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# Sexual dimorphism of physical activity on cognitive aging: Role of immune functioning

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ABSTRACT

*Objective:* Exercise is one of the most potent strategies available to support cognitive health with age, yet substantial variability exists. Sexual dimorphism is evident for brain and immune functioning, the latter being implicated as important pathway for exercise. We examined the moderating role of sex on the relationship between physical activity and systemic inflammatory and brain health outcomes in support of more personalized approaches to behavioral interventions.

*Methods:* Our discovery cohort included 45 typically aging women matched on age ( ± 5y) and education ( ± 2y) to 45 men (mean age = 72.5; Clinical Dementia Rating = 0) who completed self-reported current physical activity (Physical Activity Scale for Elderly), blood draw, neuropsychological evaluation, and brain MRI. An independent sample of 45 typically aging women and 36 men who completed the same measures comprised a replication cohort. Plasma was analyzed for 11 proinflammatory cytokine and chemokine markers via MesoScale Discovery.

*Results: Discovery cohort:* Reported physical activity did not differ between sexes (150 vs. 157, $p = 0.72$). There was a significant interaction between sex and physical activity on chemokine markers MDC, MIP-1b, MCP-4, and eotaxin-3 (ps $< 0.03$), with a similar trend for MCP-1 and INFγ (ps $< 0.09$). Men who reported greater activity demonstrated lower inflammatory markers, an effect attenuated-to-absent in women. An interaction between sex and physical activity was also observed for parahippocampal volumes ($p = 0.02$) and cognition (processing speed and visual memory; ps $< 0.04$). Again, the beneficial effect of physical activity on outcomes was present in men, but not women. Replication cohort analyses conferred a consistent effect of sex on the relationship between physical activity and immune markers; models examining neurobehavioral outcomes did not strongly replicate. Across cohorts, post-hoc models demonstrated an interaction between sex and activity-related inflammatory markers on total gray matter volume and visual memory. Men with higher inflammatory markers demonstrated poorer brain structure and function, whereas inflammatory markers did not strongly relate to neurobehavioral outcomes in women.

*Conclusions:* Greater physical activity was associated with lower markers of inflammation in clinically normal older men, but not women – an effect consistently replicated across cohorts. Additionally, men appeared disproportionately vulnerable to the adverse effects of peripheral inflammatory markers on brain structure and function compared to women. Immune activation may be a male-specific pathway through which exercise confers neurobehavioral benefit.

## 1. Introduction

Dementia is a quarter trillion-dollar public health problem with no disease modifying treatments currently available, highlighting the need to carefully examine readily available alternate approaches (Alzheimer's Association, 2018). Physical activity is one such target that

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has demonstrated some of the largest effect sizes on neurobehavioral outcomes, including age-related cognition (Barnes et al., 2003; Yaffe et al., 2009; Hörder et al., 2018; Colcombe and Kramer, 2003), white matter integrity (Kim et al., 2018; Lövdén et al., 2010; Boraxbekk et al., 2016), and even gray matter growth (Erickson et al., 2011; Wittfeld et al., 2020). For example, one recent study demonstrated an 88% reduced risk of dementia in adults with high midlife fitness compared to their less fit peers (Hörder et al., 2018). Yet, as noted in the 2017 National Academy of Medicine, other data are mixed and evidence is not yet sufficient to indicate that exercise can prevent dementia (National Academies of Sciences Engineering and Medicine, 2017). In fact, one recent large-scale trial in patients with mild-to-moderate dementia showed statistically significant functional *declines* after a 12-month exercise treatment (Lamb et al., 2018). A major gap in this literature is that we do not fully understand the mechanisms of how exercise may exert protective effects on the brain in order to identify who may stand to benefit the most.

Biological sex is not only an important moderator of brain and cognitive aging, but also of immunological functioning, and this latter system is posited as an important pathway through which exercise may benefit the brain (Cotman et al., 2007). While men demonstrate steeper memory declines in typical aging (Casaletto et al., 2019; Jack et al., 2015) and "older" appearing markers of brain epigenetics and metabolism (Horvath et al., 2016; Goyal et al., 2019), up to 2 out of 3 patients with Alzheimer's disease (AD) are women (Alzheimer's Association, 2018). Women also harbor higher levels of AD pathology and, clinically, appear to be disproportionately affected by AD risk factors (e.g., *APOEε4*, cerebral amyloid) (Buckley et al., 2019; Koran et al., 2017; Hohman et al., 2018). Interestingly, converging whole genome studies highly implicate innate immune dysfunction in the development of AD (Bis et al., 2018; Gjoneska et al., 2015; Jones et al., 2015), and there is also evidence for sexual dimorphism of the immune system. For example, there are clear sex differences in the prevalence immune-mediated diseases (e.g., 80% of autoimmune diseases are in women) (Klein and Flanagan, 2016). Women generally mount a stronger immunological response to a pathogen or injury (e.g., TBI, ischemia) particularly at younger ages compared to men, including increased transcription of toll-like receptor ligands following an immune challenge and bigger antibody production following vaccination (Klein and Flanagan, 2016; The et al., 2009; Villapol et al., 2017). The endocrine environment also plays a potent regulating role as both androgen and estrodiol response elements are present on innate immunity genes (Hannah et al., 2008) and expressed in various lymphoid tissues, lymphocytes, macrophages, and dendritic cells. While estradiol appears to have a bipotential effect with low doses enhancing inflammatory cytokine production and high levels reducing their production, androgens, including testosterone, generally suppresses immune activity in men, potentially contributing to pathogen toxicity (Hou and Wu, 1988; Rettew et al., 2008). As a result, women demonstrate faster pathogen clearance, wound healing, and return to immunological quiescence following an injury, but may ultimately be more susceptible to low grade inflammation and autoimmune diseases, while men are at risk of greater pathogen toxicity due to an insufficiently mounted immunologic response (Klein and Flanagan, 2016; The et al., 2009). Given that 1) there is compelling evidence for sexual dimorphism across brain and immunologic functioning, and 2) physical activity has been linked with both of these systems (Northey et al., 2017; Anderson et al., 2016; Fedewa et al., 2017; Fiuza-Luces et al., 2018; Duggal et al., 2019; Gleeson et al., 2011), our overarching goal was to examine the effect of biological sex on the relationship between physical activity and these age-related outcomes.

Despite these links and the surge in exercise-related brain health literature, relatively few studies have directly explored this question. In humans, there appear to be sex differences in the relationship between exercise and brain integrity and cognitive outcomes, though the exact pattern is less clear. In one of the few prospective analyses, Barha and colleagues (2019) examined the relationship of self-reported walking on 10-year cognitive and brain volume trajectories stratified by sex in the Health ABC cohort of non-demented older adults (Barha et al., 2019). In women, walking was associated with more optimal dorsolateral prefrontal volume and processing speed trajectories, while in men, walking was linked to better hippocampal volume trajectories. On the other hand, in a randomized trial of physical activity in older adults with small vessel ischemic disease, aerobic training was associated with slowed white matter hyperintensity growth in men, but not women (Dao et al., 2019). Regarding cognition, several randomized trials of aerobic activity have also demonstrated specific beneficial effects of physical activity on processing speed and executive functioning only in women (Barha et al., 2017), while others show benefits on memory only in men (Barha et al., 2017; Barha and Liu-Ambrose, 2018). Notably, the study cohorts included in these reviews were cognitively heterogenous, including a mix of both typical aging and mild cognitive impairment adults. Given that disease state may critically modulate sex effects of cognitive aging (e.g., men may decline more quickly in states of health, women may decline more quickly in states of AD), it is difficult to fully disentangle these effects. Additionally, there are fairly consistent demographic cohort differences between sexes, with women tending to be younger and less well educated, potentially reflecting generational effects on factors known to relate to cognitive aging.

In this study, we evaluated the moderating effect of biological sex on the relationship between physical activity and brain health related outcomes among typically aging older adults. Our outcomes included cognition, gray matter volume, and plasma markers of immunologic functioning between the sexes. To address cohort issues, we matched women and men on demographic factors shown to vary by sex and be related to brain health outcomes – namely, age and education. Careful characterization of the individuals who stand to benefit the most from behavioral interventions are critically needed to support personalized health approaches, shape risk-stratification techniques for clinical trials, and inform our fundamental understanding of brain health development.

