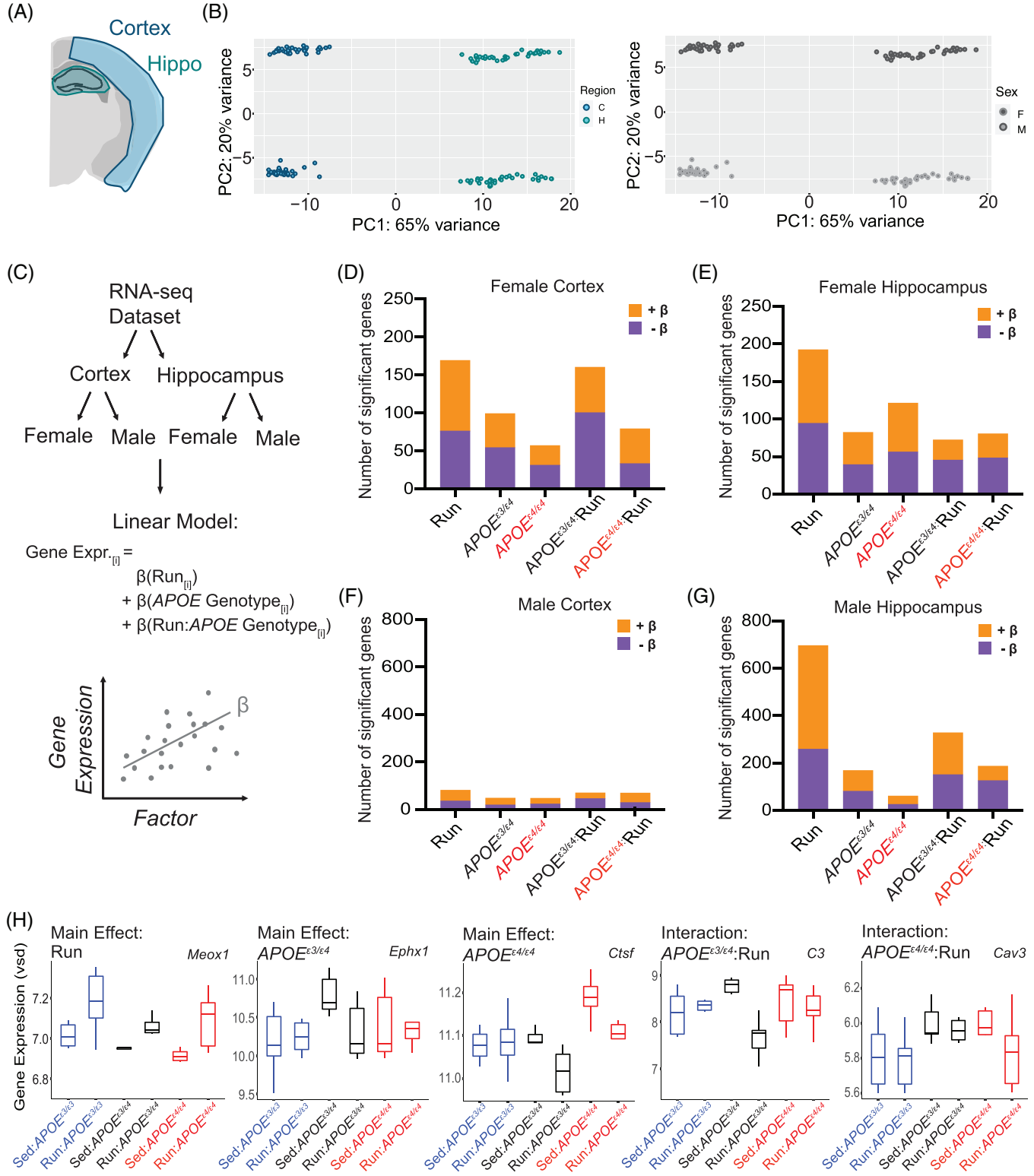

FIGURE 5 Transcriptional profiling reveals subtle changes due to apolipoprotein E (*APOE*) genotype and running in the cortex and hippocampus. A, Diagram of the cortical and hippocampal regions of the brain taken for transcriptional profiling ($n = 6$ per sex/genotype/activity). B, Principal components analysis revealed clear separations between brain regions (cortex and hippocampus, 65% variance explained), as well as by sex (female and male, 20% of variance explained). C, Schematic of the computational analysis approach; first RNA-seq was separated by brain region, next separated again by sex. Four linear models were run to examine gene expression as it varies with running, *APOE* genotype, and the interaction between *APOE* genotype:running. β-value is the association of the gene with the factor tested—positive β-value indicates a positive correlation, negative β-value indicates a negative correlation. D-G, Number of significant genes (false discovery rate corrected) for female cortex (D), female hippocampus (E), male cortex (F), and male hippocampus (G). H, Example of a significant gene for each of the main effects and interactive effects: Meox1 (Hippocampus, Male), Ephx1 (Hippocampus, Male), Ctsf (Hippocampus, Female), C3 (Hippocampus, Male), Cav3 (Cortex, Male)

