Cerebral Blood Flow as a Possible Neurobiological Mechanism Explaining the Relationship Between Physical Activity and Depressive Symptoms in Older Adults

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The antidepressant effects of physical activity and exercise in clinical and subclinical populations is well supported, but the neurobiological underpinnings of this relationship are poorly understood. Current evidence suggests that cerebral blood flow (CBF) to the anterior cingulate cortex (ACC) and hippocampus may play a role in depression and responds to chronic exercise. Leveraging baseline cross-sectional data from the Investigating Gains in Neurocognition in an Intervention Trial of Exercise (IGNITE), a multi-site aerobic exercise intervention, the present study aimed to (1) examine the relationship between moderate to vigorous physical activity (MVPA) and sub-clinical depressive symptoms, (2) examine the relationship between MVPA and CBF to the ACC and hippocampus, and (3) test CBF to the ACC and hippocampus as a statistical mediator between MVPA and depressive symptoms in a sample of 544 older adults. MVPA was measured using a wrist-worn accelerometer (Actigraph) and processed using GGIR. CBF was measured using a pseudo-continuous arterial spin labeling sequence for MRI, and regions of interest were extracted using FreeSurfer-derived masks. Aims 1 and 2 were tested using linear regression, and Aim 3 was tested using multiple mediation. All analyses controlled for age, race, gender, years of education, study site, BMI, and past smoking status. Results support a non-linear relationship between MVPA and depressive symptoms such that greater daily levels of MVPA were associated with fewer depressive symptoms, especially for those engaging in close to thirty minutes of MVPA per day. However, we did not find evidence for a relationship between MVPA and CBF to the ACC and hippocampus, nor significant mediation of CBF to the ACC and hippocampus in the

relationship between MVPA and depressive symptoms. Our results confirm the relationship between MVPA and depressive symptoms, and do not support a mediating role of CBF in the relationship between MVPA and depressive symptoms.

Table of Contents

List of Tables

List of Figures

1.0 Introduction

Research and clinical practice historically utilize the Diagnostic and Statistical Manual (DSM) for studying and treating distress related to mental illness. Because the DSM classifies psychopathology categorically, depression is most often defined categorically with symptom thresholds. Consequently, individuals with sub-clinical symptoms of depression are understudied and often go untreated; however, these symptoms can still be debilitating and cause marked impairment. Studies in this domain have revealed that subthreshold depression is associated with significant psychological distress (Rucci et al., 2003), impaired quality of life (Chachamovich, Fleck, Laidlaw, & Power, 2008), and disability in daily activities (Rucci et al., 2003). Additionally, the presence of subthreshold depression is associated with an elevated risk of the subsequent onset of clinical depression (Karsten et al., 2011). In 2019, 18.4% of adults in the United States aged 65 and over experienced at least one symptom of depression (Villarroel & Terlizzi, 2019). Therefore, expected increases in the older adult population are likely to cause significant burden on the medical, mental health, and economic systems, necessitating the exploration of health behaviors that can alleviate depressive symptoms in late life.

In addition to the abovementioned issues related to subthreshold depressive symptoms, established treatment and prevention strategies for depression should be expanded upon for several reasons. First, not all patients seeking treatment for depressive symptoms respond to common treatment approaches. For example, only 41% respond to psychotherapy (Cujpers et al., 2021). Moreover, antidepressant medications are more effective with increased depressive symptom severity, and the benefit of antidepressant medications for mild to moderate depression is minimal

or nonexistent when compared to placebo (Fournier et al., 2010). Thus, in consideration of individuals who may not respond to psychotherapy or pharmacotherapy, the development of effective and efficacious treatments for sub-clinical depression and Major Depressive Disorder would enable clinicians to treat a larger percentage of individuals suffering from depressive symptoms. Additionally, a wealth of research addresses treatment and prevention for depressive symptoms, but accessibility to mental health services including psychotherapy and pharmacotherapy in the United States is costly, especially for those without health insurance. Similarly, a nationwide shortage of mental health professionals has resulted in long wait lists and adds additional barriers to mental health resources for individuals after seeking treatment. Other barriers such as time and transportation also play a major role in preventing individuals from receiving much needed treatment.

Given the projected increase in the older adult population, poor response rates to existing treatments, and numerous barriers to treatment for depression, the present study explores physical activity as a health behavior that is associated with improvements in mood and decreased depressive symptoms. Physical activity is a modifiable, accessible lifestyle factor that improves and prevents depressive symptoms. *The present study would build upon our current understanding of the link between physical activity and depressive symptoms by exploring cerebral blood flow (CBF) as a potential biological mechanism driving this relationship to inspire public health recommendations and drive future research.*

The primary aims of the present study include:

(1) Examine the association between moderate-to-vigorous physical activity (MVPA) and depressive symptoms;

(2) Examine the association between MVPA and regional CBF (rCBF) in the ACC and hippocampus;

(3) Examine whether CBF in the ACC or hippocampus mediates the relationship between MVPA and depressive symptoms (see Figure 1 for statistical mediation model).

I predict that 1) Greater amounts of MVPA will be associated with fewer depressive symptoms; 2) greater amounts of MVPA will be associated with greater perfusion to the ACC and hippocampus; 3) variation in rCBF to the ACC and hippocampus will statistically mediate the relationship between MVPA and depressive symptoms.

1.1 Physical Activity (PA)

Physical activity (PA) is a broad category that describes any movement produced by skeletal muscle and requires energy expenditure. PA includes exercise, which is defined as physical activity for the purpose of improving cardiorespiratory fitness that is planned and generally continuous and rhythmic in nature (Caspersen, Powell, & Christenson, 1985). The term physical activity can also describe leisure, occupational or activity for the purpose of transportation (e.g., walking or biking to work). The present study will focus on aerobic physical activity, which relies on the metabolism of oxygen to supply activated muscle groups with energy (American College of Sports Medicine, 2013).

1.1.1 **Measurement**

PA can be measured objectively or subjectively. Subjective measurements of physical activity are typically self-report questionnaires in which participants recount retrospective activities and activity patterns. This type of measurement is simple, inexpensive, and efficient, yet is subject to inaccurate recollection or recording. Another subjective measure of PA involves participants recording physical activity as they complete it; however, this method can result in individuals altering their activities when instructed to monitor their activity. Current evidence suggests that device measurements (i.e., actigraphy) of physical activity may be superior to subjective measures in older adult populations (Parker, Strath, & Swartz, 2008).

Device-based measurements of physical activity aim to improve upon some of the limitations of subjective measures. Objective measures of physical activity typically involve technology in the form of a wearable device, such as an Actigraph, which is commonly worn on the wrist or hip. Actigraph devices are accelerometers and use measures of acceleration to calculate a wide range of study-specific measurements. Advancements in technology have allowed for the use of objective PA measures even in large samples. However, objective PA measurements should be considered in light of some limitations including technological issues, non-compliance, or participants changing activity patterns in response to wearing the device. Inconsistent findings from accelerometer data may be attributable to heterogeneity in measurement and analysis. Raw acceleration data must be analyzed to approximate various measures of PA, which is historically done using proprietary physical activity measurements from commercial software, such as Actilife for Actigraph devices. Reliance on proprietary software limits researchers to the code and variables generated from these companies, which may not be empirically supported. Additionally, scripts

for proprietary software are not open source, making it difficult to replicate methods across studies. In response to these concerns, researchers have begun moving away from proprietary software to open-source software, allowing researchers to define parameters and variables. Importantly, open-source software promotes transparency to allow for reproducibility. GENEactiv and GENEA data In R (GGIR), an open-source R package, is one tool gaining attention in the literature for processing and analyzing raw accelerometer data to estimate physical activity variables. Notably, using open source rather than proprietary software facilitates reproducibility of methods and results.

1.1.2 PA and Depression

Engagement in PA improves depression in clinical samples and is as effective as antidepressant medications (Kvam et al., 2016). Even low levels of PA are associated with a lower risk of depression (Pearce et al., 2022), and have demonstrated large antidepressant effects in clinical trials. A recent randomized controlled trial of 66 younger adults diagnosed with major depression found that participants randomized to an aerobic exercise intervention showed a greater reduction in depression symptom severity when compared to those randomized to a stretching control group (Brush et al., 2020). Meta-analyses examining exercise as a potential treatment for MDD found that moderate to vigorous intensity aerobic exercise reduced depressive symptoms when compared to non-active control conditions with moderate (Schuch et al., 2016) to large effect sizes (Kvam et al., 2016). PA similarly alleviates depressive symptoms in non-clinical populations as demonstrated by a meta-analysis of the effect of physical activity on depression in sub-clinical populations that included 92 studies with a total of 4310 participants. This study found that PA

reduced depressive symptoms with a medium effect size (standardized mean difference = −0.50; 95% CI: −0.93 to −0.06; Rebar et al., 2015).

While the link between depression and physical activity and exercise has been well established in the literature, the neurobiological mechanisms underlying this relationship are not fully understood, especially in subthreshold depression. The primary function of the present study is to address this knowledge gap and explore CBF as a potential biological pathway through which engaging in physical activity is associated with fewer depressive symptoms. CBF is an understudied measure of functional brain health that has been associated with both depression and physical activity, but its role in the relationship between physical activity and depressive symptoms remains a matter of speculation. Therefore, the present study aims to focus on CBF as a potential mediator between depressive symptoms and physical activity.

1.2 Cerebral Blood Flow (CBF)

The current literature linking CBF and depressive symptoms is limited by few studies with methodological limitations; however, these preliminary data support a relationship between CBF and depression as well as CBF and exercise. Interestingly, current evidence suggests that depression and physical activity are uniquely associated with specific brain regions, but sample sizes in these studies are often too small to reach convincing conclusions. Consequently, findings pertaining to global and rCBF are highly heterogeneous, with some studies resulting in significant findings, while others not, and a wide array of regions showing significant effects inconsistently across studies. As a result, a study with a large sample size exploring the relationship between CBF, depression, and exercise is warranted. The following sections aim to explain what CBF is,

why it is an important measure of brain health, and review the current state of the literature of these variables.

It is first necessary to understand the physiological importance of CBF. CBF is critical in maintaining function of the brain and has been linked to various cognitive outcomes and disease risk, such that decreased global perfusion (blood flow) in the brain, is associated with impairments in cognitive outcomes (i.e., information-processing speed, executive function, and global cognition; Poels et al., 2008), risk for dementia (Wolters et al., 2017), and risk for Alzheimer's Disease (Austin et al., 2011). Thus, the development of interventions that benefit CBF is highly relevant for supporting brain health and cognitive functioning.

CBF is a critical component for brain function. Blood flow is a mechanism by which oxygen is transported throughout the body, which is critical for neuronal oxidative metabolism of energy substrates and waste removal in the brain. Unlike other regions of the body, the brain cannot rely on anerobic metabolism for energy, so constant blood flow is essential for proper functioning. CBF is a new area of focus, so its exact mechanisms are continuing to be explored and major knowledge gaps remain. CBF can be studied in two contexts: global and regional. Global CBF refers to whole-brain blood flow at a given point in time, while regional blood flow refers to perfusion in specific regions in the brain.

As different regions of the brain are activated, energy is required to maintain function. In response to the energy demand, an increase in blood flow is directed to activated regions to meet the necessary oxygen demands. The brain uses neurovascular coupling, a mechanism linking neuronal activity to changes in blood flow via changes in vascular diameter, to ensure proper perfusion globally and in active regions (Tarumi & Zhang 2017). Like other areas of the body, blood flow to the brain depends on pressure, in this case cerebral perfusion pressure (CPP), and

resistance, in this case cerebrovascular resistance (CVR). This process is also influenced by mean arterial pressure (MAP) throughout the body. These vascular properties can be influenced by changes in vascular diameter driven by neuronal activity in the brain. For example, based on the principles of neurovascular coupling, frontal lobe activation would result in an increase in blood flow to the frontal lobe because of vasodilation to this region. This process has implications for imaging techniques such that measuring regional or global CBF can help us gain insight to brain activation in relation to any number of predictors.