## 2. Methods

### 2.1. Participants

All participants were drawn from the larger Hillblom Aging Network at University of California, San Francisco, an ongoing study characterizing the neurobehavior of typical aging. Participants represent a community-dwelling, convenience sample of the Bay Area collected between 2000 and 2019, recruited via advertisements, flyers, community outreach events, word of mouth, and through family members of patients affected by neurodegenerative disease. At screening, participants completed comprehensive neurological, and neuropsychological evaluation, including a study partner interview, all of which are reviewed at interdisciplinary case conferences, and determined to be within normative standards by board certified neurologists and neuropsychologists. Evaluation measures included detailed neurobehavioral and medical interview with participant and an informant, motor and physical exam, brain MRI review, informant-based Clinical Dementia Rating Scale, and standardized well-described neuropsychological measures covering episodic memory, executive functions, processing speed, language, visuospatial skills, and mood described in details elsewhere (see for detailed measures and further cohort description (Casaletto et al., 2019; Bott et al., 2017; Lindbergh et al., 2019; Kramer et al., 2003). Inclusion criteria: 1) no diagnosed memory or neurological condition (e.g., epilepsy, large vessel stroke), 2) no major medical (e.g., active neoplasm, HIV, dialysis), psychiatric disorder (e.g., schizophrenia), or active substance use disorder, and 2) no functional decline operationalized as Clinical Dementia Rating (CDR) of 0 via study partner interviews. This approach yields a cohort of older, functionally intact subjects who are heterogeneous in terms of

cognition, and common chronic age-related vascular risk factors and general health conditions.

#### 2.1.1. Discovery cohort

120 typically aging older adults completed the Physical Activity Scale for the Elderly (PASE) and blood draw with plasma analyzed for immune activation markers in one analytic batch. Men and women were matched on age ( ± 5 years) and education ( ± 2 years) via case control matching (IBM Corp. SPSS; Case Control Matching, Hayes package), resulting in 45 matches (N = 90 total).

#### 2.1.2. Replication cohort

We identified an additional cohort of 90 independent older adults (36 male, 45 female) from the Hillblom Aging Network who also completed the PASE and blood draw with plasma analyzed for immune markers (on the same analytic platform) *in a separate analytic batch* and who served as a validation sample. The goal of a replication cohort was to increase the rigor of our clinical study by testing the reliability of the evaluated relationships. Models demonstrating similar effect directionality and size across both cohorts may therefore be interpreted as more generalizable and robust. We opted to not match this cohort in order to optimize sample size, and instead statistically adjusted for demographic factors that differed between sexes.

The UCSF Committee on Human Research approved the study protocol, and per their guidelines, all subjects provided written informed consent.

### 2.2. Reported physical activity

Participants completed the Physical Activity Scale for the Elderly (PASE), a self-reported measure of physically-demanding activities in the past 7 days. The PASE was developed and has been well-validated to capture physical activities common in older adults, including quantification of duration, frequency, and exertion level. A total composite score was calculated according to the manual (New England Research Institutes, Inc, 1991) with higher scores indicating higher levels of physical activity (possible range 0 to 400 + ).

### 2.3. Immune activation markers

Venous blood was drawn in the morning following a 10 + hour fasting interval in EDTA-containing tubes and plasma continuously stored at −80 °C. Samples were gradually brought to room temperature and analyzed via Meso Scale Discovery (Rockville, MD, USA) V-Plex proinflammatory cytokine and chemokine kits, according to manufacturer guidelines. Analytes quantified included interleukin (IL)-6, IL-10, tumor necrosis factor-alpha (TNF-α), monocyte derived chemokine (MDC/CCL22), macrophage inflammatory protein-alpha and beta (MIP1α/CCL3 and MIP1β/CCL4), monocyte chemoattractant protein 1 and 4 (MCP-1/CCL2, MCP-4/CCL13), and interferon gamma (INFγ), eotaxin-1 and −3. Samples were run in two separate analytic batches using antibodies from two different manufacturer lots at different time points; given that discrepant analytic lots and time points are commonly not directly comparable (Casaletto et al., 2018), we elected to split the sample into independent Discovery and Replication cohorts based on batch analysis. All samples were run in duplicate and those with coefficients of variance (CV) > 20% were excluded from analyses. Final values were also examined for extreme outliers and samples with values > 5x the upper interquartile range were also excluded. Due to positive skew, all markers were log transformed to achieve normality prior to analysis.

### 2.4. Cardiovascular health

Other measures of cardiovascular health included height and weight (body mass index calculated), blood pressure (systolic and diastolic), and serum-based clinical laboratory measures of blood sugar (hemoglobin A1C) and insulin resistance (Homeostatic Model Assessment of Insulin Resistance, HOMA-IR) (Quest Diagnostics; Seacaucus, NJ).

### 2.5. Neuroimaging

Participants also completed a 3 T Magnetom Vision TIM Siemens Trio brain magnetic resonance imaging (MRI) within 180 days of their neuropsychological evaluation and blood draw. T1-weighted magnetization prepared rapid acquisition GRE structural scan was acquired (acquisition time 8 min, 53 s), sagittal orientation, field of view 160 × 240 × 256 mm and isotropic voxel resolution of 1 $mm^3$ (repetition time = 2300 ms, echo time = 2.98 ms, time inversion = 900 ms, and flip angle = 9). Before processing, all images were visually inspected for quality and those with excessive motion or other image artifact excluded. Magnetic field bias was corrected using the N3 algorithm (Sled, 1998). Tissue segmentation was performed using the unified segmentation procedure in SPM12 (Ashburner and Friston, 2005). Each participant's T1-weighted image was warped to create a study-specific template using Diffeomorphic Anatomical Registration using Exponentiated Lie algebra (DARTEL) (Ashburner, 2007); subsequently, the images were normalized and modulated in the study-specific template space using nonlinear and rigid-body registration. Images were smoothed using an 8-mm Gaussian kernel with 8-mm full width half maximum. For registration with a brain parcellation atlas, linear and nonlinear transformations between DARTEL's space and ICBM space were applied (Mazziotta et al., 1995). Quantification of volumes in specific brain regions at each time point was accomplished by transforming a standard parcellation atlas into ICBM space and summing all modulated gray matter within each parcellated region (Desikan et al., 2006). Total intracranial volume was estimated for each subject in MNI space.

*A priori* selected gray matter regions of interest (ROIs) included total gray matter volume, bilateral substructures of the medial temporal lobe (hippocampus, parahippocampus, entorhinal), and bilateral dorsolateral prefrontal cortex (caudal and rostral middle frontal gyrus), given previously reported sensitivity of these structures to exercise (Erickson et al., 2011; Wittfeld et al., 2020). Total intracranial volume was statistically regressed out of each ROI prior to analyses.

### 2.6. Neuropsychological evaluation

We selected measures of processing speed, episodic memory, and executive functions given their known changes in aging and previous reported associations with physical activity (Kramer et al., 2003; Kramer and Erickson, 2007). *Spatial processing speed* was measured using a well-validated, experimental computerized battery of visually (e.g., Dot Counting, Flanker) mediated reaction-time tasks, developed by Kerchner and colleagues (2011) (Kerchner et al., 2012). Tasks within each domain are averaged into a composite z-score, reflecting reaction time (higher values indicating *slower* performance) of participant performance compared to young adults. *Verbal episodic memory* was measured via the California Verbal Learning Test, second edition (Delis et al., 2000); total long delay (20–30 min) free recall scores were the primary metric of interest (range 0–16). *Visual episodic memory* was quantified by delayed (10-minute) free recall of a complex figure (modified Benson Figure). *Executive functions* were quantified via a composite index of digit span backwards, modified Trail Making Test, Stroop Inhibition, lexical fluency (D-words/60″), and design fluency (DKEFS Condition 1) performances; this battery of measures is described in detail elsewhere (Kramer et al., 2003; Casaletto et al., 2017).

