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Relationship between cardiorespiratory fitness, cognition, structural and functional brain health in middle-aged ∫adults

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ABBREVIATIONS

ACME	Average Causal Mediated Effect
BBHI	Barcelona Brain Health Initiative
BDFN	Brain-Derived Neurotrophic Factor
BOLD	Blood Oxygen Level Dependent
CPET	Cardiopulmonary Exercise Test
CRF	Cardiorespiratory Fitness
CSF	Cerebrospinal Fluid
CV	Cardiovascular
CVH	Cardiovascular Health
CVR	Cardiovascular Risk
DASS	Depression Anxiety Stress Scale
DMN	Default Mode Network
fMRI	Functional Magnetic Resonance Imaging
FPN	Frontoparietal Network
IGF-1	Insulin-like Growth Factor-I
MET	Metabolic Equivalent of Task
MRI	Magnetic Resonance Imaging
PA	Physical Activity
RF	Radiofrequency
SD	Standard Deviation
SN	Salience Network
VEGF	VEGF Vascular Endothelial Growth Factor
WHO	WHO World Health Organization

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Resum

En els darrers anys, l'esperança de vida ha augmentat significativament, tot i que no necessàriament acompanyada d'un augment de la salut. L'edat avançada és un dels factor de risc més rellevant pel desenvolupament de trastorns neurològics i psiquiàtrics. No obstant això, amplis estudis han establert que els canvis estructurals i funcionals produïts en el cervell i històricament associats a malalties neurodegeneratives es poden produir entre 10 i 20 anys abans que es manifestin els símptomes reals de dites patologies. Malgrat la rellevància d'abordar diferents estils de vida en el context de la prevenció i la promoció d'estratègies de salut global, els mecanismes precisos subjacents a les associacions entre l'adherència a aquests estil de vida concrets i els factors de salut cerebral front l'envelliment o en les etapes inicials de certs patologies neurodegeneratives encara no son del tot clars.

En la present tesi hem examinat els correlats mecanicistes de la relació entre l'aptitud cardiorespiratòria en la mitjana edat (40-65 anys) i les habilitats cognitives i la salut mental. En última instància, hem caracteritzat l'aptitud cardiorespiratòria com un factor neuro-protector amb mesures de salut estructural i funcional del cervell que potencialment podrien contribuir al desenvolupament d'intervencions d'estils de vida més efectives i precises per mantenir i millorar la salut cerebral durant el procés natural d'envelliment.

En el primer estudi, en una mostra de 501 persones de mitjana edat (entre 40 i 65 anys), hem estudiat si hi ha correlacions entre dos factors de salut cardiovascular modificables, com son l'aptitud cardiorespiratòria i el risc cardiovascular, i les seves relacions amb cognició. A més, hem explorat les possibles vies subjacents que poden explicar aquestes connexions. La nostra anàlisi ha demostrat que tenir una condició cardiorespiratòria més elevada està associat significativament amb millors habilitats visuoespaials i la resolució de problemes en el grup de més edat (entre 55 i 65 anys). En canvi, el risc cardiovascular es va associar negativament amb un millor raonament visuoespaial i capacitat de resolució de problemes, flexibilitat, velocitat de processament i memòria. Aquestes relacions també van ser mediades per l'estructura del cervell (concretament el gruix cortical) destacant una possible via mecanicista a través de la qual una major aptitud cardiorespiratòria i un menor risc cardiovascular poden afectar positivament la funció cognitiva en adults de mitjana edat.

En el segon estudi, vam voler caracteritzar millor els mecanismes neuronals pels quals l'aptitud cardiovascular podria influir potencialment en la salut mental a la mitjana edat. L'estudi es va dur a terme en una mostra de 418 adults sans de mitjana edat (entre

40 i 65 anys). Les nostres troballes van demostrar que una condició cardiorespiratòria més elevada està associada amb símptomes més baixos d'ansietat i estrès. A més, més connectivitat funcional dins la xarxa *Default Mode* s'associa amb millor aptitud cardiorespiratòria i puntuacions d'estrès més baixes. Tanmateix, la connectivitat funcional entre la xarxa *Frontoparietal* i la xarxa *Salience* s'associa amb una millor condició cardiorespiratòria i puntuacions d'estrès més baixes. Els nostres resultats també van indicar que la combinació d'una major integració de la xarxa *Default Mode* i una millor sincronia entre les xarxes *Salience-Frontoparietal* media la relació entre la capacitat cardiorespiratòria i l'estrès. En resum, els nostres resultats suggereixen que les diferències interindividuals en la relació entre la connectivitat funcional de les xarxes del *Model Triple Network* amb l'estrès a la mitjana edat s'expliquen parcialment per les variacions en l'aptitud cardiorespiratòria donant així suport a la importància de participar en hàbits d'estil de vida modificables que poden promoure la salut mental i cerebral al llarg de la vida.

Abstract

Over the past century, an increase in lifespan has not been accompanied by an increase in health span. Advancing age is a major risk factor for the development of neurological and psychiatric disorders. However, extensive research has established that structural and functional changes in the brain associated with neurodegenerative diseases can occur 10-20 years before symptoms appear. Despite the relevance of addressing lifestyles factors in the context of prevention and promotion of global health strategies, the precise mechanisms underlying the associations between lifestyle behaviors and brain health in the face of advancing age or even the initial stage of pathology remains unclear.

In the present thesis we have examined the relationship between cardiorespiratory fitness and cognitive and mental health outcomes in midlife and explore their mechanistic correlates using structural and functional neuroimaging. A better understanding of the role of cardiorespiratory fitness as a neuroprotective factor on brain health in midlife can potentially contribute to the development of more effective and precise lifestyles interventions to maintain and improve brain health with age.

In the first study, which included a sample of 501 middle-aged (aged 40–65 years), we explored whether correlations between cardiorespiratory fitness and cardiovascular risk and cognition are present in healthy middle-aged adults. Also, we explored the possible underlying pathways that may explain these correlations. Our results showed that higher cardiorespiratory fitness was significantly associated with better visuospatial abilities and frontal loading abstract problem-solving capabilities in the older middle-aged group (aged 55–65 years). In contrast, higher cardiovascular risk was associated with worse visuospatial reasoning and problem-solving abilities, flexibility, processing speed and memory. These relationships were mediated by brain structure (cortical thickness) highlighting a potential mechanistic pathway through which higher cardiorespiratory fitness and lower cardiovascular risk can positively impact cognitive function in midlife.

In the second study, we aimed to characterize the neural mechanisms by which cardiovascular fitness could potentially influence mental health in midlife. The study was conducted in a sample of 418 healthy middle-aged (aged 40–65 years) adults. Our findings showed that higher cardiorespiratory fitness was associated with lower anxiety and stress scores. In addition, higher within-network functional connectivity of the Default Mode Network was associated with cardiorespiratory fitness, and lower stress scores. Higher functional connectivity between the Frontoparietal Network and Salience Network was associated with higher cardiorespiratory fitness and lower stress scores.

Our findings also indicated that the combination of higher integration of the Default Mode Network and increased synchrony of Salience-Frontoparietal networks mediate the relationship between cardiorespiratory fitness and stress. In summary, our results suggest that the inter-individual differences in how functional connectivity of the Triple Network Model networks relates to stress in midlife, are partially explained by variations in cardiorespiratory fitness, supporting the importance of engaging in modifiable lifestyle behaviors that can promote cognitive and mental health in midlife.

1. Introduction

1.1 Brain Health across lifespan

Over the past century, an increase of human lifespan has occurred without a corresponding increase in health span (1,2). Advancing age is a major risk factor for the development of neurological and psychiatric disorders and the increased prevalence of these conditions are projected to account for over half of the worldwide economic impact of disability by 2030 (3). Throughout lifespan, the brain undergoes various changes that can affect its health and function. These changes can be influenced by various factors such as genetics, environmental factors, lifestyle habits, and the aging process itself.

Brain Health is defined as the development and preservation of optimal brain integrity and functioning for a given age (4). Notwithstanding, the development of pathological loss of brain health does not appear to be an obligatory consequence of aging (5). *Aging* has been defined as the gradual decline of biological functions caused by progressive dysfunction of different cellular systems responsible for repairing and maintaining the homeostasis (6). This gradual decline, along with other risk factors, can result in the emergence of cognitive decline and neurodegenerative pathologies such as dementias.

Interestingly, individual differences in the fundamental homeostatic brain mechanisms named *brain resilience* allow some individuals to cope better than others with brain pathology and hence show preserved brain function. These individual trajectories may delay the appearance of symptoms or act to reduce or eliminate the clinical and behavioral impact of neurological pathologies (7). Extensive research has established that structural and functional changes in the brain associated with neurodegenerative diseases can occur 10-20 years before symptoms appear (Beason-Held et al., 2013). There has been a significant emphasis on discovering and validating new *biomarkers* for major neurological diseases (Olsson et al., 2016), which can help identify individuals who are at risk or in the early stages of a disease before clinical symptoms become apparent (Dubois et al., 2007). During this pre-clinical period, variations in brain resilience among individuals may either delay the onset of symptoms or mitigate the clinical and behavioral effects of these diseases (Nyberg et al., 2012). Consequently, it is crucial to focus research towards identifying factors that could prevent illnesses and promote brain resilience with age.

1.2 Modifiable lifestyle factors

During the last decades, numerous theories have provided a conceptual framework to study the effects of lifestyle factors on brain health. Several lifestyle

behaviors and their interactions with biological markers have been found to be protective of age-related and pathological brain changes. Besides, the influence of both modifiable and non-modifiable lifestyle factors can interact, changing the susceptibility of an individual to develop dementia or other neurological and psychiatric disorders, as well as facilitate recovery from brain injuries or illnesses (8–10). A substantial proportion of diagnosed dementia cases could potentially be prevented through targeted changes of various modifiable lifestyle behaviors including physical activity engagement, maintaining cardiovascular health, psychological well-being, cognitively stimulating activity participation and social support across the lifespan (10,11). Similarly, the adoption of healthier diet habits (12), including specific dietary patterns such as Mediterranean diet (13–15), along with the maintenance of high-quality good sleep (16,17), and cultivating a sense of purpose in life (18,19) have been proposed to exert positive effects on brain health throughout aging and potentially mitigating the incidence of brain diseases.

In this context, several concepts like *brain reserve* and *resilience* are often used for capturing differential susceptibility to brain aging and disease and how these lifestyles could potentially impact the brain. The term *resilience* subsumes any concept that relates to the capacity of the brain to maintain cognition and function with natural aging and/or disease. However, there is variability in the mechanisms underlying resilience such as *cognitive reserve, brain maintenance* and *brain reserve* (see box 1 for definitions) (20).

Box 1. Definitions of various mechanisms underlying resilience

- *Cognitive Reserve*: refers to the individual adaptability (i.e., efficiency, capacity, flexibility) of cognitive processes that helps to explain differential susceptibility of cognitive abilities or day-to-day function to brain aging, pathology, or insult (see Figure 1) (Stern, 2012).
- *Brain Maintenance:* defined as reduced development over time of age-related brain changes and pathology based on genetics or lifestyle. This can lead to individual differences in morphologic brain decline associated with normal aging (Stern et al., 2020).
- *Brain Reserve:* it is a fixed construct that implies individual variation in the structural characteristics of the brain that allows some people to better cope with brain aging and pathology than others before clinical or cognitive changes emerge. These differences can be quantitative, such as larger brain, more neurons, or synapses. In addition, life experience can influence brain anatomy via neurogenesis, angiogenesis, promoting resistance to apoptosis, and up-regulating compounds that promote neural plasticity (Stern et al., 2020).

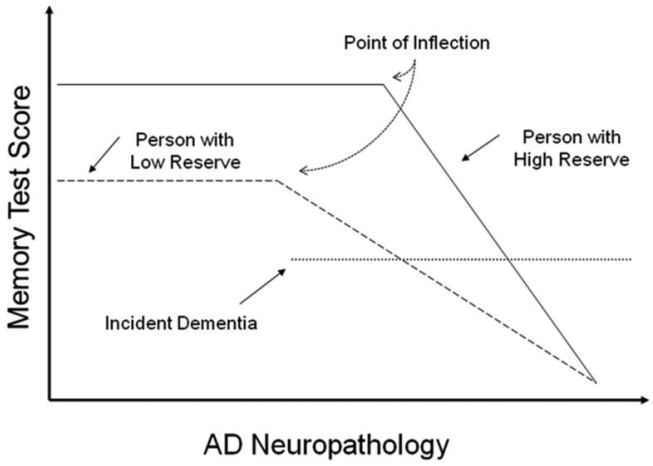

Figure 1. **Cognitive Reserve**

Theoretical illustration of how high and low cognitive reserve may mediate between Alzheimer's Disease (AD) pathology and its clinical expression. Taken from Stern, 2009 (21).

Initially presented within the framework of the *Cognitive Reserve Hypothesis* (22), lifestyle factors were proposed as potential mediators between cerebral changes and cognitive performance, suggesting their potential to mitigate clinical symptoms of neurological diseases (23). Even so, emerging evidence suggests that the impact of lifestyle variables on neuropathology and aging processes may operate through a coexisting dual mechanism involving both compensatory and neuroprotective mechanisms (23). Beyond theoretical models, this preservation has been linked to the maintenance of brain structure and connectivity patterns with advancing age (7). Moreover, it can also be associated with the emergence of compensatory mechanisms when faced with pathological alterations over lifespan (22,24,25). In summary, it has been proposed that experience-based changes in brain structure and function may act, in aging, as protective factors that contribute to intra-individual differences in the resilience to brain pathologies (26).

Despite the relevance of addressing lifestyles factors in the context of prevention and promotion of global health strategies, the precise mechanisms underlying the associations between lifestyle behaviors and brain health in the face of advancing age or even the initial stage of pathology remains unclear. Specifically, there remains a significant knowledge gap precluding the personalized prescription of specific lifestyle modifications in midlife to promote an individual's brain resilience and reduce the incidence of major neuropsychiatric and neurological diseases in advancing age.

1.3 Physical Activity, Physical Exercise, and Cardiorespiratory Fitness

Beyond overall health benefits, a correlative connection has been established between *physical activity* (see box 2 for definitions) and brain health. A Lancet commission on dementia (10) highlights that engaging in physical activity during mid-life is a modifiable lifestyle determinant capable of reducing the risk of dementia. The same group also stated that physical inactivity is one of twelve modifiable risk factors that together might explain 40% of the global dementia cases (10). In light of this evidence, excessive amounts of *sedentary behaviors* might be a risk factor for dementia and cognitive decline (27,28).

Box 2. Definitions of various exercise related terminology

- *Physical activity:* any body movement that leads to energy expenditure beyond resting levels and is initiated by skeletal muscles (Budde et al., 2016).
- *Sedentary behaviors:* certain activities in a reclining, seated, or lying prolonged position requiring very low energy expenditure (≤1.5 Metabolic equivalent of task (MET)) (Tremblay et al., 2017).
- *Physical exercise* or *exercise*: a disturbance of homeostasis through muscle activity resulting in movement and increased energy expenditure(Scheuer & Tipton, 1977). Planned, structured and goal-oriented physical activity designed to improve or maintain physical fitness (Caspersen et al., 1985). Often involves aerobic systems.
- *Cardiorespiratory fitness:* the body's ability to inhale, circulate and utilize oxygen during exercise. Gold standard measure is VO_2 max, expressed as the maximum amount of oxygen consumption in 1 minute per kilogram of body weight [mL/Kg/min] or also reported as metabolic equivalents (METs) (Hawkins et al., 2007).

Physical exercise capacity to improve cognitive function has been studied since the late 1990's (29). In the past decades, several studies have found that exercise can have immediate - and lasting - effects on brain (29–32). The interplay between exercise, mood and neuroscience is complex and the specific neurobiological effects of physical exercise are numerous and involve wide range of interrelated complex effects on brain

structure, brain function and cognition. Both animal and human studies have shown that exercise exerts positive effects on cognition through a variety of mechanisms. These encompass the capacity to mitigate the age-related atrophy of gray and white matter (33–35). Besides, it increases vascularization, dendritic spine density, and complexity within the hippocampus; along with enhance synaptic plasticity (36). Also, it increases the release of essential neurotrophic factors and trophic agents such as insulin-like growth factor-I (IGF-1), vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF), crucial for neuronal viability (37–40). Furthermore, a recent review studying dose effects of exercise in aging adults suggested that most stable and consistent improvements in cognition following exercise occur in executive functions and processing speed promoted by underling cerebral perfusion, synaptic neuroplasticity, brain structure (volume and connectivity), neurogenesis and synaptogenesis, and trophic factors (BDFN, IGF-1, and VEGF) following participation of exercise in older adults and aged rodents (see Figure 2) (41).

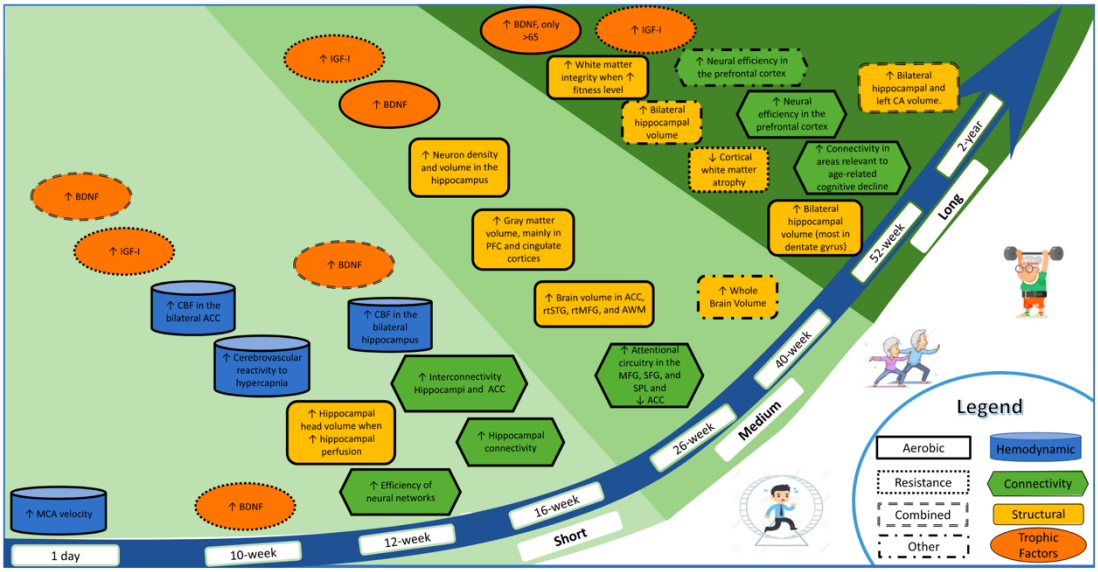

Figure 2. **Exercise effects on brain health**

The significant changes in cerebral perfusion, brain structure, connectivity, and trophic factors with short, medium, and long-term exercise interventions in older adults. MCA = middle cerebral artery; CBF= cerebral blood flow; BDNF = brain-derived neurotrophic factor; IGF-1 = insulin-like growth factor-1; VEGF = vascular endothelial growth factor; ACC= anterior cingulate cortex; PFC = prefrontal cortex; rtSTG = right superior temporal gyrus; rtMFG = right medial frontal gyrus; AWM = anterior white matter; MFG = medial frontal gyrus; SFG = superior frontal gyrus; SPL = superior parietal lobules; CA = cornus ammonis. Taken from Cabral et al., 2019 (41).

Increasing physical activity through structured exercise participation is a safe and relatively inexpensive means to modify *cardiorespiratory fitness (CRF),* a key marker of

physical health. CRF is not only associated with lower cardiovascular (CV) disease morbidity and mortality, but also with lower prevalence of risk factors for CV diseases such as diabetes, hypertension, and selected dyslipidemias. Maximal oxygen uptake (VO_2 max) is the best indicator of CRF and was defined by Hill and Lupton in 1923 as the oxygen uptake attained during maximal exercise intensity that could not be increased despite further increases in exercise workload, thereby defining the functional limits of the cardiorespiratory system. Thus, it was established that VO_2 max represents the capacity of the CV and respiratory system to transport oxygen to vital organs and skeletal muscle. A cardiopulmonary exercise test (CPET) is required to assess and measure objectively VO_2 max levels and therefore, obtain individualized levels of CRF. Besides, CRF is a complex trait determined by genetic, behavioral, and environmental factors, including exercise and physical activity (42). Importantly, CRF can be modified through exercise and such change in CRF levels can influence functional ability and CV outcomes years later in terms of mortality and brain health (43). The value of CRF as a clinical and public health tool for use in risk identification and classification is very high. Furthermore, extensive studies have explored the impact of CRF on brain and mental health (44–46). CRF has been identified as a critical mechanism explaining aerobic exercise's effect on cognitive function (47–51). Furthermore, higher CRF has been consistently related to better cognition (47,52,53), maintenance of cortical thickness and volume across the lifespan (33,52,54–56), and discrete mental health outcomes such as depression and anxiety scores (44,45,57–59). In terms of functional connectivity, CRF has been related to specific brain networks that are relevant to age-related changes in cognition and risk for neurological and psychiatric diseases (60). Specifically, Default Mode Network (DMN), the Salience Network (SN), and the Frontoparietal Network (FPN) appear most sensitive to individual differences in CRF (61).

1.4 Brain Health Metrics

1.4.1 Cognition

Cognitive changes as a normal process of aging have been well documented in the scientific literature. Cognition involves complex information processing, planning, and reasoning (62). The cognitive changes associated with aging encompass multiple domains including deficits in episodic memory, executive function, working memory, attention, and processing speed (see Box 3. Definitions of various cognitive domains) (63,64). Evidence from extensive behavioral literature suggests that there are at least three descriptive patterns of age-related change in cognition (65). (1) Processing speed, working memory and episodic memory which are basic mechanisms of cognitive information processing that tend to decline linearly across the adult lifespan (63,66,67).

(2) While implicit memory may remain relatively stable across life or show a subtle decline with age, vocabulary and semantic knowledge tend to decline in performance only very late in life. (3) Lastly, autobiographical memory and automatic memory processes tend to be stable throughout life (63,65,68).

It is important to note that the rate and degree of cognitive decline varies widely across individuals with some individuals capable of maintaining good cognitive function well into their 80's and 90's, known as *SuperAgers* (69). There are likely various reasons to explain the high level of heterogeneity in cognitive aging beyond genetics. For example, the lifestyle and environment of each individual has been proposed to strongly influence the degree and susceptibility to age-related cognitive decline (23,70–74). Importantly, in some individuals, significant pathological changes in the brain are observed in conjunction with relatively well-preserved cognitive performance. As mention before, multiple constructs have been invoked to explain this paradox of resilience, including brain reserve, cognitive reserve and brain maintenance (see Modifiable lifestyle factor section, Box 1: Definitions of various mechanisms underlying resilience) (75,76).

Box 3. Definitions of various cognitive domains

- *Attention:* ability to concentrate and focus on specific stimuli. It is a process of selectively concentrating on a discrete aspect of information (Lezak et al., 2012).
- *Processing speed:* refers to the speed with which cognitive activities are performed as well as the speed of motor responses (Lezak et al., 2012).
- *Working memory:* ability to momentarily hold information in memory while simultaneously manipulating that information (Lezak et al., 2012).
- *Memory:* refers to the psychological processes of acquiring, storing, retaining, and later retrieving information (Lezak et al., 2012).
- *Visuospatial reasoning/Problem solving:* the ability to comprehend and analyze information by sorting it into a logical structure. It is associated with an individual's ability to interpret information quickly and efficiently and filter out the irrelevant parts or slow down the process (Lezak et al., 2012).
- *Cognitive flexibility:* capacities that allow a person to successfully engage in independent, appropriate, purposive, and self-serving behavior. This includes a wide range of cognitive abilities such as self-monitor, plan, organize, reason, flexibility, and problem-solve (Lezak et al., 2012).
- *Executive functions:*
    - *Shifting*: implies shifting back and forth between multiple tasks, operations, or mental frameworks. Also referred to as ‘‘attention switching’’ or ‘‘task switching,’’ this ability is crucial to better understand both failures of cognitive control in brain-damaged patients and laboratory tasks that require participants to shift between tasks (Monsell, 1996).
    - *Updating*: requires monitoring and coding incoming information for relevance to the task and then appropriately revising the items held in working memory by replacing old, no longer relevant information with newer, more relevant information (Morris & Jones, 1990).
    - *Inhibition*: suppression inappropriate responses (Miyake et al., 2000).

1.4.2 Mental Health

The World Health Organization (WHO) conceptualizes mental health as a "state of well-being that enables people to cope with the stresses of life, realize their abilities, learn well and work well, and contribute to their community" (77). In other words, mental health is more than the absence of mental disorders, it's a complex continuum presenting distinct experiences from one person to the next, ranging in degrees of challenges and distress, and leading to potentially diverse social and clinical consequences (77). Understanding factors associated with maintenance of good mental health, especially in aging, is of public health interest (77,78).

Stress, known as a physiological and psychological response of an individual when they perceive a threat or challenge, is vital for the survival of every living organism. According to Lazarus and Folkman, "psychological stress is a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding their resources and endangering their well-being" (79). What's more, stress responses encompass emotional and cognitive aspects, such as anxiety, frustration, and rumination (79). Stress exposure disrupts homeostatic mechanisms, activating the hypothalamic-pituitary-adrenal axis (HPA) and cortisol release (80). As a result, maladaptive responses can occur, impacting on multiple biological systems, including the central nervous system (81). In young and middle-aged adults, some maladaptive responses to stress have been associated with structural and functional changes of several large-scale brain networks (82). Specifically, higher perceived stress levels are linked to disrupted communication within brain networks, including the Default Mode Network (DMN), the Salience Network (SN), and the Frontoparietal Network (FPN) (83). This phenomenon might potentially lead to impaired cognition and eventually contribute to conditions like *depression* and *anxiety* (84,85).

1.4.3 Structural and Functional Brain Health

Even at rest, the cerebral cortex organizes itself into distributed yet functionally connected intrinsic networks (86). Recent studies have revealed that measurable changes in brain health precede clinically measurable cognitive deficits by many years (87,88). Beyond theoretical models, neuroimaging studies have shown that the preservation of cognitive and mental health is associated with either the maintenance of brain structure and connectivity patterns with advancing age (7), and/or with the expression of compensatory responses in the face of pathological changes (22).

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technology that uses strong magnetic fields and radio waves to generate detailed images of the inside

of the body. It is renowned for its capacity to provide comprehensive and multi-parametric insights into brain morphology, physiology, and metabolic processes. It is often used for disease detection, diagnosis, and treatment monitoring. This technique is based on advanced technology that perturb and observe the change in the direction of the rotational axis of hydrogen atoms (protons) set in the significant portion of water found in human body tissues. MRI generates cross-sectional images of internal structures by using non-ionizing electromagnetic radiation. The body is exposed to a brief burst of radiofrequency (RF) energy, which is in the radio wave frequency range. This RF pulse is applied perpendicular to the magnetic field and is specifically tuned to the resonant frequency of hydrogen nuclei. This pulse disrupts the alignment of the protons' magnetic spins. After the RF pulse is turned off, the protons begin to return to their aligned state with the magnetic field. During this process, they emit energy signals that are detected by the MRI scan. Specialized coils within the scan pick up the emitted energy signals from protons that contains information about the density and location of hydrogen atoms within the body. Given the wealth of information contained in the signal, concerning the tissues' biochemistry and its gross structural properties, MRI is a sensitive tool for detecting and measuring subtle changes in brain anatomy and function (89).

The brain is composed of anatomically distinct elements interconnected by a complex network of connections. This structural network plays a crucial role in how neural dynamics—the processes underlying human cognitive function—unfold over time. When we talk about "*structure*" we are referring to the spatial and topological arrangement of connections between neuronal elements. Anatomically, the central nervous system structural elements can be divided up in:

1. *White Matter:* consists of myelinated nerve fibers, which are primarily responsible for transmitting signals between different regions of the nervous system. The white color is due to the high lipid content in the myelin sheath.

2. *Gray Matter:* comprises neuronal cell bodies, dendrites, and unmyelinated axons. It is where information is processed in the brain and spinal cord, and it appears gray due to the absence of myelin. The gray matter also contains the cell's cytoplasm, in which other essential structures, such as mitochondria, endoplasmic reticulum or Golgi apparatus, can be found. Gray matter is rich in areas such as the cerebellum and the cerebral cortex. In essence, the gray matter is where the processing is done, and the white matter is the channels of communication.

3. *Cerebrospinal Fluid (CSF):* is a clear, colorless fluid that surrounds and cushions the brain and spinal cord. It provides mechanical support, carries nutrients, and helps remove waste products from these vital nervous system structures.

Therefore, structural MRI facilitates the qualitative and quantitative analysis of brain tissue, enabling the characterization of the shape, size, and structural integrity of both gray and white matter components. Gray matter, rich in cell bodies like neurons and glial cells, displays distinct MRI signals compared to white matter, which primarily consists of myelinated axons and supporting glial cells. Besides, morphometric methodologies evaluate the volume and shape of gray matter structures such as subcortical nuclei or the hippocampus, along with assessing the volume, thickness, and surface area of the cerebral neocortex. Additionally, macrostructural analysis of white matter integrity involves measuring volumes of healthy and abnormal white matter, offering insights into potential inflammation, edema, or demyelination. These assessments complement microstructural evaluations obtained through diffusion-weighted MRI, resulting in a comprehensive understanding of white matter integrity.

On the other hand, the notion of "*function*" of a particular neuron or brain region is not referred to the set of behavioral or cognitive functions subserved by a given neural circuit or system, but rather to the typical patterns of activity and dynamics observed within that active neural circuit. However, it is not possible to understand brain function without invoking the concept of *brain plasticity* (90,91). The nervous system might be viewed as a continuously changing structure of which plasticity is an integral property and the obligatory consequence of any sensory, motor, signal and/or action input. At the neural system level, the brain is organized in dynamically shifting neuronal networks (91). Changes in task-related cortico-cortical and cortico-subcortical coherence and modifications of the mapping between behavior and neural activity take place in response to changes in afferent input or efferent demand. In summary, plasticity is the mechanism for development and learning, as much as a cause of pathology (91).