One theory suggests that cerebral autoregulation (CA) is the process by which the body regulates CBF to ensure that it remains stable despite changes in pressure. Although the exact details of this process remain unclear, four processes have been identified as mechanisms contributing to CA: myogenic, neurogenic, endothelial, and metabolic. Myogenic tone refers to vasoconstriction of the arteries generated by muscle tone, which directly influences pressure to control CBF. The neurogenic response also controls the diameter of vessels via various neurons secreting neurotransmitters with vasoactive properties, some influencing vasoconstriction (e.g., serotonin and neuropeptide Y) and others vasodilation (e.g., acetylcholine and nitric oxide). The metabolic mechanism contributes to autoregulation of CBF by influencing changes in vessel size to regulate carbon dioxide. With accumulation of carbon dioxide, vessels will dilate to increase CBF for waste removal. Lastly, the endothelial mechanism refers to the release of vasodilators and vasoconstrictors from endothelial tissue (Ogah & Ainslie 2009). These properties help guide current understanding of the how CBF is regulated and maintained by different mechanisms in the body despite a variable environment.

Neurovascular coupling and CA are common themes in the explanation of immediate increases and decreases in perfusion and regulation of stable flow to the brain; however, the extent

of physiological changes influencing long-term changes in CBF is a gap in the literature. Deficits in CBF are associated with aging, so most of our understanding of long-term changes in CBF can be found from studies of older adults (adults over the age of 65). Definitive conclusions as to the exact mechanisms that lead to deficits in CBF have not been reached, but prevailing theories suggest decreased cerebral metabolic rate, cerebrovascular dysfunction, neuronal and glial mitochondrial metabolism, and changes in cardiac output may be associated with impairments in CBF (Tarumi & Zhang, 2017). Deterioration of these systems over time may lead to a decreased ability to deliver blood to the brain; therefore, the development of interventions that may attenuate or even reverse such deficits would benefit improve health outcomes**.**

1.2.1 **Measurement**

Early measurements of CBF used exogenously administered diffusible tracers with the ability to pass between vascular spaces and tissue, which could be detected using imaging techniques. Positron emission tomography (PET) and dynamic susceptibility contrast (DSC)-magnetic resonance imaging (MRI) were popularly used in early research studies in the field (Petcharunpaisan, Ramalho, & Castillo, 2010). However, over recent decades, advancements in MRI techniques for measuring CBF have been developed. In contrast to classical methods, arterial spin labeling (ASL), is a non-invasive MRI technique that does not rely on exogenous tracers. ASL generates perfusion images by tagging water molecules as endogenous tracers, which can then be measured as these "labeled" exchange from the vascular space into the tissue. The images collected can then be compared to a control image, and the difference image displays perfusion, which is related to the quantification of CBF (mL) per 100 g of tissue per minute (Grade et al., 2015). These

technological advancements have proven to have clinical utility as ASL has been a useful tool for detecting disease risk (Chao et al., 2010).

1.3 CBF and Depression

Although literature linking depression and CBF is sparse and heterogeneous, existing studies suggest abnormalities in CBF may be associated with depression. Decreased global CBF has been associated with greater depressive symptomatology in adults (Alosco et al., 2013) and older adults (Lesser et al., 1994); however, no significant differences in blood flow between individuals with depression have been observed (Finkelmeyer et al., 2016). Although findings on global CBF and depression are mixed, focusing on regional blood flow may be more specific and informative. Regions that have been consistently implicated in both the depression and physical activity literature are the hippocampus and ACC, which is expanded upon in the subsequent sections; therefore, the present study aims to use a region of interest approach focused on the hippocampus and ACC.

1.3 Region of Interest (ROI) Approach

1.4.1 ACC, Hippocampus and Depression

Structural and functional deficits in the ACC and hippocampus are associated with depression. First, depression is associated with decreased gray matter volume of the hippocampus. A meta-analysis of 12 MRI studies found that hippocampal volume is reduced in individuals with

depression (Videbech & Ravnkilde, 2015). Volumetric deficits in the ACC have also been observed in individuals with depression (Caetano et al., 2006; Ballmaier et al., 2004). Regional measures of CBF as they are related to depressive outcomes are less conclusive than studies using volumetric measures; however, given the consistent findings for hippocampal and ACC volume abnormalities, it is plausible that CBF in the ACC and hippocampus is associated with depressive symptoms and is, perhaps, a mechanism through which depression is associated with structural deficits.

The current literature on the relationship between depression and regional perfusion is mixed, which may reflect methodological issues including sample size, age, cognitive status, physical health, medication status, and differences between imaging techniques and depression measurement. Consequently, regions have inconsistently emerged from individual studies as being associated with depression, and no studies have addressed the relationship between CBF and sub-clinical depression. Several studies examined the relationship between depression and CBF using PET techniques. In one study, forty-two patients with major depression based on the DSM-IIIR and a mean age of forty-one years had increased blood flow to the hippocampus, cerebellum, anterior cingulate gyrus, and basal ganglia when compared to 47 matched non-depressed controls. The Hamilton Depression Scale (HAM-D), a measure of depression severity, was associated with CBF to the hippocampus, and this relationship was moderated by gender, such that higher HAM-D scores were associated with increased CBF to the hippocampus for female patients, while higher HAM-D scores were associated with decreased CBF to the hippocampus in male patients (Videbech et al., 2002). Moreover, Lesser et al., found a 13.5% lower global CBF in thirty-nine depressed patients (based on DSM-III criteria) over the age of fifty years using PET, 39 compared to twenty psychiatrically healthy controls. HAM-D scores in this sample, however, were not

associated with CBF (Lesser et al., 1994). Lower CBF in the left hemisphere (Kanaya & Yonekawa, 1990) and right hemispheres (Lesser et al., 1994) has also been associated with depression.

Measuring CBF using PET has been shown to be an accurate measure of CBF; however, it is costly, invasive, and technically demanding. As a result, recent studies have moved away from measuring CBF using PET and have begun measuring CBF using MRI techniques, which is radiation-free and non-invasive. Current evidence suggests a strong correlation between quantified CBF obtained from PET and arterial spin labeling (ASL) MRI (Puig et al., 2019).

Using continuous ASL for MRI, Vasic and colleagues (2014) found that forty-three patients diagnosed with Major Depressive Disorder had lower rCBF in the cuneus, bilateral PHC and subgenual ACC when compared to twenty-nine healthy controls. Furthermore, higher scores on the Beck Depression Inventory were associated with higher blood flow in the middle frontal cortex, and higher scores on the HAM-D were associated with lower right parahippocampal CBF. In a randomized controlled trial examining various pharmacotherapy treatments in nineteen patients with MDD, effective treatments using selective serotonin reuptake inhibitors (SSRIs) or amesergide (AMSG) was associated with increased perfusion in the ACC (Vlassenko et al., 2004), highlighting that targeting the ACC in treatment may alleviate depressive symptoms. 80% of participants in the AMSG group and 67% showed a reduction in depression symptoms; however, changes in depression severity associated with changes in CBF were not mentioned. Notably, this study did not include a control group.

In contrast, in a study of cerebral blood flow in late life depression there was no difference in cerebral blood flow to the lateral frontal, medial frontal, cingulate, central, and parietal region

between thirty eight participants with depression (based on self-report measures) compared to 30 non-depressed controls (Colloby et al., 2018).

The relationship between CBF and sub-clinical depression is not currently known as the current literature has only focused on clinical levels of depression. The present study would directly contribute to this literature by addressing this knowledge gap in a large sample of 544 participants, a much larger sample size than any of the abovementioned studies. Heterogeneity of emerging regions among previous studies may be due to a number of factors, such as small sample size, medications, and differences in study methodology.

1.4 Aerobic Exercise and CBF

Converging evidence demonstrates that exercise can induce morphological and functional changes in the hippocampus and ACC. First, aerobic exercise increases hippocampal volume in older adults. A year-long moderate intensity aerobic exercise trial of 120 older adults resulted in a significant volumetric increase in hippocampal volume. Increases in hippocampal volume were also associated with improvements in spatial memory (Erickson et al., 2011), suggesting behavioral changes accompany exercise-induced changes in the hippocampus. Similarly, in a study of 59 adults aged 60-79, participation in a 6-month aerobic exercise trial yielded an increase in volume of the ACC (Colcombe et al., 2006). We, and others, have speculated that exercise-induced morphological changes in brain structure are driven by functional changes, such as CBF (Erickson et al., 2022).

Much of the existing literature on aerobic exercise and CBF focuses on the effects of acute exercise on CBF. A review by Ogoh and Ainslie (2009) reported increased cerebral perfusion during mild to moderate intensity exercise, which is attributable to cardiac output as well as

changes in neuronal activity and metabolism in regions associated with movement. Chronic exercise, on the other hand, is understudied in the context of CBF, and results to date have been highly heterogeneous. Indeed, a 16-week moderate to vigorous intensity supervised exercise intervention had no effect on global or rCBF in a sample of 51 participants with Alzheimer's disease (van der Kleij et al., 2018). While sixteen weeks may be insufficient for inducing changes in CBF, another 4-month aerobic exercise intervention in a sample of older adults, who reported memory impairment, observed increased perfusion to the ACC and hippocampus when compared to a control group that engaged in light stretching. However, these results should be interpreted with caution as MRI data was only collected at the conclusion of the intervention (Burdette et al., 2010). Additionally, a 12-week supervised aerobic exercise intervention in cognitively healthy sedentary adults ages 57 to 75 found higher resting CBF in the anterior cingulate in 18 participants in the exercise group when compared to a wait-list control group of 19 participants (Chapman et al., 2013: effect size – 0.42; Cohen's d = 0.939). Exercise intervention studies offer promising preliminary data to suggest a relationship between rCBF and physical activity, but study limitations, namely small samples sizes and insufficient intervention durations, hinder our ability to draw conclusions about exercise-induced changes in CBF in these regions.

Physical activity (PA) patterns have also been shown to be associated with CBF in older adults. Life-long aerobic older adult male exercisers showed higher CBF in the posterior cingulate cortex/precuneous when compared to sedentary older adult males, indicating that habitual aerobic exercise may protect against age-related decline in CBF (Thomas et al., 2013). Interestingly, CBF in the left inferior temporal gyrus, left fusiform gyrus, left inferior parietal lobule, right cerebellar tonsil, right lingual gyrus, right precuneus, and right bilateral cerebellum, and left and right hippocampus decreased after life-long endurance athletes ceased training for 10 consecutive days

(Alfini et al., 2016). Although this study did not include a control group, these findings suggest changes in CBF in response to abrupt changes in physical activity patterns.

Current literature suggests a relationship between physical activity and CBF, but study limitations (e.g., small sample sizes, variability in CBF measurement) may be leading to heterogeneity among emerging regions. Although the literature on the relationship between PA and CBF is sparse, most of the existing studies have utilized exercise interventions but fail to report participant adherence to the exercise protocol. Poor participant adherence (e.g., participants completing much less activity than prescribed by the study) can complicate the interpretation of the influence of an exercise intervention on CBF as some participants may not be reaching the level of activity claimed. Indeed, device-based measures of physical activity can measure physical activity patterns over the course of the wear time, that includes measures of duration and intensity. These variables are important for interpreting PA and exercise's relationship with CBF, making it challenging to interpret intervention results when adherence is not reported. Therefore, the present study will add to this literature base by relating device-based measured patterns of daily physical activity to CBF. Since our current understanding of MVPA and CBF is more advanced than light-intensity PA and CBF, the present study aims to focus Much of the existing literature on PA focuses on MVPA, while light-intensity physical activity is poorly understood. Prior studies linking light-intensity PA to mental health outcomes and CBF are nascent, and more research is needed prior to deriving hypotheses concerning light-intensity PA.

In sum, extant literature suggests that regional deficits in CBF to the ACC and hippocampus may be associated with depression, and physical activity may be associated with increased CBF to the ACC and hippocampus, although study limitations may be leading to heterogeneity in these programs of research. In contrast, a convincing body of evidence posits that

physical activity can prevent and improve depressive symptoms. The present study aimed to clarify these inconsistent findings using a large sample size of 544 participants with measures of depressive symptoms, CBF, and physical activity. To do so, I examined whether actigraphy-derived measures of MVPA are associated with depressive symptoms and CBF to the ACC and hippocampus. I also examined if CBF to the ACC and hippocampus statistically mediates the relationship between physical activity and depressive symptoms, a model which has never previously been tested.

2.0 Method

2.1 Participants

To test these hypotheses, I used data from the "Investigating Gains in Neurocognition in an Intervention Trial of Exercise" (IGNITE) study (PI: Erickson), which was a multisite 12-month randomized clinical trial of moderate intensity aerobic exercise for cognitively normal older adults (Erickson et al., 2019).