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**Table 1**
Discovery and Replication cohort demographic and clinical characteristics.

| | DISCOVERY COHORT* | | | REPLICATION COHORT | | |
|---|---|---|---|---|---|---|
| | Female | Male | p-value | Female | Male | p-value |
| | n = 45 | n = 45 | | n = 45 | n = 36 | |
| Age | 72.0 (8.0) | 73.0 (8.4) | 0.53 | 73.5 (6.6) | 74.4 (7.6) | 0.55 |
| Education | 17.1 (1.9) | 17.7 (1.8) | 0.11 | 16.8 (2.3) | 18.2 (2.3) | 0.006 |
| MMSE | 29.3 (1.2) | 29.1 (1.2) | 0.28 | 29.0 (1.1) | 28.8 (1.6) | 0.68 |
| BMI | 24.9 (4.6) | 25.6 (3.4) | 0.39 | 24.5 (3.8) | 26.9 (4.7) | 0.01 |
| Hemaglobin A1C | 5.46 (0.3) | 5.44 (0.4) | 0.78 | 5.53 (0.3) | 5.51 (0.3) | 0.70 |
| HOMA-IR | 1.73 (1.1) | 2.04 (1.6) | 0.32 | 2.57 (1.8) | 2.94 (1.8) | 0.38 |
| Blood pressure | 129.3 (13.8) | 132.4 (14.3) | 0.32 | 133.8 (19.7) | 128.0 (13.7) | 0.14 |
| Systolic | 72.0 (9.0) | 72.8 (8.5) | 0.69 | 71.8 (7.5) | 73.6 (8.3) | 0.32 |
| Diastolic | | | | | | |
| Reported Physical Activity (PASE) | 150.1 (96.7) | 156.9 (83.9) | 0.72 | 137 (75.3) | 120.6 (66.5) | 0.31 |

**Note**. *Case-control matched on age ( ± 5y) and education ( ± 2y). MMSE = Mini Mental Status Examination; BMI = body mass index; HOMA-IR = homeostatic model assessment of insulin resistance; PASE = Physical Activity Scale for the Elderly.

## 3. Statistical analyses

### 3.1. Discovery cohort analyses

In the age- and education-matched sample, independent Student's t-tests were conducted to examine possible remaining demographic and clinical differences between sexes. Next, to evaluate the moderating effect of sex on the relationship between reported physical activity and neurobehavioral outcomes of interest, we conducted linear regression models entering both main effects and the interaction term between sex*physical activity. Given that we captured 11 immunological markers, in order to reduce multiple comparisons, we first conducted a multivariate linear regression with all 11 markers entered as the dependent variable; follow-up analyses then evaluated each individual immune marker separately. Parallel multivariable linear regression models also evaluated the interaction of sex*physical activity on a priori selected brain structure (total GMV, medial temporal lobe subregions, dorsolateral prefrontal cortex), and cognitive (verbal/visual episodic memory, processing speed, executive functions) outcomes. To better capture the specific effect of current physical activity on outcomes of interest, all models covaried for BMI as an indicator of overall fitness/metabolic differences. Models with interaction terms reaching $\alpha < 0.05$ were further probed by testing effects of physical activity on immune/neurobehavioral outcomes stratified by sex. Standardized beta values are reported across all models.

### 3.2. Replication analyses

In the unmatched replication cohort, Student's t-tests were conducted to again evaluate demographic and clinical differences between sexes. This cohort demonstrated significant differences on education, BMI, and heart rate between males and females; as such, these measures were included as covariates in all subsequent models. To streamline analyses and avoid multiple comparisons, we replicated regression models (described above) that demonstrated small-to-medium effect sizes (interaction term parameter: $\beta > 0.30$) in the discovery cohort (Cohen, 1988).

### 3.3. Post-Hoc models

After determining that the most replicable sex-specific effect was observed on the relationship between physical activity and peripheral inflammatory markers, we aimed to explore how these exercise-related immune markers may relate to markers of central nervous system functioning (brain structure and function) in our cohorts. To do so, we first created an inflammatory composite (z-score) within each cohort that included only the chemokine markers demonstrating at least small-to-medium ($\beta > 0.30$) sex-exercise effects across cohorts: MCP4, eotaxin-3, and MIP1b. Collapsing across cohorts to increase sample size, we then examined the possible moderating role of sex on the relationship between our exercise-related immune composite and markers of brain structure and cognition. Multivariable linear regression models therefore tested the interaction between sex*exercise-related immune composite on gray matter volume and cognition, adjusting for age, education, and BMI.

## 4. Results

### 4.1. Discovery cohort

#### 4.1.1. Sample characteristics

Demographically-matched men and women did not statistically differ on overall reported physical activity levels, BMI, blood pressure, hemoglobin A1C, or HOMA-IR. Regarding inflammatory markers, men and women did not differ across most, though women showed lower IL-6 (p = 0.03) and higher INFγ (p = 0.04) (Table 2).

#### 4.1.2. Immune activation outcomes

The omnibus multivariate regression modeling sex*physical activity against all 11 immune markers reached statistical significance (F(42, 67) = 2.16, p = 0.0018). In individual models, sex significantly moderated the relationship between physical activity and several proinflammatory chemokine markers, including MDC (p = 0.03), MIP1-b (p = 0.02), eotaxin-3 (p = 0.007), and MCP-4 (p = 0.02; Table 3, Fig. 1). Models examining MCP-1 (p = 0.09) and INFγ (p = 0.06) approached, but did not reach, statistical significance. In all models, greater reported physical activity was consistently associated with lower inflammatory markers among men, but not women (Men: $\beta$ *range*: −0.59 to −0.27, all p-values < 0.07; Women: $\beta$ *range*: −0.15 to 0.19, all p-values > 0.21). There were no statistically significant sex by physical activity interactions on proinflammatory cytokine markers examined (e.g., IL-6, TNFα).

#### 4.1.3. Brain structural outcomes

Sex also significantly interacted with physical activity on parahippocampal volumes (p = 0.02; Table 4; Fig. 2). Greater physical activity demonstrated a medium, positive relationship with parahippocampal volume in men ($\beta$ = 0.43, p = 0.01), but not in women ($\beta$ = 0.03, p = 0.86). Interaction parameters were in the same direction but did not reach statistical significance for entorhinal ($\beta$ = -0.31, p = 0.35), hippocampal ($\beta$ = -0.41, p = 0.19), total gray matter ($\beta$ = -0.41, p = 0.19) or dorsolateral prefrontal cortex ($\beta$ = -0.19, p = 0.53; Table 4).

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**Table 2**
Discovery cohort plasma inflammatory marker concentrations do not substantially differ by sex.

| DISCOVERY COHORT | Female (n = 45) | Male (n = 45) | p-value |
|---|---|---|---|
| CHEMOKINES | | | |
| MDC | 955.5 (786.5, 1123.3) | 883.6 (652.0, 1073.3) | 0.27 |
| MIP1a | 26.9 (14.4, 44.0) | 23.2 (14.0, 41.4) | 0.84 |
| MIP1b | 53.6 (44.8, 77.1) | 54.8 (40.2, 64.4) | 0.20 |
| MCP-1 | 49.2 (40.2, 64.3) | 47.2 (40.0, 72.8) | 0.43 |
| MCP-4 | | 61.9 (49.1, 85.3) | 0.97 |
| Eotaxin-1 | 137.7 (120.0, 168.1) | 147 (121.6, 174.8) | 0.61 |
| Eotaxin-3 | 36.8 (21.4, 59.7) | 45.2 (20.8, 82.2) | 0.23 |
| INFγ | 3.7 (2.4, 7.2) | 3.0 (2.3, 4.0) | 0.04 |
| CYTOKINES | | | |
| IL-10 | 0.26 (0.21, 0.34) | 0.28 (0.23, 0.45) | 0.65 |
| IL-6 | 0.51 (0.40, 0.71) | 0.68 (0.43, 1.1) | 0.03 |
| TNFa | 3.3 (2.7, 4.0) | 3.3 (2.8, 3.8) | 0.75 |

**Note**. Median (interquartile range) reported.

#### 4.1.4. Neuropsychological outcomes

Lastly, there was also a significant sex by physical activity interaction on visual episodic memory (p = 0.04) and processing speed (p = 0.027; Table 4; Fig. 3). Among men, greater reported physical activity demonstrated a medium, positive association with visual memory and processing speed performances, but this effect was attenuated in women (Men: memory $\beta$ = 0.25, speed $\beta$ = −0.37, p-values < 0.12; Women: memory $\beta$ = −0.18, speed $\beta$ = 0.04, p-values < 0.82). The interaction term did not reach significance for executive functions or verbal episodic memory (all p-values > 0.15).