Functional connectivity is defined by measuring similarity between brain signals arising from two distinct regions of the brain. Is also defined as the temporal coincidence of spatially distant neurophysiological events (92). Functional connectivity analysis examines the temporal correlation in blood oxygen level dependent (BOLD) signal changes between different regions of the brain. Functional magnetic resonance imaging (fMRI) is a methodology for detecting dynamic patterns of activity in the working human brain. To study the functional connectivity, fMRI explores the answer of hemoglobin in blood flux into the variations of the neural activity. It's considered that when a specific

brain region is active, there is an increase in the blood flux towards that area that generates changes in the MRI signal, called BOLD signal (93). During an fMRI session, sequential images are taken within short time intervals. These images are processed and compared to determine the intensity changes of the signal, indicating the most active brain regions. Hence, the analysis of these fluctuations in the dependent regional BOLD, it's possible to characterize the temporal and spatial relation between different brain regions. In other words, it's assumed that brain areas that show fluctuations in BOLD signal correlated in time are functionally correlated. The levels of connectivity fluctuate over time due to the internal activity within each group of neurons and in response to signals received from various elements of the nervous system, including cortical, subcortical, and peripheral components. This continuum information exchange happens in milliseconds and depends on the excitatory or inhibitory connection in the rest of the brain. The structural connections between different brain regions are organized in a way that the efficient process and transfer of information promote the capacity to adapt and resist and provide support in a complex brain function. The concept of *resting state* refers to the neural activity that is generated within the brain in the absence of any specific stimuli or tasks and represents a measure of the brain's intrinsic activity (94).

Hence, the human brain is a complex patchwork of interconnected regions, and network approaches have become increasingly useful for understanding how functionally connected systems are. A set of functionally connected regions is referred to as a "*functional network*". Some functional networks are most detected when participants are not performing any demanding task (in the resting state); others are observed in the context of task-focused behavior; and some networks persist across both behavioral states. A set of several high-level cognitive regions such as the medial prefrontal cortex, posterior cingulate cortex, and parietal regions are known as the "*Default Mode Network" (DMN)* (95,96), a functional network that is mostly known as the "task negative" network where its regions show strongly correlated activity at rest and are deactivated during cognitive goal-directed tasks. The term *"Salience Network" (SN)* refers to a suite of brain regions whose cortical hubs are the anterior cingulate and ventral anterior insular cortices. This network, which also includes nodes in the amygdala, hypothalamus, ventral striatum, thalamus, and specific brainstem nuclei, coactivates in response to diverse experimental tasks and conditions, suggesting a domain-general function (83,97). Lastly, the *"Frontoparietal Network" (FPN)* is a large-scale brain network primarily composed of the dorsolateral prefrontal cortex and posterior parietal cortex, around the intraparietal sulcus. It is involved in sustained attention, complex problem-solving and working memory (83). These three networks integrates the *Triple*

Network Model that focuses on the dynamic cross-network interactions and coupling between DMN, SN, and FPN and their role in mental and brain health (see Figure 3: Triple Newtork Model) (83).

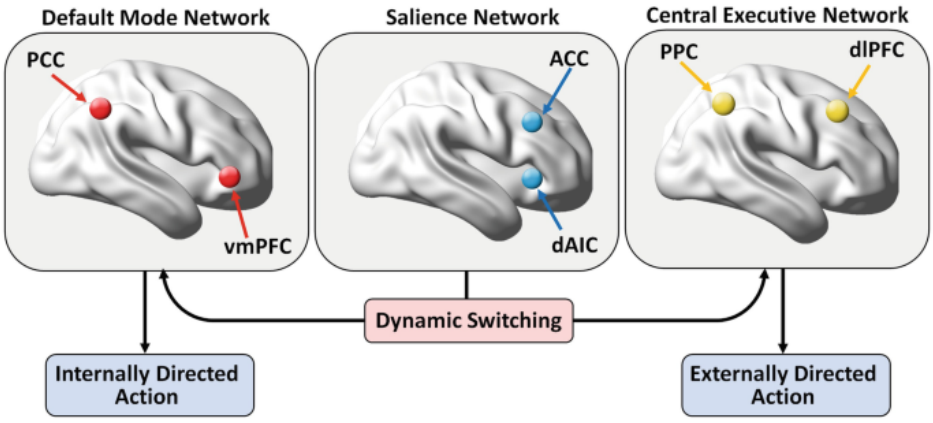

Figure 3. **Triple Network Model**

PCC = posterior cingulate cortex; vmPFC = ventromedial prefrontal cortex; ACC = anterior cingulate cortex; dAIC = dorsal anterior insular cortex; PPC = posterior parietal cortex; dlPFC = dorsolateral prefrontal cortex. Taken from Dragomir & Omurtag, 2020 (98).

Changes in brain function are also seen throughout age. Anatomically, there is a decrease in grey matter and white matter with an increase in cerebrospinal fluid space (99,100). Functionally, investigators have also reported alterations that range from decreased or increased activity in task-related brain regions, recruitment of additional brain regions, reduced hemispheric asymmetry, as well as alterations in the DMN, SN and FPN (99,101). These differential activation patterns have in general been explained as neural reorganization with increasing age or as differences in cognitive or neural strategies employed (65,100).

1.5 Thesis justification

In summary, prior research has demonstrated the importance of maintaining a healthy lifestyle to protect brain health and mitigate neurodegenerative diseases in later life (8,23,102). Nevertheless, while certain lifestyle domains provide an ecological and sustainable global health approach, their association with brain health is still under-investigated, especially during midlife. Previous studies have demonstrated that structural and functional brain changes associated with the development of neurodegenerative diseases can begin decades before the onset of symptoms (103). Therefore, individual differences in brain resilience may delay the appearance of

symptoms or act to reduce or eliminate the clinical and behavioral impact of pathologies (7). Hence, there is a strong need to focus research on factors, such as exercise or cardiovascular health, that could prevent illness and promote brain resilience in the presence of pathology and focus future lines of research towards elucidating the fundamental underlying mechanisms that potentially contribute to the maintenance of brain health across the lifespan.

Specifically, an emerging body of multidisciplinary literature has documented the beneficial influence of aerobic exercise on selective aspects of brain function. Human and animal studies have shown that aerobic exercise can enhance CRF and improve a number of aspects of brain health (31). Several cross-sectional and interventional studies have found positive associations between PA, especially aerobic exercise, and cognitive function in the elderly (104–107). However, these reported associations are inconsistent across studies (54,105,106,108–110). Although, the vast majority of the evidence related to the positive effects of exercise on brain health has been documented in young or older adults, raising questions regarding the generalizability of these findings to healthy middle-aged adults. Therefore, examining the potential neuroprotective role of CRF, and understanding the neural underlying mechanisms could potentially give valuable insights into providing lifestyle and brain health advice, prevention, and intervention across the lifespan.

This doctoral thesis has been conducted using data collected as part of the Barcelona Brain Health Initiative (BBHI) project, a prospective longitudinal cohort study with the main objective of identifying biomarkers of brain health in the healthy middle-aged population (111,112). This thesis reports results from two separate main studies. In the *first study*, our primary objective was to assess the relationships between CRF and cardiovascular risk (CVR) and cognitive function in midlife. We further aimed to examine the mechanistic correlates of these relationships through measures of brain structure using MRI, by testing whether cortical thickness mediated the relationships between each predictor (CVR and CRF) and cognitive function. In the *second study,* the main objective was to examine the relationships between CRF, mental health and functional connectivity in healthy middle-aged adults. Additionally, fMRI was used to examine the mechanistic correlates of these relationships through functional connectivity patterns. As such, this thesis provides a comprehensive analysis of the association between CRF, cognitive function and mental health and multimodal metrics of brain health.

2. Hypotheses

The overarching hypothesis of this thesis is that several of the well-established associations between cardiorespiratory fitness and brain health that exist in older age are already present in middle-aged adults.

1. Cardiorespiratory fitness (CRF) and Cardiovascular Health (CVH) are associated with better performance in cognitive tasks in midlife.
2. Higher CRF is associated with lower scores on depression, anxiety, and stress scores in midlife.
3. CRF impact on cognitive function and mental health in midlife is explained by structural and functional brain changes.

3. Objectives

3.1 Main objective

To investigate the associations and the mechanistic pathways between CRF, CVR and brain health in healthy middle-aged adults.

3.2 Secondary objectives

1. To test the associations between CRF and CVR and cognitive function in midlife.
2. To test the associations between CRF and mental health outcomes in midlife.
3. To test whether cortical thickness and functional connectivity, as measured by MRI/fMRI, mediated the relations between CRF and brain health.

4. Compendium of publications

4.1 Article 1

Associations between cardiorespiratory fitness, cardiovascular risk, and cognition are mediated by structural brain health in midlife.

Goretti España-Irla, MSc, Joyce Gomes-Osman, PhD, Gabriele Cattaneo, PhD, Sergiu Albu, PhD, María Cabello-Toscano, MSc, Javier Solana-Sanchéz, PhD, María Redondo-Camós, MSc, Selma Delgado-Gallén, MSc, Vanessa Alviarez-Schulze, MSc, Catherine Pachón-García, MSc, Josep M. Tormos, PhD, David Bartrés-Faz, PhD, Timothy P. Morris, PhD, and Álvaro Pascual-Leone, PhD.

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Journal of the American Heart Association

ORIGINAL RESEARCH

Associations Between Cardiorespiratory Fitness, Cardiovascular Risk, and Cognition Are Mediated by Structural Brain Health in Midlife

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BACKGROUND: Evidence in older adults suggests that higher cardiorespiratory fitness and lower cardiovascular risk are associated with greater cognition. However, given that changes in the brain that lead to cognitive decline begin decades before the onset of symptoms, understanding the mechanisms by which modifiable cardiovascular factors are associated with brain health in midlife is critical and can lead to the development of strategies to promote and maintain brain health as we age.

METHODS AND RESULTS: In 501 middle-aged (aged 40–65 years) adult participants of the BBHI (Barcelona Brain Health Initiative), we found differential associations among cardiorespiratory fitness, cardiovascular risk, and cognition and cortical thickness. Higher cardiorespiratory fitness was significantly associated with better visuospatial abilities and frontal loading abstract problem solving (β=3.16, *P*=0.049) in the older middle-aged group (aged 55–65 years). In contrast, cardiovascular risk was negatively associated with better visuospatial reasoning and problem-solving abilities (β=−0.046, *P*=0.002), flexibility (β=−0.054, *P*<0.001), processing speed (β=−0.115, *P*<0.001), and memory (β=−0.120, *P*<0.001). Cortical thickness in frontal regions mediated the relationship between cardiorespiratory fitness and cognition, whereas cortical thickness in a disperse network spanning multiple cortical regions across both hemispheres mediated the relationship between cardiovascular risk and cognition.

CONCLUSIONS: The relationships between modifiable cardiovascular factors, cardiorespiratory fitness, and cardiovascular risk, and cognition are present in healthy middle-aged adults. These relationships are also mediated by brain structure highlighting a potential mechanistic pathway through which higher cardiorespiratory fitness and lower cardiovascular risk can positively impact cognitive function in midlife.

Key Words: cardiorespiratory fitness ■ cardiovascular health ■ cognition ■ exercise ■ mediation ■ midlife ■ structural brain health

Understanding factors associated with maintenance of cognitive brain health in aging is of great clinical and public health interest. An increase in lifespan over the past century has not been accompanied by an increase in health span,[1] and brain-related disorders are projected to account for half of the worldwide economic impact of disability by 2030.[2] Notwithstanding, the development of pathological loss

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For Sources of Funding and Disclosures, see page 13.

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CLINICAL PERSPECTIVE

What Is New?

- We extend prior work by demonstrating that some of the well-established relationships between determinants of cardiovascular health and brain health that exist in older age are already present in middle age.
- Cardiorespiratory fitness was associated with frontal cognitive abilities, such as visuospatial problem solving, but only in individuals aged 55 years and older.
- Cardiovascular risk was associated with a wide range of cognitive abilities within the whole sample; these results suggest distinct, but synergistic effects of cardiovascular risk and cardiorespiratory fitness with cognitive brain health in healthy middle-aged adults.

What Are the Clinical Implications?

- Importantly, we advance existing knowledge by revealing that such relationships driven by distinct patterns of cortical thickness, specifically cortical thickness in frontal regions mediated the relationship between cardiorespiratory fitness and visuospatial problem solving, whereas cortical thickness in a disperse network spanning multiple cortical regions across both hemispheres mediated the relationship between cardiovascular risk and multiple domains of cognition.
- The implications of our study lie within the potential importance of engaging in modifiable lifestyle behaviors that can promote heart health, early in midlife, long before the onset of measurable cognitive decline.

Nonstandard Abbreviations and Acronyms

CPET	cardiopulmonary exercise testing
CRF	cardiorespiratory fitness
CVR	cardiovascular risk

of brain health does not appear to be an obligatory consequence of aging.[3] Several lifestyle behaviors have been found to be protective of age-related and pathological brain changes, which are referred to as the concept of cognitive reserve.[4] Cognitive reserve helps to explain why certain individuals can withstand age-related and pathological brain changes while maintaining their cognitive and physical functioning and ultimately their independence with age.[4]

Although cognitive reserve is a theoretical construct and is rarely measured directly, several modifiable sociobehavioral proxies have been found to contribute to the development of cognitive reserve.[4] For instance, maintaining an active lifestyle by engaging in physical exercise,[5,6] promoting cardiovascular health,[7] consuming nutritious foods,[8,9] assuring sufficient good-quality sleep,[10–12] and maintaining motor skills[13] are independently associated with better cognitive brain health across one's lifespan. The exact mechanisms by which modifiable sociobehavioral proxies influence the development of cognitive reserve are not fully elucidated but can be attributed to the interplay between brain reserve and brain maintenance. That is, brain reserve is defined as the neurobiological capital, or structural integrity, of the many components of the nervous system at any given point in time, whereas brain maintenance is defined as the reduced development of age-related changes over time.[4] Brain maintenance reflects the notion that the brain can be modified by experience, and many of the same lifestyle proxies that contribute to cognitive reserve also contribute to brain maintenance.[4] Finally, it is also known that measurable changes in brain structure precede clinically measurable cognitive deficits by many years,[14,15] and therefore examining the relationships between modifiable factors that may contribute to cognitive and brain reserve beginning in midlife may provide evidence to develop and refine lifestyle strategies capable of promoting or maintaining brain health in older age.

There is strong evidence that cardiovascular health in midlife is a strong predictor of cognitive health in later life.[7,16] One important domain of cardiovascular health is cardiorespiratory fitness (CRF). The gold-standard measure of CRF is the maximum rate of oxygen consumption during incremental exercise, or VO_2max, which measures the body's efficiency to intake, circulate, and use oxygen during exercise. CRF has been identified as a critical mechanism implicated in exercise's effect on cognitive function.[5,6,16–18] Furthermore, numerous studies have associated CRF itself with cognitive functions, whereby rather than having a global effect on cognition, high levels of CRF later in life seem to be related to selective enhancement of cognitive abilities more reliant on frontal brain areas such as executive and reasoning abilities.[5,6]

Another important cardiovascular health predictor is the risk of developing a future cardiovascular event, which can be calculated by measuring several factors such as hypertension, cigarette smoking, diabetes, hyperlipidemia, family history, and obesity.[19] Interestingly, cardiovascular risk (CVR) factors overlap with cognitive impairment risk factors,[20–24] further strengthening the link between cardiovascular and cognitive health. Evidence suggests that CVR later in life is associated with more diffuse patterns of gray matter atrophy and white matter lesions, thus potentially affecting cognitive abilities in a more global manner.[25–27] As such, low CVR

burden in middle age might be associated with a more global effect on cognitive health and brain structure.

In this study, our primary objective was to assess the respective relationships between CRF and CVR and cognitive function in midlife in a sample of 501 adults aged 40 to 65 years. We further aimed to examine the mechanistic correlates of these relationships in midlife through measures of brain structure using magnetic resonance imaging (MRI), by testing whether cortical thickness mediated the relationships between each predictor (CVR and CRF) and cognitive function. Although genetic predisposition influences both CRF[28,29] and CVR,[30] these 2 factors are modifiable through lifestyle changes. Therefore, further elucidating the relationships and potential mechanisms of these modifiable cognitive reserve protecting factors in midlife can contribute to the development of more effective and precise lifestyle interventions to maintain or improve cognitive brain health with age.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Design and Participants

This was a cross-sectional study that included data collected on a subset of participants enrolled in the ongoing BBHI (Barcelona Brain Health Initiative) (https://bbhi.cat/en/), who were selected to participate in phase 2 of the initiative, which involved a comprehensive in-person assessment.[31,32] For a detailed description of the cohort and study protocol see Cattaneo et al.[31,32] Inclusion criteria (assessed by a medical doctor) for this study included: (1) age between 40 and 65 years and (2) absence of any neurological or psychiatric disorders. Exclusion criteria included any person presenting with any contraindications for brain MRI and cardiopulmonary exercise testing (CPET) (see details below). We further excluded those participants who did not meet the criteria for a completed CPET evaluation (see CPET section). A cohort consort diagram from the wider BBHI study and selection criteria for this analysis is shown in Figure 1. A total of 501 participants were eligible for this analysis based on having completed a full CPET evaluation. There were incomplete data on a total of 114 subjects (74 subjects did not have full neuropsychological data and 40 subjects did not have sufficient information for the calculation of the Framingham score) and were therefore excluded from the cognitive analyses. All participants gave written informed consent before participation in any study procedures, all of which conformed to the Declaration of Helsinki for research involving human subjects. All procedures were approved by the ethics and education committee of the Institut Guttmann (Badalona, Spain). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist has been used for the reporting of the present study results.[33]

Neuropsychological Exam

Neurocognitive assessments were performed by 2 licensed neuropsychologists. Education in years was assessed via an online questionnaire, and this information was validated and corrected by a neuropsychologist or physician during the in-person assessments. Paper and pencil evaluations consisted of a battery of well-established neuropsychological tests. These included Matrix Reasoning,[34] Cancelation Test,[34] Block Design,[34] Trail Making Test B,[35] Trail Making Test A,[36] Digit Forward, Digit Backward, Letter–Number Sequencing,[37] Rey Auditory Verbal Learning Test,[38] Digit Symbol Substitution,[34] and Corsi Block-Tapping Task.[39] Tests were grouped in cognitive domains using a data-driven approach with principal component analysis. Scores on individual tests were *Z*-score normalized before their inclusion in the principal component analysis with Oblimin rotation, considering the probable correlation between latent factors.[40] Based on the sample size, the acceptable level of factor loading was set at 0.30.[41] Cognitive domains were then created as the composite sum of the *Z* scores for each test per the results from the principal component analysis. The principal component analysis indicated the presence of 5 principal components for the cognitive scores. The first factor included the Digit Symbol Test (0.65), the Cancellation Test (0.76) and the Trail Making Test A (0.80), likely reflecting visual searching, processing speed, and attentional components. The second component comprised all 3 measures of the Rey Auditory Verbal Learning Test (immediate recall=−0.85, delayed recall=−0.89, recognition=−0.81) creating a verbal memory domain. The third component contained the Digit Forward (0.81), Digit Backward (0.66), and Letter–Number Sequencing (0.68), reflecting a working memory domain. Cognitive flexibility and set-shifting abilities were reflected in the fourth component, which included the Trail Making Test B (0.91) and the Trail Making Test B-A (1.02). Finally, a visuospatial reasoning and problem-solving domain was found in the fifth component comprising Wechsler Adult Intelligence Scale Fourth Edition matrix reasoning (0.78), Block Design (0.74), and Corsi cubes (0.46).

Cardiopulmonary Exercise Testing

Before CPET evaluation, participants were assessed for potential absolute and relative contraindications for maximal exhaustive exercise following the Guidelines of

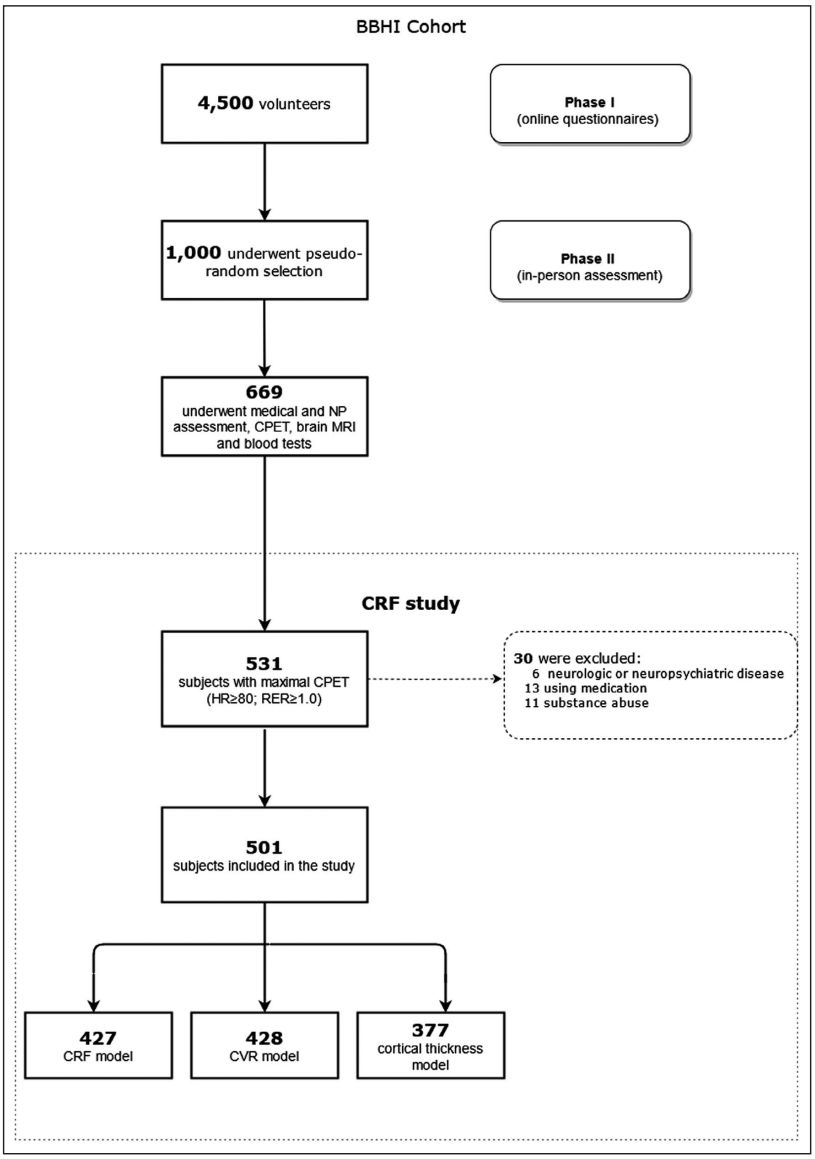

Figure 1. Cohort consort diagram from the wider BBHI (Barcelona Brain Health Initiative) study and selection criteria for the current analysis.
CPET indicates cardiopulmonary exercise testing; CRF, cardiorespiratory fitness; CVR, cardiovascular risk; HR, heart rate; MRI, magnetic resonance imaging; NP, neuropsychology assessment; and RER, respiratory exchange ratio.

the Spanish Society of Cardiology for Clinical Practice in Exercise Testing.[42] The Physical Activity Readiness Questionnaire[43] was administered to assess for safety to participate in the CPET. Additionally, participants performed baseline spirometry (Ergoflow flowsensor; Geratherm Respiratory, Bad Kissingen, Germany) and a baseline 12-lead ECG recording before the test (WAM Wireless Acquisition Module; Mortara, Milwaukee, WI). Individuals who had forced expiratory volume in 1 second of <80%, forced expiratory volume in 1 second/forced vital capacity ratio of >80%, or peak expiratory flow of >75% did not complete the CPET evaluation.

The CPET was performed using a modified Wasserman protocol[44] on a cyclometer (Ergoselect 4 model; Ergoline, Bitz, Germany) with a respiratory gas analysis system (Ergostik; Geratherm Respiratory). The modified Wasserman protocol[44] consisted of a 7-minute warm-up phase (no load), a progressive workload phase, and a 5-minute recovery phase (minimal load). The slope of the progressive increase in workload was calculated individually by dividing the expected maximum workload (calculated automatically by the Bluecherry software [Geratherm Respiratory] from height, weight, age, and sex) by 9, to derive a progressive increase in workload that would result in a maximal exercise test lasting ≈13 minutes.

Gas analysis was conducted using a tight-fitting face mask (Hans Rudolph, Shawnee, KS), and the following measures were recorded continuously: oxygen consumption, oxygen uptake (efficiency slope), and respiratory exchange ratio (VO_2/VCO_2), 12-lead ECG, heart rate (beats per minute, from a 12-lead ECG), and pulse oximetry. Blood pressure, measured manually from the left arm using a blood pressure cuff (Boso Medicus X; Boso, Jungingen, Germany) and a handheld sphygmomanometer (MDF Instruments, Agoura Hills, CA) and perceived effort, measured via the Spanish translation of the Borg scale,[45] were recorded every 2.5 minutes. Ventilatory thresholds (lactate threshold and respiratory compensation point) were calculated using the V-slope method.[44]

A test was considered complete under the following criteria: verbal manifestation of exhaustion, Borg score of ≥9, heart rate of ±10 bpm of heart rate max, or inability to maintain pedal cadence (≈70 rpm). The highest full minute VO_2 uptake (maximal oxygen consumption) value observed during the final minute of the test was accepted as the functional aerobic capacity (VO_2 plateau). Whenever a VO_2max plateau could not be detected, we applied the following 2 metrics to determine the validity of the CPET results: (1) the maximal respiratory exchange ratio (respiratory exchange ratio of ≥1.0, considered to be indicative of true maximal oxygen uptake),[46,47] and (2) the reached target heart rate ≥80% of the maximum theoretical expected heart rate (220−age). We use the term VO_2peak (oxygen uptake during peak exercise) herein because only 20.4% of participants reached a detectable VO_2 plateau. To ensure that scaling VO_2peak by total body mass did not affect the associations with our outcomes, we replicated our results using allometric scaling[48,49] (Data S1, Tables S1 through S3).

Medical Exam and Cardiovascular Risk Assessment

A medical evaluation included a structured interview, which gathered past and present medical history (including diagnosis of diabetes), medication intake (including antihypertensive drugs), alcohol and tobacco consumption, absolute and relative risk factors for the CPET, anthropometric measures (weight, height, body mass index, and waist circumference), and blood pressure. Questionnaires about education history (including number of years of formal higher education) and self-reported physical activity (including the International Physical Activity Questionnaire)[50] were filled out by each participant. A fasting blood draw was performed to measure total cholesterol (millimoles per liter) and high-density lipoprotein (millimoles per liter). The modified Framingham cardiovascular disease risk calculator was then used to calculate the 5-year risk of the development of any cardiovascular disease,[51] including the following variables: age (years), biological sex, total cholesterol, high-density lipoprotein, systolic blood pressure, treatment for hypertension, smoker status, and diabetes status. In addition, we also we calculated the modified Framingham cardiovascular disease risk calculator using a formula that was adapted specifically for the Catalan population (the Registre Gironí del Cor[52,53]). The latter is presented in Data S2, Tables S4 through S6, and Figure S1.

Structural MRI

Participants underwent a high-resolution (0.8×0.8×0.8 mm^3) 3-dimensional magnetization-prepared rapid gradient-echo T1-weighted structural brain MRI session using a 3T Siemens Magnetom Prisma machine. A total of 208 contiguous axial slices were obtained in ascending fashion (sequence parameters of repetition time=2400 ms, echo time=2.22 ms, inversion time=1000 ms, flip angle=8°, slice thickness=0.8 mm, and field of view=256 mm). Additionally, a high-resolution (0.8×0.8×0.8 mm^3) 3-dimensional SPACE T2-weighted structural brain MRI was undertaken, using the same device (sequence parameters of repetition time=3200 ms, echo time=563 ms, flip angle=120°, slice thickness=0.8 mm, and field of view=256 mm). Image quality control measures were implemented manually by a trained MRI technician.

Cortical reconstruction and volumetric segmentation were performed with the Freesurfer image analysis suite, which is documented and freely available for

download online (http://surfer.nmr.mgh.harvard.edu/). A 3-dimensional cortical surface model was created by running the recon-all processing stream with default parameters,[54] except for the addition of the T2 flag for the improvement of pial surfaces reconstruction. Therefore, inputs for this command were T1-w volumes and T2-w volumes. Briefly, automated Talairach transformation[55] and intensity normalization[56] were followed by non–brain tissue removal,[57] tessellation of the gray and white matter boundary, and automated topology correction.[58] Finally, surface deformation enabled the detection of tissue boundaries; gray–white and gray–cerebrospinal fluid (CSF) borders.[54] The cortical surfaces were then inflated and registered to a spherical atlas that used individual cortical folding patterns to match cortical geometry across subjects.[57,59,60]

Cortical Thickness Analyses

Individual cortical thickness maps were calculated as the closest distance from the gray–white matter boundary to the gray–cerebrospinal fluid boundary at each vertex on the tessellated surface.[54] Then, a Gaussian kernel of 10-mm full width at half maximum was applied to these maps. Vertex-wise general linear models were run in FreeSurfer version 6.0, with cortical thickness as the dependent variable and either CVR or CRF as the independent variables, with education, age, body mass index, socioeconomic status, waist perimeter, and biological sex as controlling predictors of no interest. A total of 5 models were fitted: Models 1 and 2 included CVR as the predictor of interest, using the Registre Gironí del Cor and Framingham scores, respectively. Models 3, 4, and 5 addressed CRF (ie, VO_2peak) as the predictor of interest. Whereas Models 1, 2, and 3 were fitted for the whole set of observations; the fitting of Models 4 and 5 were restricted to a dichotomization of the sample according to their age: younger middle-aged (aged 40–54 years) and older middle-aged (aged 55–65 years), respectively. For each model, regions where the predictor of interest significantly predicted cortical thickness were identified using a method provided by FreeSurfer (ie, mri_glmfitsim). Here, multiple comparisons correction of whole-brain vertices was performed by computing *P* values for contiguous clusters of vertices based on Monte-Carlo Null-Z simulations[61] and permutation[62] (with 10 000 iterations per simulation). This method assigns a *P* value to each resulting cluster. Consequently, we used a cluster-forming threshold of $P<0.005$ in cardiovascular risk models (ie, Models 1 and 2), and $P<0.05$ in cardiorespiratory fitness models (ie, Models 3, 4, and 5) and a cluster significance threshold of $P<0.05$ in all models.