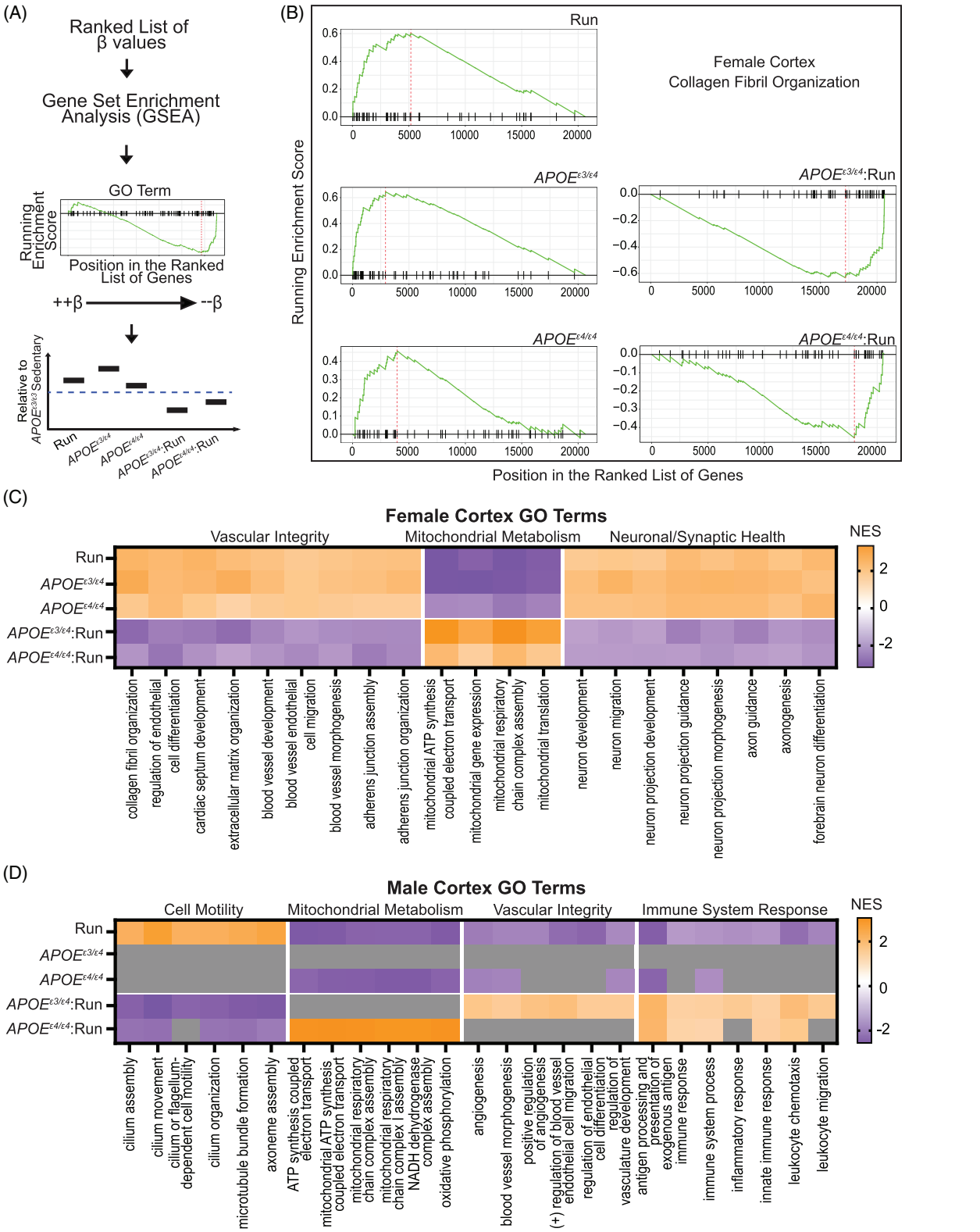

FIGURE 6 Gene set enrichment analysis (GSEA) predicts apolipoprotein E (*APOE*) genotype and running interact to mitigate main effects. A, Schematic of computational approach; *β*-values from linear models were passed through GSEA for gene ranking, GSEA plots were used to visualize results and main effects of running, *APOE* genotype, and *APOE* genotype:running were interpreted per Gene Ontology (GO) term. B, GSEA plots for "Collagen Fibril Organization" in the female cortex. Main effects of running and *APOE* ε3/ε4, and *APOE* ε4/ε4 all show positive enrichment scores, while the interactions, *APOE* ε3/ε4:run and *APOE* ε4/ε4:run reveal negative enrichment scores. C, In the female cortex data GO terms that fit the pattern shown in (B), colored by normalized enrichment score (NES), are represented specifically vascular integrity, mitochondrial metabolism, and synaptic/neuronal health. D, The pattern observed in male cortex was different than that seen in female cortex (B,C) with *APOE* ε3/ε4 appearing more similar to *APOE* ε3/ε3 (indicated by gray boxes) for enrichment terms grouped as cell motility, mitochondrial metabolism, vascular integrity, and immune system response