### 4.2. Replication sample (Unmatched)

In the replication sample, men and women were comparably aged, though women were less well educated (16.9 vs 18.3 years, p = 0.008; Table 1). Men and women in the replication sample did not differ on reported levels of physical activity, blood pressure, hemoglobin A1C, or HOMA-IR, though women had higher heart rates (68.2 vs. 63.5, p = 0.02) and lower BMIs (24.5 vs. 27.1, p = 0.007) compared to men; we adjusted for education, heart rate, and BMI in all analyses.

Only models that demonstrated small-to-medium effect sizes ($\beta$ > 0.30) (Cohen, 1988) in the discovery cohort were tested in the replication cohort. Inflammatory markers examined included: MCP-4, MCP-1, eotaxin-3, MDC, MIP1b, and INFγ; cardiovascular markers included systolic and diastolic blood pressure; brain volume regions included total GMV and all medial temporal subregions; and cognition included visual episodic memory and processing speed Table 3.

Adjusting for education, BMI, and heart rate, we continued to observe medium effect size interactions between sex by physical activity for select pro-inflammatory chemokine markers, MCP-4 ($\beta$ = 0.42, p = 0.029), MCP-1 ($\beta$ = 0.27, p = 0.17), eotaxin-3 ($\beta$ = 0.41, p = 0.016), and MIP1b ($\beta$ = 0.36, p = 0.07). Again, the beneficial relationship between physical activity and immune markers in men (MCP-4 $\beta$ = −0.29, p = 0.11; MCP-1 $\beta$ = −0.23, p = 0.21; eotaxin-3 $\beta$ = −0.30, p = 0.29; MIP1b $\beta$ = −0.30, p = 0.12) was notably attenuated in women (MCP-4 $\beta$ = 0.08, p = 0.63; MCP-1 $\beta$ = 0.01, p = 0.92; eotaxin-3 $\beta$ = 0.14, p = 0.63; MIP1b $\beta$ = 0.02, p = 0.90). The sex by activity interaction effect on other inflammatory markers was small to negligible ($\beta$ range = −0.27 to 0.03, all p-values > 0.87). Regarding gray matter volumetrics, sex by physical activity interaction terms demonstrated small benefits favoring males, but also did not reach statistical significance (GMV $\beta$ = −0.26, p = 0.31; parahippocampal $\beta$ = −0.15, p = 0.54; hippocampal $\beta$ = −0.17, p = 0.51); the sex by activity interaction term examining entorhinal volumes favored females but did not reach statistical significance ($\beta$ = 0.17, p = 0.51). Lastly, sex did not appear to meaningfully moderate the relationship between physical activity and cognition in the replication cohort (visual memory $\beta$ = 0.03, p = 0.87; processing speed $\beta$ = 0.17, p = 0.46).

### 4.3. Post-hoc: How does activity-related peripheral inflammation relate to brain and cognitive outcomes across sexes?

Adjusting for age, education, and BMI, we observed a sex by inflammatory composite (MCP-4, eotaxin-3, MIP-1b) interaction on total gray matter volume (inflammation*sex β = 0.27, p = 0.029) and visual memory (inflammation*sex β = 0.24, p = 0.02; Fig. 4). In both models, there was a stronger inverse relationship between the exercise-inflammatory composite and brain health related outcomes only in men, but not women (Men: β = -0.38, p = 0.01 (GMV); β = -0.22, p = 0.11 (visual memory); Women: β = 0.04, p = 0.73 (GMV); β = 0.10, p = 0.44 (visual memory)). Models exploring sex by inflammatory interactions on medial temporal subregions or DLPFC volumes did not reach significance (inflammation*sex β range = 0.14 to 0.17, all p-values < 0.28), nor did models examining executive functions, processing speed, and verbal memory (inflammation*sex β range = 0.03 to 0.09, all p-values < 0.92) – however, all models demonstrated the same directionality (i.e., tighter relationship between inflammation and neurobehavioral outcomes in men versus women).

## 5. Discussion

Using demographically-matched and replication samples, we show that physical activity is disproportionately related to better markers of peripheral inflammation in typically aging men, and these activity-related inflammatory proteins disproportionately associate with brain and cognitive outcomes in men (Fig. 5). In our discovery cohort, men showed a tighter relationship between reported daily physical activity and lower concentrations of MCP4, MCP1, MDC, MIP1b, INFγ, and eotaxin-3, larger parahippocampal volumes, and better visual memory and processing speed that appeared to be attenuated or absent in women. When replicated in an independent unmatched cohort, we observed similar effect sizes, particularly for plasma inflammatory markers MIP1b, MCP4, and eotaxin-3, though less consistently for neurobehavioral outcomes. Across cohorts, we then found that lower concentrations of these exercise-related inflammatory proteins were disproportionately associated with larger total gray matter volume and better visual memory in typically aging men compared to women. Taken together, our data suggest that immune regulation may be a sex-specific pathway by which men reap benefit from exercise on brain health. These data add to the emerging literature suggesting that exercise may differentially impact age-related neurological outcomes in men compared to women, and extends these works by implicating the immune system in contributing in sex-specific pathways.

It is difficult to determine from our observational design if these data indicate a *lessened* benefit in women or if typically aging men represent a high-risk group with *greater* benefit, particularly when it comes to immunologic health. Supporting the latter, females mount generally larger and faster innate and adaptive immune responses to pathogens or injury resulting in more rapid clearance, repair and wound-healing, and efficacy of vaccines across species (Klein and Flanagan, 2016). Indeed, women show greater upregulation of immune-related transcriptional factors (e.g., TLR) following an immune challenge or vaccine, greater phagocytic activity of neutrophils and macrophages, and more effective antigen-presenting activity compared to men (Hannah et al., 2008; Klein et al., 2010). Given that exercise is linked to maintenance of immune homeostasis (Duggal et al., 2019; Gleeson et al., 2011), perhaps the more tightly coupled effects observed between both physical activity and peripheral inflammation, and between peripheral inflammation and neurobehavior in our older adult men reflect this male risk bias. That is, older men may need exercise to a greater degree than women to keep either their vulnerable immune