Statistical Analysis

All statistical analyses were performed in R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). The associations between predictor variables (VO_2peak, Framingham score) and outcome measures (domain-specific cognitive performance and cortical thickness measures) were analyzed using multiple linear regression, controlling for age, education, socioeconomic status, body mass index, waist perimeter, and biological sex for the VO_2peak models and education and socioeconomic status for the CVR models (age and biological sex are factors used to calculate the Framingham score). Model assumptions were checked using Q-Q plots and fitted versus residual plots in R, and the normality of the residuals was formally checked using Shapiro-Wilk tests of normality. Outlier observations that had influence on the models were removed using Cook's distance (observed using Cook's distance of >0.5) and R's outlier package (upper limit of n=10 in any given model). To conform to the model assumptions, VO_2peak and Framingham scores were $\log^{10}$ transformed before analyses. Model fitness is presented as adjusted R^2 values, and significance was considered at the $P<0.05$ level. We present standardized β coefficients as the strength of the relationship between our predictor and outcome variables. That is, for every 1-unit increase in the predictor, there is an X standard deviation increase in the outcome. Multiple comparisons were corrected for using Bejamini and Hochberg's false discovery rate, at a *q* value of 0.05, after pooling the *P* values from the regression analyses for each predictor model. For the VO_2peak models, the cohort was dichotomized into younger middle-aged (aged 40–54 years) and older middle-aged (aged 55–65 years) groups to gain greater sensitivity to further explore age-related associations.

Mediation analysis using the R mediation package[62] was performed to assess whether cortical thickness mediated the associations between VO_2peak and Framingham and cognitive performance, taking into account all covariates (age, biological sex, socioeconomic status, education, waist perimeter, and body mass index). The total effects (effect of X [predictor variable] on Y [outcome variable]), direct effects (effect of X on Y taking into account M [mediator] [average direct effect]) and indirect effects (or mediation effect, the total effect minus the direct effect [average causal mediation effect]) are reported. The presence of statistical mediation was determined through nonparametric bootstrap confidence intervals via 1000 bootstrap resamples of the estimated indirect effect. The estimated indirect (average causal mediation effect) effect corresponds to the reduction in the independent variable effect on the dependent variable when adjusted for the mediator.

RESULTS

A total of 501 (248 women) participants with a mean±SD age of 53.58±6.96 years (range, 40–65 years)

completed the study. Our sample is generally characterized by White, highly educated, and cognitive and cardiovascularly healthy individuals. Full demographic information is found in Table 1.

Associations Between VO_2peak, Framingham, and Cognitive Functions

At the whole group level, no significant associations between VO_2peak and cognitive functions were found (Table 2). When we dichotomized our sample into younger middle-aged (aged 40–54 years) and older middle-aged (aged 55–65 years) we found no significant correlations between any cognitive domain and VO_2peak in the younger group (Table 2). However, in the older middle-aged adults, we did find a significant and positive association between VO_2peak and visuospatial reasoning and problem solving (β=3.16, *P*=0.049), which remained significant after false discovery rate corrections (false discovery rate *P*=0.0499) (Figure 2).

For CVR, we found a significant negative association between Framingham score and the following cognitive abilities: visuospatial ability (β=−0.046, *P*=0.002), processing speed (β=−0.115, *P*<0.001), flexibility (β=−0.054, *P*<0.001), and verbal memory (β=−0.120, *P*<0.001), but not working memory (β=−0.010, *P*=0.502). Full model results are seen in Table 3 and depicted in Figure 3.

Cortical Thickness

At the whole group level, higher VO_2peak was significantly associated with greater cortical thickness in the left prefrontal cortex (rostral middle frontal gyrus) (cluster-wise corrected with a vertex-wise threshold *P*<0.05, cluster-wise *P*<0.05) (Data S3, Table S7, Figure S2A). In the young middle-aged group (aged 40–54 years) VO_2peak was not associated with any specific gyrus (Data S3, Table S8), whereas in the old middle-aged group (aged ≥55 years), associations with left prefrontal regions (left rostral middle frontal) and left temporal regions (superior temporal gyrus) were seen (Data S3, Table S9, Figure S2B). Moreover, in the older middle-aged group, cortical thickness in the left prefrontal gyrus mediated the relationship between VO_2peak and visuospatial reasoning abilities (Figure 4, Data S3, Table S10).

Higher Framingham risk score was significantly associated with lower cortical thickness across different cortical regions (18 clusters) of both hemispheres including frontal, parietal, temporal, and medial (insula, cuneus) cortices (cluster-wise corrected with a vertex-wise threshold *P*<0.005, cluster-wise *P*<0.05) (Data S4, Table S11 and Figure S3). Cortical thickness significantly mediated the relation between Framingham and visuospatial problem solving, processing speed, flexibility, and memory after controlling for education and monthly incomes. In visuospatial problem solving, the following regions were significantly mediating its relationship with Framingham: left postcentral gyrus, left pars triangularis, left insula, left cuneus, left caudal anterior cingulate gyrus, left transverse temporal gyrus, and right supramarginal region. The relationship between processing speed and Framingham was significantly mediated by right cuneus, whereas flexibility

Table 1. Participant Characteristics

Age	
Age, y, mean±SD	53.58±6.96
Aged 40–54 y, n (%)	288 (54)
Aged ≥55 y, n (%)	243 (46)
Sex, n (%)	
Men	283 (53)
Women	248 (47)
Education, n (%)	
Primary	16 (3)
Secondary	125 (24)
Higher	390 (73)
Cognitive profile, mean±SD (percentile)	
Block design	12.12±3.06 (75)
Matrix reasoning	13.20±2.64 (84)
Direct digits	10.72±3.05 (63)
Indirect digits	11.16±2.62 (63)
Digit symbol	13.67±2.69 (91)
Letter–number sequencing	14.38±2.56 (91)
Cancellation test	11.41±2.76 (63)
TMT-A	11.26±2.77 (63)
TMT-B	8.66±2.16 (37)
Corsi cubes	13.99±2.52 (91)
Fitness evaluation	
VO_2peak, mL/kg per min, mean±SD	24.9±7.18
40–54 y, VO_2peak, mL/kg per min, mean±SD	26.44±7.22
55 y and above VO_2peak, mL/kg per min, mean±SD	23.28±6.57
BMI, kg/m^2, mean±SD	25.40±4.01
IPAQ, METs, min/wk	2558.13±2486.57
Cardiovascular status	
Smoker, n (%)	58 (11)
Diabetes, n (%)	7 (1)
Hypertension, n (%)	40 (8)
Systolic blood pressure, mean±SD	124±16.13
Cholesterol, mg/dL, mean±SD	177.83±75.14
HDL, mm/dL, mean±SD	54.5±24.77
Framingham 5-y risk, %, mean±SD	8.45±6.76

Percentiles extracted from the Wechsler Adult Intelligence Scale Fourth Edition toolbox (Wechsler[63]). BMI indicates body mass index; HDL, high-density lipoprotein; IPAQ, International Physical Activity Questionnaire; METs, metabolic equivalent of task; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B; and VO_2peak, oxygen uptake during peak exercise.

Table 2. Associations Between VO_2peak and Cognitive Domains

	β	SE	*P* value	R^2
Whole population				
Memory	−2.070	1.158	0.074	0.181
Working memory	−1.885	1.117	0.092	0.009
Flexibility	−0.632	0.780	0.418	0.152
Processing speed	<0.001	<0.001	0.715	0.193
Visuospatial problem solving	1.167	1.031	0.258	0.208
40–54 y				
Memory	−1.475	1.336	0.270	0.186
Working memory	−2.229	1.542	0.149	0.007
Flexibility	0.211	0.891	0.813	0.115
Processing speed	−1.035	1.374	0.452	0.164
Visuospatial problem solving	−0.709	1.347	0.598	0.129
55–65 y				
Memory	−3.231	1.971	0.102	0.102
Working memory	−1.284	1.697	0.450	−0.025
Flexibility	−0.667	1.370	0.626	0.094
Processing speed	2.053	1.844	0.267	0.132
Visuospatial problem solving	3.165	1.604	0.049*	0.160

All models are controlling for age, biological sex, body mass index, waist perimeter, socioeconomic status, and education as covariates. R^2 values are adjusted for all predictors. VO_2peak indicates oxygen uptake during peak exercise.
*Survives false discover rate corrections.

had different gyri that mediated its relationship with Framingham, in particular, left postcentral gyrus, left insula, left caudal anterior cingulate gyrus, left transverse temporal gyrus, right inferior parietal gyrus, right cuneus, right supramarginal region, and right superior frontal gyrus. Lastly, left triangularis and left and right cuneus significantly mediated the relationship between Framingham and memory (Figure 4, Data S4, Table S12A through S12D).

DISCUSSION

In the present study, we demonstrate that some of the well-established relationships between determinants of cardiovascular health and brain health that exist in older age are already present in late middle age. In our sample of healthy middle-aged adults, CRF and CVR, 2 independent clinical predictors of cardiovascular health, had distinct associations with neuropsychological metrics of cognitive brain health. CRF had domain-specific associations with cognitive abilities highly reliant on the frontal lobe, but only in individuals aged ≥55 years. In contrast, CVR had domain-general associations with various cognitive abilities within the whole sample. Importantly, mediation analyses strengthened our findings by revealing that the relationships between each predictor and cognition were driven by distinct patterns of cortical thickness. Cortical thickness in frontal regions mediated the relationship between CRF and visuospatial problem solving, whereas cortical thickness in a disperse network spanning multiple cortical regions across both hemispheres mediated the relationship between CVR and multiple domains of cognition.

We found associations between CRF and frontal-loading cognitive abilities (visuospatial reasoning) only in those aged ≥55 years. These results are supported by earlier work in older adults[6,64,65] and more recent work in middle-aged adults.[66] We extend those previous results in 2 important ways. First, although regional specificity of high CRF to the frontal lobe in older adults has been reported,[67–71] the mediating effect of cortical thickness in frontal regions on the relationship between CRF and cognition in midlife is novel, extending previous reports of a similar mediating effect in older adults.[71] Frontal regions are particularly susceptible to age-related cortical thinning,[72] and critical for visuospatial[73] problem solving and executive abilities.[74] High CRF has been shown to decrease small-vessel ischemic disease, which often preferentially affects the frontal/subcortical region of the brain,[75] providing a possible explanation for the reported regional specificity. Furthermore, white matter tracts have been implicated as an indirect path between CRF and better performance of frontal cognitive abilities.[76] Future planned studies will also examine the integrity of white matter tracts in this population.

Second, the age-specific associations between CRF and cognitive abilities can be explained in several ways. It is possible that our neuropsychological test

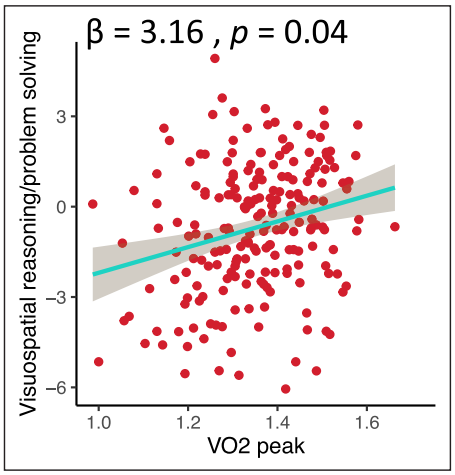

Figure 2. Significant positive relationship between VO_2peak (oxygen uptake during peak exercise) and visuospatial reasoning and problem-solving abilities in the older middle-aged group (aged 55–65 years) after controlling for age and biological sex.
Survives false discovery rate multiple comparison correction (Table 2).

battery may have been insufficiently sensitive for the younger subgroup (aged 40–54 years), and a ceiling effect may have masked potential associations between CRF and cognition. Conversely, and perhaps more likely, the relationship between CRF and neurocognitive function may be stronger in late middle age, when measurable age-related change in neurocognitive performance is more likely to be seen. Our sample of healthy adults scored in the higher percentiles for performance on these cognitive tasks (Table 1). One implication of our findings is the existence of a period from early to late middle age when it becomes particularly critical to maintain CRF to optimize cognitive brain health as we age. Longitudinal studies are needed to explore this possibility further. One potential interpretation for this finding could reflect the growing evidence that variations in brain structure and function precede the onset of behavioral symptoms of cognitive decline by years,[77–79] further strengthening the importance of engaging in modifiable lifestyle behaviors relevant for the promotion and maintenance of brain health in early midlife.

Given our analysis is cross-sectional, we can only speculate about the directionality of these results. Based on our analyses alone, in addition to our interpretations herein, it is just as plausible that higher cognitive resources lead to higher levels of fitness. Although numerous interventional studies have demonstrated that aerobic fitness training can improve cognition,[80,81] other modes of exercise have also been found to positively influence cognition.[13] Furthermore, longitudinal studies have suggested that cognitive resources themselves[82] are predictive of engagement in moderate-intensity physical exercise beyond the age of 50 years (a key modifier of CRF).[83] In addition, in a large longitudinal study, engagement in moderate physical exercise began to decline starting some 8 to 12 years before dementia diagnosis, and in those who did not have an eventual dementia diagnosis, total physical activity continued to increase through older age.[84] As previously mentioned, physical activity is one of many factors found to improve CRF.[85] Taken together, the relationship between CRF and cognition may ultimately be bidirectional, and because we cannot delineate this directionality, the result that these relationships exist in midlife in healthy adults is itself important to know for targeting through longitudinal studies beginning in midlife or earlier.

In contrast to the domain-specific associations with CRF, we found that CVR was associated with performance in many cognitive abilities, including visuospatial reasoning, but also cognitive flexibility, processing speed, and memory. Similar findings have been widely reported both in older adults[86–92] and in middle-aged adults.[7,93–97] We build on these findings by demonstrating that cortical thickness in disperse cortical regions across bihemispheric frontal, cuneus, parietal, temporal, and cingulate areas mediated the relationship between low CVR and better cognitive performance. The overlap between the clusters identified herein and cortical areas considered to be particularly sensitive to the effects of

Table 3. Associations Between Framingham and Cognitive Domains

	β	SE	*P* value	R^2
Memory	−0.120	0.016	<0.001*	0.149
Working memory	−0.010	0.015	0.502	−0.0003
Flexibility	−0.054	0.011	<0.001*	0.094
Processing speed	−0.115	0.016	<0.001*	0.120
Visuospatial problem solving	−0.046	0.015	0.002*	0.072

All models are controlling for socioeconomic status and education as covariates. R^2 values are adjusted for all predictors.
*Survives false discovery rate corrections.

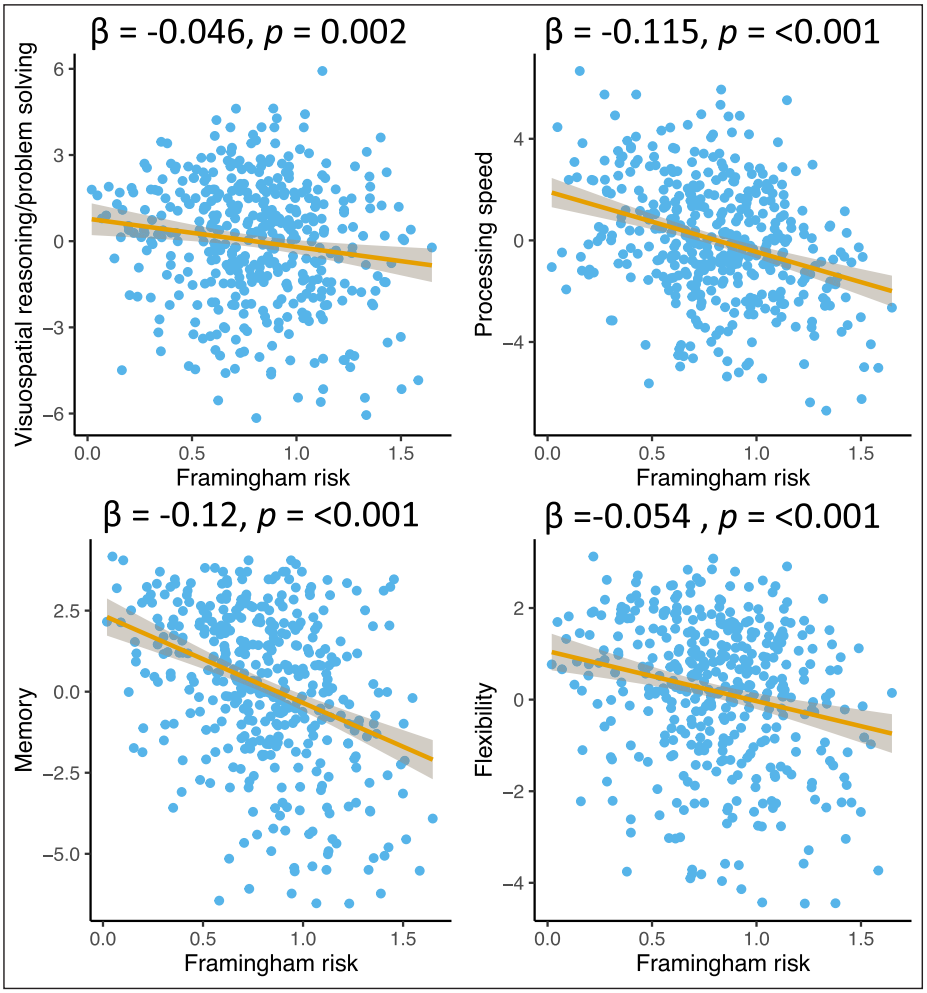

Figure 3. Significant negative relationships between cardiovascular risk (Framingham 5-year risk score) and multiple cognitive domains including flexibility, visuospatial problem-solving abilities, processing speed, and memory, after controlling for education (total number of years) and monthly incomes.
All models survive false discovery rate multiple comparison corrections (Table 3).

early cognitive impairment and Alzheimer's dementia pathology (ie, the inferior and anterior temporal lobe, inferior and superior temporal lobe, and posterior cingulate cortex),[98] supports existing evidence that cardiovascular risk factors are also cognitive risk factors.[21–24]

The region-general pattern of cortical thickness implicated in the relationship between CVR and cognition could be explained by the fact that CVR is mostly associated with small lesions in cerebral white matter that exhibit a more disperse representation over striatal, cortico–cortical, and cortical–subcortical pathways.[90] As mentioned, future studies will additionally assess the integrity of white matter tracts. Importantly, it is noteworthy that management of CVR involves not one but many healthy

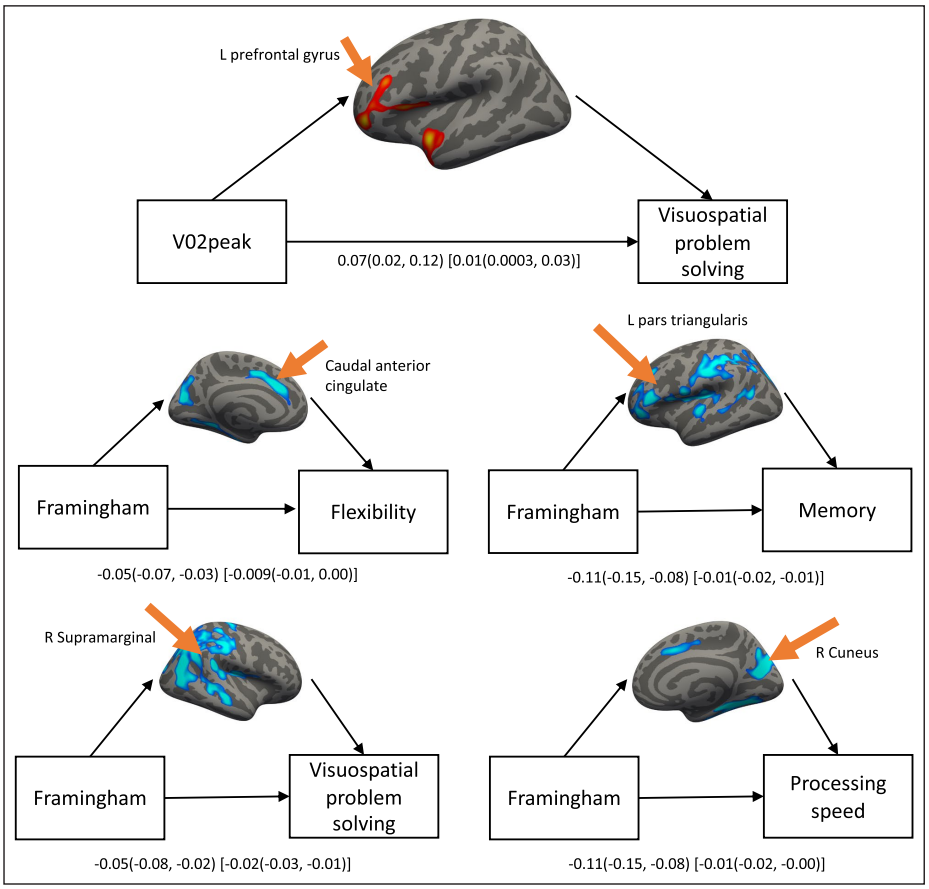

Figure 4. Cortical thickness in various regions mediated relationships between our predictors (VO_2peak and Framingham) and cognitive domains.

The relationship between each predictor and significant cortical thickness clusters (X [predictor variable] on M [mediator]) are found in Tables S7 through S9 along with full mediation model results (Table S10). Orange arrows depict the exact cluster, which mediates the relationship between X (predictor) and Y (cognitive domain outcome). The mediated effect is calculated as the difference between the estimates from the total and direct effects (see Tables S10 and S12A through S12D) which correspond to the reduction in the independent variable (X) effect on the dependent variable (Y) when adjusted for the mediator (M). The total effect (X on Y) is seen under the horizontal arrow representing the β coefficient followed by the 95% CIs in parentheses. The average causal mediation effect (X [predictor variable] on Y [outcome variable] including M [mediator]) is seen between square brackets following the direct effect. In the case of VO_2peak (oxygen uptake during peak exercise) on visuospatial problem solving (top), of the estimated total effect (0.07, note this is the unstandardized β coefficient), an estimated 0.01 is because of the mediator (cortical thickness in the left prefrontal gyrus).

behaviors (avoiding smoking, weight management, and healthy eating habits, to name a few), and we found this collective effort to be manifested by diffuse patterns of brain structure that likely support the wide range of cognitive abilities that were associated with CVR.

One important point that distinguishes our results from previous studies is that our sample was particularly healthy from a heart-health perspective. For instance, our sample had a relatively high group average in CRF (24.9±7.18 mL/kg per minute) (see age

and gender norms for the American population[99]) and a low group average for CVR (estimated to be ≈8% risk of a future cardiovascular event in 5 years). The fact that in this overall healthy sample, individual variations in CRF and CVR were still associated with cognitive behavior, and brain structure demonstrates that these established biomarkers of heart health in older adults may also be sufficiently sensitive for better understanding cognitive trajectories in early and late middle age. It is pertinent to highlight though that these same characteristics of this sample may affect generalizability to other populations. The BBHI sample is by design particularly young and healthy, because our data are cross-sectional samples of this longitudinal cohort study that aims to better understand and characterize neurobiological determinants of cognitive brain health from middle to late life. As such, this sample is exposed to known environmental factors reported to strongly contribute to cognitive brain health, such as adherence to a Mediterranean diet, engagement in physical activity, and leisure activities. It is also important to note that this is a mostly White sample, which is relevant because cardiovascular risk has differential associations with other racial and ethnic groups, particularly in Black and Latino individuals and other minority groups. As such, comprehensive and inclusive brain health strategies must also address this knowledge gap by examining such associations between determinants of cardiovascular health and cognitive brain health in other racial and ethnic groups in midlife.

Importantly, although cognitive brain health is a top health-related priority for people when they reach older age,[100] our findings highlight the relevance of creating a cognitive brain health plan in middle age. Given growing evidence demonstrating changes in the brain related to the onset of neurodegenerative disorders begin some 10 to 20 years before the onset of symptoms,[77–79] it is critical that strategies to mitigate age-related cognitive decline and promote cognitive brain health need to be introduced decades earlier in midlife. CRF and CVR are both modifiable factors, and thus our results could potentially suggest that by adopting lifestyle changes that promote heart health in middle age, it may be possible to actively steer the course of one's cognitive trajectory in later life. Our results (Data S5) also reproduce the ubiquitous association between greater CRF and greater time practicing physical activity (Figure S4). Thus, engagement in a physically active lifestyle is a potential strategy (among many, including diet, sleep, and other cognitively stimulating activities), that are likely to have a positive effect on cognitive brain health even in midlife. Albeit these conclusions need to be supported by longitudinal and interventional studies.

Although our results are complimentary to several previous and large population studies investigating associations between cardiovascular outcomes and cognitive brain health, our study has unique strengths. We add to previous research by examining not just 1 but 2 independent predictors (CRF, CVR), and by using a detailed and comprehensive neuropsychological assessment in over 500 healthy middle-aged adults free from clinically detectable cognitive deficits. Finally, given that the relationship between cardiovascular health and cognition is likely to be underpinned by brain structure, we also advance previous studies by using neuroimaging and analytical methods to demonstrate the mediating effect of brain structure on the relationships between CRF/CVR and cognition.

There are also limitations to our study. Because of the cross-sectional nature of our results, it was not possible to make any kind of inference about casual relationships. In addition, the normalization of VO_2peak to total body mass (referred to as simple ratio standard) can produce confounding results because of individual differences in adiposity levels.[48] We aimed to minimize this source of bias by including waist circumference as a covariate in all analyses. Furthermore, we replicated our results using allometric scaling of VO_2peak to ensure that scaling to total body mass did not confound the associations with cognition.[48,49] Future studies are encouraged to measure adiposity levels and normalize VO_2peak to fat-free mass. Considerations about biological sex interactions are critical in this work given reported differences in CRF,[101–103] CVR,[104] and trajectories of cognitive performance[105] between men and women. We will address biological sex interactions in a future planned study. Finally, we did not assess other potential factors that influence the relationships seen such as diet, physical activity levels, and motor skills.

Taken together, our findings show that even in younger and healthy middle-aged adults with relatively high CRF and low CVR, relationships between these modifiable factors that may contribute to cognitive/brain reserve and cognition exist. Furthermore, we shed light on a potential mechanistic pathway (cortical thickness) that may contribute to this relationship. The implications of our study lie within the potential importance of engaging in modifiable lifestyle behaviors that can promote heart health, early in midlife, long before the onset of measurable cognitive decline, which can be assessed in future longitudinal and interventional study designs.

ARTICLE INFORMATION

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Disclosures

Dr Pascual-Leone is a cofounder of Linus Health and TI Solutions AG; serves on the scientific advisory boards for Starlab Neuroscience, Neuroelectrics, Magstim Inc., and MedRhythms; and is listed as an inventor on several issued and pending patents on the real-time integration of noninvasive brain stimulation with electroencephalography and magnetic resonance imaging. The remaining authors have no disclosures to report.