Participants aged 65 to 80 were recruited through local media (television, radio, newspapers), promotional flyers, targeted postcard mailings, announcements to local aging and senior citizen agencies, bus advertisements, online media, university registries, and announcements through local churches, synagogues, and mosques websites in the Boston, Kansas City, and Pittsburgh areas. Recruitment of racial and ethnic minorities was in proportion to each of the three sites. Eligibility criteria was determined to recruit participants who were generally inactive (engaging in no more than 20 minutes of moderate-intensity aerobic exercise on 3 or more days of the week week), cognitively normal within broad limits, ambulatory and able to safely exercise regularly at a moderate intensity. Inclusion and exclusion criteria are outlined at length in the IGNITE protocol paper and in the appendix of this document (Erickson et al., 2019). Participants were excluded for a current Major Depressive episode based on DSM-V criteria or scored 9 or higher on the 15-item self-report Geriatric Depression Scale, which is the clinical cutoff for moderate depression. Since the primary aims of the parent IGNITE grant focus on cognitive outcomes, investigators elected to exclude on the basis of depression to reduce variability and confounding effects on cognition.

A detailed consort diagram outlining participant recruitment is provided in the Appendix on page 36.

2.2 Measures

2.2.1 Cerebral Blood Flow (CBF)

Cerebral blood flow (CBF) was assessed using a Pseudo-Continuous Arterial Spin Labeling (pCASL) approach for magnetic resonance imaging (MRI). MRI data was collected at the MR Research Centers at the University of Pittsburgh and Northeastern University using a Siemens PRISMA 3T Scanner with a 64-channel coil and at the University of Kansas Medical Center using a Siemens Skyra 3T scanner with a 32-channel coil. A 3D GRASE pCASL sequence at a resolution of 3.1 x 3.1 x 2.5 mm, TE/TR = 22.08/4430 ms, 48 slices, post-label delay 2s, with background suppression, 10 measurements for labeling and control, 4 segment readout was used. All sequences were monitored for quality assurance and standardization prior to initiation of the study. pCASL data was analyzed using a region of interest (ROI) approach focusing on the hippocampus and ACC.

2.2.2 Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale is a widely used self- report measure of depression and anxiety originally designed for inpatient hospital populations. The HADS is frequently used in epidemiological research to evaluate the prevalence of clinically relevant symptoms of depression and anxiety in older adults (Zenebe et al., 2022). This scale is comprised of 14 items that were scored on a four-point severity scale (0-3), higher scores represent increased

symptom severity. Seven items on the scale evaluate depressive symptomatology and 7 evaluate anxious symptomatology, resulting in depression and anxiety subscale scores. The maximum score for each subscale is 21. Cutoffs for each subscale are normal (0-7), mild (8-10), moderate (11-14), and severe (15-21) (Zigmond & Snaith, 1983); however, lower scores have been recommended for older adults. Eriksen and colleagues (2019) found a cutoff of ≥ 4 (compared to ≥ 8) is best for identifying a depressive episode in older adults on the HADS-depression (HADS-D) subscale.

A major consideration when assessing depression and anxiety in older adults is the overlap in somatic symptomatology associated with depression and advanced age. As such, it is important for depression self-report measures to distinguish symptoms that are solely attributed to psychological symptoms rather than those that can also be explained by advanced age, such as insomnia, fatigue, and changes in appetite. Unlike other commonly utilized metrics for depression, such as the Beck Depression Inventory, the HADS does not include somatic symptoms of depression to ensure that scores do not reflect symptoms independently related to advanced age rather than emotional distress.

All items show good reliability; however, in a Norwegian sample of older adults, three items on the depression subscale explained very little of the construct variance, which caused low internal consistency for the depression subscale, while the anxiety subscale demonstrated good internal consistency. The anxiety and depression subscales demonstrate discriminant, convergent, and construct validity (Siversten, Helvik, Gjøra, & Haugan, 2022).

2.2.2 Physical Activity (PA)

The present study aims to examine MVPA. Much of the existing PA literature focuses on MVPA, so the literature on MVPA and depressive symptoms is more consistent and convincing when compared to other metrics of PA. In consideration of minimal existing data on CBF, the logical first step in exploring the relationship between PA, CBF and depressive symptoms is to first address MVPA.

Actigraphy. Physical activity was assessed using an accelerometer device (ActigraphG Link) worn on the non-dominant wrist for seven consecutive days. Participants were instructed to wear the device as much as possible and encouraged to leave it on for all activities including sleeping and showering unless it caused discomfort. This device measures raw acceleration, energy expenditure, Metabolic Equivalent of Task rates, steps, physical activity intensity, activity bouts, sedentary bouts, body position, sleep latency, total sleep time, and sleep efficiency. Actigraph data was processed using GGIR software.

Participants received the wearable Actigraph watch from exercise staff following the completion of assessments during one of their visits. Participants were asked to complete a log indicating any time the watch was not being worn. The screen of the Actigraph was intentionally blank, ensuring that participants did not receive feedback from the device. Participants were instructed to wear the device as much as possible for a period of seven days but were allowed take it off if it caused discomfort.

GGIR. GGIR is an R package with an Open source LGPL 2 license on CRAN since 2013 (Migueles et al., 2019) containing five parts that are completed sequentially. Raw data begins in part one where it is calibrated according to local gravity, acceleration metrics are calculated, and

non-wear time is detected. In the second part, non-wear time is detected, and a basic description of accelerations are computed. Output in this step includes .csv files of measurements per day of physical activity (e.g., quantities, levels), data quality report, report per recording. Parts three and four are primarily relevant for sleep variables in which it attempts to detect sustained inactivity consistent with sleep and detect sleep period time. The fifth and final part merges the physical activity and sleep data for each participant (Migueles et al., 2019).

Quality control measures are completed to confirm protocol compliance, device wear time, calibration errors, and potential scoring artifacts.

The variable used for these analyses from the GGIR output is total average minutes per day calculated from the seven-day wear period.

2.3 Statistical Analysis

Before pCASL data was analyzed, BASIL (Chappell et al., 2009) and Human Connectome Project (Van Essen et al., 2012) methods were used for registering individual brain images to MNI space using non-linear registration and to create a voxel-wise comparison of perfusion to quantify CBF. FreeSurfer was used to create regions of interest of the ACC and hippocampus, and these ROIs were then used to extract average CBF values.

All statistical analyses were run using R version 4.2.2 (Development Core Team, 2016). To test Aim 1, multivariate regression was used to test for an association between physical activity (independent variable) and depressive symptoms (dependent variable) (See Figure 1; path c). To test Aim 2, separate multivariate multiple regressions were used to analyze the relationship between MVPA (independent variable) and CBF to each subregion of the ACC and hippocampus (dependent variable) (path a). Age, sex, years of education, study site, BMI, and previous smoking

status were added into both regressions as covariates and p value < .05 was considered statistically significant. Aims 1 and 2 were tested using the "stats" package in R. I tested to ensure that the regression assumptions were met and ran appropriate diagnostic tests for outliers. When the assumptions of linear regression were not met, non-linear terms were explored.

To test Aim 3, rCBF in the ACC and hippocampus were tested as parallel statistical mediators using a multiple mediation model. Testing multiple mediators using this approach allows for the testing of one region as a mediator in the presence of the other. In doing so, complementary indirect paths can be tested, which may highlight how CBF to all subregions may uniquely or jointly contribute to the relationship between PA and depressive symptoms (Preacher & Hayes, 2008). This model tested the relationship between predictor X (MVPA) on the outcome Y (depressive symptoms) through the parallel mediators M (CBF to the ACC and hippocampus) while controlling for age, sex, years of education, study side, BMI, and past smoking status. Standard errors were resampled with 5,000 iterations to create bootstrapped confidence intervals for all paths. The "lavaan" R package for structural equation modeling (SEM) with bootstrapping (5000 iterations) was used to test Aim 3. Indirect effects of X on Y through M were calculated as the product of path a and path b, and a 95% confidence interval was used to determine statistical significance.

2.4 Covariates

All analysis were controlled for age, gender, race, years of education, and study site. Because of prior studies showing links between both cardiovascular health and CBF (Gruhn et al.,

2001; Pase et al., 2012), we included body mass index (BMI; derived from participant height and weight) and past smoking status as covariates.

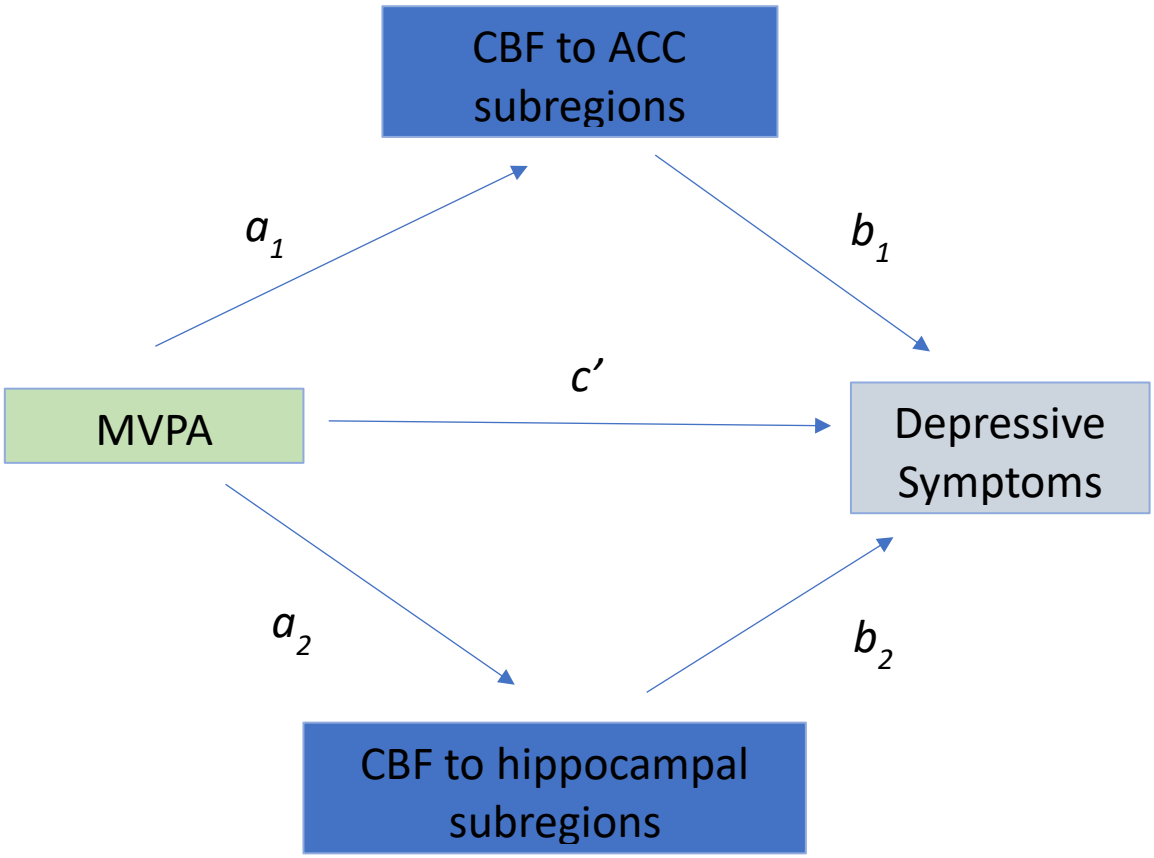

Figure 1. Mediation model.

3.0 Results

3.1 Participants

Of the 648 participants in the IGNITE sample, 608 had CBF data, and 588 had actigraphy data. After excluding seven participants due to poor image quality for the pCASL sequence, which, in most cases, was due to excessive motion in the scanner, the resulting sample of participants with CBF, actigraphy, and depressive symptom data was 544. The sample that was used for the present study is predominantly female (71%), white (77%), well-educated, and overweight (see Table 1). Race was categorized into white and nonwhite groups.

Table 1. Demographic Characteristics of the Sample

Characteristics	**n (%) or Mean [SD]**
Gender	
Female	386 (71)
Male	158 (29)
Race	
African American/ Black	98 (18)
Asian	9 (1.7)
Caucasian/White	420 (77)
Bi-racial	1 (<1)
Refused to answer	7 (1.3)
Other	8 (1.5)
Age (years)	69.77 [3.70]
Education	16.31 [2.20]
VO_2peak (ml/kg/min)	21.88 [5.07]
VO_2 Percentile	46 [27.85]
BMI (kg/m^2)	29.54 [5.71]

MVPA (average minutes/day)	31.13(26.91)

3.2 HADS-D Scores

HADS-D scores for this sample were right-skewed indicating the majority of the sample had few depressive symptoms (M = 3.5, SD = 3.3). Figure 1 illustrates the distribution of HADS-D scores.