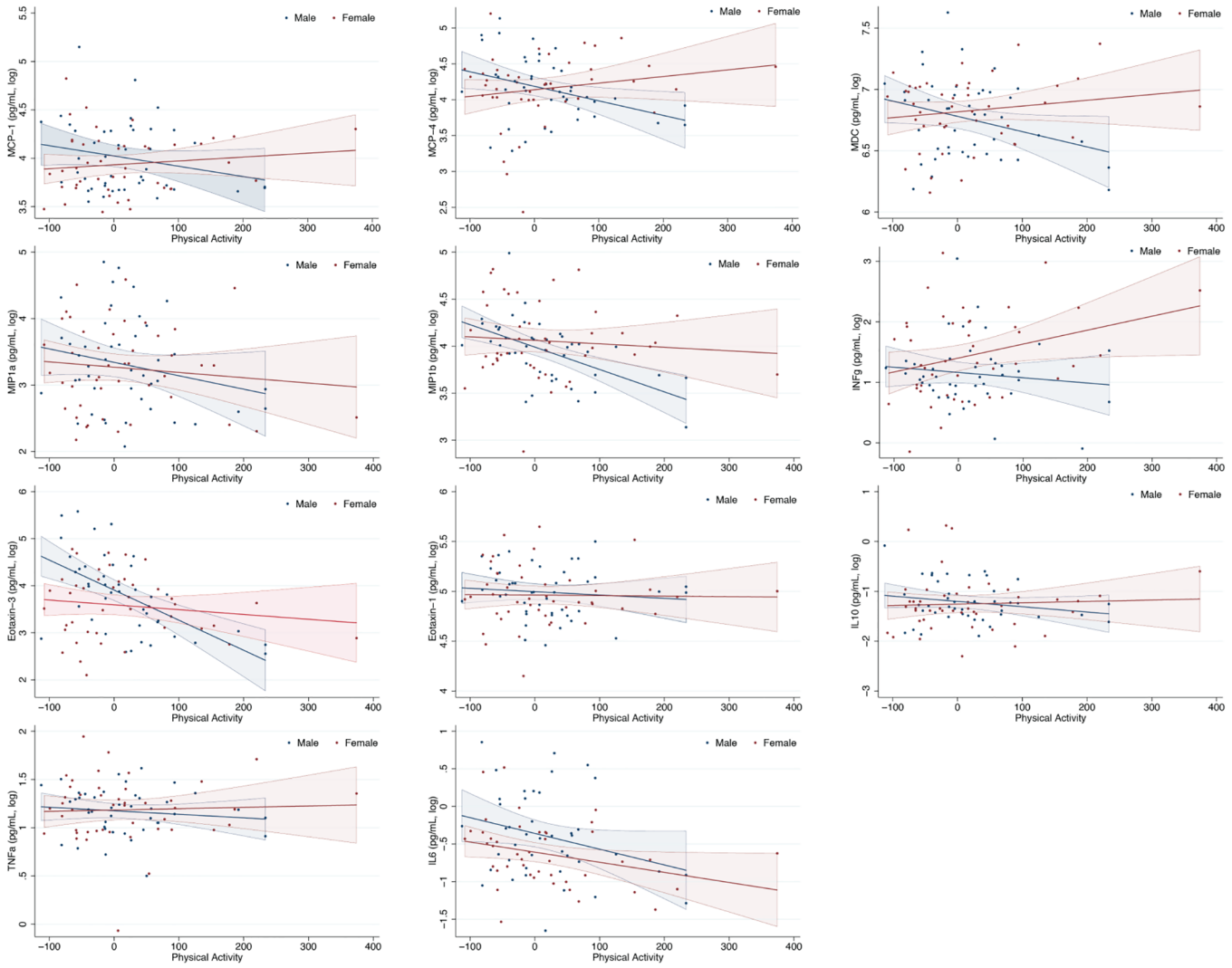


**Fig. 1.** Men demonstrate a stronger relationship between reported physical activity and peripheral inflammatory markers compared to women (Discovery cohort models). ***Note****. Interaction terms for MDC, MIP-1b, MCP-4, eotaxin-3 (all p-values* $<$ *0.05), and MCP-1 and INFy (p-values* $<$ *0.09) demonstrated small-to-medium effects (all* $\beta$ *values* $>$ *0.32; MCP-4, MIP-1b, eotaxin-3, and MCP-1 demonstrated similar effect sizes in replication cohort).*

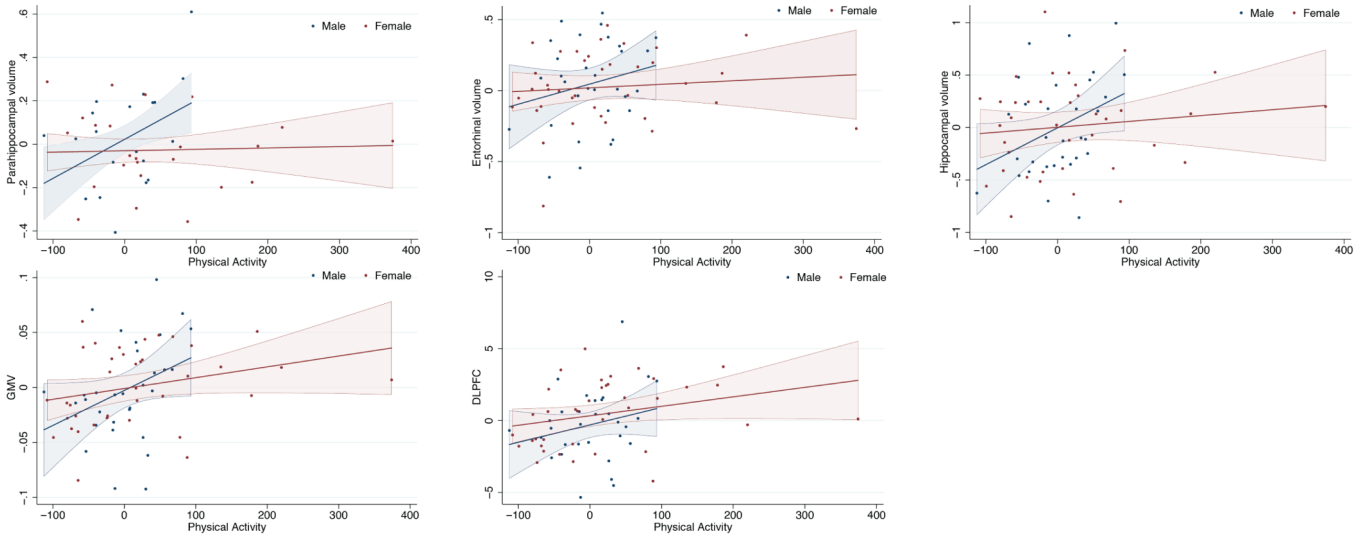


**Fig. 2.** Men demonstrate a stronger relationship between reported physical activity and brain volume outcomes compared to women (Discovery cohort models). ***Note****. Interaction terms for parahippocampal (p = 0.02), hippocampal (p = 0.19) and gray matter volume (GMV; p = 0.19) demonstrated small-to-medium effects (all* $\beta$ *values* $>$ *0.41).*