Transcriptomic approaches have revolutionized our understanding of ADRDs and have therefore become a hypothesis-generating tool for identifying the molecular pathways impacted by genetic and environmental risk factors. Therefore, we used transcriptional profiling to identify interactions between *APOE* genotype and running. Our data revealed a reversal of NES direction from the main effects and the interaction of *APOE* genotype and running. This was unexpected, as we saw similar patterns of positive (or negative) enrichment for (1) running compared to sedentary, (2) *APOE* ε3/ε4 compared to *APOE* ε3/ε3, and (3) *APOE* ε4/ε4 compared to *APOE* ε3/ε3. These results contradict the assumption that running would have the opposite effect on the brain as *APOE* ε4, particularly when considering each of these terms collectively (vascular, immune, mitochondrial, neuronal/synaptic). We propose that there is a possibility for overcompensation for the *APOE* ε4 allele. While evidence shows *APOE* ε4 causes gains and losses of APOE function across many processes, it is unknown whether there is a preemptive response that has not been considered. Further, the *APOE* ε3/ε4 genotype may be responding to early aging phenotypes different than *APOE* ε4/ε4 genotype. Precise experimentation on this phenomenon is needed in both mice and humans to better understand which *APOE* ε4-specific pathways are mitigated by running. Last, while these models are key for interpretation of APOE biology, other important pathological interactions (e.g., amyloid or tau) are not present in this study. Future studies are necessary to interrogate the interactions among *APOE*, exercise, and hallmark ADRD pathologies to provide further translatable outcomes.

Advancements in RNA sequencing have made it cheaper and faster to sequence the brains of ADRD patients (Religious Orders Study/Memory and Aging Project [ROSMAP], Mayo Clinic [MAYO], Alzheimer's Disease Sequencing Project [ADSP]). Recently, research programs have explored whether *APOE* influences the human cerebral transcriptome. In three largescale Accelerating Medicines Partnership–Alzheimer's Disease studies, reports revealed few to no gene expression changes in multiple brain regions in *APOE* ε4+ cases compared to noncarriers (ROSMAP: syn8456629, MAYO: syn8466812, Mount Sinai Brain Bank [MSBB]: syn8484987)[72–74] (Figure S12). The ROSMAP dataset analysis showed no differences due to *APOE* ε4 status across the dorsolateral prefrontal cortex region.[72] The MAYO dataset showed a significant differential expression (DE) of only 173 genes between *APOE* ε3/ε4 and *APOE* ε3/ε3, and a significant DE of only 88 genes between *APOE* ε4/ε4 and *APOE* ε3/ε3 in the temporal cortex.[73] The MSBB reported fewer than five genes DE between all *APOE* genotype comparisons in the frontal pole region, parahippocampal gyrus region, frontal superior temporal gyrus region, and inferior frontal gyrus region.[74] Our mouse data align more closely with these human studies, possibly due to litter-matched mice, and further analyses using GSEA showed subtle changes that escaped detection through traditional DE analysis. Moving forward, our data show the importance of including heterozygous genotypes (e.g., *APOE* ε3/ε4) and varying degrees of chronic voluntary exercise (e.g., low, medium, high) in mouse studies to improve the alignment to ADRD in human studies.

The *APOE* ε4 allele emerged as our early hominin ancestors adapted to changes in habitat and food availability to include more aerobic exercise such as running.[75] The *APOE* ε4 allele was beneficial for storage of fats, increasing cholesterol. While the *APOE* ε4 conferred longer lifespan 200,000 years ago, the diet and exercise of an individual was drastically different.[75] Currently, Western culture sees some of the highest rates of ADRD, due to the interaction between *APOE* ε4 and our environment, and as we show, running. This work supports that *APOE* ε4 interacts with running in a genotype- and sex-specific manner to influence peripheral and central risk factors for diseases such as ADRDs.

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CONFLICTS OF INTEREST

The authors declare they have no competing interests or disclosures.

AUTHOR CONTRIBUTIONS

Kate E. Foley and Gareth R. Howell conceived and designed this project. Kate E. Foley, Cory A. Diemler, Amanda A. Hewes performed mouse experiments. Kate E. Foley performed IF, experimental analysis, and bioinformatic analysis. Kate E. Foley and Gareth R. Howell consulted for statistical approach and analysis. Kate E. Foley and Gareth R. Howell wrote and prepared this manuscript. All authors read and approved the final manuscript.

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