Supplementary Material

Data S1–S5
Tables S1–S12
Figures S1–S4
References [108–110]

REFERENCES

1. Crimmins EM. Lifespan and healthspan: past, present, and promise. *Gerontologist*. 2015;55:901–911. DOI: 10.1093/geront/gnv130.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Medicine*. 2006;3:e442. DOI: 10.1371/journal.pmed.0030442.
3. Pascual-Leone A, Freitas C, Oberman L, Horvath JC, Halko M, Eldaief M, Bashir S, Vernet M, Shafi M, Westover B, et al. Characterizing brain cortical plasticity and network dynamics across the age-span in health and disease with TMS-EEG and TMS-fMRI. *Brain Topogr*. 2011;24:302–315. DOI: 10.1007/s10548-011-0196-8.
4. Stern Y, Arenaza-Urquijo EM, Bartrés-Faz D, Belleville S, Cantilon M, Chetelat G, Ewers M, Franzmeier N, Kempermann G, Kremen WS, et al. Whitepaper: defining and investigating cognitive reserve, brain reserve, and brain maintenance. *Alzheimers Dement*. 2020;16:1305–1311. DOI: 10.1016/j.jalz.2018.07.219.
5. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. *Psychol Sci*. 2003;14:125–130. DOI: 10.1111/1467-9280.t01-1-01430.
6. Pentikäinen H, Savonen K, Ngandu T, Solomon A, Komulainen P, Paajanen T, Antikainen R, Kivipelto M, Soininen H, Rauramaa R. Cardiorespiratory fitness and cognition: longitudinal associations in the FINGER study. *J Alzheimers Dis*. 2019;68:961–968. DOI: 10.3233/JAD-180897.
7. Gardener H, Wright CB, Dong C, Cheung K, DeRosa J, Nannery M, Stern Y, Elkind MSV, Sacco RL. Ideal cardiovascular health and cognitive aging in the Northern Manhattan Study. *J Am Heart Assoc*. 2016;5:e002731. DOI: 10.1161/JAHA.115.002731.
8. Féart C, Samieri C, Allès B, Barberger-Gateau P. Potential benefits of adherence to the Mediterranean diet on cognitive health. *Proc Nutr Soc*. 2013;72:140–152. DOI: 10.1017/S0029665112002959.
9. Hardman RJ, Kennedy G, Macpherson H, Scholey AB, Pipingas A. Adherence to a Mediterranean-style diet and effects on cognition in adults: a qualitative evaluation and systematic review of longitudinal and prospective trials. *Front Nutr*. 2016;3:22. DOI: 10.3389/fnut.2016.00022.
10. Pace-Schott EF, Spencer RMC. Age-related changes in the cognitive function of sleep. *Prog Brain Res*. 2011;191:75–89. DOI: 10.1016/B978-0-444-53752-2.00012-6.
11. Scullin MK, Bliwise DL. Sleep, cognition, and normal aging: integrating a half-century of multidisciplinary research. *Perspect Psychol Sci*. 2015;10:97–137. DOI: 10.1177/1745691614556680.
12. Spira AP, Chen-Edinboro LP, Wu MN, Yaffe K. Impact of sleep on the risk of cognitive decline and dementia. *Curr Opin Psychiatry*. 2014;27:478–483. DOI: 10.1097/YCO.0000000000000106.
13. Ludyga S, Gerber M, Pühse U, Looser VN, Kamijo K. Systematic review and meta-analysis investigating moderators of long-term effects of exercise on cognition in healthy individuals. *Nat Hum Behav*. 2020;4:603–612. DOI: 10.1038/s41562-020-0851-8.
14. Launer LJ. The epidemiologic study of dementia: a life-long quest? *Neurobiol Aging*. 2005;26:335–340. DOI: 10.1016/j.neurobiolaging.2004.03.016.
15. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, Iwatsubo T, Jack CR, Kaye J, Montine TJ, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7:280–292. DOI: 10.1016/j.jalz.2011.03.003.
16. Newson RS, Kemps EB. Cardiorespiratory fitness as a predictor of successful cognitive ageing. *J Clin Exp Neuropsychol*. 2006;28:949–967. DOI: 10.1080/13803390591004356.
17. Wendell CR, Gunstad J, Waldstein SR, Wright JG, Ferrucci L, Zonderman AB. Cardiorespiratory fitness and accelerated cognitive decline with aging. *J Gerontol A Biol Sci Med Sci*. 2014;69:455–462. DOI: 10.1093/gerona/glt144.
18. Zhu N, Jacobs DR, Schreiner PJ, Yaffe K, Bryan N, Launer LJ, Whitmer RA, Sidney S, Demerath E, Thomas W, et al. Cardiorespiratory fitness and cognitive function in middle age: the CARDIA study. *Neurology*. 2014;82:1339–1346. DOI: 10.1212/WNL.0000000000000310.
19. Gorelick PB, Furie KL, Iadecola C, Smith EE, Waddy SP, Lloyd-Jones DM, Bae H-J, Bauman MA, Dichgans M, Duncan PW, et al.; American Heart Association/American Stroke Association. Defining optimal brain health in adults: a presidential advisory from the American Heart Association/American Stroke Association. *Stroke*. 2017;48:e284–e303. DOI: 10.1161/STR.0000000000000148.
20. Hörder H, Johansson L, Guo X, Grimby G, Kern S, Östling S, Skoog I. Midlife cardiovascular fitness and dementia: a 44-year longitudinal population study in women. *Neurology*. 2018;90:e1298–e1305. DOI: 10.1212/WNL.0000000000005290.
21. Launer LJ, Ross GW, Petrovitch H, Masaki K, Foley D, White LR, Havlik RJ. Midlife blood pressure and dementia: the Honolulu-Asia aging study. *Neurobiol Aging*. 2000;21:49–55. DOI: 10.1016/S0197-4580(00)00096-8.
22. Mielke MM, Rosenberg PB, Tschanz J, Cook L, Corcoran C, Hayden KM, Norton M, Rabins PV, Green RC, Welsh-Bohmer KA, et al. Vascular factors predict rate of progression in Alzheimer disease. *Neurology*. 2007;69:1850–1858. DOI: 10.1212/01.wnl.0000279520.59792.fe.
23. Ott A, Breteler MM, de Bruyne MC, van Harskamp F, Grobbee DE, Hofman A. Atrial fibrillation and dementia in a population-based study. The Rotterdam Study. *Stroke*. 1997;28:316–321. DOI: 10.1161/01.STR.28.2.316.
24. Skoog I, Lernfelt B, Landahl S, Palmertz B, Andreasson LA, Nilsson L, Persson G, Odén A, Svanborg A. 15-year longitudinal study of blood pressure and dementia. *Lancet*. 1996;347:1141–1145. DOI: 10.1016/S0140-6736(96)90608-X.
25. Flicker L. Cardiovascular risk factors, cerebrovascular disease burden, and healthy brain aging. *Clin Geriatr Med*. 2010;26:17–27. DOI: 10.1016/j.cger.2009.12.005.
26. Leritz EC, McGlinchey RE, Kellison I, Rudolph JL, Milberg WP. Cardiovascular disease risk factors and cognition in the

elderly. *Curr Cardiovasc Risk Rep*. 2011;5:407–412. DOI: 10.1007/s12170-011-0189-x.
27. Levin BE, Llabre MM, Dong C, Elkind MSV, Stern Y, Rundek T, Sacco RL, Wright CB. Modeling metabolic syndrome and its association with cognition: the Northern Manhattan study. *J Int Neuropsychol Soc*. 2014;20:951–960. DOI: 10.1017/S1355617714000861.
28. Hoppeler H. Deciphering VO_2, max: limits of the genetic approach. *J Exp Biol*. 2018;221:jeb164327. DOI: 10.1242/jeb.164327.
29. Williams CJ, Williams MG, Eynon N, Ashton KJ, Little JP, Wisloff U, Coombes JS. Genes to predict VO_2max trainability: a systematic review. *BMC Genom*. 2017;18:831. DOI: 10.1186/s12864-017-4192-6.
30. Kathiresan S, Srivastava D. Genetics of human cardiovascular disease. *Cell*. 2012;148:1242–1257. DOI: 10.1016/j.cell.2012.03.001.
31. Cattaneo G, Bartrés-Faz D, Morris TP, Sánchez JS, Macià D, Tarrero C, Tormos JM, Pascual-Leone A. The Barcelona Brain Health Initiative: a cohort study to define and promote determinants of brain health. *Front Aging Neurosci*. 2018;10:321. DOI: 10.3389/fnagi.2018.00321.
32. Cattaneo G, Bartrés-Faz D, Morris TP, Solana Sánchez J, Macià D, Tormos JM, Pascual-Leone A. The Barcelona Brain Health Initiative: cohort description and first follow-up. *PLoS One*. 2020;15:e0228754. DOI: 10.1371/journal.pone.0228754.
33. Cuschieri S. The STROBE guidelines. *Saudi J Anaesth*. 2019;13:S31–S34. DOI: 10.4103/sja.SJA_543_18.
34. Wechsler D. Wechsler Adult Intelligence Scale-Fourth Edition (WAIS-IV) [database record]. *APA PsycTests*. 2008.
35. Reitan RM, Wolfson D. *The Halstead-Reitan Neuropsycholgical Test Battery: Therapy and Clinical Interpretation*. Neuropsychological Press; 1985.
36. Shimoyama I, Ninchoji T, Uemura K. The finger-tapping test. A quantitative analysis. *Arch Neurol*. 1990;47:681–684. DOI: 10.1001/archneur.1990.00530060095025.
37. Pena-Casanova J, Quinones-Ubeda S, Quintana-Aparicio M, Aguilar M, Badenes D, Molinuevo JL, Torner L, Robles A, Barquero MS, Villanueva C, et al. Spanish Multicenter Normative Studies (NEURONORMA Project): norms for verbal span, visuospatial span, letter and number sequencing, trail making test, and symbol digit modalities test. *Arch Clin Neuropsychol*. 2009;24:321–341. DOI: 10.1093/arclin/acp038.
38. Rey A. *L'examen Clinique en psychologie*. Presses Universitaires de France; 1958.
39. Tamayo F, Casals-Coll M, Sánchez-Benavides G, Quintana M, Manero RM, Rognoni T, Calvo L, Palomo R, Aranciva F, Peña-Casanova J,. et al.*Neurologia*. 2012;27:319–329. DOI: 10.1016/j.nrl.2011.12.020.
40. Lee S, Jennrich RI. A study of algorithms for covariance structure analysis with specific comparisons using factor analysis. *Psychometrika*. 1979;44:99–113. DOI: 10.1007/BF02293789.
41. Hair JF, Anderson RE, Tatham RL, Black WC. *Multivariate Data Analysis*. 5th ed. Prentice Hall; 1998.
42. Arós F, Boraita A, Alegría E, Alonso AM, Bardají A, Lamiel R, Luengo E, Rabadán M, Alijarde M, Aznar J, et al. [Guidelines of the Spanish Society of Cardiology for clinical practice in exercise testing]. *Rev Esp Cardiol*. 2000;53:1063–1094.
43. Adams R. Revised Physical Activity Readiness Questionnaire. *Can Fam Physician*. 1999;45:992, 995, 1004–1005.
44. Wasserman K, Hansen J, Sue D, Whipp B, Casaburi R. *Principles of Exercise Testing and Interpretation*. 4th ed. Williams and Wilkins; 2004.
45. Borg GAV, Noble BJ. Perceived exertion. *Exerc Sport Sci Rev*. 1974;2:131–153. DOI: 10.1249/00003677-197400020-00006.
46. Aspenes ST, Nilsen TIL, Skaug E-A, Bertheussen GF, Ellingsen Ø, Vatten L, Wisløff U. Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Med Sci Sports Exerc*. 2011;43:1465–1473. DOI: 10.1249/MSS.0b013e31820ca81c.
47. Paterson DH, Cunningham DA, Koval JJ, St Croix CM. Aerobic fitness in a population of independently living men and women aged 55–86 years. *Med Sci Sports Exerc*. 1999;31:1813–1820. DOI: 10.1097/00005768-199912000-00018.
48. Lolli L, Batterham AM, Weston KL, Atkinson G. Size exponents for scaling maximal oxygen uptake in over 6500 humans: a systematic review and meta-analysis. *Sports Med*. 2017;47:1405–1419. DOI: 10.1007/s40279-016-0655-1.
49. Vanderburgh PM, Mahar MT, Chou CH. Allometric scaling of grip strength by body mass in college-age men and women. *Res Q Exerc Sport*. 1995;66:80–84. DOI: 10.1080/02701367.1995.10607658.
50. Roman-Viñas B, Serra-Majem L, Hagströmer M, Ribas-Barba L, Sjöström M, Segura-Cardona R. International Physical Activity Questionnaire: reliability and validity in a Spanish population. *Eur J Sport Sci*. 2010;10:297–304. DOI: 10.1080/17461390903426667.
51. Anderson KM, Odell PM, Wilson PWF, Kannel WB. Cardiovascular disease risk profiles. *Am Heart J*. 1991;121:293–298. DOI: 10.1016/0002-8703(91)90861-B.
52. Marrugat J, Solanas P, D'Agostino R, Sullivan L, Ordovas J, Cordón F, Ramos R, Sala J, Masià R, Rohlfs I, et al. Coronary risk estimation in Spain using a calibrated Framingham function. *Rev Esp Cardiol*. 2003;56:253–261. DOI: 10.1016/s0300-8932(03)76861-4.
53. Marrugat J, Vila J, Baena-Díez JM, Grau M, Sala J, Ramos R, Subirana I, Fitó M, Elosua R. Relative validity of the 10-year cardiovascular risk estimate in a population cohort of the REGICOR study. *Rev Esp Cardiol*. 2011;64:385–394. DOI: 10.1016/j.recesp.2010.12.011.
54. Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci USA*. 2000;97:11050–11055. DOI: 10.1073/pnas.200033797.
55. Talairach J, Tournoux P. *Co-Planar Stereotaxic Atlas of the Human Brain*. Thieme Medical Publishers; 1988. Available at: https://www.thieme.com/books-main/neurosurgery/product/414-co-planar-stereotaxic-atlas-of-the-human-brain. Accessed October 13, 2020.
56. Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging*. 1998;17:87–97. DOI: 10.1109/42.668698.
57. Ségonne F, Dale AM, Busa E, Glessner M, Salat D, Hahn HK, Fischl B. A hybrid approach to the skull stripping problem in MRI. *NeuroImage*. 2004;22:1060–1075. DOI: 10.1016/j.neuroimage.2004.03.032.
58. Fischl B, Liu A, Dale A. Automated manifold surgery: constructing geometrically accurate and topologically correct models of the human cerebral cortex. *IEEE Trans Med Imaging*. 2001;20:70–80. DOI: 10.1109/42.906426.
59. Desikan RS, Ségonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *NeuroImage*. 2006;31:968–980. DOI: 10.1016/j.neuroimage.2006.01.021.
60. Fischl B, Sereno MI, Dale AM. Cortical surface-based analysis: II: inflation, flattening, and a surface-based coordinate system. *NeuroImage*. 1999;9:195–207. DOI: 10.1006/nimg.1998.0396.
61. Hagler DJ, Saygin AP, Sereno MI. Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. *NeuroImage*. 2006;33:1093–1103. DOI: 10.1016/j.neuroimage.2006.07.036.
62. Tingley D, Yamamoto T, Hirose K, Keele L, Imai K. Mediation: R package for causal mediation analysis. *UCLA Statistics/American Statistical Association*. 2014. Available at: https://dspace.mit.edu/handle/1721.1/91154. Accessed March 19, 2021.
63. Wechsler D. *WAIS-IV. Escala de Inteligencia de Wechsler Para Adultos-IV. Manual de Aplicación y Corrección*. NCS Pearson, Inc.; 2012.
64. Barnes DE, Yaffe K, Satariano WA, Tager IB. A longitudinal study of cardiorespiratory fitness and cognitive function in healthy older adults. *J Am Geriatr Soc*. 2003;51:459–465. DOI: 10.1046/j.1532-5415.2003.51153.x.
65. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults. *Psychol Sci*. 2003;14:125. DOI: 10.1111/1467-9280.t01-1-01430.
66. Boots EA, Schultz SA, Oh JM, Larson J, Edwards D, Cook D, Koscik RL, Dowling MN, Gallagher CL, Carlsson CM, et al. Cardiorespiratory fitness is associated with brain structure, cognition, and mood in a middle-aged cohort at risk for Alzheimer's disease. *Brain Imaging Behav*. 2015;9:639–649. DOI: 10.1007/s11682-014-9325-9.
67. Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, Elavsky S, Marquez DX, Hu L, Kramer AF. Aerobic exercise training increases brain volume in aging humans. *J Gerontol A Biol Sci Med Sci*. 2006;61:1166–1170. DOI: 10.1093/gerona/61.11.1166.
68. Erickson KI, Prakash RS, Voss MW, Chaddock L, Hu L, Morris KS, White SM, Wójcicki TR, McAuley E, Kramer AF. Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus*. 2009;19:1030–1039. DOI: 10.1002/hipo.20547.
69. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, Kim JS, Heo S, Alves H, White SM, et al. Exercise training increases size of hippocampus and improves memory. *Proc Natl Acad Sci USA*. 2011;108:3017–3022. DOI: 10.1073/pnas.1015950108.

70. Erickson KI, Leckie RL, Weinstein AM. Physical activity, fitness, and gray matter volume. *Neurobiol Aging*. 2014;35(suppl 2):S20–S28. DOI: 10.1016/j.neurobiolaging.2014.03.034.
71. Weinstein AM, Voss MW, Prakash RS, Chaddock L, Szabo A, White SM, Wojcicki TR, Mailey E, McAuley E, Kramer AF, et al. The association between aerobic fitness and executive function is mediated by prefrontal cortex volume. *Brain Behav Immun*. 2012;26:811–819. DOI: 10.1016/j.bbi.2011.11.008.
72. Salat DH, Buckner RL, Snyder AZ, Greve DN, Desikan RSR, Busa E, Morris JC, Dale AM, Fischl B. Thinning of the cerebral cortex in aging. *Cereb Cortex*. 2004;14:721–730. DOI: 10.1093/cercor/bhh032.
73. Watson CE, Chatterjee A. A bilateral frontoparietal network underlies visuospatial analogical reasoning. *NeuroImage*. 2012;59:2831–2838. DOI: 10.1016/j.neuroimage.2011.09.030.
74. Nissim NR, O'Shea AM, Bryant V, Porges EC, Cohen R, Woods AJ. Frontal structural neural correlates of working memory performance in older adults. *Front Aging Neurosci*. 2017;08:328. DOI: 10.3389/fnagi.2016.00328.
75. Erkinjuntti T, Inzitari D, Pantoni L, Wallin A, Scheltens P, Rockwood K, Desmond DW. Limitations of clincal criteria for the diagnosis of vascular dementia in clinical trials. Is a focus on subcortical vascular dementia a solution? *Ann N Y Acad Sci*. 2000;903:262–272. DOI: 10.1111/j.1749-6632.2000.tb06376.x.
76. Oberlin LE, Verstynen TD, Burzynska AZ, Voss MW, Prakash RS, Chaddock-Heyman L, Wong C, Fanning J, Awick E, Gothe N, et al. White matter microstructure mediates the relationship between cardiorespiratory fitness and spatial working memory in older adults. *NeuroImage*. 2016;131:91–101. DOI: 10.1016/j.neuroimage.2015.09.053.
77. Bateman RJ, Xiong C, Benzinger TLS, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, et al. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. *N Engl J Med*. 2012;367:795–804. DOI: 10.1056/NEJMoa1202753.
78. Beason-Held LL, Goh JO, An Y, Kraut MA, O'Brien RJ, Ferrucci L, Resnick SM. Changes in brain function occur years before the onset of cognitive impairment. *J Neurosci*. 2013;33:18008–18014. DOI: 10.1523/JNEUROSCI.1402-13.2013.
79. Younes L, Albert M, Moghekar A, Soldan A, Pettigrew C, Miller MI. Identifying change points in biomarkers during the preclinical phase of Alzheimer's disease. *Front Aging Neurosci*. 2019;11:74. 10.3389/fnagi.2019.00074.
80. Gomes-Osman J, Cabral DF, Morris TP, McInerney K, Cahalin LP, Rundek T, Oliveira A, Pascual-Leone A. Exercise for cognitive brain health in aging. *Neurol Clin Pract*. 2018;8:257–265. DOI: 10.1212/CPJ.0000000000000460.
81. Kramer AF, Colcombe S. Fitness effects on the cognitive function of older adults: a meta-analytic study-revisited. *Perspect Psychol Sci*. 2018;13:213–217. DOI: 10.1177/1745691617707316.
82. Cheval B, Orsholits D, Sieber S, Courvoisier D, Cullati S, Boisgontier MP. Relationship between decline in cognitive resources and physical activity. *Health Psychol*. 2020;39:519–528. DOI: 10.1037/hea0000857.
83. Lin X, Zhang X, Guo J, Roberts CK, McKenzie S, Wu W-C, Liu S, Song Y. Effects of exercise training on cardiorespiratory fitness and biomarkers of cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials. *J Am Heart Assoc*. 2015;4:e002014. DOI: 10.1161/JAHA.115.002014.
84. Sabia S, Dugravot A, Dartigues J-F, Abell J, Elbaz A, Kivimäki M, Singh-Manoux A. Physical activity, cognitive decline, and risk of dementia: 28 year follow-up of Whitehall II cohort study. *BMJ*. 2017;357:j2709. DOI: 10.1136/bmj.j2709.
85. Perissiou M, Borkoles E, Kobayashi K, Polman R. The effect of an 8 week prescribed exercise and low-carbohydrate diet on cardiorespiratory fitness, body composition and cardiometabolic risk factors in obese individuals: a randomised controlled trial. *Nutrients*. 2020;12:482. DOI: 10.3390/nu12020482.
86. Barnes DE, Covinsky KE, Whitmer RA, Kuller LH, Lopez OL, Yaffe K. Predicting risk of dementia in older adults: the late-life dementia risk index. *Neurology*. 2009;73:173–179. DOI: 10.1212/WNL.0b013e3181a81636.
87. Reitz C, Tang MX, Schupf N, Manly JJ, Mayeux R, Luchsinger JA. A summary risk score for the prediction of Alzheimer disease in elderly persons. *Arch Neurol*. 2010;67:835–841. DOI: 10.1001/archneurol.2010.136.
88. Exalto LG, Biessels GJ, Karter AJ, Huang ES, Katon WJ, Minkoff JR & Whitmer RA. Risk score for prediction of 10 year dementia risk in individuals with type 2 diabetes: a cohort study. *Lancet Diabetes Endocrinol*. 2015;1:183–190. DOI: 10.1016/S2213-8587(13)70048-2.
89. Anstey KJ, Cherbuin N, Herath PM, Qiu C, Kuller LH, Lopez OL, Wilson RS, Fratiglioni L. A self-report risk index to predict occurrence of dementia in three independent cohorts of older adults: the ANU-ADRI. *PLoS One*. 2014;9:e86141. DOI: 10.1371/journal.pone.0086141.
90. Gorelick PB, Scuteri A, Black SE, DeCarli C, Greenberg SM, Iadecola C, Launer LJ, Laurent S, Lopez OL, Nyenhuis D, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42:2672–2713. DOI: 10.1161/STR.0b013e3182299496.
91. Baumgart M, Snyder HM, Carrillo MC, Fazio S, Kim H, Johns H. Summary of the evidence on modifiable risk factors for cognitive decline and dementia: a population-based perspective. *Alzheimers Dement*. 2015;11:718–726. DOI: 10.1016/j.jalz.2015.05.016.
92. Qiu C, Fratiglioni L. A major role for cardiovascular burden in age-related cognitive decline. *Nat Rev Cardiol*. 2015;12:267–277. DOI: 10.1038/nrcardio.2014.223.
93. Tarraf W, Kaplan R, Daviglus M, Gallo LC, Schneiderman N, Penedo FJ, Perreira KM, Lamar M, Chai A, Vásquez PM, et al. Cardiovascular risk and cognitive function in middle-aged and older hispanics/latinos: results from the Hispanic Community Health Study/Study of Latinos (HCHS/SOL). *J Alzheimers Dis*. 2020;73:103–116. DOI: 10.3233/JAD-190830.
94. Joosten H, Van Eersel MEA, Gansevoort RT, Bilo HJG, Slaets JPJ, Izaks GJ. Cardiovascular risk profile and cognitive function in young, middle-aged, and elderly subjects. *Stroke*. 2013;44:1543–1549. DOI: 10.1161/STROKEAHA.111.000496.
95. Reis JP, Loria CM, Launer LJ, Sidney S, Liu K, Jacobs DR, Zhu N, Lloyd-Jones DM, He K, Yaffe K. Cardiovascular health through young adulthood and cognitive functioning in midlife. *Ann Neurol*. 2013;73:170–179. DOI: 10.1002/ana.23836.
96. Kivipelto M, Ngandu T, Laatikainen T, Winblad B, Soininen H, Tuomilehto J. Risk score for the prediction of dementia risk in 20 years among middle aged people: a longitudinal, population-based study. *Lancet Neurol*. 2006;5:735–741. DOI: 10.1016/S1474-4422(06)70537-3.
97. Exalto LG, Quesenberry CP, Barnes D, Kivipelto M, Biessels GJ, Whitmer RA. Midlife risk score for the prediction of dementia four decades later. *Alzheimers Dement*. 2014;10:562–570. DOI: 10.1016/j.jalz.2013.05.1772.
98. Pettigrew C, Soldan A, Zhu Y, Wang M-C, Moghekar A, Brown T, Miller M, Albert M. Cortical thickness in relation to clinical symptom onset in preclinical AD. *Neuroimage Clin*. 2016;12:116–122. DOI: 10.1016/j.nicl.2016.06.010.
99. Ferguson B. ACSM's guidelines for exercise testing and prescription 9th Ed. 2014. *J Can Chiropr Assoc*. 2014;58:328.
100. National Association of Area Agencies on Aging NC on A and UH The 2015 United States of Aging Survey.
101. Al-Mallah MH, Juraschek SP, Whelton S, Dardari ZA, Ehrman JK, Michos ED, Blumenthal RS, Nasir K, Qureshi WT, Brawner CA, et al. Sex differences in cardiorespiratory fitness and all-cause mortality: the Henry Ford ExercIse Testing (FIT) Project. *Mayo Clin Proc*. 2016;91:755–762. DOI: 10.1016/j.mayocp.2016.04.002.
102. Wang C-Y, Haskell WL, Farrell SW, Lamonte MJ, Blair SN, Curtin LR, Hughes JP, Burt VL. Cardiorespiratory fitness levels among US adults 20–49 years of age: findings from the 1999–2004 National Health and Nutrition Examination Survey. *Am J Epidemiol*. 2010;171:426–435. DOI: 10.1093/aje/kwp412.
103. Weltman A, Weltman JY, Hartman ML, Abbott RD, Rogol AD, Evans WS, Veldhuis JD. Relationship between age, percentage body fat, fitness, and 24-hour growth hormone release in healthy young adults: effects of gender. *J Clin Endocrinol Metab*. 1994;78:543–548. DOI: 10.1210/jcem.78.3.8126124.
104. Mosca L, Barrett-Connor E, Wenger NK. Sex/gender differences in cardiovascular disease prevention what a difference a decade makes. *Circulation*. 2011;124:2145–2154. DOI: 10.1161/CIRCULATIONAHA.110.968792.
105. McCarrey AC, An Y, Kitner-Triolo MH, Ferrucci L, Resnick SM. Sex differences in cognitive trajectories in clinically normal older adults. *Psychol Aging*. 2016;31:166–175. DOI: 10.1037/pag0000070.

106. Vanderburgh PM, Katch FI, Schoenleber J, Balabinis CP, Elliott R. Multivariate allometric scaling of men's world indoor rowing championship performance. *Med Sci Sports Exerc*. 1996;28:626–630. DOI: 10.1097/00005768-199605000-00015.
107. Yu CCW, McManus AM, Au CT, So HK, Chan A, Sung RYT, Li AM. Appropriate scaling approach for evaluating peak VO_2 development in Southern Chinese 8 to 16 years old. *PLoS One*. 2019;14:e0213674. DOI: 10.1371/journal.pone.0213674.
108. Román Viñas B, Ribas Barba L, Ngo J, Serra ML. Validación en población catalana del cuestionario internacional de actividad física. *Gac Sanit*. 2013;27:254–257. DOI: 10.1016/j.gaceta.2012.05.013.

4.1.1 Supplemental Material

Supplemental Material

Data S1. CRF allometric scaling models.

VO_2 peak was allometrically scaled using the procedure described by Vanderburgh et al[49,108]and seen in multiple CRF papers.[48,109]Firstly, VO_2 peak and body weight were log-transformed. A log-linear regression model was constructed using log (VO_2 peak) as the dependent and log (body weight) as independent variables. The interaction effect of biological sex was tested and found to significantly modulate the association between body mass and VO_2 peak, justifying the need for biological sex specific exponent. For that reason, regressions were performed separately for men and women to ensure the models were appropriate. Homoscedasticity was assessed by plotting the standardized residuals against the standardized predicted value. The resulting beta coefficients were used as the allometric exponents. Thus, VO_2 peak can then be allometrically scaled using the following equation: [109]

$$\text{allometrically scaled peak } VO_2 = \frac{\text{unscaled peak } VO_2}{\text{body mass}^{\text{exponent}}}$$

In addition, Pearson correlation analysis was used to examine the association of the scaled VO_2 peak with non-scaled VO_2 peak to verify the effectiveness of the allometric scaling approach for controlling for body size within the sample.

There was a very strong correlation between VO_2 peak and allometric VO_2 ($r = .92$, $p < 0.001$), suggesting that total body mass did not strongly affect our VO_2 peak measure and therefore, the results have remained practically stable.

CRF models has been replicated using the new VO_2 scaled value and the results are seen on Table S1, S2 and S3.

Data S2. Cardiovascular risk as measured by the Catalan-adjusted Framingham risk score (REGICOR).

To ensure our results were valid when adjusted for the Catalan population, we repeated our analyses with the REGICOR risk score[53]. The REGICOR (Registre Gironí del Cor) function is an adaptation of the Framingham function to the incidence of ischemic heart disease and prevalence of local risk factors taking into account the different epidemiological characteristics of Spanish population. The Framingham-based REGICOR CV risk function provides a good prediction of the incidence of the coronary events of the general population of a region in the northwest of Spain and having a high long-term follow-up rate[53].

We found similar results both for the cognitive analyses and the cortical thickness analysis.

Data S3. Individual results for the cortical thickness analyses with the VO_2 peak groups and mediation analyses.

We run these models to illustrate the relationship between each significant cluster and VO_2 peak. Significant correlations were seen between VO2 peak and left rostral middle frontal gyrus (r mean=0.118). The older middle age group (55 and above) showed that left rostral middle frontal gyrus (r mean= 0.172) and left superior temporal gyrus (r mean= 0.169) were positively associated to VO_2 peak.

The results also shown that cortical thickness significantly mediated the relationship between CRF 55 and above years old group and visuo-spatial problem solving, after controlling for age, biological sex, monthly incomes, education, waist perimeter and body mass index.

Data S4. Individual plots and table for the cortical thickness analyses with the cardiovascular risk (Framingham) score and mediation analyses.

We run these models to illustrate the relationship between each significant cluster and cardiovascular risk (Framingham score). Distributed clusters across multiple cortical regions were associated with cardiovascular risk (Framingham 5-year risk score). Those specific clusters were left post central (r mean= -0.170), left pars triangularis (r mean= -0.170), left insula (r mean= -0.170), left cuneus gyrus (r mean= -0.172), left lingual (r mean= -0.164), left caudal anterior cingulate gyrus (r mean= -0.184), left superior parietal gyrus (r mean= -0.158), left inferior parietal gyrus (r mean= -0.160), left transverse temporal gyrus (r mean= -0.169). left rostral middle frontal (r mean=-0.161) and left precentral gyrus (r mean= -0.170). On the right hemisphere, the correlations were in right inferior parietal gyrus (r mean= -0.176), para hippocampal region (r mean= -0.184), right cuneus (r mean= -0.192), right supramarginal gyrus (r mean= -0.175), right precentral gyrus (r mean= -0.165), right lateral occipital gyrus (r mean= -0.160), and right superior frontal gyrus (r mean= -0.163).

The results also shown that cortical thickness significantly mediated the relation between CVH and visuo-spatial problem solving, processing speed, flexibility, and memory, after controlling for education and monthly incomes.

Data S5. Self-reported physical activity and its association with cardiorespiratory fitness.

