Fitness, independent of physical activity is associated with cerebral blood flow in adults at risk for Alzheimer's disease

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Abstract

Patterns of decreased resting cerebral blood flow (CBF) within the inferior temporal gyri, angular gyri, and posterior cingulate are a feature of aging and Alzheimer's disease (AD) and have shown to be predictive of cognitive decline among older adults. Fitness and physical activity are both associated with many indices of brain health and may positively influence CBF, however, the majority of research to date has examined these measures in isolation, leaving the potential independent associations unknown. The purpose of this study was to determine the unique contributions of fitness and physical activity when predicting CBF in cognitively healthy adults at risk for AD. One hundred participants (63% female) from the Wisconsin Registry for Alzheimer's Prevention underwent a maximal exercise test, physical activity monitoring, and a 3-D arterial spin labeling magnetic resonance imaging scan. For the entire sample, fitness was significantly associated with CBF while accounting for physical activity, age, gender, APOE ε4, family history of AD, education, and handedness ($p = .026$). Further, fitness explained significantly more variance than the combined effect of the covariates on CBF (R^2 change $= .059$; $p = .047$). These results appear to be gender dependent, our data suggest fitness level, independent of physical activity, is associated with greater CBF in regions that are known to decline with age and AD for female ($p = .011$), but not male participants.

Keywords Cardiorespiratory fitness · Physical fitness · $\dot{V}O_{2peak}$ · Arterial spin labeling · Accelerometer

Introduction

Decreased resting cerebral blood flow (CBF) is a feature of aging and Alzheimer's disease (AD) (Austin et al. 2011; Okonkwo et al. 2014a). Reductions in CBF measured by arterial spin labeling (ASL) have shown to be predictive of cognitive decline and conversion to AD among older adults (Chao et al. 2010; Wang et al. 2013), and can be detected in cognitively normal adults harboring genetic and/or familial risk for AD (Okonkwo et al. 2014a; Thambisetty et al. 2010;

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Wierenga et al. 2013). However, inconsistencies in the literature are present with some studies reporting increased CBF, typically in anterior brain regions (e.g. anterior cingulate) (Wierenga et al. 2014). Specific brain regions that are susceptible to declining CBF include the inferior temporal gyri, angular gyri, and posterior cingulate (Chen et al. 2011; Wang et al. 2013). For these reasons, ASL-measured CBF is a promising method for investigating early pathophysiological brain changes in preclinical AD and may be a potential target for interventions aimed at delaying the progression of the disease (Wierenga et al. 2014).

Fitness and physical activity are both positively associated with many indices of brain health (Erickson et al. 2014; Okonkwo et al. 2014b) and may favorably influence CBF (Thomas et al. 2013; Zimmerman et al. 2014). However, whether fitness and physical activity differentially affect CBF remains unclear (Chapman et al. 2013; Pereira et al. 2007). Aerobic fitness is defined as the ability to perform moderate-to-high intensity exercise for prolonged periods of time (ACSM 2013; Caspersen et al. 1985) and is objectively measured by a maximal exercise test where the highest rate of oxygen consumption ($\dot{V}O_{2peak}$) serves as the gold-standard fitness measure (ACSM 2013). Physical activity is any bodily movement that results in energy expenditure (Caspersen et al. 1985) and can be objectively measured through accelerometry, which records the duration and intensity of movement (Lyden et al. 2014). Although fitness and physical activity are related, they are separate physiological and behavioral measures that may explain unique variance when predicting various health outcomes (Lee et al. 2010).

Limitations of previous research investigating relationships between fitness, physical activity, and brain health include unstandardized fitness testing (Dougherty et al. 2018) and the use of self-reported physical activity questionnaires which are subject to recall and social desirability biases (Dyrstad et al. 2014). Importantly, the vast majority of research to date has examined these measures in isolation, leaving the potential independent contributions that fitness and physical activity may have towards mitigating the pathophysiology of AD largely unknown. Further, there is a paucity of studies that have investigated gender differences related to potential AD therapeutics (Dougherty et al. 2017a; Nebel et al. 2018). The purpose of this study was to determine the unique variance explained by fitness and physical activity when predicting CBF in an older adult population at-risk for AD, and ascertain whether this effect is gender dependent.