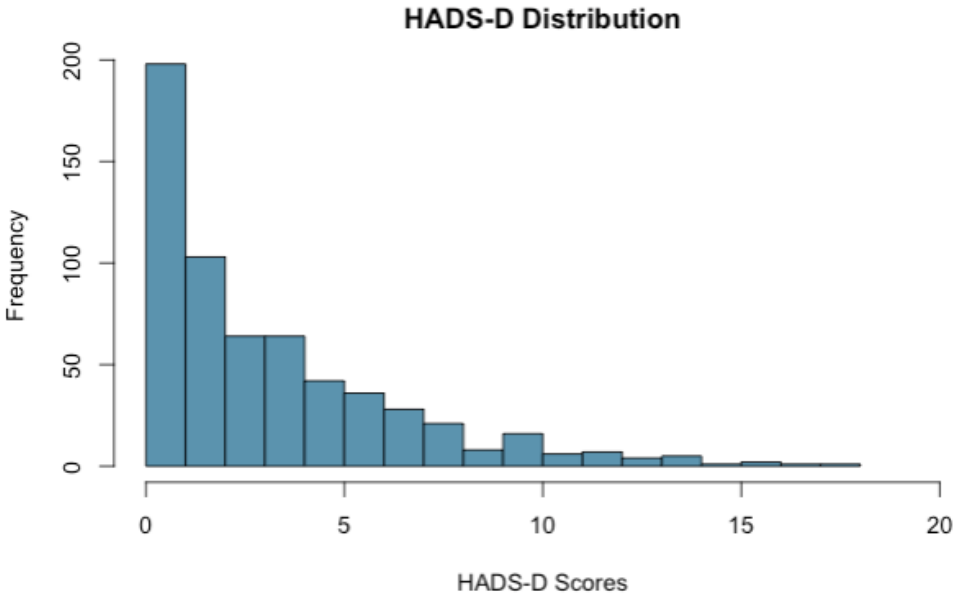

Figure 2. Frequency of HADS-D scores.

3.3 Aim 1

The relationship between depressive symptoms and MVPA in this sample was nonlinear, likely due to the distribution of the HADS-D scores, which was evident by non-normally distributed residuals in the model. Non-linear transformations were explored, and the best fitting

model resulted when taking the square root of HADS-D scores. After this transformation, the distribution of residuals in the model were more normally distributed, and, consistent with my hypothesis, MVPA was significantly associated with depressive symptoms (β = -0.003, p = .029, *adjusted* R^2 = 0.038) when controlling for age, race, years of education, study site, BMI, and smoking status.

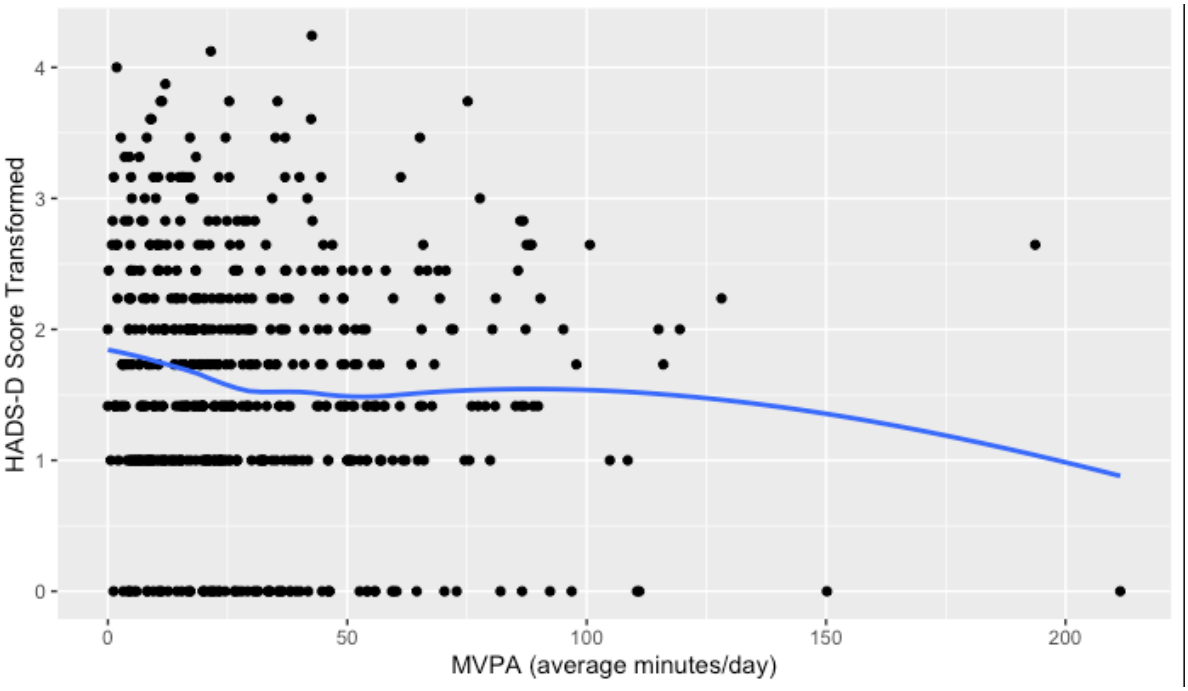

Figure 3. Nonlinear relationship between MVPA and transformed (square root) HADS-D scores

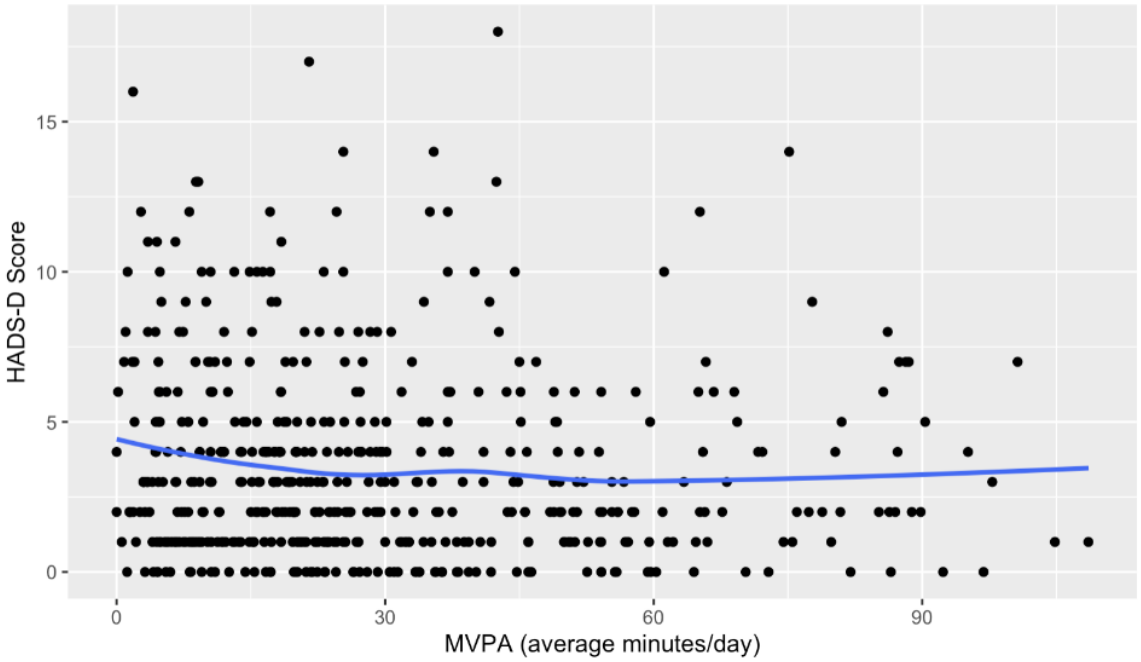

Figure 4. Relationship between MVPA and non-transformed HADS scores with MVPA outliers removed.

3.4 Aim 2

Inconsistent with my hypothesis, however, rCBF in any region of the ACC and hippocampus was not significantly associated with MVPA. This relationship did not reach significance after controlling for age, race, years of education, study site, BMI, and past smoking status. All p-values exceeded .05 and assumptions of linear regression were satisfied. Results for Aims 1 and 2 are summarized in Table 2.

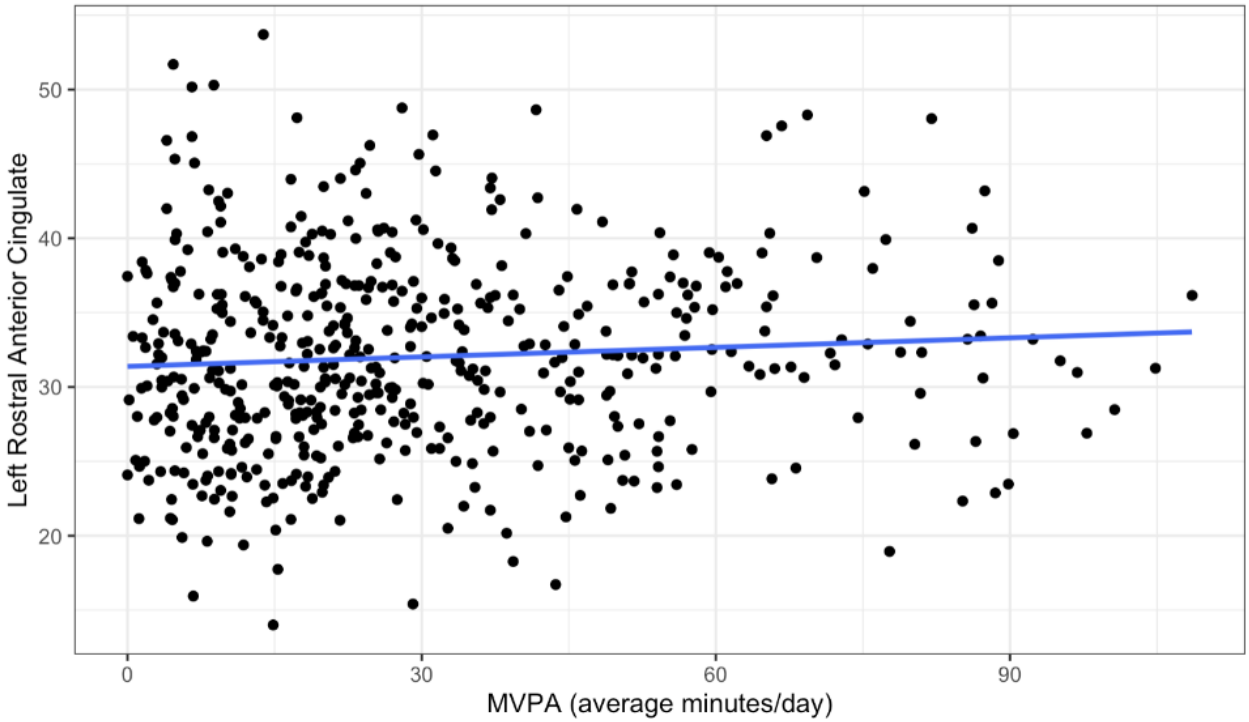

Figure 5. Relationship between MVPA and CBF in the left rostral ACC excluding outliers.