Self-reported physical activity was assessed using the International Physical Activity Questionnaire (IPAQ), validated for the Spanish/Catalan population [50,110]. Data collected from the self-administered IPAQ surveys were summed within each physical activity domain (walking, moderate-intensity and vigorous-intensity activities) to estimate the total metabolic equivalent of task (MET) in minutes/week spent performing physical activity related to occupational, transportation, household, and leisure activities. The questionnaire was scored and analysed using established methods, available on the IPAQ website (www.ipaq.ki.se). Here, data collected with the IPAQ have been reported as a continuous measure. Total scores have been calculated for walking, moderate-intensity activities, and vigorous-intensity activities, for each domain (work, transport, domestic and garden, and leisure) and for overall total physical activity MET-minutes/week score, calculated as: Total physical activity MET-minutes/week = sum of Total (Walking + Moderate + Vigorous) MET-minutes/week scores.

Engagement in physical activity as measured by the total number of METs-min/week including 'walking', 'moderate activity' and 'vigorous activity' explained 46% of the variance in VO_2 peak in our cohort ($\beta = 3.61$, SE = 0.71, $p = <.001$, $R^2=0.46$).

Table S1. Associations between CRF whole group allometric scaling values and cognitive domains.

	β	SE	P	R2
Memory	-0.007	0.003	0.023	0.115
Working memory	-0.002	0.002	0.387	0.006
Flexibility	-0.001	0.002	0.487	0.142
Processing speed	-0.0006	0.003	0.839	0.163
Visuo-spatial problem solving	0.005	0.002	0.046	0.198

All CRF allometric scaled models are controlling for age, education and socioeconomic status as a covariate. R2 are adjusted for all predictors.

Table S2. Associations between CRF_40_55 group allometric scaling values and cognitive domains.

	β	SE	P	R2
Memory	-0.007	0.003	0.025	0.115
Working memory	-0.001	0.003	0.062	-0.002
Flexibility	0.0008	0.002	0.704	0.106
Processing speed	-0.006	0.003	0.232	0.103
Visuo-spatial problem solving	0.001	0.003	0.731	0.083

All CRF allometric scaled models are controlling for age, education and socioeconomic status as a covariate. R2 are adjusted for all predictors.

Table S3. Associations between CRF_55 and above group allometric scaling values and cognitive domains

	β	SE	P	R2
Memory	-0.007	0.003	0.022	0.115
Working memory	-0.002	0.004	0.539	0.006
Flexibility	-0.001	0.003	0.706	0.142
Processing speed	0.005	0.005	0.330	0.117

5	Left	Cuneus	1051.47
6	Left	Inferior parietal	617.95
7	Left	Insula	458.83
8	Left	Superior parietal	436.36
9	Left	Middle temporal	358.97
1	Right	Inferior parietal	9013.89
2	Right	Precuneus	1799.52
3	Right	Superior temporal	1708.11
4	Right	Para hippocampal	1537.87
5	Right	Lateral occipital	935.62
6	Right	Superior frontal	491.33
7	Right	Precentral	401.51

Table S7. Associations between VO_2 peak and anatomical regions of cortical thickness in the whole sample.

Cluster	Hemisphere	Anatomical ROI	Size
1	Left	Rostral middle frontal	1465.59

Table S8. Associations between VO_2 peak and anatomical regions of cortical thickness in the 40-54 years old group.

- No significant results.

Table S9. Associations between VO_2 peak and anatomical regions of cortical thickness in the 55 and above years old group.

Cluster	Hemisphere	Anatomical ROI	Size
1	Left	Rostral middle frontal	1634.39
2	Left	Superior temporal	1168.92

Outcomes	Total effect	ADE	ACME

Visuo-spatial problem solving	0.011	0.004	0.007**	0.169

All CRF allometric scaled models are controlling for age, education and socioeconomic status as a covariate. R2 are adjusted for all predictors.

Table S4. Associations between Regicor and cognitive domains.

	β	SE	*P*	R^2
Memory	-2.587	0.392	<0.001	0.128
Working memory	-0.558	0.368	0.130	0.004
Flexibility	-1.105	0.269	<0.001	0.081
Processing speed	-2.331	0.394	<0.001	0.091
Visuo-spatial problem solving	-1.253	0.365	0.0006	0.079

All models are controlling for monthly incomes and education as covariates. R^2 are adjusted for all predictors. *survives false discovery rate (FDR) corrections

Table S5. Regicor Standardized beta coefficients.

	β	*P*
Memory	-0.303	<0.001
Working memory	-0.074	0.130
Flexibility	-0.193	<0.001
Processing speed	-0.274	<0.001
Visuo-spatial problem solving	-0.160	0.0006

All models are controlling for age and education as covariates. R^2 are adjusted for all predictors. *survives FDR corrections

Table S6. Associations between Regicor and anatomical regions of cortical thickness.

Cluster	Hemisphere	Anatomical ROI	Size
1	Left	Postcentral	2525.44
2	Left	Insula	2343.16
3	Left	Pars triangularis	1995.18
4	Left	Superior frontal	1103.54

Outcomes	Total effect	ADE	ACME
	Beta (95%CI)	Beta (95%CI)	Beta (95%CI)
Visuospatial problem solving			
Left postcentral gyrus	-0.05(-0.08, -0.02)*	-0.03(-0.07, 0.00)*	-0.01(-0.02, 0.00)*
Left parstriangularis	-0.05(-0.08, -0.02)*	-0.03(-0.07, 0.00)*	-0.01(-0.02, 0.00)*
Left insula	-0.05(-0.08, -0.02)*	-0.03(-0.07, 0.00)	-0.01(-0.02, -0.01)*
Left cuneus	-0.05(-0.08, -0.02)*	-0.04(-0.07, -0.01)*	-0.01(-0.02, 0.00)*
Left lingual	-0.05(-0.08, -0.02)*	-0.04(-0.08, -0.02)*	-0.002(-0.01, 0.01)
Left caudal anterior cingulate gyrus	-0.05(-0.08, -0.02)*	-0.03(-0.07, 0.00)*	-0.01(-0.02, -0.01)*
Left superior parietal	-0.05(-0.08, -0.01)*	-0.04(-0.08, -0.01)*	-0.006(-0.01, 0.00)
Left inferior parietal	-0.05(-0.08, -0.02)*	-0.04(-0.08, -0.01)*	-0.005(-0.01, 0.01)
Left transverse temporal gyrus	-0.05(-0.08, -0.02)*	-0.04(-0.07, -0.01)*	-0.009(-0.01, 0.00)*
Left rostral middle frontal gyrus	-0.05(-0.08, -0.02)*	-0.04(-0.08, -0.01)*	-0.004(-0.01, 0.00)
Left precentral gyrus	-0.05(-0.08, -0.02)*	-0.04(-0.08, -0.01)*	-0.002(-0.01, 0.01)
Right inferior parietal gyrus	-0.05(-0.08, -0.02)*	-0.04(-0.08, -0.01)*	-0.009(-0.02, 0.00)*
Right parahippocampal region	-0.05(-0.08, -0.02)*	-0.05(-0.08, -0.02)*	-0.0001(-0.011, 0.01)
Right cuneus	-0.05(-0.08, -0.02)*	-0.04(-0.07, -0.01)*	-0.01(-0.02, 0.00)
Right supramarginal gyrus	-0.05(-0.08, -0.02)*	-0.03(-0.07, 0.00)*	-0.01(-0.02, -0.01)*
Right precentral gyrus	-0.05(-0.08, -0.02)*	-0.05(-0.08, -0.01)*	-0.0002(-0.008, 0.01)
Right lateral occipital gyrus	-0.05(-0.08, -0.02)*	-0.05(-0.08, -0.02)*	0.0008(-0.006, 0.01)
Right superior frontal gyrus	-0.05(-0.08, -0.02)*	-0.04(-0.08, -0.02)*	-0.001(-0.10, 0.01)

Visuo-spatial problem solving	**Beta (95%CI)**	**Beta (95%CI)**	**Beta (95%CI)**
Left rostral middle frontal gyrus	0.07(0.02, 0.12)*	0.05(0.006, 0.11)*	0.01(0.0003, 0.03)*
Left superior temporal gyrus	0.07(0.02, 0.12)*	0.06(0.02, 0.11)*	0.007(-0.005, 0.02)

Table S10. Each model was adjusted for age, biological sex, monthly incomes, education, waist perimeter and body mass index. ADE = average direct effect; ACME = average causal mediation effect. Statistical significance at $p < 0.05$ and 95% CI not including 0.

Table S11. Associations between Framingham and anatomical regions of cortical thickness.

Cluster	Hemisphere	Anatomical ROI	Size
1	Left	Post central	3518.9
2	Left	Pars triangularis	1928.37
3	Left	Insula	1428.72
4	Left	Cuneus	1181.96
5	Left	Lingual	845.33
6	Left	Caudal anterior cingulate	833.6
7	Left	Superior parietal	589.08
8	Left	Inferior parietal	587.35
9	Left	Transverse temporal	509.91
10	Left	Rostral middle frontal	376.1
11	Left	Precentral	357.23
1	Right	Inferior parietal	9432.87
2	Right	Parahippocampal	1962.3
3	Right	Cuneus	1806.08
4	Right	Supramarginal	1367.08
5	Right	Precentral	568.03
6	Right	Lateral occipital	462.1
7	Right	Superior frontal	448.49

Table S12A. Each model was adjusted for monthly incomes and education. ADE = average direct effect; ACME = average causal mediation effect. Statistical significance at $p < 0.05$ and 95% CI not including 0.

Outcomes	Total effect	ADE	ACME
	Beta (95%CI)	**Beta (95%CI)**	**Beta (95%CI)**
Processing speed			
Left postcentral gyrus	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.08)*	-0.005(-0.18, 0.01)
Left parstriangularis	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.07)*	-0.008(-0.02, 0.00)
Left insula	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.08)*	-0.004(-0.01, 0.01)
Left cuneus	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.08)*	-0.003(-0.01, 0.00)
Left lingual	-0.11(-0.14, -0.08)*	-0.11(-0.14, -0.09)*	0.003(-0.005, 0.01)
Left caudal anterior cingulate gyrus	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.08)*	-0.005(-0.01, 0.00)
Left superior parietal gyrus	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.08)*	-0.004(-0.01, 0.00)
Left inferior parietal gyrus	-0.11(-0.14, -0.08)*	-0.11(-0.14, -0.08)*	-0.001(-0.01, 0.01)
Left transverse temporal gyrus	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.07)*	-0.006(-0.01, 0.00)
Left rostral middle frontal gyrus	-0.11(-0.14, -0.08)*	-0.11(-0.14, -0.08)*	-0.001(-0.009, 0.01)
Left precentral gyrus	-0.11(-0.14, -0.08)*	-0.11(-0.15, -0.08)*	0.0009(-0.006, 0.01)
Right inferior parietal gyrus	-0.11(-0.14, -0.08)*	-0.10(-0.13, -0.07)*	-0.01(-0.02, 0.00)
Right parahippocampal region	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.08)*	-0.005(-0.01, 0.01)
Right cuneus	-0.11(-0.14, -0.08)*	-0.10(-0.13, -0.07)*	-0.01(-0.02, 0.00)*
Right supramarginal gyrus	-0.11(-0.14, -0.08)*	-0.10(-0.14, -0.08)*	-0.006(-0.01, 0.00)
Right precentral gyrus	-0.11(-0.14, -0.08)*	-0.11(-0.14, -0.08)*	-0.001(-0.009, 0.00)

Right parahippocampal region	-0.05(-0.07, -0.03)*	-0.04(-0.06, -0.02)*	-0.007(-0.01, 0.00)
Right cuneus	-0.05(-0.07, -0.03)*	-0.04(-0.06, -0.02)*	-0.009(-0.01, 0.00)*
Right supramarginal gyrus	-0.05(-0.07, -0.03)*	-0.04(-0.06, -0.01)*	-0.01(-0.02,0.00)*
Right precentral gyrus	-<0.001(-<0.001, -0.03)*	-<0.001(-<0.001, -0.03)*	-<0.001(-<0.001, -0.00)
Right lateral occipital gyrus	-0.05(-0.07, -0.03)*	-0.05(-0.07, -0.03)*	-0.0009(-0.00, 0.00)
Right superior frontal gyrus	-0.05(-0.07, -0.03)*	-0.03(-0.06, -0.01)*	-0.01(-0.02, 0.00)

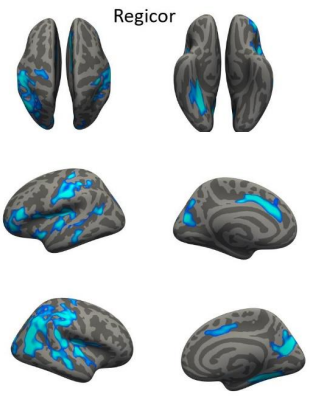
Table S12C. Each model was adjusted for monthly incomes and education. ADE = average direct effect; ACME = average causal mediation effect. Statistical significance at $p < 0.05$ and 95% CI not including 0.

Outcomes	Total effect	ADE	ACME
	Beta (95%CI)	**Beta (95%CI)**	**Beta (95%CI)**
Memory			
Left postcentral gyrus	-0.11(-0.15, -0.08)*	-0.10(-0.14, -0.06)*	-0.01(-0.02, 0.00)
Left pars triangularis	-0.11(-0.15, -0.08)*	-0.10(-0.13, -0.06)*	-0.01(-0.02, -0.01)*
Left insula	-0.11(-0.15, -0.08)*	-0.10(-0.14, -0.07)*	-0.008(-0.01, 0.00)
Left cuneus	-0.11(-0.15, -0.08)*	-0.10(-0.14, -0.07)*	-0.009(-0.02, 0.00)*
Left lingual	-0.11(-0.15, -0.08)*	-0.11(-0.14, -0.08)*	-0.002(-0.01, 0.01)
Left caudal anterior cingulate gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.14, -0.07)*	-0.006(-0.01, 0.00)
Left superior parietal gyrus	-0.11(-0.15, -0.08)*	-0.12(-0.15, -0.08)*	-0.004(-0.00, 0.01)
Left inferior parietal gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.15, -0.07)*	-0.001(-0.01, 0.01)

Left transverse temporal gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.14, -0.07)*	-0.004(-0.01, 0.00)
Left rostral middle frontal gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.14, -0.07)*	-0.004(-0.01, 0.00)
Left precentral gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.15, -0.08)*	-0.0007(-0.00, 0.01)
Right inferior parietal gyrus	-0.11(-0.15, -0.08)*	-0.10(-0.14, -0.06)*	-0.01(-0.02, 0.00)
Right parahippocampal region	-0.11(-0.15, -0.08)*	-0.11(-0.14, -0.07)*	-0.006(-0.01, 0.00)
Right cuneus	-0.11(-0.15, -0.08)*	-0.10(-0.13, -0.06)*	-0.01(-0.02, 0.00)*
Right supramarginal gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.14, -0.07)*	-0.006(-0.01, 0.00)
Right precentral gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.15, -0.08)*	-0.001(-0.01, 0.00)
Right lateral occipital gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.15, -0.08)*	-0.0007(-0.00, 0.01)
Right superior frontal gyrus	-0.11(-0.15, -0.08)*	-0.11(-0.15, -0.08)*	-0.0007(-0.00, 0.01)

Table S12D. Each model was adjusted for monthly incomes and education. ADE = average direct effect; ACME = average causal mediation effect. Statistical significance at $p < 0.05$ and 95% CI not including 0.

Figure S1. REGICOR and cortical thickness.



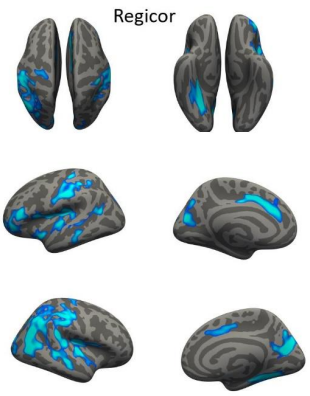
When using the Catalan-population adjusted Framingham risk score, we see similar patterns of associations with cortical thickness compared to those when using Framingham.

Right lateral occipital gyrus	-0.11(-0.14, -0.08)*	-0.11(-0.14, -0.08)*	0.002(-0.007, 0.01)
Right superior frontal gyrus	-0.11(-0.14, -0.08)*	-0.11(-0.14, -0.08)*	-0.001(-0.01, 0.01)

Table S12B. Each model was adjusted for monthly incomes and education. ADE = average direct effect; ACME = average causal mediation effect. Statistical significance at $p < 0.05$ and 95% CI not including 0.

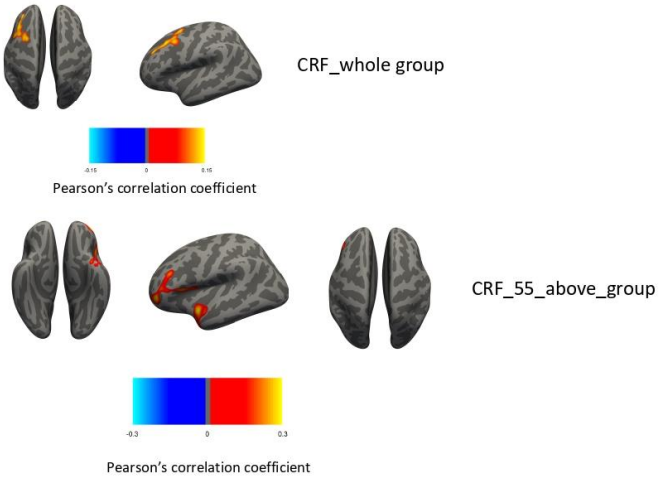
Outcomes	Total effect	ADE	ACME
	Beta (95%CI)	**Beta (95%CI)**	**Beta (95%CI)**
Flexibility			
Left postcentral gyrus	-0.05(-0.07, -0.03)*	-0.03(-0.06, -0.01)*	-0.01(-0.02, 0.00)*
Left pars triangularis	-0.05(-0.07, -0.03)*	-0.04(-0.07, -0.02)*	-0.007(-0.01, 0.00)
Left insula	-0.05(-0.07, -0.03)*	-0.04(-0.06, -0.02)*	-0.007(-0.01, 0.00)*
Left cuneus	-0.05(-0.07, -0.03)*	-0.04(-0.07, -0.02)*	-0.005(-0.01, 0.00)
Left lingual	-0.05(-0.07, -0.03)*	-0.05(-0.07, -0.03)*	-0.001(-0.008, 0.01)
Left caudal anterior cingulate gyrus	-0.05(-0.07, -0.03)*	-0.04(-0.06, -0.02)*	-0.009(-0.01, 0.00)*
Left superior parietal gyrus	-0.05(-0.07, -0.03)*	-0.05(-0.07, -0.03)*	-0.001(-0.009, 0.00)
Left inferior aprietal gyrus	-0.05(-0.07, -0.03)*	-0.05(-0.07, -0.02)*	-0.001(-0.009, 0.01)
Left transverse temporal gyrus	-0.05(-0.07, -0.03)*	-0.04(-0.06, -0.02)*	-0.008(-0.01, 0.00)*
Left rostral middle frontal gyrus	-0.05(-0.07, -0.03)*	-0.04(-0.07, -0.02)*	-0.003(-0.009, 0.00)
Left precentral gyrus	-0.05(-0.07, -0.03)*	-0.04(-0.07, -0.02)*	-0.002(-0.008, 0.00)
Right inferior parietal gyrus	-0.05(-0.07, -0.03)*	-0.03(-0.06, -0.01)*	-0.01(-0.02,0.00)*

Figure S1. REGICOR and cortical thickness.



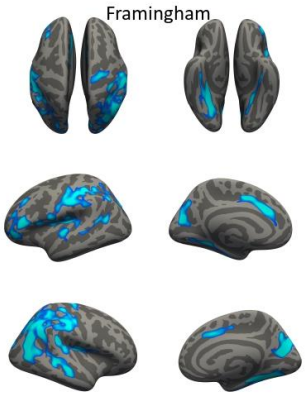
When using the Catalan-population adjusted Framingham risk score, we see similar patterns of associations with cortical thickness compared to those when using Framingham.

Figure S2. CRF and cortical thickness.



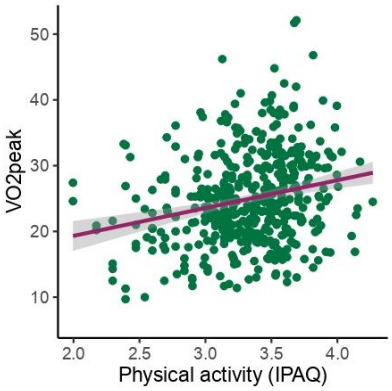
(A). Significant correlations were seen between VO2 peak and left rostral middle frontal gyrus (r mean=0.118). **(B)** The older middle age group (55 and above) showed that left rostral middle frontal gyrus (r mean= 0.172) and left superior temporal gyrus (r mean= 0.169) were positively associated to VO_2 peak.

Figure S3. Framingham and cortical thickness.



All plots illustrating the relationship between each significant cluster and cardiovascular risk (Framingham score). Distributed clusters across multiple cortical regions were associated with cardiovascular risk (Framingham 5-year risk score). Those specific clusters were left post central (r mean= -0.170), left pars triangularis (r mean= -0.170), left insula (r mean= -0.170), left cuneus gyrus (r mean= -0.172), left lingual (r mean= -0.164), left caudal anterior cingulate gyrus (r mean= -0.184), left superior parietal gyrus (r mean= -0.158), left inferior parietal gyrus (r mean= -0.160), left transverse temporal gyrus (r mean= -0.169). left rostral middle frontal (r mean=-0.161) and left precentral gyrus (r mean= -0.170). On the right hemisphere, the correlations were in right inferior parietal gyrus (r mean= -0.176), para hippocampal region (r mean= -0.184), right cuneus (r mean= -0.192), right supramarginal gyrus (r mean= -0.175), right precentral gyrus (r mean= -0.165), right lateral occipital gyrus (r mean= -0.160), and right superior frontal gyrus (r mean= -0.163).

Figure S4. Physical activity and cardiorespiratory fitness.



A significant positive association between physical activity levels (total weekly MET [metabolic equivalent of task]) and $V0_2$ peak, controlling for age, biological sex, education, monthly incomes, BMI (body mass index), and waist was found.

4.2 Article 2

Functional connectivity mediates the relationship between cardiorespiratory fitness and stress in midlife.

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Functional connectivity mediates the relationship between cardiorespiratory fitness and stress in midlife.

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ABSTRACT

Background: Increasing evidence suggests that the relation between mental health and physical health is bidirectional and underpinned by complex neural systems. Cardiovascular fitness is a key measure of physical health but its relation to mental health is insufficiently examined. Characterizing the neural mechanisms by which cardiovascular fitness influences mental health could inform the development of strategies to promote mental health and minimize the risk of mental disorders.

Methods and results: The relation between cardiorespiratory fitness, functional brain connectivity and mental health was studied in 418 healthy middle-aged (aged 40–65 years) adult participants of the Barcelona Brain Health Initiative (BBHI). Higher cardiorespiratory fitness, measured by VO_2 peak, was associated with lower symptoms of anxiety ($\beta = -0.111$, $p = 0.017$) and stress ($\beta = -0.242$, $p = 0.002$) scores, evaluated by the Depression Anxiety and Stress Scale (DASS-21) and its three subscales (stress, anxiety, and depression). Higher within-network functional connectivity of the Default Mode Network (DMN) was associated with higher VO_2 peak ($\beta = 0.195$, $p = 0.002$), and lower stress scores ($\beta = -0.126$, $p = 0.011$). In addition, higher functional connectivity between the Frontoparietal Network (FPN) and Salience Network (SN) was associated with higher VO_2 peak ($\beta = 0.187$, $p = 0.002$), and lower stress scores ($\beta = -0.123$, $p = 0.016$). Both within-DMN [ACME = −0.02 (-0.04,-0.00), $p = 0.040$] and between FPN-SN [ACME = −0.01 (-0.04,-0.00), $p = 0.036$] functional connectivity mediated the relationship between cardiorespiratory fitness and stress.

Conclusions: The relationship between the cardiorespiratory fitness and stress in middle-aged adults is mediated by functional connectivity of several intrinsic resting-state networks. These results highlight a potential mechanistic pathway through which higher cardiorespiratory fitness can positively impact brain health in midlife.

1. Introduction

Mental health is more than the absence of mental disorders such as anxiety or depression. As the World Health Organization describes it, "mental health is a state of well-being that enables people to cope with the stresses of life, realize their abilities, learn well and work well, and

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contribute to their community" (World Health Organization, 2023). Unfortunately, the prevalence and burden of stress in society is expected to rise remarkably in the near future stemming from several sources including the COVID-19 pandemic. Such stressors could generate emotional dysregulation in certain individuals and potentially lead in mental disorders and serious health and social consequences for years to come (American Psychological Association, 2020). Therefore, understanding factors associated with maintenance of good mental health, especially stress, is of public health interest (Shapero et al., 2019; World Health Organization, 2023).

Stress, known as a physiological and psychological response of an individual when they perceive a threat or challenge, is vital for the survival of every living organism. According to Lazarus and Folkman, "psychological stress is a particular relationship between the person and the environment that is appraised by the person as taxing or exceeding their resources and endangering their well-being" (Lazarus & Folkman, 1984). What's more, stress responses encompass emotional and cognitive aspects, such as anxiety, frustration, and rumination (Lazarus & Folkman, 1984). Stress exposure disrupts homeostatic mechanisms, activating the hypothalamic-pituitary-adrenal axis (HPA) and cortisol release (Hellhammer, Wüst, & Kudielka, 2009). As a result, maladaptive responses can occur, impacting on multiple biological systems, including the central nervous system (Yaribeygi, Panahi, Sahraei, Johnston, & Sahebkar, 2017). This phenomenon might potentially lead to inappropriate cognitive behavior and eventually contribute to conditions like depression and anxiety (Dias-Ferreira et al., 2009; Marin et al., 2011).

In young and middle-aged adults, some maladaptive responses to stress have been associated with structural and functional changes of several large-scale brain networks (Hermans, Henckens, Joëls, & Fernández, 2014). Concretely, higher perceived stress levels are linked to disrupted communication within brain networks, including the Default Mode Network (DMN), the Salience Network (SN), and the Frontoparietal Network (FPN). A proposed model known as the Triple Network Model suggests that psychiatric disorders (beyond stress and perceived stress) are characterized by abnormal interactions among these same brain networks (DMN, SN and FPN) (Menon, 2011). The DMN is primarily responsible for self-referential mental activities such as processing emotions, reflecting on the past and future, and is typically deactivated when attention is directed towards the external environment (Buckner, Andrews-Hanna, & Schacter, 2008). On the other hand, the SN is believed to play a role in evaluating the salience of sensory and emotional stimuli (Uddin, 2015), facilitating the transition from internal cognition of the DMN to more external cognitive processes, such as working memory and goal-directed thinking, which are characteristic of the FPN (Goulden et al., 2014; Uddin, Yeo, & Spreng, 2019). According to this model, dysfunction arises when regions of the SN fail to accurately assign significance to relevant stimuli, leading to an inappropriate engagement of the FPN and difficulties in disengaging the DMN. Stress is linked to increased connectivity within the DMN and FPN, along with SN and amygdala interactions (Taren et al., 2015). Whereas, decreased functional connectivity between SN-FPN have been linked to stress related pathologies affecting emotional and cognitive processes (Basten, Stelzel, & Fiebach, 2011; Etkin, Prater, Schatzberg, Menon, & Greicius, 2009; Menon, 2011; Young et al., 2017). Also, stress-induced cortisol levels have been associated with increased connectivity within SN, but with decreased coupling of DMN at both local (within network) and global (with brain regions outside the network) levels (Zhang et al., 2019). Conversely, anxiety disorders often exhibit reduced within-network connectivity in the DMN, while the SN tends to exhibit increased within connectivity (Northoff, 2020). In summary, individual anatomical and functional differences within intrinsic large scale brain networks can therefore contribute to individual variations in psychological resilience (Bolsinger, Seifritz, Kleim, & Manoliu, 2018; Cabello-Toscano et al., 2022).

Specific modifiable health and lifestyle behaviors have been found to be protective of age-related and pathological brain changes (Di Marco et al., 2014; Frankish & Horton, 2017; Livingston et al., 2017). These protective lifestyles such as dietary patterns, good sleep quality, socialization, weight and blood pressure control, psychological well-being and cognitive activity help to explain why certain individuals can withstand age-related and pathological brain changes (Livingston et al., 2017, 2020). Furthermore, these modifiable lifestyles have been shown to interact with biomarkers, potentially promoting mechanisms of brain resilience (Arenaza-Urquijo, Wirth, & Chételat, 2015; Landau et al., 2012; Livingston et al., 2020; Wirth, Villeneuve, La Joie, Marks, & Jagust, 2014). Of several modifiable lifestyle factors, physical activity has seen a lot of attention in the scientific literature regarding its neuroprotective effect across the lifespan (Vecchio et al., 2018). Increasing physical activity through structured exercise participation is a safe and relatively inexpensive means to modify cardiorespiratory fitness (CRF), a key marker of physical health. CRF is a modifiable measure of the body's ability to intake, circulate and utilize oxygen during incremental exercise (Hawkins, Raven, Snell, Stray-Gundersen, & Levine, 2007). Extensive studies have explored the impact of CRF on brain and mental health (Blumenburg et al., 2021; Lindegård, Wastensson, Hadzibajramovic, & Grimby-Ekman, 2019; Pozuelo-Carrascosa et al., 2017). For example, higher CRF has been consistently related to reduced cardiovascular risk and all-cause mortality (Kodama et al., 2009), improved cognition (S. Colcombe & Kramer, 2003; España-Irla et al., 2021; Kramer & Colcombe, 2018), maintenance of cortical thickness and volume across the lifespan (S. J. Colcombe et al., 2003, 2006; Erickson et al., 2009; España-Irla et al., 2021; Szabo et al., 2011), and discrete mental health outcomes such as depression and anxiety scores (Blumenburg et al., 2021; Bueno-Antequera & Munguía-Izquierdo, 2020a, 2020b; Lindegård et al., 2019; Ruegsegger & Booth, 2018). In terms of functional connectivity, CRF has been related to specific brain networks that are relevant to age-related changes in cognition and risk for neurological and psychiatric diseases (Voelcker-Rehage & Niemann, 2013). Specifically, the DMN, FPN and SN appear most sensitive to individual differences in fitness (Voss, Erickson, et al., 2010). In older adults, high levels of CRF have been linked to selective enhancement of coping strategies in front of stressful situations (Gerber & Pühse, 2009), which, in healthy young adults, different copying styles have been suggested to be linked with specific functional connectivity profiles of regions belonging to the DMN and SN (Santarnecchi et al., 2018). Previously, CRF has been also associated with increased functional connectivity of the DMN, specifically in older adults (Voss, Erickson, et al., 2010; Voss et al., 2016). A recent study concluded that increased within- and between-network connectivity of DMN, FPN and SN following aerobic exercise training (a key modifier of CRF) may promote improvements in cognitive performance in older individuals with and without cognitive impairments (Won, Nielson, & Smith, 2023).