Methods

Participants

One hundred cognitively healthy adults from the Wisconsin Registry for Alzheimer's Prevention (WRAP) participated in this study. WRAP is a longitudinal registry composed of more than 1500 late-middle-aged adults who were cognitively normal, and free of major medical conditions (e.g. neurological diseases, psychiatric disorders) at study entry (Sager et al. 2005). For the current study, participants were determined to be cognitively healthy using the mini-mental state examination (MMSE ≥24), and were excluded for any of the following: documented vascular disease, type I or II diabetes mellitus, severe untreated hypertension (>200/100 mmHg), and the inability to safely walk on a treadmill. All 100 participants completed a maximal exercise test, wore a triaxial accelerometer during waking hours for one week, and underwent ASL magnetic resonance imaging (MRI). Similar to the overall WRAP cohort, this study's sample was enriched with persons who have a family history (FH) of AD (71%) or who were APOE ε4 positive (43%). The University of Wisconsin Institutional Review Board approved all study procedures and informed consent was obtained from all individual participants.

Fitness assessment

Exercise tests were conducted by a certified exercise physiologist along with a trained exercise specialist following a resting twelve-lead electrocardiogram (ECG) assessment. The exercise test was completed on a motor driven treadmill using a modified Balke protocol (Balke and Ware 1959). Comfortable walking speeds were determined during a warm-up period and kept constant for the duration of testing. The majority of participants walked at 3.5 miles per hour. However, if the participant indicated that this speed was uncomfortable, a slower walking speed was chosen. Following a 2-min warm-up at 0% grade, the grade of the treadmill was increased by 2.5% every 2 min until the participant reached volitional exhaustion or indicated they could no longer continue.

During the exercise test, oxygen consumption ($\dot{V}O_2$), carbon dioxide production ($\dot{V}CO_2$), minute ventilation ($\dot{V}E$), respiratory exchange ratio (RER) and work rate were obtained using a metabolic cart (TrueOne® 2400 Parvomedics, Sandy, UT) and a two-way non-rebreathing mask. Heart rate (HR) was continuously measured through a twelve-channel ECG device (Schiller CS-200 Exercise Stress System, Baar, Switzerland). The metabolic cart was calibrated using standard procedures within 4 h of the exercise test to ensure accuracy. Inclusion criteria for a valid exercise test was determined based on American College of Sports Medicine (ACSM) criteria of meeting at least two of the following: (1) RER ≥ 1.1, (2) change in $\dot{V}O_2 < 200$ ml with an increase in work, (3) rating of perceived exertion (RPE) of 17 or greater (Borg 1982), and (4) achieving at least 90% of age predicted maximal heart rate (220-age) (ACSM 2013). These methods were implemented to avoid potential age-driven relationships

when volitional effort was not achieved (Dougherty et al. 2018). Aerobic fitness was defined as the highest oxygen consumption ($\dot{V}O_{2peak}$, ml/kg/min) value recorded during the final stage of the exercise test when criteria were met.

Physical activity assessment

All participants wore a triaxial GT3X+ accelerometer (Actigraph, Pensacola, FL) on their hip for 7 consecutive days following the graded exercise test. Participants were instructed to wear the device during all waking hours, with the exception of when showering, swimming, or bathing. Standard accelerometry inclusion criteria consisted of at least 10 h of valid wear time per day for a minimum of 3 weekdays and 1 weekend day (Troiano et al. 2008). Accelerometer data (in 1-s epochs) were processed using the validated Sojourn-3 Axis method which uses an artificial neural net to identify boundaries between activities of different intensities and to estimate metabolic equivalents (METs) for each bout (Lyden et al. 2014). To calculate minutes spent in different intensity categories of physical activity, estimated METs were determined for each bout interval and then separated into physical activity categories accordingly: <1.5 METs = sedentary, 1.5–2.99 METs = light, 3–6 METs = moderate and > 6 METs = vigorous. Based on current physical activity recommendations (2018 Physical Activity Guidelines Advisory Committee; Piercy et al. 2018), our chosen physical activity measure was time spent engaged in moderate-to-vigorous physical activity which was expressed as a percentage of total accelerometer wear time to account for individual differences.