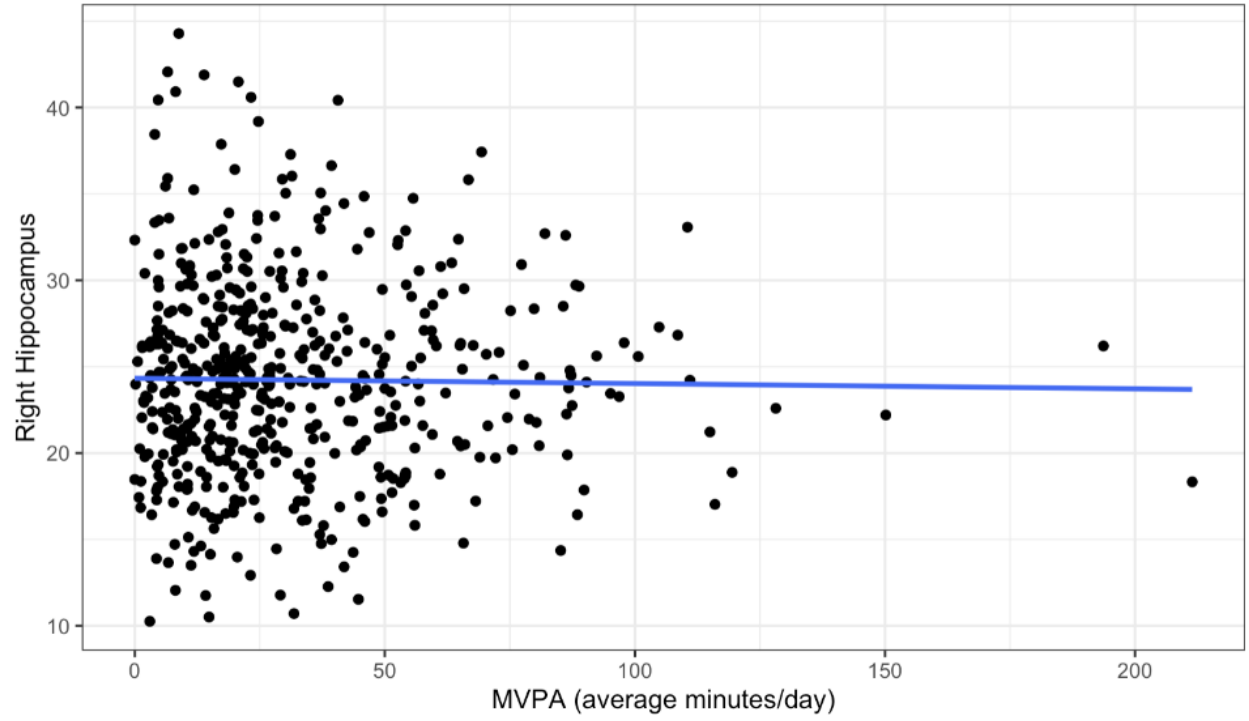

Figure 6. Relationship between MVPA and CBF to the right hippocampus.

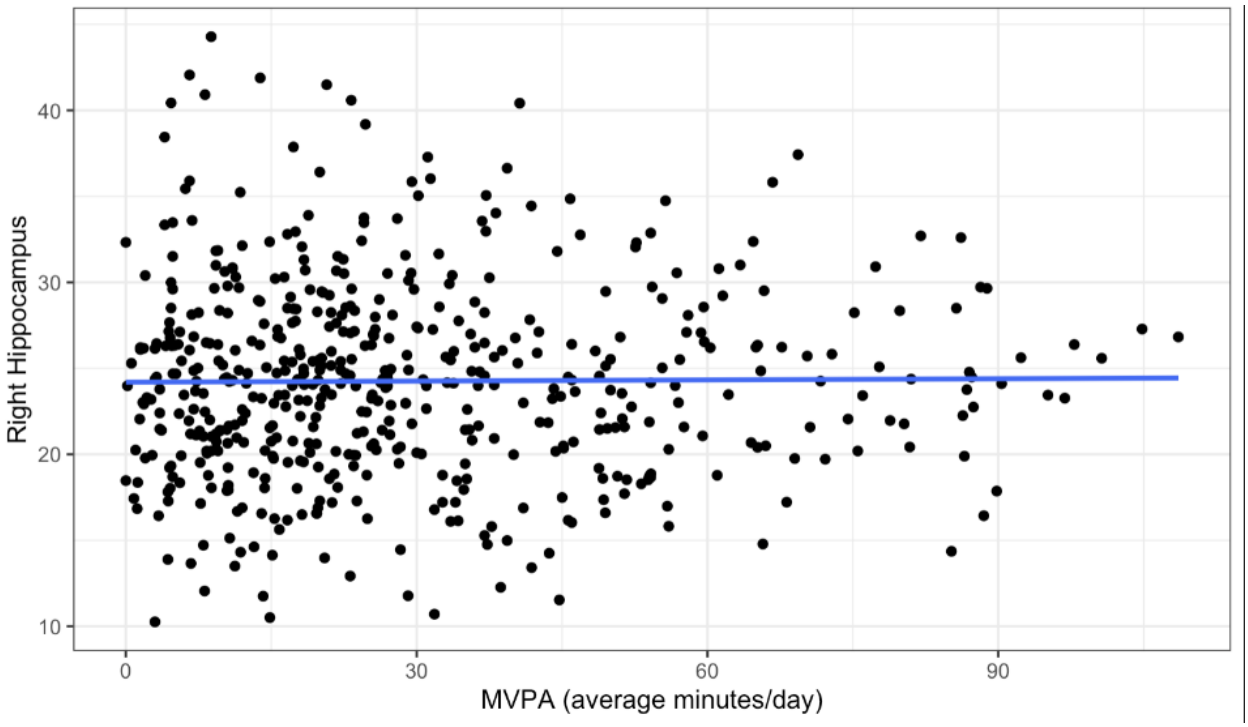

Figure 7. Relationship between MVPA and CBF to the Right Hippocampus excluding outliers.

Table 2. Results Summary: Aims 1 and 2

Characteristics	β	p	Adjusted R^2
Aim 1[#]	-0.0034	.03*	0.038
Aim 2			
Left Hippocampus	-0.003	0.73	0.10
Right Hippocampus	-0.001	0.85	0.10
Left Parahippocampal	0.000	0.94	0.09
Right Parahippocampal	-0.001	0.87	0.08
Left Caudal ACC	0.009	0.42	0.06
Right Caudal ACC	0.008	0.49	0.08
Left Rostral ACC	0.011	0.29	0.08
Right Rostral ACC	0.011	0.28	0.08

*Statistically significant
[#]HADS-D scores (dependent variable) were transformed (square root) due to non-normality of residuals

3.5 Aim 3

Also inconsistent with my hypothesis, rCBF did not significantly mediate the relationship between depressive symptoms and MVPA when controlling for age, race, years of education, study site, BMI, and smoking status in this sample (for all regions). The confidence interval for the multiple mediation model included zero.

3.6 Missing Data

The final sample of 544 participants excluded 104 participants from the full sample due to missing CBF data, poor image quality, or missing actigraphy data. Analyses show that excluded participants had slightly higher BMI (M = 30.87, SD = 6.88), which may be attributable to discomfort in the scanner. A higher percentage of participants who identified as African American/Black (n = 24; 23%) and a lower percentage of participants who identified as White (n = 71; 68%) had missing data than had all measures, but these differences were not statistically significant (p = .051). Excluded participants did not differ by age or years of education.

3.7 Sensitivity Analyses

Diagnostic tests for Aim 1 and Aim 2 were run to test for outliers, leverage, multicollinearity, Cook's distance, and heteroscedasticity of the residuals using the following R packages: "olsrr," "car", and the R base function, "plot()." Participants who showed to have significant influence for Aims 1 and 2 were removed, and analyses were rerun. Aim 1 was no longer significant after removing these participants (β = -0.010, p = .062). The zero-order

correlations between MVPA and the left (β = 0.02, *p*-value = 0.048) and right rostral ACC (β = 0.02, *p*-value = 0.03) were significant after removing participants with significant influence in Aim 2, but no regions remained significant or trended towards significance with the inclusion of the covariates**.**

As part of quality control for accelerometer data, we set levels of valid days of data. Four valid wear days (three weekdays and one weekend day) are used in the literature as the minimum threshold of a wear period that are considered representative of the participant's lifestyles (Migueles et al., 2017). Participants with fewer days than this acceptable threshold were removed for a sensitivity analysis. After rerunning with this subsample of 533, Aim 1 remained significant (β = -0.003, p = 0.039) and no analyses within Aim 2 reached significance. All *p*-values for Aim 2 (zero order and with covariates) exceeded 0.05. For Aim 3, the 95% confidence interval when conducting the multiple mediation analysis was -0.021 to 0.

4.0 Discussion

The present study aimed to examine the relationships between MVPA, depressive symptoms, and rCBF by testing rCBF as a possible mediator in the relationship between MVPA and depressive symptoms in a sample of sedentary and generally psychologically-well older adults.

4.1 Aim 1

The first aim was to examine the association between MVPA and depressive symptoms, with the hypothesis more MVPA was associated with fewer depressive symptoms. Our results supported this nonlinear relationship, such that going from close to 0 minutes of MVPA to about 30 minutes per day was associated with the steepest decrease in depressive symptoms, which tapered off at the highest levels of MVPA. To our knowledge, the present study is the first of its kind to examine the relationship between device-based measurement of MVPA and sub-threshold depressive symptoms in a sample of older adults. According to a review by Kandola and colleagues (2019), many other studies have addressed related questions but relied on self-reported measures of physical activity. Therefore, for the first time, the present study found a significant non-linear relationship between actigraphy-based MVPA and depressive symptoms in a sample of older adults. This finding has meaningful public health implications such that fewer than thirty minutes of MVPA per day is associated with significantly fewer depressive symptoms in older adults who do not meet current diagnostic criteria for MDD. The older adult population is at increased risk for depressive symptoms and is growing rapidly, placing strain on the healthcare system. Physical activity, a relatively affordable and accessible lifestyle behavior,r can be utilized to help bolster

mood in a group that may face depression-related impairment but might not be eligible to receive treatment based on current health insurance practices.

A significant relationship between PA and depressive symptoms in a sub-clinical sample is consistent with existing literature summarized by the meta-analysis by Rebar and colleagues (2015) confirming MVPA promotes mood in older adults with sub-clinical depressive symptoms. However, the non-linear relationship of this finding suggests that low amounts of MVPA (fewer than thirty minutes per day) are associated with more depressive symptoms. Around about thirty minutes per day, this relationship seems to taper, such that more MVPA than thirty minutes per day is not associated with fewer depressive symptoms. Two reviews identified that three thirty-minute aerobic exercise sessions per week at 60-80% of maximum heart rate (Perraton, Kumar, & Machotka, 2010) for a total of ninety minutes per week or three to four thirty-to-forty-minute moderate aerobic exercise sessions (Stanton & Reaburn, 2014) are necessary to achieve antidepressant effects. The results of the present study seem to align with the existing literature as the antidepressant association of MVPA tapers off around an average of thirty minutes per day. However, these results are difficult to compare as Perraton, Kumar, & Machotka (2010) and Stanton & Reaburn (2014) concentrated their reviews on supervised exercise interventions in depressed samples in which exercise was often accumulated in supervised bouts. Indeed, intervention data from IGNITE can be used to test the dose-response relationship between supervised exercise bouts and depressive symptoms.

The present study focused on every minute counts for MVPA, which is consistent with the 2018 Physical Activity Guidelines for Americans (Physical Activity Guidelines for Americans, 2nd Edition, 2018). While exploring these relationships through the every-minute-counts perspective is informative, PA accumulated in bouts may represent more planned, structured PA,

which may differentially be associated with sub-clinical depressive symptoms when compared to PA not accumulated in bouts. Comparing exercise accumulated in bouts versus not will elucidate optimal exercise patters to promote mood.

Notably, Aim 1 was no longer significant after removing participants with significant leverage. The effect size for this relationship was originally small, so it appears that removing highly influential data compromised the significance of the results. Although previous data consistently supports the antidepressant effects of physical activity, the effect size in our sample is likely smaller than the medium effect size presented in previous studies.

4.2 Aim 2

The second aim of the present study was to examine the association between MVPA and rCBF in the ACC and hippocampus, and I hypothesized that higher levels MVPA would be associated with increased rCBF. Inconsistent with the hypothesis, there was not a significant relationship between MVPA and rCBF. These findings are the first to show that actigraph-measured MVPA is not associated with CBF to the ACC and hippocampus in a large sample of self-reported sedentary older adults. Although CBF is an important marker of brain health, MVPA might not be an important lifestyle factor for promoting CBF in late life.

The findings of the present study are consistent with the findings of van der Kleij and colleagues (2018) in their sample of Alzheimer's Disease patients. Conversely, the results of Aim 2 contradict the finds of Burdette and colleagues (2010) and Chapman and colleagues (2013), both of which included samples of cognitively normal, non-depressed sedentary older adults. Contradictory findings may be due to methodological differences as Burdette and colleagues (2010) and Chapman and colleagues (2013) both tested for changes or differences in CBF

following a supervised exercise intervention, which is notably different than MVPA. It may be true that differences in CBF may not be associated MVPA, which can be accumulated through lifestyle (e.g., walking up stairs, walking to the bus stop) but are more specifically related to aerobic exercise, which is planned, structured physical activity for the purpose of improving fitness. Moreover, these differences may also be due to methodological limitations from these studies, such as small sample size and adherence to the protocol.