Nevertheless, several questions regarding how CRF, functional connectivity of intrinsic resting state networks and perceived mental health in healthy middle-aged adults remain to be addressed. Advancing age is the major risk factor for the development of neurological and psychiatric brain disorders, and aging is associated with increased prevalence of mental health conditions (Barnett et al., 2012). Converging evidence has reported that aging is also associated with reduced functional connectivity of DMN (Mevel, Chételat, Eustache, & Desgranges, 2011), FPN (Campbell, Grady, Ng, & Hasher, 2012) and SN (Lee, Kim, Katz, & Mather, 2020), which are indices of age-related deterioration in brain functional network organization. Focus on lifestyles habit-related changes such as CRF in healthy middle-aged adults and characterizing the neural mechanisms by which CRF influences psychological stress could inform the development of strategies to promote mental health and minimize the risk of mental disorders across the lifespan.

Our primary objective was to evaluate the relation between CRF and mental health in midlife, in a sample of 418 healthy adults aged 40–65 years. We further examined the mechanistic correlates of these relationships through measures of brain function using functional

magnetic resonance imaging (fMRI), by testing whether the functional connectivity of the triple network model, DMN, the SN and the FPN, mediated the relationships between CRF and mental health. We hypothesized that higher CRF would be associated with lower scores on depression, anxiety, and stress scales. Whereas higher within network connectivity and increased between network connectivity would be associated with better scores in mental health outcomes. Given the beneficial effects of exercise on functional network connectivity in older adults, we hypothesized that there would be positive significant correlations between the triple network model and levels of CRF. Specifically, CRF would be correlated with increased functional connectivity within and between DMN, FPN and SN. Lastly, we tested whether the relation between CRF and mental health was mediated by the functional connectivity of and between the DMN, the SN and the FPN.

2. Methods

2.1. Study design and participants

This was a cross-sectional study which included data collected on participants enrolled in the ongoing Barcelona Brain Health Initiative who answered online questionnaires (Phase I) and completed an in-person assessment (Phase II). For a detailed description of the cohort and study protocol see (Cattaneo et al., 2018, 2020). Participants' inclusion criteria (assessed by a medical doctor) were: (1) age between 40 and 65 years, (2) absence of any neurological or neuropsychiatric disorders at the time of recruitment which was firstly pre-screened and self-reported by phone in the time of the recruitment and secondly confirmed by a physician evaluation in their first in-person appointment. The neuropsychologist who was performing the neuropsychological batteries asked about past diagnostic mental health history and was also re-confirming the lack of any psychiatric disease or substance abuse before the in-person evaluation. Participants were asked to report new diagnosis upon their appearance, and every year were query them for new diagnoses and about the number of times they have visited their general practitioner. We excluded any person presenting with any contraindications for functional magnetic resonance imaging (fMRI, see details in MRI section below) and cardiopulmonary exercise testing (CPET, see details in CPET section below). We further excluded those participants who did not meet the criteria for a completed CPET test (see CPET section). All in-person assessment measures were collected within maximum 3 months of each other (26,77 ± 41.68 days). Online questionnaires and in-person assessments were not paralleled in time and were not equal for all participants (12,67 ± 4.68 months). All participants gave written informed consent before participation in any study procedures, all of which conformed to the Declaration of Helsinki for research involving human subjects. All participants gave written informed consent, and the local ethics committee (*Comité d'Ètica i Investigació Clínica de la Unió Catalana d'Hospitals*) approved the study protocol.

2.2. Cardiopulmonary exercise testing (CPET)

Prior to any CPET, participants were evaluated by a physician for potential absolute and relative contraindications for maximal exhaustive exercise following the Guidelines of the Spanish Society of Cardiology for Clinical Practice in Exercise Testing (Arós et al., 2000). The Physical Activity Readiness Questionnaire (PAR-Q (Adams, 1999): was administered to identify any potential health risks associated with exercise. Additionally, participants performed baseline spirometry (Ergoflow flowsensor, Geratherm Respiratory, Bad Kissingen, Germany) and a baseline 12-lead electrocardiogram (EKG) recording before the test (WAM Wireless Acquisition Module, Mortara, Milwaukee, Wisconsin, USA). Individuals who had forced expiratory volume in 1 s (FEV1) of <80%, FEV1/forced vital capacity (FVC) ratio of >80% or peak expiratory flow (PEF) of >75% did not complete the CPET test.

The CPET was conducted using a modified Wasserman protocol on a cyclometer (Ergoselect 4 model, Ergoline, Bitz, Germany) with respiratory gas analysis system Ergostik, Geratherm Respiratory, Bad Kissingen, Germany). The modified Wasserman protocol (Wasserman, Hansen, Sue, Whipp, & Casaburi, 2004) consisted of a 7-min warm-up phase (no load), a progressive workload phase and a 5-min recovery phase (no load). The slope of the progressive increase in workload was calculated individually by dividing the expected maximum workload (calculated automatically by the Bluecherry software (Geratherm Respiratory, Bad Kissingen, Germany) from height, weight, age, and sex) by 9, to derive a progressive increase in workload that would result in a maximal exercise test lasting ~13 min.

During the test the following measures were recorded continuously; gas analysis, via a tight-fitting face mask (Hans Rudolph, Germany) which included oxygen consumption, oxygen uptake (efficiency slope) and respiratory exchange ratio (RER; VO_2/VCO_2), 12-lead EKG, heart rate (beats per minute, from 12-lead EKG) and pulse oximetry. Blood pressure was measured manually from the left arm using a blood pressure cuff (Boso medicus X, Jungingen, Germany) and a hand-held sphygmomanometer (MDF Instruments, Agoura Hills, CA, USA). The perceived effort was evaluated using the Spanish translation of the Borg scale (Borg, 1974) of perceived effort that was recorded every 2 min.

A test was considered complete under the following criteria: verbal manifestation of exhaustion, Borg score of ≥9, heart rate of ±10bpm of HRmax or inability to maintain pedal cadence (~70RPM). The highest full minute VO_2 uptake observed during the final minute of the test was accepted as the functional aerobic capacity (VO_2 peak/plateau). Whenever a VO_2 max plateau could not be detected, the maximal respiratory exchange ratio (RER) (RER of ≥1.0, considered to be indicative of true maximal oxygen uptake (Aspenes et al., 2011; Paterson, Cunningham, Koval, & St Croix, 1999) and the reached target heart rate ≥85% of the maximum theoretical expected HR (220 – age) were applied to determine validity of the CPET results. Because only 14.6% of participants reached a detectable $V0_2$plateau, the term $V0_2$peak is used herein.

2.3. Mental health assessment

Mental health outcomes were evaluated by the Depression Anxiety and Stress Scale (DASS-21) (Henry & Crawford, 2005; Osman et al., 2012). The DASS-21 measures aspects of key negative emotional states of depression, anxiety, and stress, rather than discrete diagnoses of each condition. Descriptively, depression is characterized by cognitive triad of negative automatic thinking, negative self-schemas, and errors in logic with particular emphasis on symptoms such as anhedonia, inertia, lack of interest, hopelessness, and devaluation of live (Beck & Beck, 1967). Anxiety is a future-oriented mood state associated with preparation for possible, upcoming negative events, with particular emphasis on autonomic arousal symptoms, skeletal muscle effects, situational anxiety, and subjective experience of anxious effect (Barlow, 2004). Finally, stress is related to levels of chronic non-specific arousal, difficulty in relaxing, and being easily agitated, over-reactive, and impatient (Lovibond & Lovibond, 1995).

The DASS-21 instrument consists of 21-point Likert-style item self-report questionnaire and is sub-divided by three subscales (seven items each): depression, anxiety, and stress. Participants are asked to score every item on a scale from 0 (did not apply to me at all) to 3 (applied to me very much). Sum scores are computed by adding up the scores on the items per (sub)scale and multiplying them by a factor 2. Sum scores for the total DASS-total scale thus range between 0 and 120, and those for each of the subscales may range between 0 and 42. Scores ≥60 (for DASS-total), ≥21 (for the depression subscale), ≥15 (for the anxiety subscale) and ≥26 (for the stress subscale) are labeled as "high" or "severe" (Brown, Chorpita, Korotitsch, & Barlow, 1997; Lovibond & Lovibond, 1995). The construct validity of the DASS-21 in non-clinical samples has been demonstrated to be satisfactory. The findings from

confirmatory factor analysis (CFA) suggest that while the three scales of the DASS-21 measure a significant shared factor related to general psychological distress, they also capture distinct variances specific to each scale. Furthermore, the DASS-21 scales exhibit high levels of reliability (Henry & Crawford, 2005).

2.4. MRI acquisition parameters

Magnetic resonance imaging (MRI) data were acquired in a 3 T Siemens scanner (MAGNETOM Prisma) with 32-channel head coil, at the Unitat d'Imatge per Ressonància Magnètica IDIBAPS (Institut d'Investigacions Biomèdiques August Pi i Sunyer*) at Hospital Clínic de Barcelona, Barcelona*. MRI session included accelerated multiband sequences adapted from the Human Connectome Project and provided by the Center of Magnetic Resonance Research at the University of Minnesota. For all participants, a high-resolution T1-weighted structural image was obtained with a magnetization prepared rapid acquisition gradient-echo (MPRAGE) three-dimensional protocol and a total of 208 contiguous axial slices obtained in ascending fashion [repetition time (TR) = 2400 ms, echo time (TE) = 2.22 ms, inversion time = 1000 ms, flip angle = 8°, field of view (FOV) = 256 mm and 0.8 mm isotropic voxel]. Additionally, a high-resolution 3-dimensional SPACE T2 weighted acquisition was undertaken [TR = 3200ms, TE = 563ms, flip angle = 120°, 0.8 mm isotropic voxel, FOV = 256 mm]. In the same session, they also underwent 10-min resting-state functional MRI (rs-fMRI) multiband (anterior-posterior phase-encoding; acceleration factor = 8) interleaved acquisitions [T2*weighted EPI scans, TR = 800 ms, TE = 37 ms, 750 vol, 72 slices, slice thickness = 2 mm, FOV = 208 mm]. All the MRI images were examined by a senior neuroradiologist (N·B) in order to detect any clinically significant pathology (none found). Then, all the acquisitions were visually inspected before analysis (M.C.-T. and L.M.-P.) to ensure that they did not contain MRI artifacts or excessive motion.

2.5. MRI preprocessing

The rs-fMRI preprocessing pipeline comprised spatial standardization and nuisance correction by making use of functions from FMRIB Software Library (FSL; version 5.0.11; https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/), FreeSurfer (version 6.0; https://surfer.nmr.mgh.harvard.edu) and Statistical Parametric Mapping (SPM12; https://www.fil.ion.ucl.ac.uk/spm/). To start with, the first 10 scans were removed to ensure magnetization equilibrium. After that, all images were field inhomogeneity corrected (*FSL topup tool*) and realigned to a reference image (*FSL MCFLIRT*) and then standardized into native T1-weighted space (*SPM Coregister*). Finally, normalization (*SPM Normalize*) of all fMRI images to Montreal Neuroscience Institute (MNI152) standard space was performed to ensure among-subjects comparability. As for nuisance correction, different components were defined and manually removed from the rs-fMRI images by the "*fsl_regfilt*" tool implemented in FSL. These components correspond to (i) motion regressors of rotation, translation, and their derivatives, as estimated during scans' realignment, (ii) a drift estimated by a discrete cosine transform (DCT) as a low-pass frequency filter (<0.01), and (iii) signals from white matter (WM) and cerebrospinal fluid (CSF). In order to extract these, CSF and WM masks were obtained from automatic subcortical segmentation of brain volume, based upon the existence of an atlas containing probabilistic information on the location of structures (Fischl et al., 2002). This step was part of the FreeSurfer *'recon-all'* processing stream, which was run with default parameters, except for the addition of the T2 flag for the improvement of pial surfaces reconstruction. That is to say, both T1-and T2-weighted images were used for processing anatomical information. As head movement may affect rs-fMRI results (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012, 2014; 2015; van Dijk, Sabuncu, & Buckner, 2012), in-scanner head motion was considered in al statistical analyses. Mean frame-wise displacement (FWD) was calculated for every subject and used as a covariate in all subsequent analyses. FWD was computed as in Power et al., 2012, using the vectors of rotation and translation estimated during scans' realignment as part of the preprocessing pipeline. Acquisitions with greater than 0.5 mm scan-to-scan displacement were flagged and 3 subjects were eventually excluded for having greater than 50% of volumes flagged at this threshold. This cut off was determined based on preserving at least 5 min of scanning time (Van Dijk et al., 2010).

2.6. Functional connectivity measures

A node-based approach was adopted to quantify individual resting state functional connectivity (rs-FC) within intrinsic resting state networks (RSNs) as defined in the Schaefer-Yeo atlas of 100 nodes and 7 networks (Schaefer et al., 2018; Thomas Yeo et al., 2011); https://github.com/ThomasYeoLab/CBIG/tree/master/stable_projects/brain_parcellation/Schaefer2018_LocalGlobal). Based on previous evidence of age-associated declines in rs-FC (Chan, Park, Savalia, Petersen, & Wig, 2014; Geerligs, Maurits, Renken, & Lorist, 2014; Grady, 2012; Voss et al., 2016) and fitness-related modulation of network integrity (Voss, Erickson, et al., 2010; Voss et al., 2016; Voss, Prakash, et al., 2010) DMN, FPN and SN were selected a priori as our networks of interest. Particularly, as our hypotheses focused on these networks, only 49 out of the 100 nodes were included in FC calculations. Blood-oxygen-level-dependent signal after preprocessing was extracted and averaged across all voxels falling within each region of interest (ROI; i.e., node). Then, ROI-to-ROI connectivity were computed as Pearson correlations and subsequently Fisher-z transformed. The resulting 49 × 49 connectivity matrices per subject were averaged into three within and three between network connectivity measures. Within network rs-FC values were computed as the average rs-FC connecting all the nodes within the same network, while between network rs-RC values were computed as the average rs-FC connecting a pair of networks. Based on previous evidence of age-associated declines in rs-FC (Chan et al., 2014; Geerligs et al., 2014; Grady, 2012; Voss et al., 2016) and fitness-related modulation of network integrity (Voss, Erickson, et al., 2010; Voss et al., 2016; Voss, Prakash, et al., 2010) (Fig. 1).

2.7. Statistical analysis

All statistical analyses were performed in R v.3.6.3 (R Foundation for Statistical Computing, Vienna, Austria). The associations between our predictor variables (V02peak) and outcome measures (mental health questionnaires and functional connectivity measures) were analyzed using multiple linear regressions. In the first model, exploring the association between VO_2 peak and mental health we controlled for age, education, socioeconomic status (SES), waist circumference, biological sex and time between assessments. Instead, when we looked at the relation between cardiovascular fitness and functional connectivity, conducted within and between all three networks (DMN, SN, FPN), we corrected for age, sex, education, waist circumference, SES, and mean frame-wise displacement (FWD). Assumptions of linearity, independence of residuals, multicollinearity and normality were met in all models. Model assumptions were checked using Q-Q plots and fitted vs residual plots in R and the normality of the residuals was formally checked using Shapiro-Wilk tests of normality. In cases of possible heteroscedasticity, a Breusch-Pagan test was conducted. All variables were properly normalized in models where tests of the homoscedasticity assumption were violated. Models are presented as adjusted R2 values and significance is considered at the $p < 0.05$ level. We present standardized beta coefficients (β) as the strength of the relationship between our predictor and outcome variables. That is, for every one-unit increase in the predictor, there is an x standard deviation increase in the outcome. The Benjamini-Hochberg method has been employed to control the False Discovery Rate (FDR), minimizing the chances of erroneous rejections, and enhancing the possibility of valid discoveries

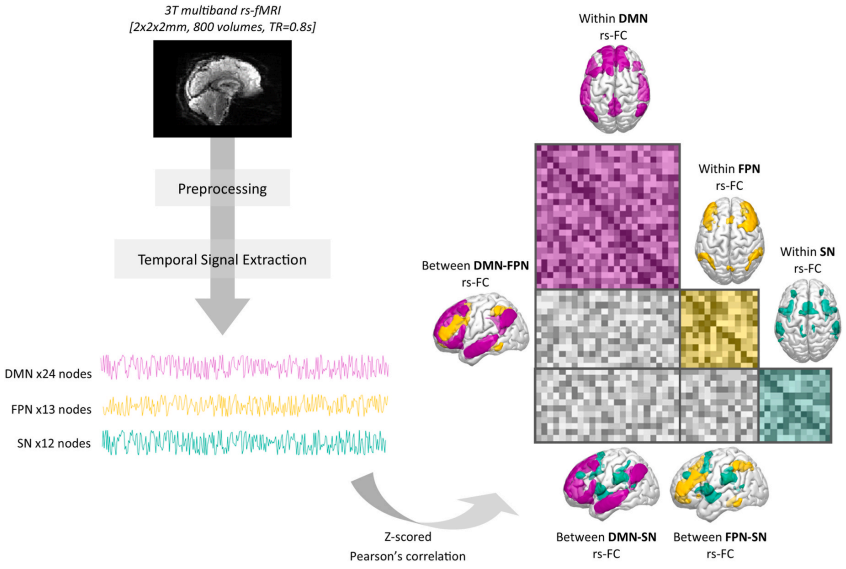

Fig. 1. Acquisition and preprocessing fMRI data methodology. Firstly, the participants underwent resting-state functional MRI (rs-fMRI) multiband (anterior-posterior phase-encoding; acceleration factor = 8) interleaved acquisitions (T2*weighted EPI scans, 2 × 2x2mm, 800 vol, TR = 800 ms). Secondly, after the fMRI data preprocessing, blood-oxygen-level-dependent signal was extracted and averaged across all voxels falling within each region of interest (ROI; i.e., node). Then, ROI-to-ROI connectivity were computed as Pearson correlations and subsequently Fisher-z transformed. The resulting 49 × 49 connectivity matrices per subject were averaged into three within and three between network connectivity measures as defined in the Schaefer-Yeo atlas. Lastly, within network rs-FC values were computed as the average rs-FC connecting all the nodes within the same network while between network rs-RC values were computed as the average rs-FC connecting a pair of networks. Graphical representation of the volumes shaping each network were created with Surf Ice tool (https://www.nitrc.org/projects/surfice/). DMN is represented in pink, FPN in yellow and SN in light blue. Abbreviations: Default Mode Network, DMN; Frontoparietal Networks, FPN; Salience Network, SN.

within our sample. It is favored for multiple comparison correction due to its effective balance between FDR control and increased statistical power. Furthermore, its adaptability to different study designs, widespread acceptance, and broad applicability across various fields contribute to its popularity and utilization in our study. In resume, multiple comparisons were corrected for using Bejamini and Hochberg's FDR (Benjamini & Hochberg, 1995), Multiple comparisons were corrected at a *q* value of 0.05, after pooling the *p* values from the regression analyses for each outcome model.

After the first round of analyses, mediation analyses using the R package 'mediation' (Tingley, Yamamoto, Hirose, Keele, & Imai, 2014) were performed to assess whether functional connectivity within DMN and between SN-FPN mediated the associations between CRF and stress, taking into account all covariates (current age, biological sex, education, SES, time between assessments and FWD). The total effects (effect of X on Y), direct effects (effect of X on Y taking into account M(ADE)) and indirect effects (or 'mediation effect', the total effect minus the direct effect (ACME)) are reported. The presence of statistical mediation was determined through nonparametric bootstrap confidence intervals via 1000 bootstrap resamples of the estimated indirect effect. The estimated indirect (ACME) effect corresponds to the reduction in the independent variable effect on the dependent variable when adjusted for the mediator. For the direct and total effects, the estimate is interpreted as per 1-unit (1 CRF unit) increase.

3. Results

A total of 418 (197 female) participants with a mean ± standard deviation (SD) of 53.21 ± 6.85 years (range 40–65 years) completed the study. All data was collected prior to the COVID-19 global pandemic. Our sample is generally characterized by highly educated (70%), and mentally healthy individuals. Range scores for Depression, Anxiety and Stress sub-scales of the DASS suggest on average the sample reports between normal and mild levels for the population. Full demographic information is found in Table 1.

Table 1
Participant characteristics.

Age (Mean ± SD), y	53.21 ± 6.85
Biological sex (N (%))	
Male	221 (52)
Female	197 (48)
Education (N (%))	
Primary	7 (2)
Secondary	119 (28)
Higher	292 (70)
BMI (N (%))	
Normal	225 (54)
Overweight	143 (34)
Obesity	50 (12)
Mental Health profile (mean ± SD)	
Total DASS	15.85 ± 15.86
Depression	4.61 ± 5.86
Anxiety	3.15 ± 4.90
Stress	8.08 ± 7.20
Fitness evaluation	
VO_2peak (Mean ± SD), mL/kg/min	25.07 ± 7.00

SD: standard deviation. See methods section for range values of DASS scores for interpretation.

3.1. Associations between VO_2peak and mental health

A significant negative correlation between VO_2peak and anxiety ($\beta = -0.111$, $p = 0.017$) and stress ($\beta = -0.242$, $p = 0.002$) sub-scales was found, which all remained significant after false discovery rate corrections (false discovery rate $p = 0.025$; $p = 0.006$, respectively) (see Table 2 and Fig. 2).

3.2. Associations between VO_2 peak and functional connectivity

VO_2 peak was positively associated with the connectivity strength within the Default Mode Network (DMN) ($\beta = 0.195$, $p = 0.002$*), Salience Network (SN) ($\beta = 0.143$, $p = 0.026$*), and Frontoparietal Network (FPN) ($\beta = 0.133$, $p = 0.036$*), which all remained significant after false discovery rate corrections (false discovery rate $p = 0.006$, $p = 0.39$, $p = 0.041$, respectively) (Table 3a, Fig. 3). For functional connectivity between networks, VO_2 peak was positively associated with DMN – FPN ($\beta = 0.130$, $p = 0.041$*), DMN – SN ($\beta = 0.67$, $p = 0.006$*) and FPN – SN ($\beta = 0.187$, $p = 0.002$*) which both remained significant after false discovery rate corrections (false discovery rate $p = 0.041$, $p = 0.012$, $p = 0.006$, respectively) (Table 3b, Fig. 3).

3.3. Associations between functional connectivity and mental health

The DMN within functional connectivity was negatively associated with stress (($\beta = -0.126$, $p = 0.011$) which remained significant after false discovery rate corrections (false discovery rate $p = 0.048$) (Fig. 4), whereas the relationships with the rest of mental health constructs were not statistically significant (Table 4a).

The FPN within functional connectivity was negatively associated with stress (($\beta = -0.112$, $p = 0.025$) which did not remain significant after false discovery rate corrections (false discovery rate $p = 0.050$) (Fig. 4), whereas the relationships with the rest of mental health constructs were not statistically significant (Table 4b).

Whereas the relationships between SN within connectivity with the mental health constructs were not statistically significant (Table 4c).

The connectivity between DMN and SN with mental health constructs were not statistically significant whereas the connectivity between FPN and SN and the connectivity between DMN and FPN were negatively associated with stress ($\beta = -0.123$, $p = 0.016$; $\beta = -0.102$, $p = 0.041$, respectively) which FPN-SN remained significant after false discovery rate corrections (false discovery rate $p = 0.048$) whereas DMN-FPN did not remain significant after controlling by multiple comparisons (false discovery rate $p = 0.061$) (Table 4d, 4e and 4f).

3.4. Mediation results

The mediation results showed that the DMN within connectivity ($p = 0.040$) and the connectivity between FPN – SN ($p = 0.036$) mediated the relationship between VO_2 peak and stress (Fig. 5). The relationship between CRF and functional connectivity (X [predictor variable] on M ([mediator]) are found in Table 5 along with full mediation model results.

Table 2
Associations between CRF and mental health.

	β	SE	*P*	R^2
Depression	−0.099	0.055	0.073	0.057
Anxiety	−0.111	0.046	0.017[a]	0.043
Stress	−0.242	0.066	0.002[a]	0.076

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status, and time between assessments. R^2 is adjusted for all predictors.
[a] Survives FDR corrections.

4. Discussion

In the present study, in line with previous hypotheses, higher CRF was found to be associated with lower scores on anxiety and stress scales. Additionally, significant correlations were observed between CRF and the triple network model, indicating that the functional connectivity interactions of the DMN, SN, and FPN partially explain the relationship between CRF and mental health. Specifically, CRF was positively associated with rs-FC of the DMN and between DMN-SN and FPN-SN in middle-aged adults. Furthermore, mediation analyses revealed that within connectivity of DMN and between FPN-SN mediated the relationship between CRF and stress.

Firstly, our results are in line with previous reports, showing that low CRF is related to anxiety (Williams, Carroll, Veldhuijzen van Zanten, & Ginty, 2016), and stress (Blumenburg et al., 2021; Lindegård et al., 2019). We extend previous knowledge in an important way. Although the effect of CRF on mental health in clinical population has been reported (Bueno-Antequera & Munguía-Izquierdo, 2020a, 2020b; Gujral et al., 2019), the effect of these relations in healthy middle-aged adults is novel. Under certain assumptions and extending previous reports (Dishman et al., 2012), our result could promote further studies in healthy middle-aged adults on the effect of interventions to improve or maintain CRF as a means to protect against the onset of mental illnesses, poorer cognitive functioning and lower emotional well-being as a result of a differential chronic exposure of stressful major life events during midlife (Thoits, 2010) whereby declines in CRF typically accelerates (Ades & Toth, 2005). Furthermore, research has indicated that age differences significantly influence daily stress patterns and impacts cognitive strategies to cope with them (Stawski, Sliwinski, Almeida, & Smyth, 2008). Our results shed light in how CRF can mitigate the negative consequences of aging on brain health providing valuable insights into the factors associated with overall well-being and mental health throughout adulthood.

Our findings linking CRF in midlife to the functional connectivity of the DMN, FPN and SN, and subsequently stress, are in line with previous reports that have suggested that physical activity (as measured via self-report or objective monitoring) and aerobic exercise might be effective at preserving or strengthening functional connectivity within and between large-scale brain networks (Damoiseaux et al., 2008; Stillman, Donofry, & Erickson, 2019; Tomasi & Volkow, 2012). Specifically, reduced intra- and inter-network signal coherence may underlie the development and progression of cognitive emotion regulation difficulties which can potentially lead to the onset of anxiety and depressive symptoms in older adults. The salient system plays an important role in attentional capture of biologically and cognitively relevant events and in the subsequent engagement of frontoparietal systems for working memory and higher-order cognitive control (Gallen et al., 2016; Seeley et al., 2007), since the dynamic interactions between these two networks regulate shifts in attention and access to domain-general and domain-specific cognitive resources (Kelly, Uddin, Biswal, Castellanos, & Milham, 2008) and that these processes have important implications for psychopathology disorders involving dysfunctional saliency processing which can lead to aberrant allocation of attentional resources and consequently to diminished goal-relevant cognitive capabilities (Menon, 2011). It is plausible that an imbalanced connectivity between the FPN and SN may indicate a top-down regulation problem in the triple network paradigm and may explain why perceived stress and other mental health complains are characterized by disengaging from irrational internal thoughts or correcting their internal concept with external evidence leading eventually in deficits in regulating and modulating mood (Gürsel et al., 2020). It is important therefore to identify interventions and specific modifiable lifestyles that may slow or reverse these functional changes and contribute to psychological resilience in middle-aged adults (Cabello-Toscano et al., 2022; Stillman et al., 2019). What's more, the DMN, FPN and SN appear to be most sensitive to the deleterious effects of aging relative to other large scale

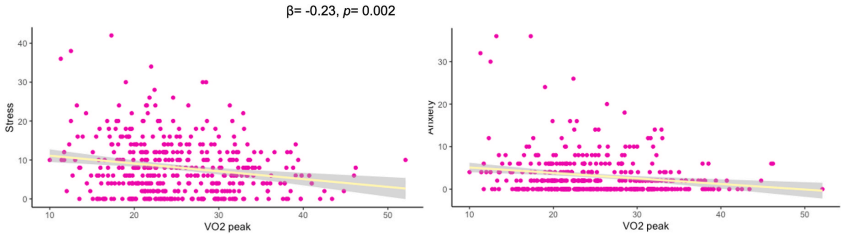

Fig. 2. Significant negative relationships between VO_2 peak and mental health constructs after controlling for age, biological sex, education, waist circumference, and socioeconomic status. Survive false discovery rate multiple comparison corrections.

Table 3a
Associations between CRF and within functional connectivity.

	β	SE	P	R^2
Salience network	0.143	0.001	0.026[a]	0.06
Default mode network	0.195	0.001	0.002[a]	0.056
Frontoparietal network	0.133	0.001	0.036[a]	0.035

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status and fwd mean. R^2 is adjusted for all predictors.
[a] survives FDR corrections.

networks (Andrews-Hanna et al., 2007; Bluhm et al., 2008; Campbell et al., 2012; Damoiseaux et al., 2008; Lee et al., 2020; Razlighi et al., 2014; Voss, Prakash, et al., 2010). Additionally, these same networks are also sensitive to change via aerobic exercise (Talukdar et al., 2018; Voelcker-Rehage & Niemann, 2013; Voss et al., 2016; Voss, Prakash, et al., 2010). For example, prior studies showed that greater CRF predicts greater within DMN and FPN functional connectivity in older adults (Voss, Erickson, et al., 2010; Voss et al., 2016) and that these changes may subserve improvements in cognitive performance in older individuals with and without cognitive impairments (Won et al., 2023). We extend those results to a healthy middle-aged population.