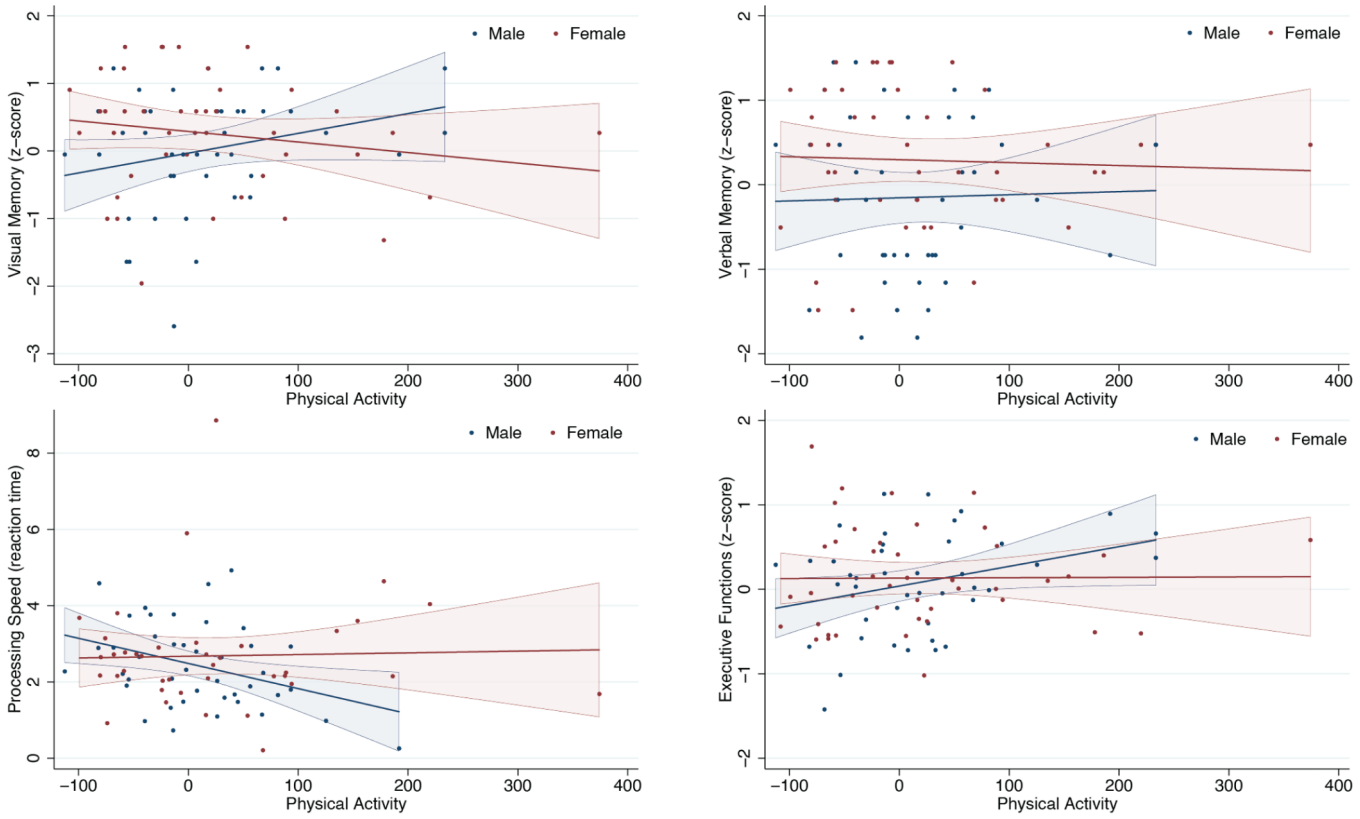


**Fig. 3.** Men demonstrate a stronger relationship between reported physical activity and cognitive outcomes compared to women (Discovery cohort models). ***Note.*** *Interaction terms for visual memory ($p = 0.04$) and processing speed ($p = 0.07$) demonstrated small-to-medium effect sizes ((all $\beta$ values $> 0.37$).*

systems in check and/or mount an appropriate immunologic response, whereas low physical activity is simply less detrimental to women's already robust immune system. Further supporting this line of thinking and as related to the brain, we found a stronger relationship between activity-related inflammation and brain structure and function in men compared to women. These data suggest that immune dysregulation may be particularly detrimental to the aging male brain. Taken together, in states of health, perhaps women's more inherently robust immune system is less important for and/or confers a female-specific resilience to the effects of typical brain aging, whereas men need greater support (in the form of exercise) to maintain the same level of immune and cognitive functioning.

On the other hand, although converging lines of evidence clearly indicate that females benefit from exercise (Hörder et al., 2018; Yaffe et al., 2001), it is possible that the degree or pattern may differ, and/or that women require a larger amount of exercise to gain the same benefits as men. In female ovariectomized mice, Berchtold and colleagues (2001) showed that not only was estrogen loss associated with reduced voluntary exercise, but the beneficial effect of exercise on hippocampal neurotrophic expression (i.e., mRNA BDNF) was significantly attenuated in the absence of estrogen (Berchtold et al., 2001). Importantly, estrogen replacement resulted in improved exercise-induced BDNF levels in female hippocampi, though the effect was not entirely restored to baseline levels. Other studies additionally show greater hippocampal long-term potentiation and improved memory following voluntary exercise in male compared to female mice (Titterness et al., 2011; Barha et al., 2017). Consistent with our findings, these data suggest that men may show greater neurobehavioral benefits following exercise, but the effects may be (at least in part) hormone-mediated.

Other clinical and animal studies have been less clear and perhaps indicate network-specificity to the observed sex-exercise effects on the brain. Though certainly not the case in all studies, there may be an emerging pattern suggesting a female-specific relationship between physical activity and dorsolateral prefrontal cortices and speeded/executive functioning tasks, with men demonstrating greater hippocampal and related memory-specific benefits (Barha et al., 2019); (Titterness et al., 2011; Goldstein, 2001). This network specificity may be consistent with the reported relative abundance of estrogen receptors not only in the hippocampus but also particularly, the prefrontal cortex (i.e., up to 50% of ER?? receptors) (Donahue et al., 2000; Wang et al., 2010; Laing and Secombes, 2004). Our data recapitulate disproportionate effects of physical activity on medial temporal and memory functioning in men compared to women; yet, we did not observe a female-specific effect on prefrontal or executive/speeded tasks, or any other outcomes. One major study design difference was our utilization of demographic matching which aimed to remove sex-specific variance in age and educational differences that were not directly controlled for in other studies that used stratified modeling (i.e., developed models in males and females separately versus direct testing of sex-specific slopes) (Barha et al., 2019). Additionally, we intentionally captured a cognitively homogeneous cohort of high-functioning, typically aging older adults, which differs from some previous works that included individuals with mild cognitive impairment. This cognitive homogeneity may be advantageous given the known differential sex effects on brain aging (i.e., women demonstrating more optimal trajectories in states of health but more precipitous declines in states of disease (e.g., Alzheimer's disease); nonetheless, it will be critical to parse out disease state specific effects of exercise by sex.

Regarding the specific inflammatory markers, we found that MIP1b, MCP4, and eotaxin-3 and to a lesser extent, MCP1, demonstrated the most consistent sex-specific relationships with physical activity across cohorts. Although it is difficult to draw pathway-specific interpretations from circulating protein levels, we do note that each of these markers are C–C motif chemokines involved in monocyte, granulocyte, and lymphocyte recruitment, migration, and activation (Huber et al., 2018; Bettcher et al., 2019). Interestingly, plasma levels of MCP1 have been

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**Table 3**
Sex moderates the relationship between reported physical activity and peripheral inflammatory markers (Discovery cohort models).

| | **MDC** | | | **MIP1b** | | | **MIP1a** | | | **Eotaxin-1** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value |
| BMI | 0.29 | 0.006, 0.04 | 0.007 | −0.21 | −0.037, −0.0006 | 0.04 | 0.12 | −0.02, 0.06 | 0.26 | −0.08 | −0.02, 0.009 | 0.45 |
| Sex | 0.19 | −0.01, 0.23 | 0.07 | 0.12 | −0.06, 0.23 | 0.24 | −0.04 | −0.35, 0.23 | 0.70 | −0.08 | −0.16, 0.07 | 0.45 |
| PASE | −0.30 | −0.002, 9.8e-5 | 0.07 | −0.59 | −0.004, −0.001 | 0.0005 | −0.26 | −0.005, 0.001 | 0.14 | −0.14 | −0.002, 0.007 | 0.45 |
| Sex* PASE | **0.36** | **0.0001, 0.003** | **0.03** | **0.39** | **0.0003, 0.004** | **0.019** | 0.13 | −0.002, 0.005 | 0.47 | 0.09 | −0.001, 0.001 | 0.61 |

| | **Eotaxin-3** | | | **MCP-1** | | | **MCP-4** | | | **INFγ** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value |
| BMI | 0.05 | −0.52, 0.09 | 0.16 | −0.07 | −0.02, 0.01 | 0.55 | 0.09 | −0.01, 0.04 | 0.41 | 0.08 | −0.02, 0.05 | 0.46 |
| Sex | −0.14 | −0.52, 0.09 | 0.16 | −0.11 | −0.22, 0.07 | 0.30 | 0.02 | −0.18, 0.22 | 0.83 | 0.24 | 0.06, 0.61 | 0.02 |
| PASE | −0.70 | −0.009, −0.003 | < 0.001 | −0.28 | −0.002, 0.0003 | 0.12 | −0.35 | −0.004, −1.4e5 | 0.048 | −0.10 | −0.003, 0.002 | 0.60 |
| Sex* PASE | **0.45** | **0.002, 0.009** | **0.006** | **0.30** | **−0.0003, 0.003** | **0.09** | **0.41** | **0.0004, 0.005** | **0.02** | **0.32** | **−0.0002, 0.006** | **0.064** |

| | **IP10** | | | **IL-6** | | | **TNFa** | | | **IL-10** | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value |
| BMI | 0.10 | −0.01, 0.04 | 0.38 | 0.19 | −0.003, 0.05 | 0.07 | 0.03 | −0.01, 0.02 | 0.82 | 0.12 | −0.01, 0.04 | 0.28 |
| Sex | 0.15 | −0.07, 0.34 | 0.18 | −0.19 | −0.40, 0.02 | 0.07 | 0.03 | −0.11, 0.15 | 0.79 | −0.01 | −0.23, 0.20 | 0.91 |
| PASE | 0.06 | −0.002, 0.002 | 0.75 | −0.33 | −0.004, 3.5e5 | 0.054 | −0.11 | −0.001, 0.001 | 0.55 | −0.17 | −0.003, 0.001 | 0.35 |
| Sex* PASE | −0.03 | −0.003, 0.002 | 0.85 | 0.09 | −0.002, 0.003 | 0.60 | 0.12 | −0.001, 0.002 | 0.52 | 0.17 | −0.001, 0.004 | 0.33 |

**Note**. Male = 1, Female = 2; B = standardized beta parameters; bolded models reproduced similar effect sizes in replication cohort. BMI = body mass index; PASE = Physical Activity Scale for the Elderly.