While literature on the influence of acute bouts of exercise on CBF is more developed and consistent, the results from the present study highlight the muddiness of the literature examining the relationship between chronic and lifestyle PA and CBF. The present study may serve to rule out a relationship between CBF and MVPA in a sample of self-reported sedentary older adults; however, IGNITE intervention data can be leveraged to attempt to replicate the findings of Burdette and colleagues (2010) and Chapman and colleagues (2013) who found higher rCBF in participants engaging in regular chronic exercise.

A criticism of previous work is small sample sizes that result in insufficient power to detect associations between PA and CBF; however, it is unclear what a sufficient sample size is. Using an effect size from Chapman and colleagues (2013), I conducted a power analysis to examine if IGNITE's sample size would leverage sufficient power to test the primary aims. A Pearson's correlation of 0.42 was calculated from Chapman and colleagues' (2013) intervention results showing increased CBF to the ACC following a supervised aerobic exercise intervention. Using this effect size and the sample size of 544 of the present study, this analysis is sufficiently powered to detect an association between MVPA and rCBF (power > 0.99). Similarly, based on Vasic and colleagues' (2015) results exploring rCBF differences between participants diagnosed with depression and healthy controls, a similar medium effect size can be deduced (statistics reported

were insufficient to calculate an effect size). Using a more conservative Pearson's correlation of 0.3 with the sample size of 544 similarly results in power greater than 0.99. Therefore, it is assumed that the present study was sufficiently powered to detect associations, which has been a limitation of studies examining CBF.

4.3 Aim 3

The third aim was to examine whether rCBF in ACC or hippocampal subregions mediates the relationship between MVPA and depressive symptoms, and I predicted that this mediation model would be significant. Inconsistent with my hypothesis, however, mediation was not significant for any subregion of the ACC or hippocampus. Given the statistical power of this sample, these results expand upon our current understanding of the biological mechanisms through which physical activity is associated with depressive symptoms. rCBF is an intuitive, yet untested pathway through which MVPA alleviates depression; however, our results do not support it as such in this particular sample of psychologically-well sedentary older adults. Thus, rCBF may not play a role in the link between MVPA and depressive symptoms, and alternative brain regions and mechanisms should be considered. To our knowledge, the present study is the first to test rCBF as a mediator in the relationship between MVPA and depressive symptoms.

The present study tested statistical mediation from cross-sectional data. Although mediation can be tested cross sectionally, testing mediation from multiple time points is more widely used and accepted. While our results did not support statistical mediation, future work using the IGNITE intervention should test whether potential exercise induced changes mood is associated changes in CBF before ruling out this model entirely in sample of this kind. Moreover,

future work in clinical samples (e.g., AD, mild cognitive impairment, clinical depression) should also test this pathway, as it may differ in clinical populations compared not cognitively and psychological normal samples.

4.4 Strengths and Limitations

The present study has notable strengths. First, few studies have examined the relationship between CBF and MVPA using accelerometer data, a device-based measure of PA. Subjective measures of PA, such as questionnaires, are widely used but recall bias may negatively affect the validity of these measures, particularly in older adult populations with some memory impairments (VandeBunte et al., 2022). Similarly, some studies, such as the exercise interventions discussed by Burdette and colleagues (2010), utilize ratings of perceived exertion (RPE) to measure exercise intensity, which does not allow for standardization of intensity measures across participants. Conversely, accelerometers are a standardized measure PA volume at varying levels of intensity, providing a volume of MVPA per wear period (seven days in IGNITE), which can be distinguished from light-intensity PA. Therefore, device-based measurements of PA are useful for eliminating recall bias and standardizing the measurement of PA intensity across participants.

Using data from IGNITE is another strength as it affords the present study a large sample size and sufficient power to test these aims. While few studies examining CBF have had sufficient statistical power to test rCBF or statistical mediation, the present study has necessary data to overcome this limitation. In the CBF literature, no sample size has exceeded 100 participants. Small sample sizes and low power increase the likelihood of Type II errors, which may be driving inconsistent findings within this literature base. Thus, the present study with a sample size of 544 decreases the likelihood of a Type II error.

Additionally, while many studies dichotomize depression status (having depression versus no depression), the present study used continuous scores from the HADS-D to examine the relationship between depressive symptoms, PA and CBF, which overcomes the criticism related to the categorization of a cluster of heterogenous symptoms into a single diagnosis.

The present study should also be considered in light of some limitations. First, due to the cross-sectional nature of the data, results are correlational, so causality cannot be inferred. Similarly, temporal precedence of the mediation model cannot be inferred. Moreover, temporality of these variables will remain a matter of speculation, but findings from the present study can inspire testable hypotheses based on the IGNITE intervention data, which can be used to address this issue of temporality.

Secondly, a diagnosis of Major Depressive Disorder or a score of 9 (the clinical cutoff for moderate depression) on the Geriatric Depression Scale were exclusion criteria for the IGNITE study. As a result, the IGNITE sample excluded participants with clinically significant levels of depression. However, some participants scored above the clinical threshold of 8 for mild depression on the HADS-D (n = 101) and the recommended adjusted cutoff or mild depression in order adults (n = 307). This exclusion criteria led to a smaller range of depressive scores than are expected in the general population, which may impose limitations on the statistical plan. For example, almost half of the sample (n = 300) had a score of 3 or less on the HADS-D. Although our sample is generally psychologically well, results from the present study have meaningful implications for subclinical depression because 86.5% (n = 525) of our sample endorsed that they at least occasionally experienced at least one depressive symptom.

4.5 Conclusion

Although CBF is plausible biological pathway through which engagement in MVPA may be associated with fewer depressive symptoms, our results do not support this pathway. However, limitations of the sample and use of cross-sectional data may have limited our ability to detect mediation.

In sum, the results from this study confirm that higher levels of MVPA are associated with fewer depressive symptoms even in in self-reported sedentary older adults with subclinical levels of depressive symptoms. The lack of significance for Aims 2 and 3, however, prompt the need for future studies to continue to explore the relationship between MVPA, depressive symptoms, and the potential role of CBF. Non-significant results between MVPA and rCBF are highly informative that engagement of MVPA might not be associated with CBF; however, future work should examine MVPA accumulated in bouts and test whether exercise induced changes in CBF are possible and associated with behavioral changes (e.g., mood, cognition). Indeed, intervention data from IGNITE can be leveraged to test this mediation model.

The present study provides an important and incremental step in our knowledge of whether subclinical depressive symptoms relate to CBF and whether engaging in greater physical activity or exercise is associated with reduced depressive symptoms through CBF pathways. Future work should continue to explore this pathway and other pathways that could serve as a target for alleviating symptoms of depression in older adults. As the older adult population continues to increase exponentially, identifying and promoting physical activity as a low-cost evidence-based lifestyle factor for improving quality of life could have far-reaching impacts.

Appendix A Tables and Figures in Main Document

Table 3. Demographic Characteristics of the Sample

Characteristics	**n (%) or Mean [SD]**
Gender	
Female	386 (71)
Male	158 (29)
Race	
African American/ Black	98 (18)
Asian	9 (1.7)
Caucasian/White	420 (77)
Bi-racial	1 (<1)
Refused to answer	7 (1.3)
Other	8 (1.5)
Age (years)	69.77 [3.70]
Education	16.31 [2.20]
VO_2peak (ml/kg/min)	21.88 [5.07]
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MVPA (average minutes/day)	31.13(26.91)