Neuroimaging protocol

The MRI scans were acquired in the axial plane on a GE X750 Discovery 3.0 T scanner with an eight-channel phased array head coil (General Electric, Waukesha, WI). Three-dimensional (3-D) T1-weighted inversion recovery-prepared spoiled gradient echo scans were collected using the following parameters: inversion time (TI)/echo time (TE)/repetition time (TR) = 450 ms/3.2 ms/8.2 ms, flip angle = 12°, slice thickness = 1 mm no gap, field of view (FOV) = 256, matrix size = 256 × 256. Resting CBF assessments were made using background suppressed pseudocontinuous ASL (pcASL) (Ye et al. 2000; Dai et al. 2008). The protocol featured a 3-D fast spin echo spiral sequence that utilizes a stack of variable-density spiral 4-ms readout and 8 interleaves. Scan parameters included TE/TR = 10.5 ms/4900 ms, slice thickness = 4 mm no gap, FOV = 240, matrix size = 128 × 128, number of excitations = 3, and labeling radiofrequency (RF) amplitude = 0.24 mG. Multislice spin labeling was implemented using a single coil that eliminates off-resonance errors (Garcia et al. 2005) and post-labeling delay was 2025 ms. The three acquisitions that

comprise the pcASL sequence (i.e., NEX = 3) were averaged to improve signal-to-noise ratio. The pcASL sequence also included a fluid-suppressed proton density (PD) acquisition, with the same imaging sequence/image slab location as the pcASL but without the RF-labeling preparation, for CBF flow quantitation and image registration. The entire pcASL sequence—all 3 excitations plus PD scan—lasts 4 min and 27 s. To preserve the fidelity of the CBF assessment, scanning was done after a minimum 4-h fast from food, tobacco, caffeine, and medications with vasomodulatory properties. We have previously reported excellent test-retest reliability ($r > 0.95$) of this pcASL procedure (Xu et al. 2010).

Image processing

The CBF images were processed using SPM12 (www.fil.ion.ucl.ac.uk/spm). The procedure essentially involved registering each participant's co-localized PD image to their T1 volume, applying the derived transformation matrix to their average quantitative CBF map, then spatially normalizing the T1 volume and associated CBF image to the Montreal Neurological Institute (MNI) template, with resampling to a $2 \times 2 \times 2$ mm^3 voxel size. The normalized CBF maps were then smoothed using an 8-mm full-width at half-maximum Gaussian kernel. To focus our analyses on brain regions known to be implicated in AD and reduce the risk of false-positive errors, we used the Alzheimer's Disease Neuroimaging Initiative (ADNI) FDG Meta-ROI suite to extract CBF values from five AD-relevant regions of interest (ROI) (Landau et al. 2011). The Meta-ROI suite was developed based on a meta-analysis and has shown to discriminate between cognitively healthy, mild cognitive impairment (MCI) and AD participants (Landau et al. 2011). The ROIs identified included the left and right inferior temporal gyri, the left and right angular gyri, and the posterior cingulate. A composite calculated by averaging extractions from all five ROIs served as our CBF measure of interest. Although originally developed for FDG PET data (Landau et al. 2011), the ADNI Meta-ROI suite has shown to be sensitive to age and AD-related CBF changes (Chen et al. 2011; Wang et al. 2013). Additionally, global CBF was extracted to account for individual CBF variability. The composite CBF measure is expressed in both absolute (ml/100 g/min) and relative terms (i.e., scaled to the global CBF measure to account for individual CBF variability). The mean ± SD time between the fitness / physical activity assessment and MRI was 0.32 ± 0.35 years.