**Table 4**
Sex moderates the relationship between reported physical activity and neurobehavioral outcomes (Discovery cohort models).

| COGNITIVE OUTCOMES | Visual Processing Speed* | | | Executive Functions | | | Verbal Memory | | | Visual Memory | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value |
| BMI | −0.02 | −0.08, 0.07 | 0.87 | 0.05 | −0.03, 0.04 | 0.65 | 0.02 | −0.04, 0.05 | 0.83 | 0.03 | −0.04, 0.05 | 0.82 |
| Sex | 0.11 | −0.29, 0.88 | 0.32 | 0.07 | −0.18, 0.34 | 0.55 | 0.25 | 0.05, 0.84 | 0.03 | 0.13 | −0.15, 0.61 | 0.24 |
| PASE | −0.44 | −0.01, −0.0003 | 0.04 | 0.35 | −0.5.6e5, 0.005 | 0.055 | 0.03 | −0.003, 0.004 | 0.88 | 0.30 | −0.001, 0.006 | 0.09 |
| Sex*PASE | **0.40** | **−0.0005, 0.02** | **0.065** | −0.26 | −0.005, 0.0008 | 0.15 | −0.05 | −0.005, 0.004 | 0.80 | **−0.37** | **−0.01, −0.0001** | **0.043** |

| BRAIN VOLUME OUTCOMES | GMV | | | DLPFC | | | Parahippocampal | | | Entorhinal | | | Hippocampus | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value | B | 95% CI | p-value |
| BMI | −0.12 | −0.003, 0.001 | 0.34 | −0.15 | −0.02, 0.05 | 0.23 | 0.10 | −0.006, 0.01 | 0.42 | −0.12 | −0.02, 0.008 | 0.34 | 0.11 | −0.01, 0.04 | 0.39 |
| Sex | −0.05 | −0.02, 0.02 | 0.66 | 0.10 | −0.08, 1.6 | 0.49 | −0.21 | −0.16, −0.01 | 0.09 | −0.14 | −0.21, 0.06 | 0.28 | −0.05 | −0.27, 0.18 | 0.41 |
| PASE | 0.64 | 1.0e−5, 0.001 | 0.04 | 0.43 | −0.005, 0.03 | 0.17 | 0.82 | 0.0004, 0.003 | 0.009 | 0.40 | −0.001, 0.003 | 0.21 | 0.57 | −0.00004, 0.007 | 0.08 |
| Sex*PASE | −0.41 | −0.001, 0.0001 | 0.19 | −0.19 | −0.03, 0.01 | 0.53 | **−0.72** | **−0.003, −0.0003** | **0.02** | −0.31 | −0.003, 0.001 | 0.35 | −0.41 | −0.006, 0.001 | 0.19 |

**Note.** Male = 1, Female = 2; *higher values indicate slower reaction time; B = standardized beta parameters; bolded models were reproduced in replication cohort. BMI = body mass index; PASE = Physical Activity Scale for the Elderly; GMV = total gray matter volume; DLPFC = dorsolateral prefrontal cortex.

linked to risk of AD diagnosis and longitudinal memory-specific declines in clinically normal adults by our group (Bettcher et al., 2016; Westin et al., 2012), and CSF (but not plasma) MCP1 has been shown to predict conversion from MCI and AD dementia in older adults (Bettcher et al., 2018). Additionally, we have also demonstrated that, among a larger panel of inflammatory markers, peripheral MIP1b concentrations were among the most closely associated with cerebrospinal fluid levels in healthy older adults (r = 0.55) and were associated with higher CSF p-tau levels (Craig-Schapiro et al., 2011), suggesting that the relationships observed here may have implications for central nervous system inflammation and neurodegeneration. Additionally, eotaxins have increasingly been implicated in aging and neurodegenerative disease (Bettcher et al., 2019). Both in serum and CSF, eotaxin-3 levels relate to age (Choi et al., 2008), differ between normal and cognitively impaired patients, (Bettcher et al., 2018; O'Bryant et al., 2011) and serum concentrations have been proposed as a potential adjunct biomarker for AD (Leung et al., 2013); yet other studies of peripheral eotaxin-3 levels have failed to find group differences across cognitively impaired cohorts (Westin et al., 2012); (Bettcher et al., 2018; Moffat, 2005). Similarly, MCP-4 is known to increase with age and relate to chronic inflammatory diseases in the periphery, though CSF and peripheral levels have not tracked closely with cognition in older adults (Bettcher et al., 2016); (Bettcher et al., 2018). Taken together, these peripheral proteins appear to show some relevant signals to age-related CNS function and AD risk, suggesting that systemic immune cell activation and recruitment may be pathway through which exercise relates to cognitive aging. Nonetheless, these are observational, clinical data, and future mechanistic works are greatly needed to parse out the immune-specific pathways (likely via network-based proteomics) represented in the periphery that may be moderated by exercise and driving CNS age-related health.

Although we are among the first to directly implicate the immune system as a potential sex-specific pathway between exercise and brain health, our understanding of other mechanisms driving this relationship are nascent and ultimately a complex interplay among chromosomal, hormonal, metabolic, and psychosocial contextual (e.g., health-seeking behavior, exposure) factors are likely implicated. A limitation to our study is that these retrospective data did not capture menopausal status or hormone replacement therapy, though the majority of women in our study were likely in post-menopausal stages (> 94% of cohort were 60 + years old). As noted previously, estrogen availability may be a critical moderator of how the female brain gains benefits of exercise (Berchtold et al., 2001). Additionally, estradiol importantly moderates immune responses with heightened inflammation at low levels but attenuated inflammation at high levels (Klein and Flanagan, 2016). On the other hand, androgen levels, including testosterone, also decline with age, and their decreases are associated with more adverse brain and cognitive outcomes (Lv et al., 2016; Janowsky, 2006; Bianchi et al., 2012) and also (given their immunosuppressive role) greater states of age-related chronic inflammation (Klein and Flanagan, 2016). In-depth characterization of how sex hormone levels, including menopausal state and hormone replacement therapy, and age interact with physical activity and the immune system to impact the aging male and female brain are ongoing but clearly a high-need area of research. Furthermore, sex chromosomes are known to directly impact immune functioning (Libert et al., 2010; Smith-Bouvier et al., 2008; Davis et al., 2019) and recent elegant experiments manipulating sex organs across sex chromosomal genotypes indicate that the X chromosome may drive female-linked longevity (Davis et al., xxxx) and cognitive resilience in wildtype and AD mouse models, independently of sex hormones (Dubal and Rogine, 2017); (Ali et al., 2013) . Careful exploration of the synergistic and divergent effects of sex-relevant biologies (e.g., hormonal and chromosomal) is critically needed to disentangle these important emerging brain, behavior, and immune relationships.