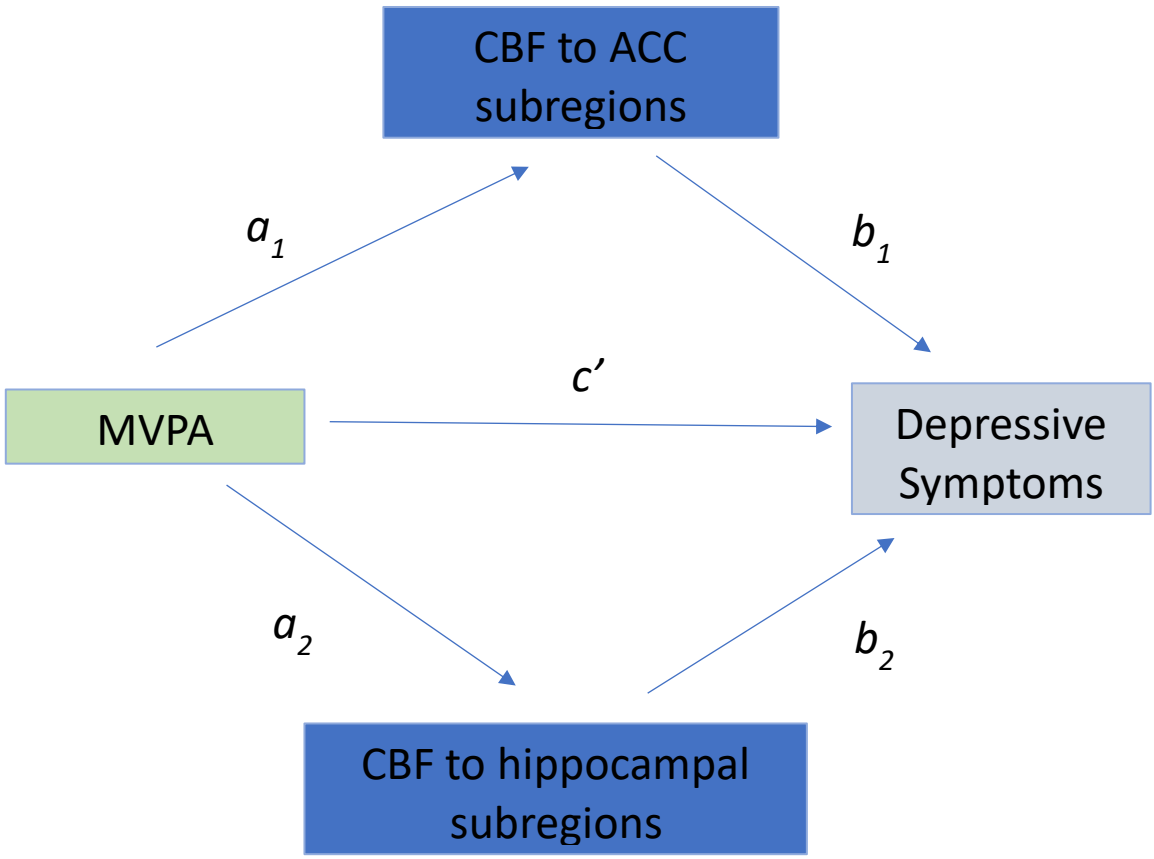

Figure 8. Mediation model

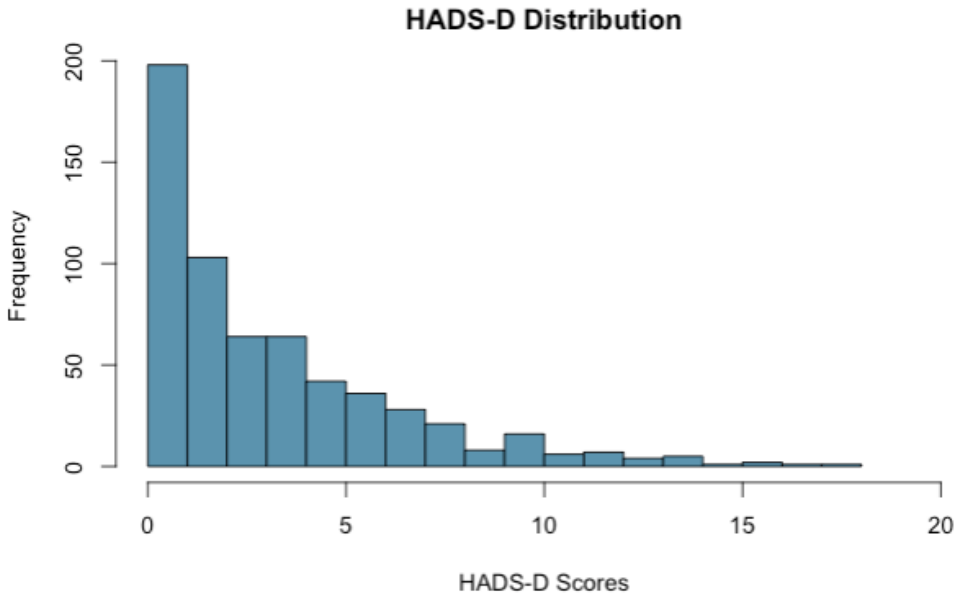

Figure 9. Frequency of HADS-D scores

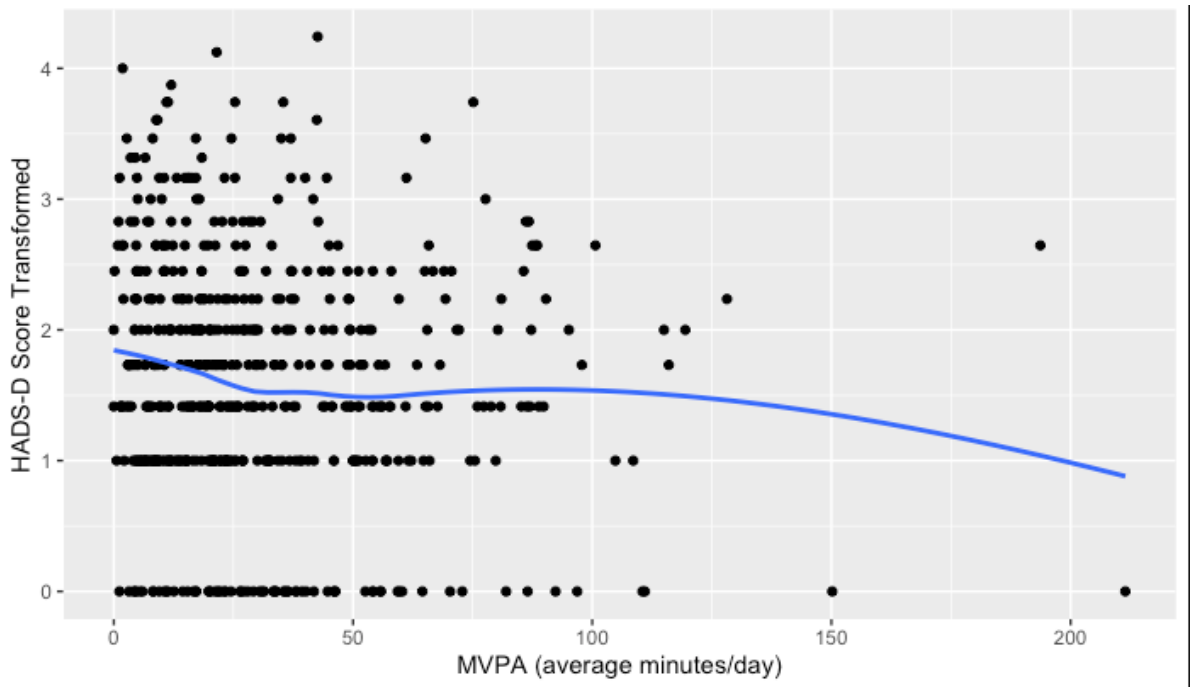

Figure 10. Nonlinear relationship between MVPA and transformed (square root) HADS-D scores

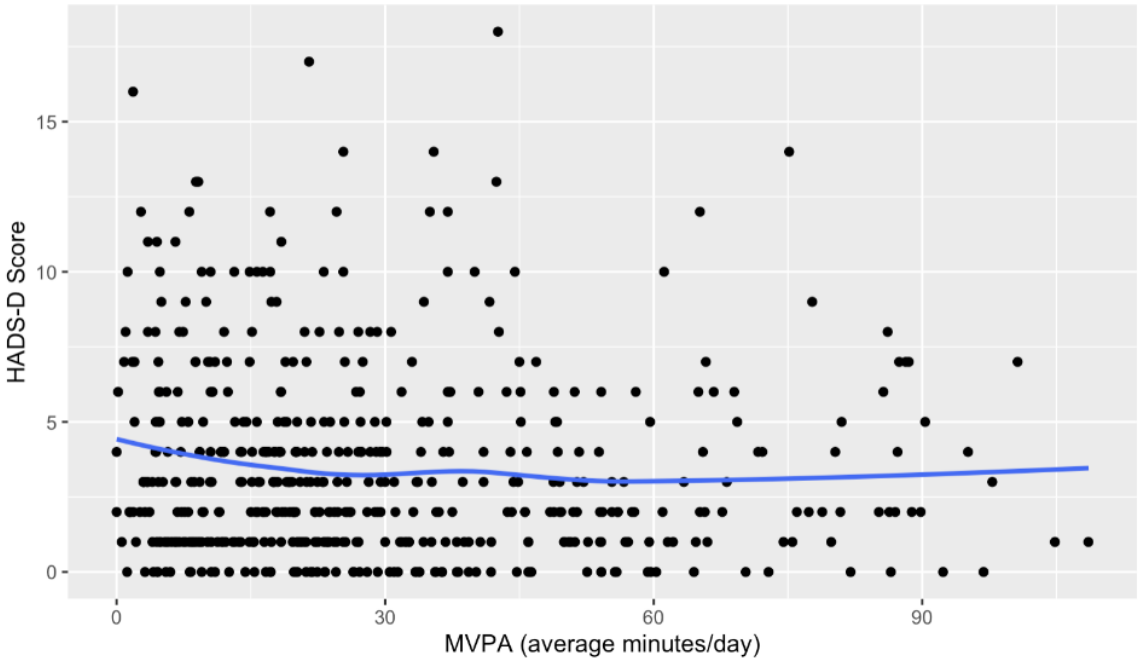

Figure 11. Relationship between MVPA and non-transformed HADS scores with MVPA outliers removed

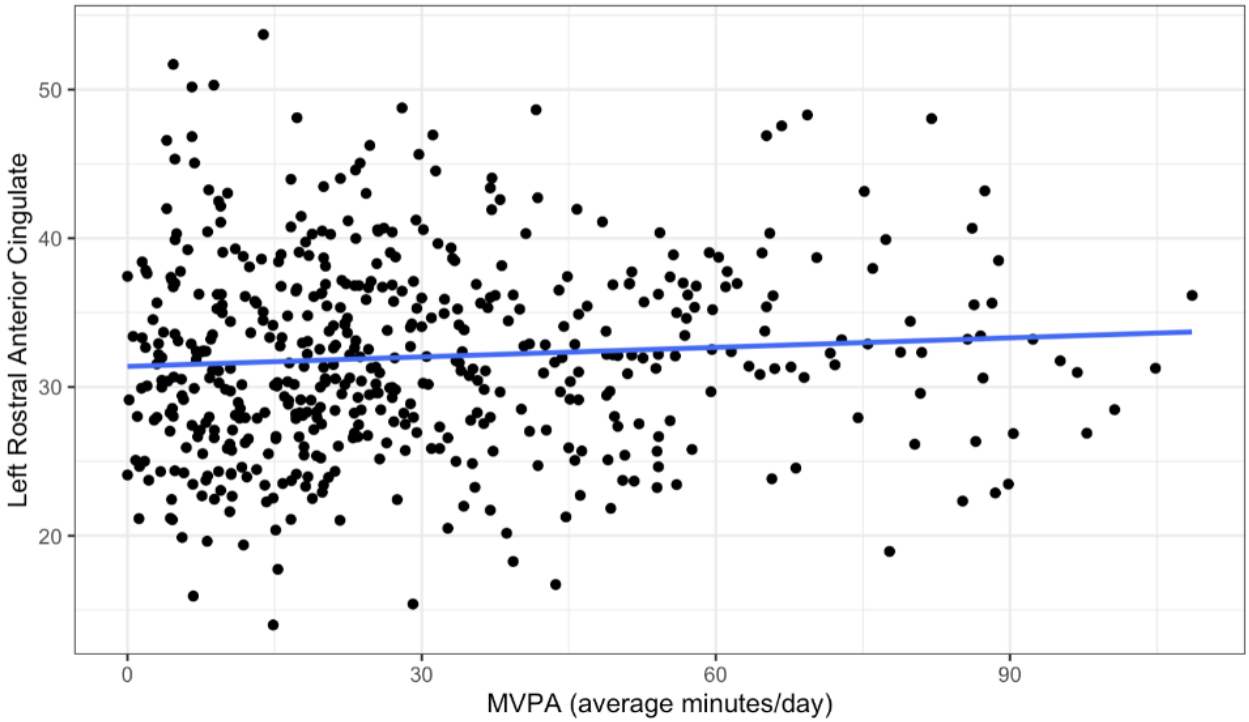

Figure 12. Relationship between MVPA and CBF in the left rostral ACC excluding outliers