Statistical analyses

A model assumption test revealed that physical activity was non-normally distributed (positive skewness). To address the normality violation we subjected the physical activity variable to a log base 10 transformation. Independent-sample t tests (or χ^2 tests, as applicable) were used to compare demographics

between males and females. Pearson correlations were used to determine the associations between age, fitness, physical activity, and CBF. Hierarchical multiple linear regression was used to assess the independent relationships between CBF and both fitness and physical activity while adjusting for the following covariates: age, gender, APOE-ε4 status, FH status, education and handedness with the significance level set at 0.05. Model 1 included the covariates predicting CBF, while Model 2 added fitness and physical activity to determine if these variables increased the total amount of variance explained (R^2 values). To visualize relationships, participants were separated into fitness tertiles. Controlling for the previously described covariates, differences in CBF across the fitness tertiles were tested using pairwise contrasts and Cohen's d effect sizes ($d = M_1 - M_2 / SD_{pooled}$) were used to examine the magnitude of the differences. All analyses were first conducted within the entire sample, and then separated for males and females. Our statistical package was IBM SPSS, version 25.

Results

One hundred cognitively healthy (MMSE = 29.13 ± 1.0) participants (mean age = 63.6 ± 5.9 years, 63% female) completed the study. On average, participants displayed a $\dot{V}O_{2peak}$ (ml/kg/min) of 26.5 ± 6.3 and engaged in 1.31 ± .64 h of moderate-vigorous physical activity per day. The average absolute and relative composite CBF values were 38.13 ± 8.37 ml/100 g/min, and 1.15 ± .07 respectively. Additional participant characteristics are listed in Table 1. Age was

negatively associated with fitness ($r = -.41$; $p < .001$), physical activity ($r = -.22$; $p = .027$) and CBF ($r = -.21$; $p = .035$; Fig. 1). The modest relationship between fitness and physical activity ($r = .35$; $p < .001$) suggests that these variables were not completely overlapping constructs and, therefore, may explain unique variance with respect to CBF.

For the entire sample ($n = 100$) Model 1 did not predict CBF ($p = .158$). In Model 2, adding fitness and physical activity significantly increased the overall variance explained (R^2 change = .059; $p = .047$; Table 2) and resulted in a significant model predicting CBF ($p = .050$). Model 2 revealed fitness was significantly and positively associated with CBF ($p = .026$; Table 3) while accounting for variance explained by physical activity, age, gender, APOE ε4 status, FH status, education and handedness. In contrast, physical activity was not ($p = .629$). For females ($n = 63$), Model 1 did not predict CBF ($p = .155$), however, Model 2 increased the overall variance explained (R^2 change = .099; $p = .036$) and resulted in a significant model predicting CBF ($p = .039$) demonstrating, fitness ($p = .011$) but not physical activity ($p = .574$) was positively associated with CBF (Table 4). Neither Model 1 ($p = .975$) nor Model 2 ($p = .874$) significantly predicted CBF in the male participants. To visualize this relationship female participants were separated into low (mean = 19.5 ml/kg/min), moderate (mean = 24.1 ml/kg/min), and high (mean = 29.8 ml/kg/min), fitness tertiles ($n = 21, 21, 21$). Mean CBF was highest in Tertile 3 (high fitness) and lowest in Tertile 1 (low fitness) ($d = 1.4$). Participants in Tertile 2 (moderate fitness) displayed greater CBF compared to participants in Tertile 1 ($d = .70$), and participants in Tertile 3 exhibited higher CBF than participants in Tertile 2 ($d = .49$; Fig. 2).

Table 1 Characteristics of study participants

Variable	Entire sample, $n = 100$	Females, $n = 63$	Males, $n = 37$	p value
Age, years, mean (SD)	63.64 (5.9)	63.55 (5.9)	63.78	.852
Gender, n	–	63	37	–
APOE ε4 positive, %	43	37	54	.089
Family history of AD, %	71	67	78	.217
Body mass index, kg/m^2	27.89 (5.42)	27.55 (6.0)	28.46 (4.3)	.418
Education, years	16.4 (2.5)	16.0 (2.4)	17.2 (2.6)	.014
Handedness, % Right	84	92	70	.012
Mini-Mental State Examination	29.13 (1.0)	29.17 (1.1)	29.05 (.9)	.564
Fitness, $\dot{V}O_{2peak}$, ml/kg/min	26.5 (6.3)	24.4 (5.0)	30.0 (6.7)	.000
Total actigraph daily wear time, hours	15.4 (0.99)	15.3 (.99)	15.5 (.98)	.293
Daily moderate-vigorous physical activity, hours	1.31 (0.64)	1.28 (.71)	1.45 (.48)	.089
Daily moderate-vigorous physical activity, %	8.53 (4.27)	8.0 (4.7)	9.4 (3.3)	.107
ROI Composite CBF, ml/100 g/min	38.13 (8.37)	40.30 (7.32)	34.44 (8.85)	.001
Relative ROI Composite CBF, %	1.15 (.07)	1.16 (.07)	1.13 (.08)	.099