Prior research suggests that disruptions in the communication and dynamic interaction of DMN, FPN and SN, known as the Triple Network Model, have been implicated in numerous neuropsychiatric disorders (Menon, 2011; Northoff, 2020; Whitfield-Gabrieli & Ford, 2012). However, in our sample, only stress was negatively associated with functional connectivity of the DMN and between FPN-SN, which is potentially explained by the relative healthiness of our sample and low scoring in DASS-21 scale. Based on the age range (midlife) and the health status (few participants reporting high or severe levels of any

Table 3b
Associations between CRF and functional connectivity between networks.

	β	SE	P	R^2
Default mode network - Frontoparietal	0.130	0.001	0.041[a]	0.049
Default mode network - Salience	0.167	0.001	0.006[a]	0.113
Frontoparietal network - Salience	0.187	0.001	0.002[a]	0.101

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status and fwd mean. R^2 is adjusted for all predictors.
[a] survives FDR corrections

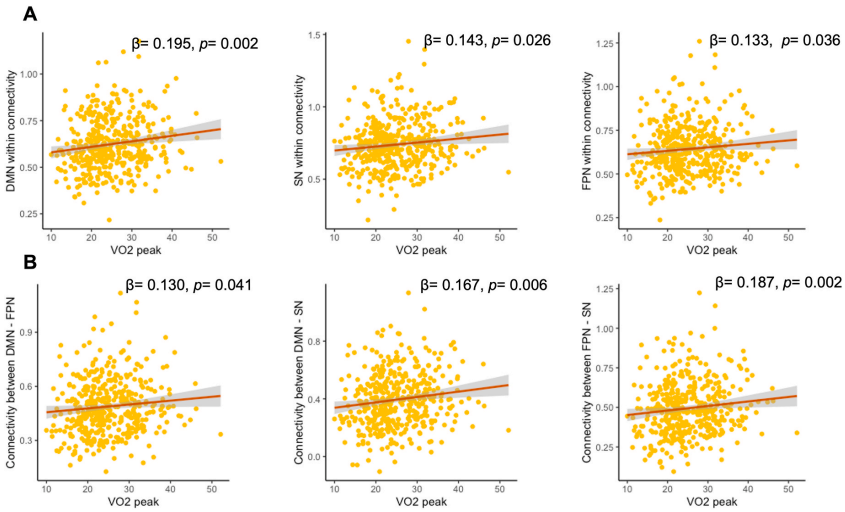

Fig. 3. **(A)** Significant positive relationship between VO_2 peak and within connectivity and **(B, C)** connectivity between networks after controlling for age, biological sex, education, socioeconomic status, waist circumference, and mean FWD. Survives false discovery rate multiple comparison correction.

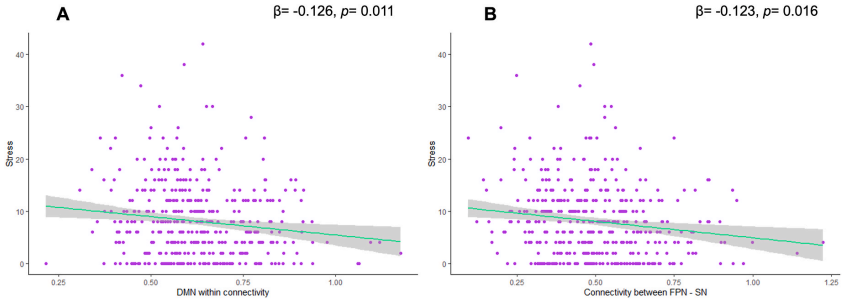

Fig. 4. **(A)** Significant negative relationship between within DMN connectivity and stress and **(B)** the connectivity between FPN-SN and stress after controlling for age, biological sex, education, waist circumference, socioeconomic status, time between assessments and mean FWD. Survive false discovery rate multiple comparison corrections.

Table 4a
Associations between within DMN connectivity and mental health.

	β	SE	*P*	R^2
Depression	−0.055	2.093	0.269	0.05
Anxiety	−0.018	1.781	0.718	0.027
Stress	−0.126	2.529	0.011[a]	0.057

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status, time between assessments and fwd mean. R^2 is adjusted for all predictors.
[a] Survives FDR corrections.

Table 4b
Associations between within FPN connectivity and mental health.

	β	SE	*P*	R^2
Depression	−0.083	2.049	0.096	0.054
Anxiety	−0.050	1.745	0.322	0.029
Stress	−0.112	2.485	0.025[a]	0.054

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status, time between assessments and fwd mean. R^2 is adjusted for all predictors.
[a] Not survives FDR corrections.

Table 4c
Associations between within SN connectivity and mental health.

	β	SE	*P*	R^2
Depression	−0.055	1.781	0.271	0.05
Anxiety	0.003	1.515	0.943	0.027
Stress	−0.078	2.163	0.122	0.048

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status, time between assessments and fwd mean. R^2 is adjusted for all predictors..

Table 4d
Associations between DMN-SN and mental health.

	β	SE	P	R^2
Depression	−0.067	1.575	0.183	0.049
Anxiety	0.009	1.369	0.857	0.027
Stress	−0.096	1.952	0.062	0.050

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status, time between assessments and fwd mean. R2 is adjusted for all predictors. *survives FDR corrections.

Table 4e
Associations between FPN-SN and mental health.

	β	SE	P	R^2
Depression	−0.094	1.776	0.065	0.056
Anxiety	−0.030	1.507	0.559	0.028
Stress	−0.123	2.142	0.016[a]	0.056

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status, time between assessments and fwd mean. R^2 is adjusted for all predictors.
[a] survives FDR corrections

DASS-21 sub-scale) observed in our sample, an alternative interpretation considers the concept that there exists underlying brain changes that occur prior to the clinical or behavioral manifestation of certain symptoms (Beason-Held et al., 2013). These results suggest that the combination of higher integration of the DMN and increased synchrony of SN-FPN can potentially lead to a better mental health status during midlife. In other words, life stressors may lead to individual variations in the functional connectivity of specific intrinsic resting state networks, as revealed by our results, before the onset of clinically significant symptoms related to depression and anxiety. Our results strengthen the importance of engaging in modifiable lifestyle behaviors relevant for the promotion and maintenance of brain health in early midlife (Di Marco et al., 2014). That is, our results potentially suggest that the inter-individual differences in how FC of the triple-network model networks relates to stress in our sample of middle-aged adults are partly explained by variations in CRF.

Thus, considering stress as a potential precursor for depression and anxiety (Dias-Ferreira et al., 2009; Marin et al., 2011), our findings indicate a selective association (and mediation) between functional connectivity and stress, which may capture a relationship at the early stages of development within our middle-aged and relatively healthy population. If poor CRF in midlife subsequently predicts worse outcomes in depression and anxiety later in life, our results provide mechanistic

Table 4f
Associations between DMN-FPN and mental health.

	β	SE	P	R^2
Depression	−0.032	1.919	0.519	0.049
Anxiety	−0.001	1.632	0.981	0.027
Stress	−0.102	2.323	0.041[a]	0.052

All models are controlling for age, biological sex, education, waist circumference, socioeconomic status, time between assessments and fwd mean. R^2 is adjusted for all predictors.
[a] Not survives FDR corrections.

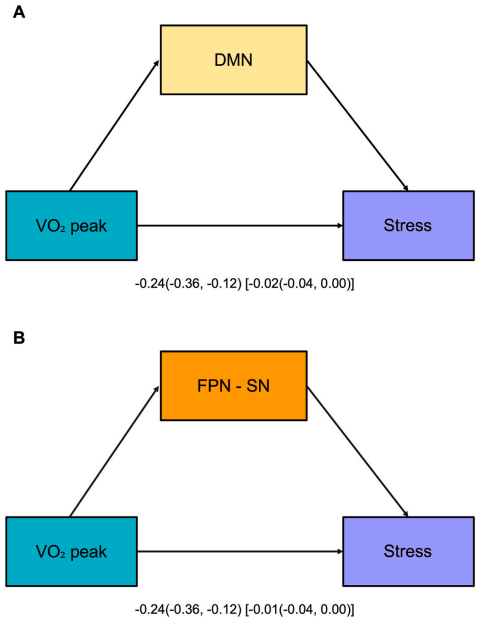

Fig. 5. (A) Within DMN connectivity mediated the relationship between X (predictor) and Y (mental health). **(B)** The connectivity between FPN and SN mediated the relationship between X (predictor) and Y (mental health). The total effect (X on Y) is seen under the horizontal arrow representing the β coefficient followed by the 95% CIs in parentheses. The average causal mediation effect (X [predictor variable] on Y [outcome variable] including M [mediator]) is seen between square brackets following the direct effect. The mediated effect is calculated as the difference between the estimates from the total and direct effects (see Table 4) which correspond to the reduction in the independent variable(X) effect on the dependent variable (Y) when adjusted for the mediator (M).

Table 5
Mediation model between V0₂ peak, functional connectivity, and stress.

	Total effect	ADE	ACME
Outcomes	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Default mode network	−0.24 (-0.36, −0.12)*	−0.22 (-0.34, −0.10)*	−0.02 (-0.04,-0.00)*
Prefrontal - Salience network	−0.24 (-0.36, −0.12)*	−0.22 (-0.35, −0.10)*	−0.01 (-0.04,-0.00)*

The model is adjusted for age, education, biological sex, waist circumference, FDW, time between assessments and socioeconomic status. ADE = average direct effect; ACME = average causal mediation effect. Statistical significance at $p < 0.05$ and 95% CI not including 0.

evidence supporting the importance of promoting and maintaining CRF as individuals age, as it relates to mental health outcomes in the aging process. One theoretical framework for how these interactions arise could include variations in coping strategies. For example, coping strategies have been found to attenuate the impact of perceived stress on mental health (Cabello-Toscano et al., 2022). What is more, functional segregation of the FPN and DMN were found to modulate the impact of perceived stress on mental health. In other words, their results highlight the role of DMN and FPN connectivity balance, as part of the triple network model, in attenuating the impact of perceived stress (Cabello-Toscano et al., 2022). Importantly high levels of CRF later in life are linked to selective enhancement of coping strategies in front of stressful situations (Gerber & Pühse, 2009). On this basis, our results suggest the importance of maintaining good levels of CRF in midlife to confront plausible midlife stressors. Future longitudinal investigations are necessary to test if changes/maintenance of CRF are linked to long-term changes/maintenance of mental health.

Our results should be interpreted in light of several considerations. Firstly, our results are derived from cross-sectional data yet the relation between lifestyle and the brain is likely bi-directional (Audiffren & André, 2019; Cheval et al., 2020, Morris et al., 2022) (see supplementary materials for extended analyses) and it was not possible to make any kind of inference about causal relationships. Second, our sample is generally characterized by white, highly educated, and healthy from a cardiovascular standpoint. Future studies are encouraged to measure these relations in a more heterogeneous population. Third, the stress evaluation was based only on a neuropsychological scale without complementary assessment of biological markers as hair and salivary cortisol levels. Therefore, our results are limited to associations with perceived stress and mental health outcomes. Fourth, the lack of measures of adiposity is also an important limitation in our study due to weight status has been widely associated with mental health outcomes in adults (Avila et al., 2015; Rajan & Menon, 2017) and excess adiposity has been also related to altered FC patterns within and/or between the DMN, FPN and SN (Donofry, Stillman, & Erickson, 2020). Further studies are encouraged to measure adiposity levels and normalize VO_2 peak to fat-free mass and its relationships with mental health and functional connectivity patterns. Fifth, intrinsic resting scale networks have shown a significant variability in psychiatric and neurological disorders providing a whole-brain approach to understand how large-scale networks relate to behaviors. The ease of interpretation of large-scale network connectivity and their ubiquitous use in the literature allows us to study and subsequently report the results of functional networks as they relate to fitness and mental health to a field of researchers that may not be that familiar with functional connectivity analyses. Nevertheless, several approaches with rs-FC could have been taken in this study and are highly recommended in future investigations including ROI-to-ROI and seed-based analyses. Sixth, an additional potential limitation was related to time between questionnaires and in-person assessments. To help account for this limitation we used this time gap as a covariate in all our analyses, given how brain connectivity, mental health scores, and exercise fitness can change over time. Notwithstanding we cannot exclude the possibility that the temporal period between assessments introduced some bias in the results. Lastly, we did not assess other potential factors that may influence the observed relationships such as cognition, diet, sleep patterns, physical activity levels, sedentary behaviors, and motor skills. We did analyze the correlation in our sample between self-reported levels of physical activity measured by the International Physical Activity Questionnaire (IPAQ) and CRF values. The result was a significant positive correlation between self-reported physical activity levels and CRF values (Supplementary Material Section 1) providing evidence of the modifiable nature of CRF.

Taken together, our findings show a significant relation between CRF and mental health in healthy middle-aged adults, which is mediated by functional brain connectivity of the DMN and inter-connectivity of FPN-SN. Furthermore, we shed light on a potential mechanistic pathway (within connectivity of DMN and between FPN-SN) that may contribute to this relationship. The implications of our study lie within the potential importance of engaging in modifiable lifestyles behaviors that can promote brain and mental health in middle-aged adults. In conclusion, maintaining high CRF may be a modifiable interventional target to confront typical life stresses during midlife.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mhpa.2023.100552.

References

Adams, R. (1999). Revised physical activity readiness questionnaire. *Canadian Family Physician Medecin De Famille Canadien, 45*(995), 1004–1005, 992.

Ades, P. A., & Toth, M. J. (2005). Accelerated decline of aerobic fitness with healthy aging. *Circulation, 112*(5), 624–626. https://doi.org/10.1161/CIRCULATIONAHA.105.553321

American Psychological Association, (APA). (2020). *Stress in America™ 2020: A national mental health crisis*. https://www.apa.org/news/press/releases/stress/2020/report-october.

Andrews-Hanna, J. R., Snyder, A. Z., Vincent, J. L., Lustig, C., Head, D., Raichle, M. E., et al. (2007). Disruption of large-scale brain systems in advanced aging. *Neuron, 56*(5), 924–935. https://doi.org/10.1016/j.neuron.2007.10.038

Arenaza-Urquijo, E. M., Wirth, M., & Chételat, G. (2015). Cognitive reserve and lifestyle: Moving towards preclinical Alzheimer's disease. *Frontiers in Aging Neuroscience, 7*, 134. https://doi.org/10.3389/fnagi.2015.00134

Arós, F., Boraita, A., Alegría, E., Alonso, A. M., Bardají, A., Lamiel, R., et al. (2000). [Guidelines of the Spanish Society of Cardiology for clinical practice in exercise testing]. *Revista Espanola De Cardiologia, 53*(8), 1063–1094.

Aspenes, S. T., Nilsen, T. I. L., Skaug, E.-A., Bertheussen, G. F., Ellingsen, Ø., Vatten, L., et al. (2011). Peak oxygen uptake and cardiovascular risk factors in 4631 healthy women and men. *Medicine & Science in Sports & Exercise, 43*(8), 1465–1473. https://doi.org/10.1249/MSS.0b013e31820ca81c

Audiffren, M., & André, N. (2019). The exercise-cognition relationship: A virtuous circle. *Journal of Sport and Health Science, 8*(4), 339–347. https://doi.org/10.1016/j.jshs.2019.03.001

Avila, C., Holloway, A. C., Hahn, M. K., Morrison, K. M., Restivo, M., Anglin, R., et al. (2015). An overview of links between obesity and mental health. *Current Obesity Reports, 4*(3), 303–310. https://doi.org/10.1007/s13679-015-0164-9

Barlow, D. H. (2004). *Anxiety and its disorders: The nature and treatment of anxiety and panic*. Guilford press.

Barnett, K., Mercer, S. W., Norbury, M., Watt, G., Wyke, S., & Guthrie, B. (2012). Epidemiology of multimorbidity and implications for health care, research, and medical education: A cross-sectional study. *Lancet (London, England), 380*(9836), 37–43. https://doi.org/10.1016/S0140-6736(12)60240-2

Basten, U., Stelzel, C., & Fiebach, C. J. (2011). Trait anxiety modulates the neural efficiency of inhibitory control. *Journal of Cognitive Neuroscience, 23*(10), 3132–3145. https://doi.org/10.1162/jocn_a_00003

Beason-Held, L. L., Goh, J. O., An, Y., Kraut, M. A., O'Brien, R. J., Ferrucci, L., et al. (2013). Changes in brain function occur years before the onset of cognitive impairment. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 33*(46), 18008–18014. https://doi.org/10.1523/JNEUROSCI.1402-13.2013

Beck, A. T., & Beck, A. T. (1967). *Depression: Clinical, experimental and theoretical aspects theoretical aspects*. New York: Harper and Row. New York: Harper and Row.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B, 57*(1), 289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bluhm, R. L., Osuch, E. A., Lanius, R. A., Boksman, K., Neufeld, R. W. J., Théberge, J., et al. (2008). Default mode network connectivity: Effects of age, sex, and analytic approach. *NeuroReport, 19*(8), 887–891.

Blumenburg, W. T., Frederick, J. M., Cross, B. L., Culver, M. N., McMillan, N. K., Montoye, A. H., et al. (2021). Physical fitness, but not physical activity, is associated with mental health in apparently healthy young adults. *The Journal of Sports Medicine and Physical Fitness*. https://doi.org/10.23736/S0022-4707.21.13115-9

Bolsinger, J., Seifritz, E., Kleim, B., & Manoliu, A. (2018). Neuroimaging correlates of resilience to traumatic events-A comprehensive review. *Frontiers in Psychiatry, 9*, 693. https://doi.org/10.3389/fpsyt.2018.00693

Borg, G. A. (1974). Perceived exertion. *Exercise and Sport Sciences Reviews, 2*, 131–153.

Brown, T. A., Chorpita, B. F., Korotitsch, W., & Barlow, D. H. (1997). Psychometric properties of the depression anxiety stress scales (DASS) in clinical samples. *Behaviour Research and Therapy, 35*(1), 79–89. https://doi.org/10.1016/s0005-7967(96)00068-x

Buckner, R. L., Andrews-Hanna, J. R., & Schacter, D. (2008). The brain's default network: Anatomy, function, and relevance to disease. *Annals of the New York Academy of Sciences - Wiley Online Library, 1124*(Issue 1), 1–38.

Bueno-Antequera, J., & Munguía-Izquierdo, D. (2020a). Exercise and depressive disorder. *Advances in Experimental Medicine and Biology, 1228*, 271–287. https://doi.org/10.1007/978-981-15-1792-1_18

Bueno-Antequera, J., & Munguía-Izquierdo, D. (2020b). Exercise and schizophrenia. *Advances in Experimental Medicine and Biology, 1228*, 317–332. https://doi.org/10.1007/978-981-15-1792-1_21

Cabello-Toscano, M., Vaqué-Alcázar, L., Cattaneo, G., Solana-Sánchez, J., Bayes-Marin, I., Abellaneda-Pérez, K., et al. (2022). Functional brain connectivity prior to the COVID-19 outbreak moderates the effects of coping and perceived stress on mental health changes. A first year of COVID-19 pandemic follow-up study. *Biological Psychiatry. Cognitive Neuroscience and Neuroimaging, S2451–9022*(22). https://doi.org/10.1016/j.bpsc.2022.08.005, 00188-4.

Campbell, K. L., Grady, C. L., Ng, C., & Hasher, L. (2012). Age differences in the frontoparietal cognitive control network: Implications for distractibility. *Neuropsychologia, 50*(9), 2212–2223. https://doi.org/10.1016/j.neuropsychologia.2012.05.025

Cattaneo, G., Bartrés-Faz, D., Morris, T. P., Sánchez, J. S., Macià, D., Tarrero, C., et al. (2018). The Barcelona brain health initiative: A cohort study to define and promote determinants of brain health. *Frontiers in Aging Neuroscience, 10*, 321. https://doi.org/10.3389/fnagi.2018.00321

Cattaneo, G., Bartrés-Faz, D., Morris, T. P., Solana Sánchez, J., Macià, D., Tormos, J. M., et al. (2020). The Barcelona brain health initiative: Cohort description and first follow-up. *PLoS One, 15*(2), Article e0228754. https://doi.org/10.1371/journal.pone.0228754

Chan, M. Y., Park, D. C., Savalia, N. K., Petersen, S. E., & Wig, G. S. (2014). Decreased segregation of brain systems across the healthy adult lifespan. *Proceedings of the National Academy of Sciences of the United States of America, 111*(46), E4997–E5006. https://doi.org/10.1073/pnas.1415122111

Cheval, B., Orsholits, D., Sieber, S., Courvoisier, D., Cullati, S., & Boisgontier, M. P. (2020). Relationship between decline in cognitive resources and physical activity. *Health Psychology: Official Journal of the Division of Health Psychology, American Psychological Association, 39*(6), 519–528. https://doi.org/10.1037/hea0000857

Colcombe, S. J., Erickson, K. I., Raz, N., Webb, A. G., Cohen, N. J., McAuley, E., et al. (2003). Aerobic fitness reduces brain tissue loss in aging humans. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences, 58*(2), 176–180. https://doi.org/10.1093/gerona/58.2.m176

Colcombe, S. J., Erickson, K. I., Scalf, P. E., Kim, J. S., Prakash, R., McAuley, E., et al. (2006). Aerobic exercise training increases brain volume in aging humans. *Journals of Gerontology - Series A Biological Sciences and Medical Sciences, 61*(11). https://doi.org/10.1093/gerona/61.11.1166. Article 11.

Colcombe, S., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: A meta-analytic study. *Psychological Science, 14*(2), 125–130. https://doi.org/10.1111/1467-9280.t01-1-01430

Damoiseaux, J. S., Beckmann, C. F., Arigita, E. J. S., Barkhof, F., Scheltens, P., Stam, C. J., et al. (2008). Reduced resting-state brain activity in the "default network" in normal aging. *Cerebral Cortex (New York, N.Y.: 1991, 18*(8), 1856–1864. https://doi.org/10.1093/cercor/bhm207

Di Marco, L. Y., Marzo, A., Muñoz-Ruiz, M., Ikram, M. A., Kivipelto, M., Ruefenacht, D., et al. (2014). Modifiable lifestyle factors in dementia: A systematic review of longitudinal observational cohort studies. *Journal of Alzheimer's Disease: JAD, 42*(1), 119–135. https://doi.org/10.3233/JAD-132225

Dias-Ferreira, E., Sousa, J. C., Melo, I., Morgado, P., Mesquita, A. R., Cerqueira, J. J., et al. (2009). Chronic stress causes frontostriatal reorganization and affects decision-

making. *Science (New York, N.Y.), 325*(5940), 621–625. https://doi.org/10.1126/science.1171203
van Dijk, K. R. A., Sabuncu, M. R., & Buckner, R. L. (2012). The influence of head motion on intrinsic functional connectivity MRI. *NeuroImage, 59*(1). https://doi.org/10.1016/j.neuroimage.2011.07.044
Dishman, R. K., Sui, X., Church, T. S., Hand, G. A., Trivedi, M. H., & Blair, S. N. (2012). Decline in cardiorespiratory fitness and odds of incident depression. *American Journal of Preventive Medicine, 43*(4), 361–368. https://doi.org/10.1016/j.amepre.2012.06.011
Donofry, S. D., Stillman, C. M., & Erickson, K. I. (2020). A review of the relationship between eating behavior, obesity and functional brain network organization. *Social Cognitive and Affective Neuroscience, 15*(10), 1157–1181. https://doi.org/10.1093/scan/nsz085
Erickson, K. I., Prakash, R. S., Voss, M. W., Chaddock, L., Hu, L., Morris, K. S., et al. (2009). Aerobic fitness is associated with hippocampal volume in elderly humans. *Hippocampus, 19*(10). https://doi.org/10.1002/hipo.20547. Article 10.
España-Irla, G., Gomes-Osman, J., Cattaneo, G., Albu, S., Cabello-Toscano, M., Solana-Sanchéz, J., et al. (2021). Associations between cardiorespiratory fitness, cardiovascular risk, and cognition are mediated by structural brain health in midlife. *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease, 10*(18), Article e020688. https://doi.org/10.1161/JAHA.120.020688
Etkin, A., Prater, K. E., Schatzberg, A. F., Menon, V., & Greicius, M. D. (2009). Disrupted amygdalar subregion functional connectivity and evidence of a compensatory network in generalized anxiety disorder. *Archives of General Psychiatry, 66*(12), 1361–1372. https://doi.org/10.1001/archgenpsychiatry.2009.104
Fischl, B., Salat, D. H., Busa, E., Albert, M., Dieterich, M., Haselgrove, C., et al. (2002). Whole brain segmentation: Automated labeling of neuroanatomical structures in the human brain. *Neuron, 33*(3). https://doi.org/10.1016/S0896-6273(02)00569-X
Frankish, H., & Horton, R. (2017). Prevention and management of dementia: A priority for public health. *Lancet (London, England), 390*(10113), 2614–2615. https://doi.org/10.1016/S0140-6736(17)31756-7
Gallen, C. L., Baniqued, P. L., Chapman, S. B., Aslan, S., Keebler, M., Didehbani, N., et al. (2016). Modular brain network organization predicts response to cognitive training in older adults. *PLoS One, 11*(12), Article e0169015. https://doi.org/10.1371/journal.pone.0169015
Geerligs, L., Maurits, N. M., Renken, R. J., & Lorist, M. M. (2014). Reduced specificity of functional connectivity in the aging brain during task performance. *Human Brain Mapping, 35*(1), 319–330. https://doi.org/10.1002/hbm.22175
Gerber, M., & Pühse, U. (2009). Review article: Do exercise and fitness protect against stress-induced health complaints? A review of the literature. *Scandinavian Journal of Public Health, 37*(8), 801–819. https://doi.org/10.1177/1403494809350522
Goulden, N., Khusnulina, A., Davis, N. J., Bracewell, R. M., Bokde, A. L., McNulty, J. P., et al. (2014). The salience network is responsible for switching between the default mode network and the central executive network: Replication from DCM. *NeuroImage, 99*, 180–190. https://doi.org/10.1016/j.neuroimage.2014.05.052
Grady, C. (2012). The cognitive neuroscience of ageing. *Nature Reviews Neuroscience, 13*(7), 491–505. https://doi.org/10.1038/nrn3256
Gujral, S., Aizenstein, H., Reynolds, C. F., Butters, M. A., Grove, G., Karp, J. F., et al. (2019). Exercise for depression: A feasibility trial exploring neural mechanisms. *American Journal of Geriatric Psychiatry: Official Journal of the American Association for Geriatric Psychiatry, 27*(6), 611–616. https://doi.org/10.1016/j.jagp.2019.01.012
Gürsel, D. A., Reinholz, L., Bremer, B., Schmitz-Koep, B., Franzmeier, N., Avram, M., et al. (2020). Frontoparietal and salience network alterations in obsessive–compulsive disorder: Insights from independent component and sliding time window analyses. *Journal of Psychiatry & Neuroscience : JPN, 45*(3), 214–221. https://doi.org/10.1503/jpn.190038
Hawkins, M. N., Raven, P. B., Snell, P. G., Stray-Gundersen, J., & Levine, B. D. (2007). Maximal oxygen uptake as a parametric measure of cardiorespiratory capacity. *Medicine & Science in Sports & Exercise, 39*(1), 103–107. https://doi.org/10.1249/01.mss.0000241641.75101.64
Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology, 34*(2), 163–171. https://doi.org/10.1016/j.psyneuen.2008.10.026
Henry, J. D., & Crawford, J. R. (2005). The short-form version of the Depression Anxiety Stress Scales (DASS-21): Construct validity and normative data in a large non-clinical sample. *British Journal of Clinical Psychology, 44*(Pt 2), 227–239. https://doi.org/10.1348/014466505X29657
Hermans, E. J., Henckens, M. J. A. G., Joëls, M., & Fernández, G. (2014). Dynamic adaptation of large-scale brain networks in response to acute stressors. *Trends in Neurosciences, 37*(6), 304–314. https://doi.org/10.1016/j.tins.2014.03.006
Kelly, A. M. C., Uddin, L. Q., Biswal, B. B., Castellanos, F. X., & Milham, M. P. (2008). Competition between functional brain networks mediates behavioral variability. *NeuroImage, 39*(1), 527–537. https://doi.org/10.1016/j.neuroimage.2007.08.008
Kodama, S., Saito, K., Tanaka, S., Maki, M., Yachi, Y., Asumi, M., et al. (2009). Cardiorespiratory fitness as a quantitative predictor of all-cause mortality and cardiovascular events in healthy men and women: A meta-analysis. *JAMA, 301*(19), 2024–2035. https://doi.org/10.1001/jama.2009.681
Kramer, A. F., & Colcombe, S. (2018). Fitness effects on the cognitive function of older adults: A meta-analytic study-revisited. *Perspectives on Psychological Science: A Journal of the Association for Psychological Science, 13*(2), 213–217. https://doi.org/10.1177/1745691617707316
Landau, S. M., Marks, S. M., Mormino, E. C., Rabinovici, G. D., Oh, H., O'Neil, J. P., et al. (2012). Association of lifetime cognitive engagement and low β-amyloid deposition. *Archives of Neurology, 69*(5), 623–629. https://doi.org/10.1001/archneurol.2011.2748
Lazarus, R. S. L., & Folkman, S. (1984). *Stress, appraisal, and coping*. Springer Publishing Company.
Lee, T.-H., Kim, S. H., Katz, B., & Mather, M. (2020). The decline in intrinsic connectivity between the salience network and locus coeruleus in older adults: Implications for distractibility. *Frontiers in Aging Neuroscience, 12*, 2. https://doi.org/10.3389/fnagi.2020.00002
Lindegård, A., Wastensson, G., Hadzibajramovic, E., & Grimby-Ekman, A. (2019). Longitudinal associations between cardiorespiratory fitness and stress-related exhaustion, depression, anxiety and sleep disturbances. *BMC Public Health, 19*(1), 1726. https://doi.org/10.1186/s12889-019-8081-6
Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., et al. (2020). Dementia prevention, intervention, and care: 2020 report of the lancet commission. *Lancet (London, England), 396*(10248), 413–446. https://doi.org/10.1016/S0140-6736(20)30367-6
Livingston, G., Sommerlad, A., Orgeta, V., Costafreda, S. G., Huntley, J., Ames, D., et al. (2017). Dementia prevention, intervention, and care. *Lancet (London, England), 390*(10113), 2673–2734. https://doi.org/10.1016/S0140-6736(17)31363-6
Lovibond, S. H., & Lovibond, P. F. (1995). *Manual for the depression anxiety stress scales* (2nd ed.). Psychology Foundation of Australia.
Marin, M.-F., Lord, C., Andrews, J., Juster, R.-P., Sindi, S., Arsenault-Lapierre, G., et al. (2011). Chronic stress, cognitive functioning and mental health. *Neurobiology of Learning and Memory, 96*(4), 583–595. https://doi.org/10.1016/j.nlm.2011.02.016
Menon, V. (2011). Large-scale brain networks and psychopathology: A unifying triple network model. *Trends in Cognitive Sciences, 15*(10), 483–506. https://doi.org/10.1016/j.tics.2011.08.003
Mevel, K., Chételat, G., Eustache, F., & Desgranges, B. (2011). The default mode network in healthy aging and Alzheimer's disease. *International Journal of Alzheimer's Disease*. , Article 535816. https://doi.org/10.4061/2011/535816, 2011.
Morris, T. P., Burzynska, A., Voss, M., Fanning, J., Salerno, E. A., Prakash, R., … Kramer, A. F. (2022). Brain structure and function predict adherence to an exercise intervention in older adults. *Medicine and Science in Sports and Exercise*. https://doi.org/10.1249/MSS.0000000000002949
Northoff, G. (2020). Anxiety disorders and the brain's resting state networks: From altered spatiotemporal synchronization to psychopathological symptoms. *Advances in Experimental Medicine and Biology, 1191*, 71–90. https://doi.org/10.1007/978-981-32-9705-0_5
Osman, A., Wong, J. L., Bagge, C. L., Freedenthal, S., Gutierrez, P. M., & Lozano, G. (2012). The Depression Anxiety Stress Scales-21 (DASS-21): Further examination of dimensions, scale reliability, and correlates. *Journal of Clinical Psychology, 68*(12), 1322–1338. https://doi.org/10.1002/jclp.21908
Paterson, D. H., Cunningham, D. A., Koval, J. J., & St Croix, C. M. (1999). Aerobic fitness in a population of independently living men and women aged 55-86 years. *Medicine & Science in Sports & Exercise, 31*(12), 1813–1820. https://doi.org/10.1097/00005768-199912000-00018
Power, J. D., Barnes, K. A., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2012). Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *NeuroImage, 59*(3). https://doi.org/10.1016/j.neuroimage.2011.10.018
Power, J. D., Mitra, A., Laumann, T. O., Snyder, A. Z., Schlaggar, B. L., & Petersen, S. E. (2014). Methods to detect, characterize, and remove motion artifact in resting state fMRI. *NeuroImage, 84*. https://doi.org/10.1016/j.neuroimage.2013.08.048
Power, J. D., Schlaggar, B. L., & Petersen, S. E. (2015). Recent progress and outstanding issues in motion correction in resting state fMRI. *NeuroImage, 105*. https://doi.org/10.1016/j.neuroimage.2014.10.044
Pozuelo-Carrascosa, D. P., Martínez-Vizcaíno, V., Sánchez-López, M., Bartolomé-Gutiérrez, R., Rodríguez-Martín, B., & Notario-Pacheco, B. (2017). Resilience as a mediator between cardiorespiratory fitness and mental health-related quality of life: A cross-sectional study. *Nursing and Health Sciences, 19*(3), 316–321. https://doi.org/10.1111/nhs.12347
Rajan, T., & Menon, V. (2017). Psychiatric disorders and obesity: A review of association studies. *Journal of Postgraduate Medicine, 63*(3), 182–190. https://doi.org/10.4103/jpgm.JPGM_712_16
Razlighi, Q. R., Habeck, C., Steffener, J., Gazes, Y., Zahodne, L. B., MacKay-Brandt, A., et al. (2014). Unilateral disruptions in the default network with aging in native space. *Brain and Behavior, 4*(2), 143–157. https://doi.org/10.1002/brb3.202
Ruegsegger, G. N., & Booth, F. W. (2018). Health benefits of exercise. *Cold Spring Harbor Perspectives in Medicine, 8*(7), a029694. https://doi.org/10.1101/cshperspect.a029694
Santarnecchi, E., Sprugnoli, G., Tatti, E., Mencarelli, L., Neri, F., Momi, D., et al. (2018). Brain functional connectivity correlates of coping styles. *Cognitive, Affective, & Behavioral Neuroscience, 18*(3), 495–508. https://doi.org/10.3758/s13415-018-0583-7
Schaefer, A., Kong, R., Gordon, E. M., Laumann, T. O., Zuo, X.-N., Holmes, A. J., et al. (2018). Local-global parcellation of the human cerebral cortex from intrinsic functional connectivity MRI. *Cerebral Cortex, 28*(9). https://doi.org/10.1093/cercor/bhx179
Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 27*(9), 2349–2356. https://doi.org/10.1523/JNEUROSCI.5587-06.2007
Shapero, B. G., Chai, X. J., Vangel, M., Biederman, J., Hoover, C. S., Whitfield-Gabrieli, S., et al. (2019). Neural markers of depression risk predict the onset of depression. *Psychiatry Research: Neuroimaging, 285*, 31–39. https://doi.org/10.1016/j.pscychresns.2019.01.006