Another major limitation of our study was the use of self-report for physical activity that did not explicitly differentiate activity intensity

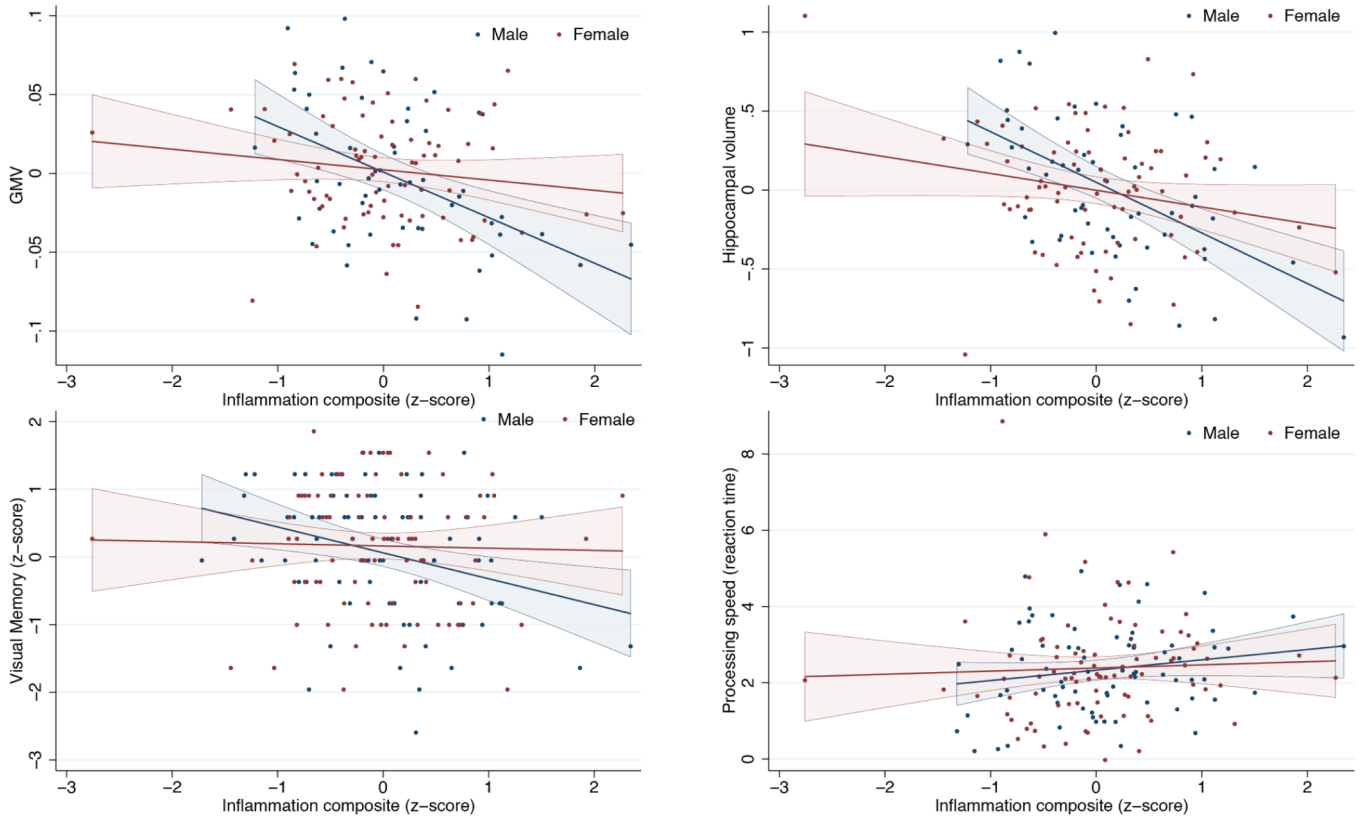


**Fig. 4.** Post-hoc models exploring the differential relationship between activity-related inflammatory markers (MCP4, eotaxin-3, and MIP1b) and brain structure and function across sexes. ***Note.*** *Interaction terms for gray matter volume (GMV; $p = 0.03$) and visual memory ($p = 0.02$) demonstrated small effect sizes (all $\beta$ values $> 0.24$).*

(e.g., aerobic vs resistance training). We cannot know if sex differences in reporting bias exist or impact our findings. That is, it is certainly possible that either women overestimated their activity resulting in an apparent greater male benefit at seemingly similar activity levels, or that men are engaged in more intensive physical activities that are not qualitatively captured on our self-report measure (e.g., jogging at the same frequency but a faster speed). We also did not systematically capture common medication regimens known to target immune-mediated processes (e.g., NSAIDs, corticosteroids, TNF inhibitors) (Tsuboi et al., 1995; Wallen et al., 1991), and may be an important confounder to evaluate in future works. Additionally, the observational design of the study inherently limits determination of causality, as it is possible that older adults with poorer overall functioning (e.g., poorer immune, cardiovascular, neurobehavioral health) are engaging in less physical

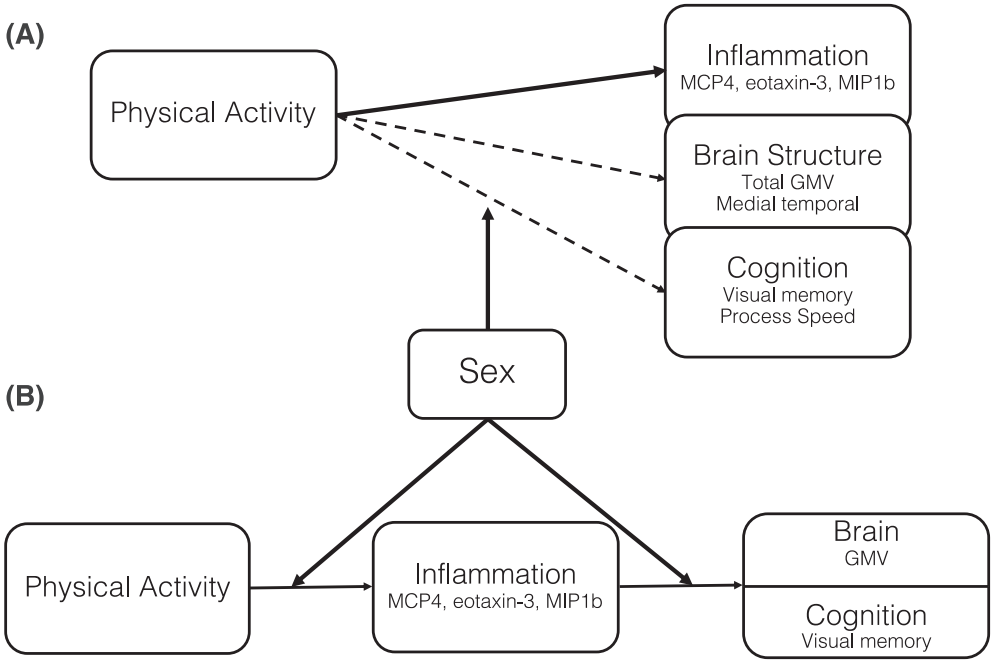


**Fig. 5.** Summary schematic of primary study findings: (A) Initial models in the Discovery Cohort indicated that sex moderated the relationship between physical activity and markers of immune, brain and cognitive health, with men demonstrating a stronger relationship that appeared attenuated-to-absent in women; however, the most consistent effect reproduced in the Replication Cohort was demonstrated for inflammatory markers; (B) Taking together both the initial and post-hoc models, greater reported physical activity related to lower markers of inflammation in men only, and lower markers of inflammation related to better brain and cognitive outcomes in men only.

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activity. Lastly, although we utilized an independent-sample replication design, the sample sizes were small, making it difficult to meaningfully test more complex models (e.g., mediating effects). Future large cohort studies and, ultimately, randomized controlled trials that include fluid biological markers as well as neurobehavioral outcomes are critically needed to begin to parse out these mechanistic relationships.

Our data suggest sexual dimorphism influences how physical activity confers effects on the body and the brain. Namely, the beneficial effect of physical activity on brain health indicators may be more closely related to regulation of immune functioning for men, but not women. These data underscore the importance of personalized medicine approaches to better understand how and in whom certain behavioral interventions may be most efficacious. The critical role of biological sex on the development of the peripheral and central nervous systems develop and risk for disease with age is an exciting area of study that will likely lead to highly fruitful avenues of understanding both disease risk and resiliency more broadly.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2020.05.014.

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