APOE ε4, apolipoprotein E ε4 allele; *AD*, Alzheimer's disease; $\dot{V}O_{2peak}$, peak oxygen consumption; *ROI*, region of interest; *CBF*, resting cerebral blood flow

*Values are represented as mean and standard deviation, unless otherwise indicated
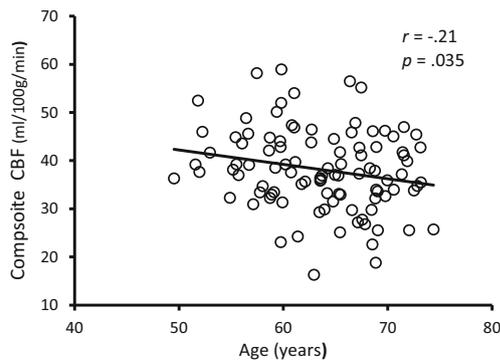
Fig. 1 **Age and CBF.** The relationship between age and CBF. CBF = resting cerebral blood flow

Discussion

This study concomitantly examined objectively measured fitness and accelerometer-determined physical activity to explore the unique associations with CBF among adults at risk for AD. We found that within the full sample, as well among females, fitness, independent of physical activity was positively associated with CBF in regions known to decline with age and the progression of AD (Chen et al. 2011; Wang et al. 2013). Notably, hierarchical multivariable regression demonstrated that fitness accounted for significantly more variance than the combined effect of the covariates on CBF. This finding was most pronounced in the female participants where fitness level accounted for approximately the same amount of variance (10%) as the covariates. This finding suggests that in our sample, fitness level was a stronger predictor of CBF than the known risk-factors included in Model 1. Interestingly, tertile analyses suggest that fitness might exert a threshold-response relationship with CBF. Female participants categorized in the low fitness tertile (19.5 ml/kg/min) showed the lowest CBF compared to the moderate (24.1 ml/kg/min) and high fitness (29.8 ml/kg/min) groups.

Table 3 Hierarchical multiple linear regression predicting CBF in the entire sample (n = 100)

Model	Independent predictors	β (SE)	Standardized β	p value
1	Age	−.001 (.001)	−.078	.467
	Gender	−.040 (.017)	−.270	.018
	APOE ε4	−.008 (.015)	−.052	.625
	Family History of AD	.016 (.017)	.105	.335
	Education	.006 (.003)	.220	.048
	Handedness	.032 (.021)	.165	.125
2	Age	.001 (.001)	.050	.668
	Gender	−.059 (.018)	−.402	.001
	APOE ε4	−.015 (.016)	−.106	.328
	Family History of AD	.023 (.017)	.148	.175
	Education	.005 (.003)	.188	.112
	Handedness	.032 (.020)	.166	.115
	Physical Activity	.019 (.039)	.056	.629
	Fitness	.003 (.001)	.295	.026

Fitness, $\dot{V}O_{2peak}$; *Physical activity*, moderate-vigorous; *APOE ε4*, apolipoprotein E ε4 allele; *AD*, Alzheimer's disease; *CBF*, resting cerebral blood flow; *β*, beta coefficient; *SE*, standard error

There is increasing recognition that CBF is an important preclinical biomarker of AD (Austin et al. 2011; Okonkwo et al. 2014a). In a large-scale study, decreased CBF was found to be the earliest pathophysiological event, preceding traditional AD biomarkers (e.g. amyloid, tau) (Iturria-Medina et al. 2016). Further, patterns of decreased CBF have been shown to predict conversion from MCI to AD (Chao et al. 2010), are associated with an accelerated rate of cognitive decline (Benedictus et al. 2017), and can differentiate cognitively normal adults, MCI and AD patients (Johnson et al. 2006). Notably, studies that used the same ADNI composite measure which includes the inferior temporal gyri, angular