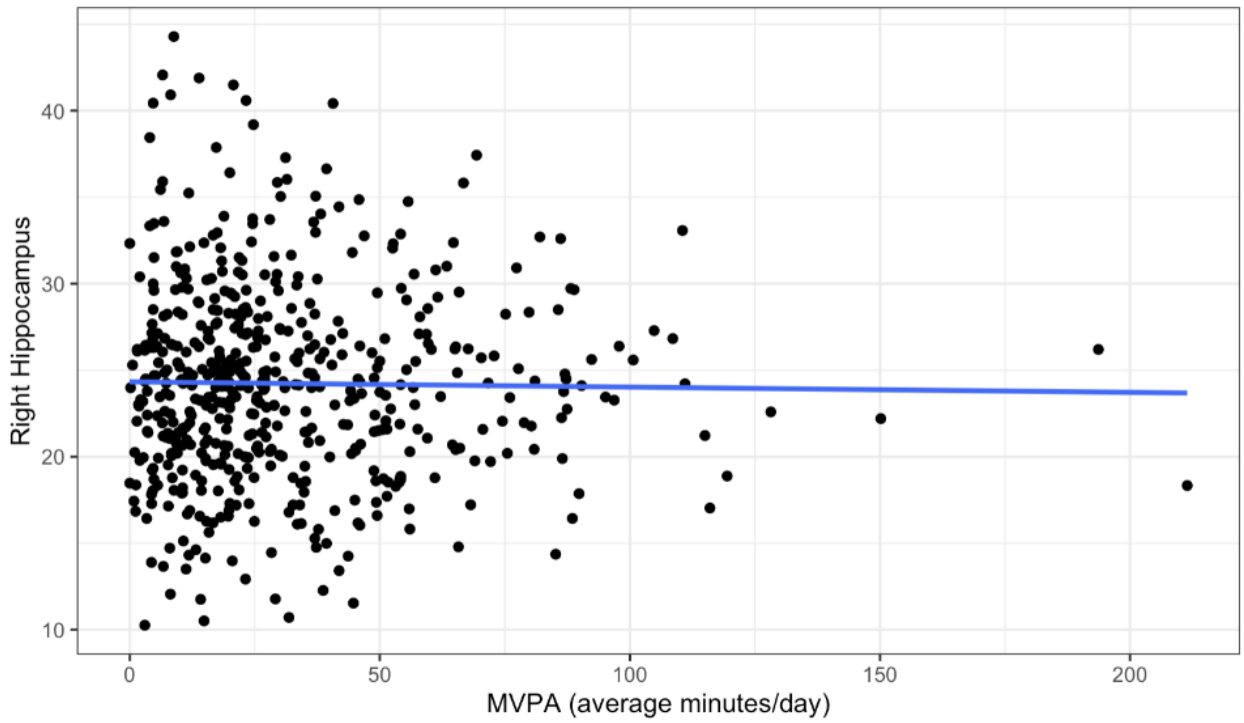

Figure 13. Relationship between MVPA and CBF to the right hippocampus

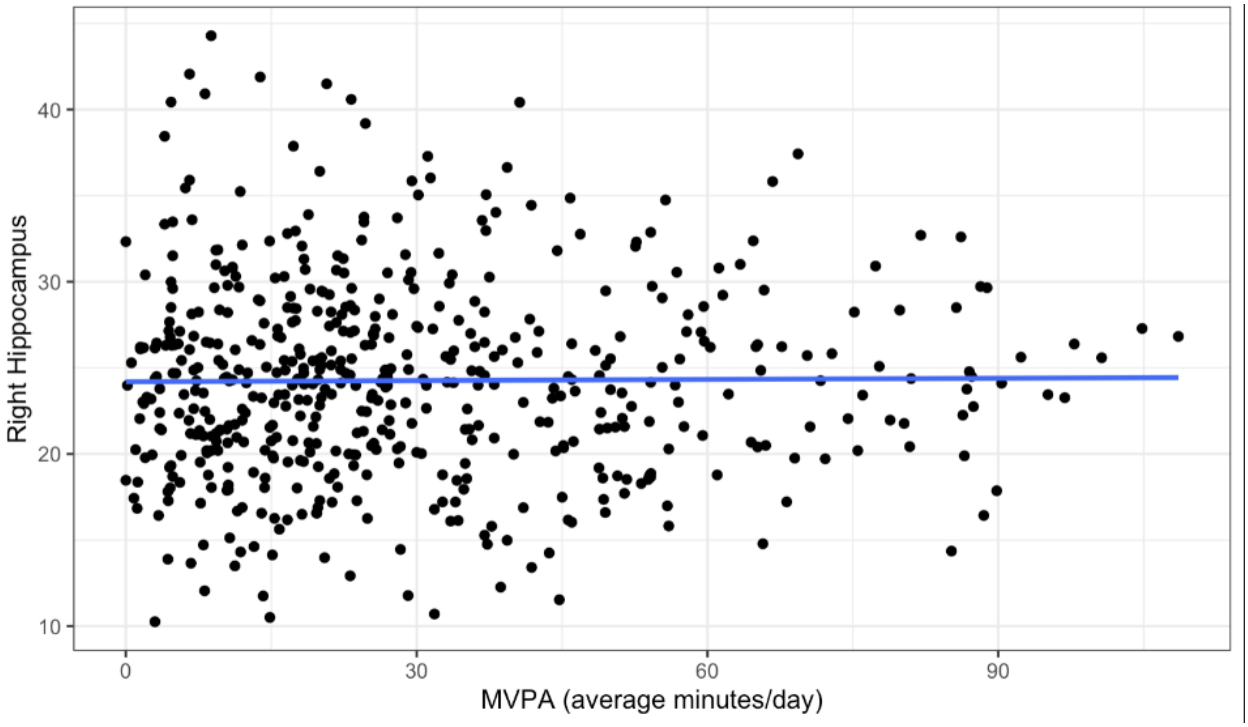

Figure 14. Relationship between MVPA and CBF to the Right Hippocampus excluding outliers

Table 4. Results Summary: Aims 1 and 2

Characteristics	β	p	Adjusted R^2
Aim 1[#]	-0.0034	.03*	0.038
Aim 2			
Left Hippocampus	-0.003	0.73	0.10
Right Hippocampus	-0.001	0.85	0.10
Left Parahippocampal	0.000	0.94	0.09
Right Parahippocampal	-0.001	0.87	0.08
Left Caudal ACC	0.009	0.42	0.06
Right Caudal ACC	0.008	0.49	0.08
Left Rostral ACC	0.011	0.29	0.08
Right Rostral ACC	0.011	0.28	0.08

*Statistically significant
[#]HADS-D scores (dependent variable) were transformed (square root) due to non-normality of residuals

Appendix B Supplemental Figures

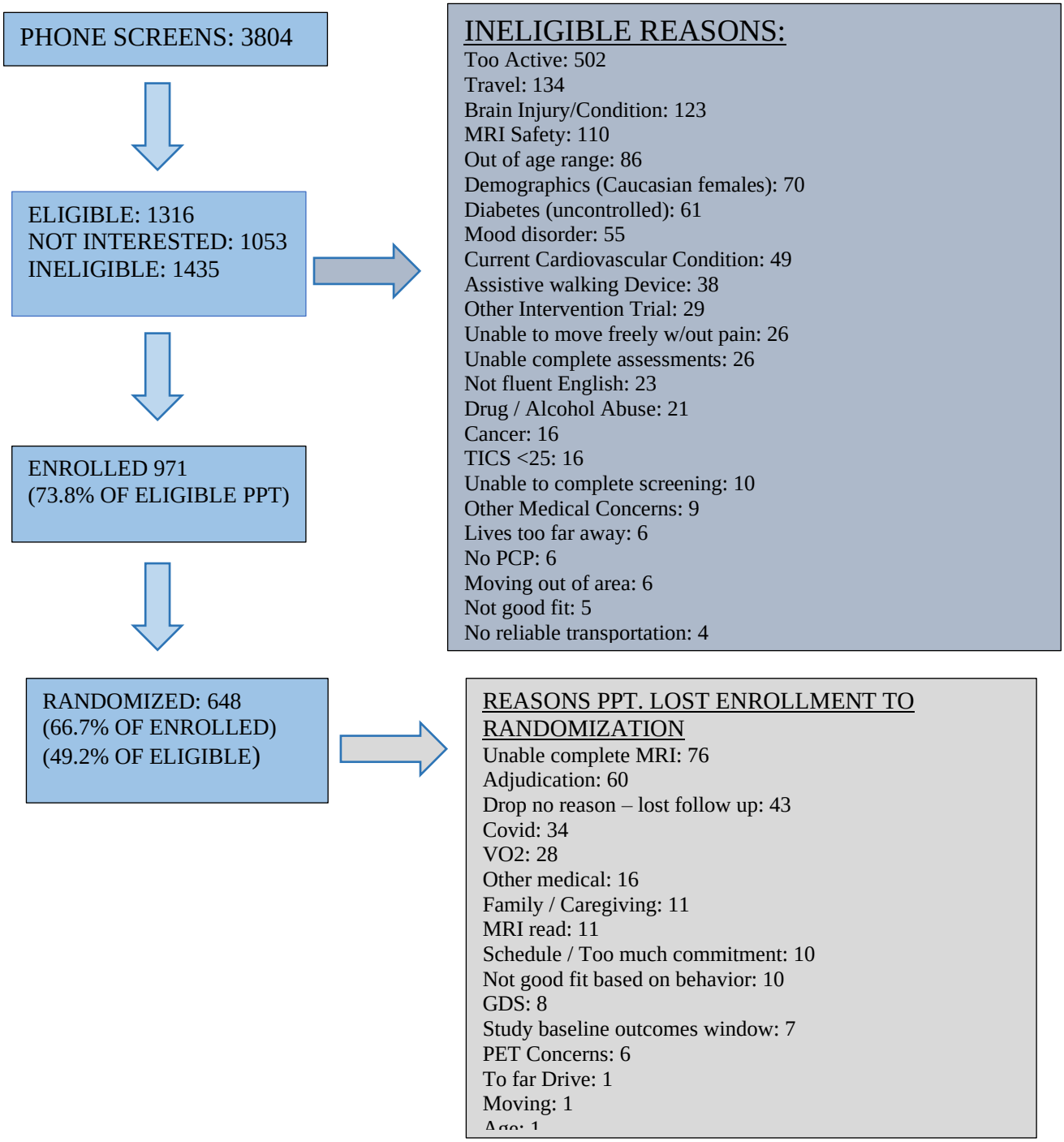

Figure S1: Consort Diagram – IGNITE Intervention

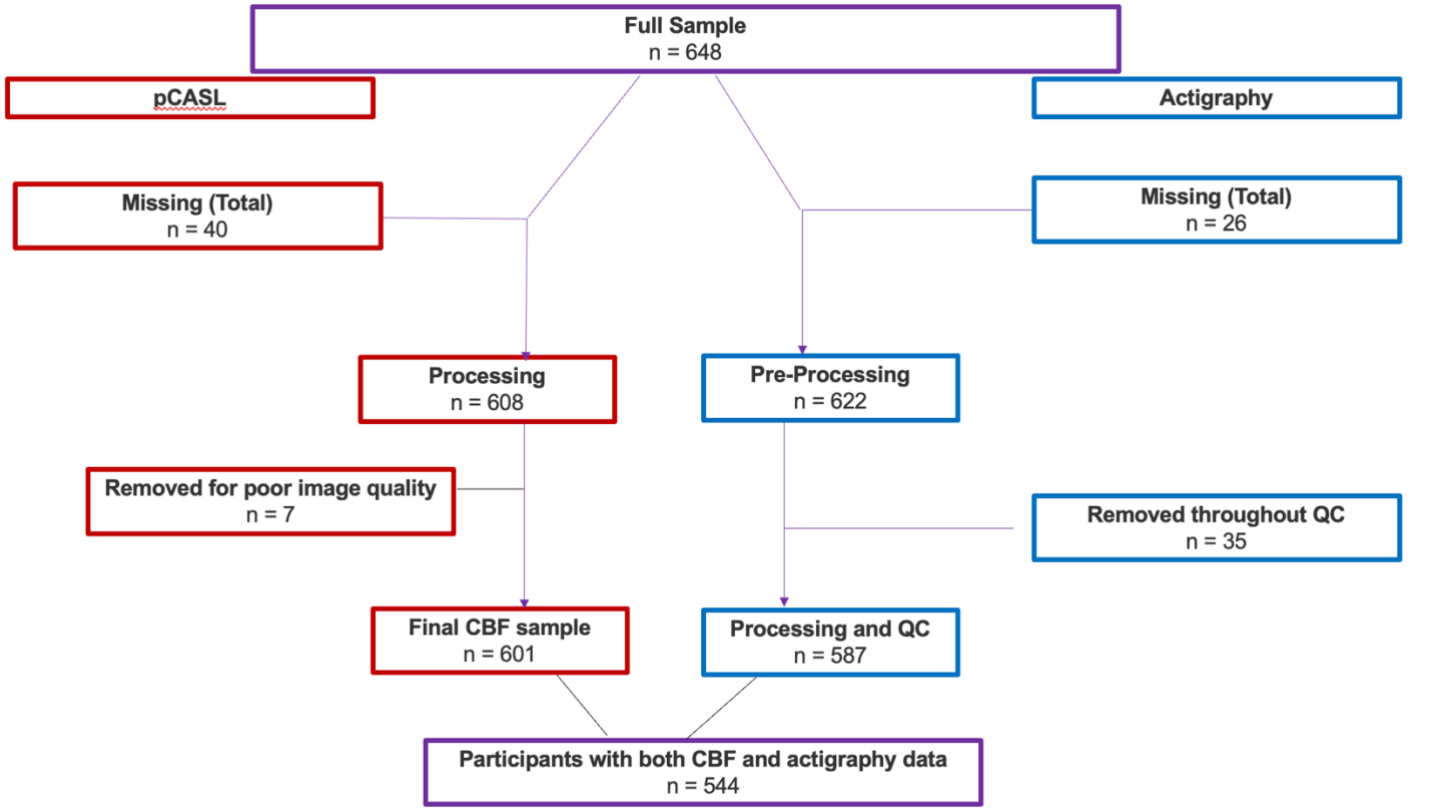

Figure S2: Consort Diagram – Present Study

Table S1: IGNITE Inclusion and Exclusion Criteria

Inclusion Criteria	Exclusion Criteria
• Men and women 65 – 80 yrs • Ambulatory without pain or the use of assisted walking devices • Able to speak and read English • Medical clearance by primary care physician (PCP) • Living in community for duration of the study • Reliable means of transportation • No diagnosis of a neurological disease • Eligible to undergo MRI • Telephone Interview of Cognitive Status[9] score >25 • Cognitive adjudication decision of cognitively normal	• Current diagnosis of an Axis I or II disorder including Major Depression • History of major psychiatric illness including schizophrenia (not including general anxiety disorder or depression (Geriatric Depression Scale [GDS] ≥9)[10] • Current treatment for cancer – except non-melanoma skin cancer • Neurological condition (MS, Parkinson's, Dementia) or brain injury (Stroke) • Type I Diabetes, Insulin-dependent Type II Diabetes, uncontrolled Type II diabetes (defined as an HbA1c level > 10) • Current alcohol or substance abuse or treatment for abuse in the past 5 years • Current treatment for congestive heart failure, angina, uncontrolled arrhythmia, deep vein thrombosis (DVT) or another cardiovascular event • Myocardial infarction, coronary artery bypass grafting, angioplasty or other cardiac condition in the past year • Regular use of an assisted walking device • Presence of metal implants (pacemaker, stents) that are MR ineligible • Inability to complete the MRI scan • Claustrophobia • Color Blindness • Not fluent in English • Not medically cleared by PCP • Engaging in >20 minutes of moderate intensity physical activity per day for 3 days or more per week • Not local or able to travel 3 times per week to the exercise facility • Travelling consecutively for 3 weeks or more during the study • Telephone Interview of Cognitive Status[9] score <25 • Cognitive adjudication decision of memory impairment • Unwillingness to be randomized to one of the three groups • Current participation in an ongoing trial likely to influence exercise ability or cognitive function (e.g., mindfulness training).

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