Table 2 Hierarchical multiple linear regression predicting CBF

	Model	R	R²	Std. error of the estimate	Change statistics				
					R2 change	F change	df1	df2	Sig. F change
All n = 100	1	.305	.093	.070	–	–	6	93	–
	2	.390	.152	.069	.059	3.15	2	91	.047
Females[a] n = 63	1	.358	.128	.067	–	–	5	57	–
	2	.477	.227	.064	.099	3.52	2	55	.036
Males[a] n = 37	1	.160	.025	.080	–	–	5	31	–
	2	.307	.094	.080	.069	1.104	2	29	.345

Model 1 = age, gender, APOE-ε4 status, FH status, education, handedness

Model 2 = age, gender, APOE-ε4 status, FH status, education, handedness, physical activity, fitness

[a] Model does not include gender

Table 4 Hierarchical multiple linear regression predicting CBF in Females (n = 63)

Model	Independent predictors	β (SE)	Standardized β	p value
1	Age	−.002 (.002)	−.164	.226
	APOE ε4	−.018 (.020)	−.125	.369
	Family History of AD	.027 (.019)	.184	.176
	Education	.007 (.004)	.248	.055
	Handedness	.039 (.031)	.156	.215
2	Age	.000 (.002)	.023	.878
	APOE ε4	−.029 (.019)	−.204	.141
	Family History of AD	.032 (.019)	.223	.096
	Education	.004 (.004)	.149	.267
	Handedness	.035 (.030)	.139	.248
	Physical Activity	−.024 (.043)	−.080	.574
	Fitness	.006 (.002)	.426	.011

Fitness, $\dot{V}O_{2peak}$; *Physical activity*, moderate-vigorous; *APOE ε4*, apolipoprotein E ε4 allele; *AD*, Alzheimer's disease; *CBF*, resting cerebral blood flow; *β*, beta coefficient; *SE*, standard error

gyri, and the posterior cingulate have found CBF in these regions to be negatively associated with age, cognition and AD severity (Chen et al. 2011; Wang et al. 2013). Therefore, treatments that promote CBF and/or delay decline in these regions may mitigate the progression of cognitive decline and AD. That said, it is worth noting that, depending on the brain region, there are data to suggest that an increase in CBF is sometimes observed in AD (Wierenga et al. 2014) and is hypothesized to be the result of compensatory mechanisms (Wierenga et al. 2014; Hays et al. 2016).

Emerging evidence suggests that both fitness and physical activity may be associated with CBF in regions afflicted by aging and AD (Alsop et al. 2010; Wang et al. 2013; Wierenga et al. 2014). Similar to the present findings, estimated fitness mediated the relationship between age and CBF, that is,
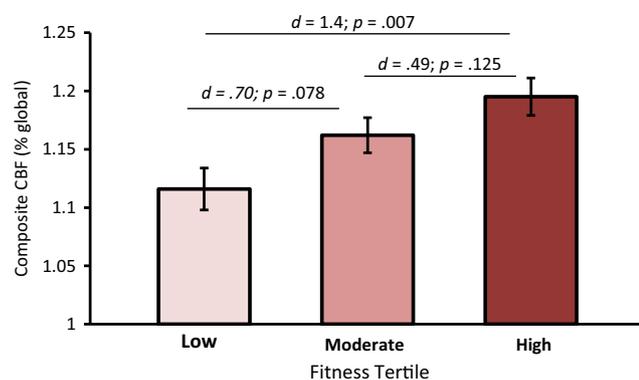

Fig. 2 Fitness and CBF for females. Female participants were separated into equal tertiles (n = 21, 21, 21) based on fitness level (Low = 19.5 ml/kg/min; Moderate = 24.1 ml/kg/min; High = 29.8 ml/kg/min). Panels show adjusted means and standard error. Effect sizes (d) reflect adjusted means. CBF = resting cerebral blood flow

greater fitness levels negated age-related CBF decline (Zimmerman et al. 2014). Research investigating life-long exercisers (i.e. master athletes) found that these older adults displayed greater fitness and CBF than age-matched sedentary controls (Thomas et al. 2013) and interestingly, cessation of exercise decreased CBF in a similar sample of master athletes (Alfini et al. 2016). Further, 12-weeks of structured physical activity has shown to increase CBF in both young and old adults, however, whether fitness adaptations are necessary to impart this benefit remains unclear (Chapman et al. 2013; Pereira et al. 2007). The extant research has demonstrated a link between fitness, physical activity, and CBF. However, until now, it was uncertain whether fitness and physical activity differentially predict CBF.

In the current study, we observed a modest correlation between fitness and physical activity, replicating what has been observed in similar populations (Burzynska et al. 2014; Voss et al. 2016). Although separate measures, physical activity has been shown to influence fitness levels (Haskell et al. 2007), and therefore fitness has been regarded as a measure of habitual physical activity. Indeed, increasing moderate-vigorous physical activity has been shown to improve fitness (Skinner et al. 2000), and inactivity (i.e. bedrest) has been shown to decrease fitness (Saltin et al. 1968). Further, exercise training performed throughout a lifetime has a strong influence on fitness levels in later life (Carrick-Ranson et al. 2014). Although physical activity is a key factor, other aspects that contribute to fitness include social, environmental, behavioral, physiological, and genetic components (Bouchard and Rankinen 2001; Eaton et al. 1995; Tager et al. 1998). In fact, genetics may account for up to 50% of the individual variability in fitness adaptations (Bouchard et al. 2011, 1999). Because multiple factors contribute to the trait attribute of fitness, delineating the independent contributions of fitness and physical activity is necessary in order to understand the unique health benefits each may confer.

In our sample of older adults at-risk for AD, we found that fitness was significantly associated with CBF, especially among females. These findings demonstrate fitness is a key contributor to CBF, and suggest engagement in activities aimed at improving fitness (e.g. exercise training) may be a promising treatment towards abating age-related CBF decline. Interestingly, there appears to be a gradient where a ~5 ml/kg/min increase in fitness, which is typically observed following several months of exercise training (Kohrt et al. 1991; Skinner et al. 2000), imparts substantial benefit (Fig. 2). This may be due to exercise training enhancing cardiovascular function (Seals et al. 1984) which promotes peripheral arterial health (Seals et al. 2008) and may lead to improved CBF regulation (Tarumi and Zhang 2017; Barnes and Corkery 2018). Another study of older female adults similarly reported that fitness is positively associated with several metrics of cerebral vascular function (i.e. mean arterial pressure, cerebrovascular

conductance) (Brown et al. 2010). Other hypothesized exercise-related mechanisms for improved CBF include increasing neurotrophic factors, angiogenesis, vascular function and neurovascular coupling (Davenport et al. 2012). An alternative interpretation of our findings is that genetic and environmental factors that contribute to fitness levels have a stronger influence on CBF in older adulthood than current physical activity. It could be that participants who have greater fitness due to genetic influences exhibit a healthier CBF profile compared to those with lower fitness levels because of hereditary differences (Bouchard et al. 2011). It is also plausible that a lifetime of physical activity, which has been shown to contribute to higher fitness levels in later life (Carrick-Ranson et al. 2014), may be more influential than present physical activity behavior with respect to CBF.

The gender specific findings observed in the current study may be due to a multitude of factors. There is a large body of literature that describes gender differences in cardiovascular health (see reviews: Miller et al. 2013; Joyner et al. 2015) including blood pressure regulation (Barnes 2017), cerebral metabolic rate of oxygen and cerebral blood flow (Lu et al. 2010). Our gender finding is consistent with our group's previous work which reported a positive association between fitness and hippocampal volume in female but not male participants (Dougherty et al. 2017a). Further, meta-analyses of exercise trials have found evidence for gender specific effects on cognition (Colcombe and Kramer 2003; Barha et al. 2017), with data suggesting that superior cognitive response in females may be the result of differences in exercise induced hormonal glucometabolic and hypothalamic-pituitary-adrenal axis responses (Baker et al. 2010). This may explain the gender findings reported from the epidemiological Canadian Study of Health and Aging where moderate to high exercise participation resulted in reduced risk of cognitive impairment for females but not males (Middleton et al. 2008). Alternatively, the present study's results may be explained by gender differences of the current sample. Examining the demographic information (Table 1), males had significantly greater years of education which may translate to increased cognitive reserve (Stern et al. 2012), limiting the potential influence of fitness on cerebral hemodynamics.

Our findings compliment previous research that has investigated both fitness and physical activity simultaneously. Epidemiological studies that examined both measures have routinely found fitness to be a stronger predictor of mortality (Kampert et al. 1996; Park et al. 2009; Wei et al. 2000) even after adjusting for activity level (Lee et al. 2010; Myers et al. 2004). Similar findings have been reported with cardiovascular disease, where fitness appears to be a superior prognostic indicator (DeFina et al. 2015). However, these studies have relied on self-reported physical activity questionnaires, which have shown large variability in comparison to objectively measured physical activity (Dyrstad et al. 2014; Prince et al. 2008).

Research investigating both fitness and physical activity in relation to cognitive health is less clear. The cardiorespiratory fitness hypothesis, first described by Kramer et al. (1999) posits the cognitive benefit to exercise training is due to the physiological adaptations in one's cardiovascular system (i.e. increased fitness). To date, meta-analytic data do not support this theory (Angevaren et al. 2008; Colcombe and Kramer 2003; Etnier et al. 2006). This may be in part due to variability in fitness testing methods (Dougherty et al. 2018), because there is evidence for the cardiorespiratory fitness hypothesis when applying strict physiological testing criteria (Billinger et al. 2017).

Evidence from our group and others has linked objectively-measured fitness and accelerometer-determined physical activity to many indices of brain health (Dougherty et al. 2017a, b, 2016; Makizako et al. 2015; Hayes et al. 2015), however, research investigating fitness and physical activity concomitantly is limited. In a similar older adult sample, Voss and colleagues reported that fitness was positively associated with functional connectivity in regions afflicted by aging (i.e. default mode network), and this finding remained significant after accounting for accelerometer-determined physical activity (Voss et al. 2016; but see also Burzynska et al. 2014). In agreement with previous research, our findings suggest that fitness has a significant impact on brain health that is not explained by current physical activity behavior.

Future longitudinal studies that assess changes in fitness and physical activity will be needed to better elucidate causality and directionality of the observed relationships. Although our sample's fitness levels were representative of national normative data (Kaminsky et al. 2015), physical activity was higher than the reported national average (Troiano et al. 2008). Further, we cannot rule out the potential of fluctuations in physical activity behaviors, which would influence our accelerometer measures; however, this does not present a concern with fitness testing. Lastly, due to our sample's demographic make-up (i.e., predominantly highly-educated non-Hispanic Whites) generalizability of our findings to other ethnic groups may be limited. Studies that include diverse populations are needed to determine whether our findings extend to other ethnic groups.

In summary, the present study provides evidence that fitness, independent of physical activity, is a significant predictor of CBF in regions that decline with age, and disease progression (Chen et al. 2011; Wang et al. 2013). While others have reported that estimated fitness levels (Zimmerman et al. 2014) and habitual physical activity (Thomas et al. 2013) are associated with maintained CBF in older adulthood, our findings suggest fitness is uniquely associated with preserved CBF, particularly among cognitively normal older female adults at risk for AD. This is an important finding as it is now well accepted that the pathophysiology of AD, including reductions in CBF, precedes cognitive impairment (Iturria-Medina

et al. 2016; Jack et al. 2010; Wierenga et al. 2014), highlighting the necessity to discover efficacious treatments before cognitive symptoms ensue. This work makes a significant contribution towards research investigating the gender specific mechanisms through which fitness and physical activity reduce the risk of cognitive decline by mitigating AD-related brain changes during the preclinical stage of the disease.

Compliance with ethical standards

Conflict of interest Ryan J. Dougherty, Elizabeth A. Boots, Jacob B. Lindheimer, Aaron J. Stegner, Stephanie Van Riper, Dorothy F. Edwards, Catherine L. Gallagher, Cynthia M. Carlsson, Howard A. Rowley, Barbara B. Bendlin, Sanjay Asthana, Bruce P. Hermann, Mark A. Sager, Sterling C. Johnson, Ozioma C. Okonkwo, and Dane B. Cook declare no conflicts of interest.

Ethical approval and informed consent All procedures followed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.

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