Stawski, R. S., Sliwinski, M. J., Almeida, D. M., & Smyth, J. M. (2008). Reported exposure and emotional reactivity to daily stressors: The roles of adult-age and global perceived stress. *Psychology and Aging, 23*(1), 52–61. https://doi.org/10.1037/0882-7974.23.1.52

Stillman, C. M., Donofry, S. D., & Erickson, K. I. (2019). Exercise, fitness and the aging brain: A review of functional connectivity in aging. *Archives de Psychologie, 3*(4). Article 4 https://archivesofpsychology.org/index.php/aop/article/view/98.

Szabo, A. N., McAuley, E., Erickson, K. I., Voss, M., Prakash, R. S., Mailey, E. L., et al. (2011). Cardiorespiratory fitness, hippocampal volume, and frequency of forgetting in older adults. *Neuropsychology, 25*(5), 545–553. https://doi.org/10.1037/a0022733

Talukdar, T., Nikolaidis, A., Zwilling, C. E., Paul, E. J., Hillman, C. H., Cohen, N. J., et al. (2018). Aerobic fitness explains individual differences in the functional brain connectome of healthy young adults. *Cerebral Cortex (New York, N.Y.: 1991, 28*(10), 3600–3609. https://doi.org/10.1093/cercor/bhx232

Taren, A. A., Gianaros, P. J., Greco, C. M., Lindsay, E. K., Fairgrieve, A., Brown, K. W., et al. (2015). Mindfulness meditation training alters stress-related amygdala resting state functional connectivity: A randomized controlled trial. *Social Cognitive and Affective Neuroscience, 10*(12), 1758–1768. https://doi.org/10.1093/scan/nsv066

Thoits, P. A. (2010). Stress and health: Major findings and policy implications. *Journal of Health and Social Behavior, 51*(Suppl), S41–S53. https://doi.org/10.1177/0022146510383499

Thomas Yeo, B. T., Krienen, F. M., Sepulcre, J., Sabuncu, M. R., Lashkari, D., Hollinshead, M., et al. (2011). The organization of the human cerebral cortex estimated by intrinsic functional connectivity. *Journal of Neurophysiology, 106*(3). https://doi.org/10.1152/jn.00338.2011

Tingley, D., Yamamoto, T., Hirose, K., Keele, L., & Imai, K. (2014). *mediation: R package for causal mediation analysis*. UCLA Statistics/American Statistical Association. https://dspace.mit.edu/handle/1721.1/91154.

Tomasi, D., & Volkow, N. D. (2012). Aging and functional brain networks. *Molecular Psychiatry, 17*(5), 471–558. https://doi.org/10.1038/mp.2011.81

Uddin, L. Q. (2015). Salience processing and insular cortical function and dysfunction. *Nature Reviews Neuroscience, 16*(1), 55–61. https://doi.org/10.1038/nrn3857

Uddin, L. Q., Yeo, B. T. T., & Spreng, R. N. (2019). Towards a universal taxonomy of macro-scale functional human brain networks. *Brain Topography, 32*(6), 926–942. https://doi.org/10.1007/s10548-019-00744-6

Van Dijk, K. R. A., Hedden, T., Venkataraman, A., Evans, K. C., Lazar, S. W., & Buckner, R. L. (2010). Intrinsic functional connectivity as a tool for human connectomics: Theory, properties, and optimization. *Journal of Neurophysiology, 103*(1), 297–321. https://doi.org/10.1152/jn.00783.2009

Vecchio, L. M., Meng, Y., Xhima, K., Lipsman, N., Hamani, C., & Aubert, I. (2018). The neuroprotective effects of exercise: Maintaining a healthy brain throughout aging. *Brain Plasticity, 4*(1), 17–52. https://doi.org/10.3233/BPL-180069

Voelcker-Rehage, C., & Niemann, C. (2013). Structural and functional brain changes related to different types of physical activity across the life span. *Neuroscience & Biobehavioral Reviews, 37*(9 Pt B), 2268–2295. https://doi.org/10.1016/j.neubiorev.2013.01.028

Voss, M. W., Erickson, K. I., Prakash, R. S., Chaddock, L., Malkowski, E., Alves, H., et al. (2010). Functional connectivity: A source of variance in the association between cardiorespiratory fitness and cognition? *Neuropsychologia, 48*(5), 1394–1406. https://doi.org/10.1016/j.neuropsychologia.2010.01.005

Voss, M. W., Prakash, R. S., Erickson, K. I., Basak, C., Chaddock, L., Kim, J. S., et al. (2010). Plasticity of brain networks in a randomized intervention trial of exercise training in older adults. *Frontiers in Aging Neuroscience, 2*, 32. https://doi.org/10.3389/fnagi.2010.00032

Voss, M. W., Weng, T. B., Burzynska, A. Z., Wong, C. N., Cooke, G. E., Clark, R., et al. (2016). Fitness, but not physical activity, is related to functional integrity of brain networks associated with aging. *NeuroImage, 131*, 113–125. https://doi.org/10.1016/j.neuroimage.2015.10.044

Wasserman, K., Hansen, J., Sue, D., Whipp, B., & Casaburi, R. (2004). *Principles of exercise testing and interpretation* (4th ed.). Williams and Wilkins.

Whitfield-Gabrieli, S., & Ford, J. M. (2012). Default mode network activity and connectivity in psychopathology. *Annual Review of Clinical Psychology, 8*, 49–76. https://doi.org/10.1146/annurev-clinpsy-032511-143049

Williams, S. E., Carroll, D., Veldhuijzen van Zanten, J. J. C. S., & Ginty, A. T. (2016). Anxiety symptom interpretation: A potential mechanism explaining the cardiorespiratory fitness-anxiety relationship. *Journal of Affective Disorders, 193*, 151–156. https://doi.org/10.1016/j.jad.2015.12.051

Wirth, M., Villeneuve, S., La Joie, R., Marks, S. M., & Jagust, W. J. (2014). Gene-environment interactions: Lifetime cognitive activity, APOE genotype, and β-amyloid burden. *Journal of Neuroscience: The Official Journal of the Society for Neuroscience, 34*(25), 8612–8617. https://doi.org/10.1523/JNEUROSCI.4612-13.2014

Won, J., Nielson, K. A., & Smith, J. C. (2023). Large-scale network connectivity and cognitive function changes after exercise training in older adults with intact cognition and mild cognitive impairment. *Journal of Alzheimer's Disease Reports, 7*(1), 399–413. https://doi.org/10.3233/ADR-220062

World Health Organization. (2023). Mental health: Strengthening our response. https://www.who.int/news-room/fact-sheets/detail/mental-health-strengthening-our-response.

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T. P., & Sahebkar, A. (2017). The impact of stress on body function: A review. *EXCLI Journal, 16*, 1057–1072. https://doi.org/10.17179/excli2017-480

Young, C. B., Raz, G., Everaerd, D., Beckmann, C. F., Tendolkar, I., Hendler, T., et al. (2017). Dynamic shifts in large-scale brain network balance as a function of arousal. *Journal of Neuroscience, 37*(2), 281–290. https://doi.org/10.1523/JNEUROSCI.1759-16.2016

Zhang, W., Hashemi, M. M., Kaldewaij, R., Koch, S. B. J., Beckmann, C., Klumpers, F., et al. (2019). Acute stress alters the "default" brain processing. *NeuroImage, 189*, 870–877. https://doi.org/10.1016/j.neuroimage.2019.01.063

4.2.1 Supplemental Material

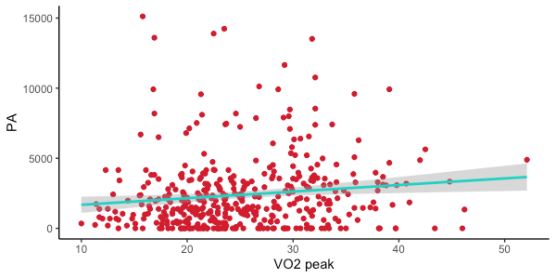
Supplementary material

Functional connectivity mediates the relationship between cardiorespiratory fitness and stress in midlife.

1. Self-reported physical activity and its association with cardiorespiratory fitness

Self-reported physical activity was assessed using the International Physical Activity Questionnaire (IPAQ), validated for the Spanish/Catalan population [50,107]. Data collected from the self-administered IPAQ surveys were summed within each physical activity domain (walking, moderate-intensity and vigorous-intensity activities) to estimate the total metabolic equivalent of task (MET) in minutes/week spent performing physical activity related to occupational, transportation, household, and leisure activities. The questionnaire was scored and analysed using established methods, available on the IPAQ website (www.ipaq.ki.se). Here, data collected with the IPAQ have been reported as a continuous measure. Total scores have been calculated for walking, moderate-intensity activities, and vigorous-intensity activities, for each domain (work, transport, domestic and garden, and leisure) and for overall total physical activity MET-minutes/week score, calculated as: Total physical activity MET-minutes/week = sum of Total (Walking + Moderate + Vigorous) MET-minutes/week scores.

Engagement in physical activity as measured by the total number of METs-min/week including 'walking', 'moderate activity' and 'vigorous activity' was significantly associated with VO_2 peak in our cohort after controlling for age, biological sex, education, monthly incomes, and waist ($\beta = 0.19$, SE = 23.06, $p = 0.002$, $R2=0.045$).



Supplementary figure 1. A significant positive association between physical activity levels (total weekly MET) and $V0_2$ peak, controlling for age, biological sex, education, monthly incomes, and waist was found.

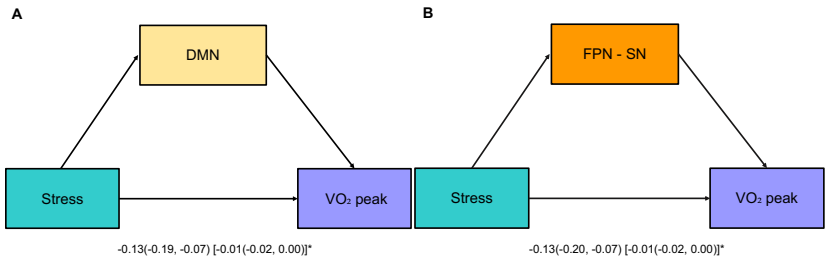
2. *Mediation models to test the bidirectional hypothesis.*

The mediation results showed that the DMN within connectivity (p=0.016) and the connectivity between FPN – SN (p=0.01) mediated the relationship between stress and VO_2 peak and stress. The relationship between CRF and functional connectivity (X [predictor variable] on M ([mediator]) are found in Table 5 along with full mediation model results.

Supplementary table 1. Mediation model between stress functional connectivity and $V0_2$ peak.

	Total effect	ADE	ACME
Outcomes	Beta (95% CI)	Beta (95% CI)	Beta (95% CI)
Default mode network	-0.14(-0.20, -0.08)	-0.13(-0.19, -0.07)	-0.01(-0.02, -0.00)*
Prefrontal - Salience network	-0.14(-0.20, -0.08)	-0.13(-0.20, -0.07)	-0.01(-0.02,- 0.00)*

The model is adjusted for age, education, biological sex, waist circumference, FDW, and socioeconomic status. ADE = average direct effect; ACME = average causal mediation effect. Statistical significance at $p < 0.05$ and 95% CI not including 0.



Supplementary figure 2. A) Within DMN connectivity mediated the relationship between X (mental health) and Y (CRF). **(B)** The connectivity between FPN and SN mediated the relationship between X (mental health) and Y (CRF). The total effect (X on Y) is seen under the horizontal arrow representing the β coefficient followed by the 95% CIs in parentheses. The average causal mediation effect (X [predictor variable] on Y [outcome variable] including M [mediator]) is seen between square brackets following the direct effect. The mediated effect is calculated as the difference between the estimates from the total and direct effects (see Supplementary Table 1) which correspond to the reduction in the independent variable(X) effect on the dependent variable (Y) when adjusted for the mediator (M).

5. Overall summary of results

In the *first study*, our primary objective was to assess the respective relationships between CRF and cardiovascular risk (CVR) and cognitive function in midlife in a sample of 501 adults (248 female) with a mean ± standard deviation (SD) age of 53.58±6.96 years (range 40 to 65 years). We further aimed to examine the mechanistic correlates of these relationships in midlife through measures of brain structure using MRI, by testing whether cortical thickness mediated the relationships between each predictor (CVR and CRF) and cognitive function.

Firstly, we examined the associations between VO_2 peak, cardiovascular risk (measured by Framingham) and cognitive function. At the whole group level, no significant associations between VO_2 peak and cognitive functions were found. When we dichotomized our sample into younger middle-aged (aged 40–54 years) and older middle-aged (aged 55–65 years) we found no significant correlations between any cognitive domain and VO_2 peak in the younger group. However, in the older middle-aged adults, we did find a significant and positive association between VO_2 peak and visuospatial reasoning and problem solving (β=3.16, P=0.049), which remained significant after false discovery rate corrections (false discovery rate P=0.0499). All models were controlled for age, biological sex, body mass index, waist perimeter, socioeconomic status, and education as covariates. For CVR, we found a significant negative association between Framingham score and the following cognitive abilities: visuospatial ability (β=−0.046, P=0.002), processing speed (β=−0.115, P<0.001), flexibility (β=−0.054, P<0.001), and verbal memory (β=−0.120, P<0.001), but not working memory (β=−0.010, P=0.502).

Secondly, we studied the potential mechanistic pathway through which higher cardiorespiratory fitness and lower cardiovascular risk can positively impact cognitive function in midlife. At the whole group level, higher VO_2 peak was significantly associated with greater cortical thickness in the left prefrontal cortex (rostral middle frontal gyrus) (cluster-wise corrected with a vertex-wise threshold P<0.05, cluster-wise P<0.05). In the young middle-aged group (aged 40–54 years) VO_2 peak was not associated with any specific gyrus, whereas in the old middle-aged group (aged ≥55 years), associations with left prefrontal regions (left rostral middle frontal) and left temporal regions (superior temporal gyrus) were seen. Moreover, in the older middle-aged group, cortical thickness in the left prefrontal gyrus mediated the relationship between VO_2 peak and visuospatial reasoning abilities [ACME= 0.01(0.0003,0.03)]. Moreover, higher Framingham risk score was significantly associated with lower cortical thickness across different cortical regions (18 clusters) of both hemispheres including frontal, parietal, temporal, and medial (insula, cuneus) cortices (cluster-wise corrected with a vertex-wise threshold P<0.005, cluster-

wise P<0.05). Cortical thickness significantly mediated the relation between Framingham and visuospatial problem solving, processing speed, flexibility, and memory after controlling for education and monthly income. In visuospatial problem solving, the following regions were significantly mediating its relationship with Framingham: left postcentral gyrus, left pars triangularis, left insula, left cuneus, left caudal anterior cingulate gyrus, left transverse temporal gyrus, and right supramarginal region. The relationship between processing speed and Framingham was significantly mediated by right cuneus, whereas flexibility had different gyri that mediated its relationship with Framingham, in particular, left postcentral gyrus, left insula, left caudal anterior cingulate gyrus, left transverse temporal gyrus, right inferior parietal gyrus, right cuneus, right supramarginal region, and right superior frontal gyrus. Lastly, left triangularis and left and right cuneus significantly mediated the relationship between Framingham and memory.

In the *second study* the main objective was to examine the relationships between CRF, mental health and functional connectivity in healthy middle-aged adults. A total of 418 (197 female) participants with a mean ± SD of 53.21 ± 6.85 years (range 40-65 years) completed the study. Data collected from the CPET was used to value cardiorespiratory fitness (VO_2 peak), Depression Anxiety and Stress Scale (DASS-21) and its three subscales (stress, anxiety, and depression) were used to score mental health states and lastly, fMRI data was collected to examine the mechanistic correlates of these relationships in midlife through functional connectivity patterns of the Triple Model Network, mainly composed by the DMN, FPN and SN.

We first examined the associations between VO_2 peak and mental health controlling for age, biological sex, education, waist circumference, socioeconomic status, and time between assessments. A significant negative correlation between VO_2 peak and anxiety (β= -0.111, p=0.017) and stress (β= -0.242, p= 0.002) sub-scales was found, which all remained significant after false discovery rate corrections (false discovery rate p=0.025; p= 0.006, respectively).

Secondly, we studied the associations between VO_2 peak and functional connectivity within and between networks controlling all the models for age, biological sex, education, waist circumference, socioeconomic status, and frame wise displacement (FDW) mean value. VO_2 peak was positively associated with the connectivity strength within the DMN (β= 0.195, p= 0.002*), Salience Network (SN) (β= 0.143, p= 0.026*), FPN (β= 0.133, p= 0.036*), which all remained significant after false discovery rate corrections (false discovery rate p=0.006, p=0.39, p=0.041, respectively). For functional connectivity between networks, VO_2 peak was positively associated with

DMN-to-FPN connectivity (β= 0.130, p= 0.041*), DMN–to-SN (β= 0.67, p= 0.006*) and FPN–to-SN (β= 0.187, p= 0.002*) which both remained significant after false discovery rate corrections (false discovery rate p=0.041, p=0.012, p=0.006, respectively).

Thirdly, we examined the associations between functional connectivity and mental health using age, biological sex, education, waist circumference, socioeconomic status, time between assessments and FDW mean as covariates. The DMN within functional connectivity was negatively associated with stress ((β= -0.126, p=0.011) which remained significant after false discovery rate corrections (false discovery rate p=0.048), whereas the relationships with the rest of mental health constructs were not statistically significant. The FPN within functional connectivity was negatively associated with stress ((β= -0.112, p=0.025) which did not remain significant after false discovery rate corrections (false discovery rate p=0.050), whereas the relationships with the rest of mental health constructs were not statistically significant. Whereas the relationships between SN within connectivity with the mental health constructs were not statistically significant. The connectivity between DMN and SN with mental health constructs were not statistically significant whereas the connectivity between FPN and SN and the connectivity between DMN and FPN were negatively associated with stress (β=-0.123, p=0.016; β=-0.102, p=0.041, respectively) which FPN-SN remained significant after false discovery rate corrections (false discovery rate p=0.048) whereas DMN-FPN did not remain significant after controlling by multiple comparisons (false discovery rate p=0.061).

Lastly, we run the mediation analyses between DMN within connectivity and between FPN-SN with CRF and stress levels. The mediation results showed that the DMN within connectivity [ACME= -0.02(-0.04,-0.00), p= 0.040] and the connectivity between FPN – SN [ACME= -0.01(-0.04,-0.00), p= 0.036] mediated the relationship between VO_2 peak and stress. Both models were adjusted for age, education, biological sex, waist circumference, FDW, time between assessments and socioeconomic status.

6. Overall summary of the discussion

Mainly, the global results from this thesis show that certain connections between cardiovascular health and brain health, typically observed in older individuals, are already evident in middle age stages. In our initial study, we demonstrate that CRF and CVR displayed distinct associations with cognitive health. Importantly, the main takeaway from the first study was that there appears to the existence of a period from early (40-55 years old) to late middle age (55-65 years old) when it becomes particularly critical to maintain CRF to optimize cognitive and brain health as we age. That is, the main results of CRF on cognition, mediated by cortical thickness in the prefrontal cortex was only apparent in the late middle-aged group. These findings support the pre-established notion that variations in brain structure and function precede the onset of behavioral symptoms of cognitive decline by years (103,113), but also adding a new critical time frame where to target preventive interventions. Together, further strengthening the importance of engaging in modifiable lifestyle behaviors relevant for the promotion and maintenance of cardiovascular health in early midlife.

Secondly, the findings also suggest regional specific associations between CRF, CVR and brain health. CRF has shown more specific associations in cognition and brain structure (frontal areas) while CRV was associated with cortical thickness in disperse cortical regions and several cognitive domains. The regional specificity of cognitive domains and cortical thickness patterns concerning cardiovascular health predictors may be due to the distinct neurobiological pathways associated with exercise-related cognitive enhancements through CRF and CVH (41). From this standpoint, CRF has been shown to decrease small-vessel ischemic diseases which often preferentially affects the frontal/subcortical region of the brain (114). In contrast, CVR has been mostly associated with small lesions in cerebral white matter that exhibit a more disperse representation over striatal, cortico–cortical, and cortical–subcortical pathways (115). Together, the present findings regarding the overlap between the clusters identified herein and cortical areas considered to be particularly sensitive to the effects of early cognitive impairment and Alzheimer's dementia (Pettigrew et al., 2016), supports existing evidence that cardiovascular health factors such as CRF and CVR are also cognitive protective factors during midlife (116–119).

Regarding the second study on mental health outcomes, we demonstrated that higher CRF levels are associated with lower scores in mental health during midlife. We extend previous knowledge in an important way. Although the effect of CRF on mental health in clinical population has been widely reported (57,58,120), the effect of these relations in healthy middle-aged adults is novel. Significantly, our findings could potentially promote further studies in healthy middle-aged adults on the effect of

interventions to enhance or preserving CRF as a means to mitigate the onset of mental illnesses as a result of chronic exposures to stressful major life events during midlife (121) whereby interestingly, the decline of CRF levels typically accelerates (122). Based on our findings, it is implied that maintaining optimal levels of CRF during midlife may serve as a valuable strategy to confront plausible midlife stressors. Thus, in light of considering stress as a potential precursor for depression and anxiety (84,85), our findings indicate a selective association between functional connectivity and stress. This specific association may capture a relationship at the early stages of development within healthy middle-aged population. If low levels of CRF in midlife subsequently predict worse outcomes in depression and anxiety later in life, our findings provide mechanistic evidence supporting the importance of promoting and sustaining CRF as individuals age, particularly in regard to mental health outcomes across the lifespan.

Importantly, the second study also reveals a link between CRF in midlife to the functional connectivity of the DMN, FPN and SN, and subsequently to stress scores. These correlations have important implications for psychopathology disorders involving disrupted saliency processing, potentially leading to impaired cognitive processes (83). What's more, previous research has suggested that the disruptions in the dynamic interplay of the triple network model are implicated in numerous neuropsychiatric disorders (83,123,124). The results are in line with prior work that suggests the existence of underlying brain changes that occur prior to the clinical or behavioral manifestation of certain symptoms (103). Life stressors may lead to individual variations in the functional connectivity of specific intrinsic resting state networks before the onset of clinically significant symptoms related to depression and anxiety. That is, the inter-individual differences in how functional connectivity of the triple network model networks relates to stress in our sample of middle-aged adults are partly explained by variations in CRF scores. It is essential therefore to identify interventions and specific modifiable lifestyles that may slow or reverse these functional changes and contribute to psychological resilience in middle-aged adults such as engaging in aerobic exercise programs (a key strategy to enhance CRF) and cardiovascular health interventions (125,126).

Nevertheless, the results should be interpreted in light of several considerations since there are certain general limitations to the studies. Firstly, given the cross-sectional nature of both research studies, it was not possible to make any kind of inference about casual relationships and, additionally, we can only speculate about the directionality of these results. Based on the analyses alone, in addition to the interpretations herein, it is just as plausible that higher cognitive resources or resilience lead to higher levels of fitness or better cardiovascular health. Furthermore, longitudinal studies have suggested

that cognitive resources themselves are predictive of engagement in physical exercise interventions (127). Secondly, the sample of individuals studied in my thesis is generally characterized by white, highly educated, and healthy from a cardiovascular standpoint. Thirdly, although numerous interventional studies have demonstrated that aerobic fitness training can improve brain health (30,53), other modes of exercise have also been found to positively influence the brain diminishing age effects (41) and should been extensively studied in healthy middle aged population in the future. Fourth, weight status and BMI measures have been widely associated with mental health status (128,129), CRF and functional connectivity patterns in adults (130). Therefore, even though all statistical models have been controlled for specific covariates in both studies, the lack of measures of adiposity is a limitation in this thesis.

The findings outlined in this doctoral thesis highlight the significance of adopting modifiable lifestyles behaviors that can promote and maintain cardiorespiratory and cardiovascular health in midlife, which in turn have the potential to positively impact brain health. The study of the mechanisms underlying the relationship between these modifiable cardiorespiratory factors and cognitive and mental health outcomes provides new avenues for the development of innovative strategies to promote brain health across the lifespan.

7. Conclusions

The conclusions of the present thesis are:

- CRF is associated with overall well-being, cognition, and mental health throughout adulthood.
- Significant associations between determinants of cardiovascular health and brain health previously reported in older age are already present in middle age. Therefore, engaging in modifiable lifestyle behavior during midlife can potentially maintain and promote brain health in later life.
- Variation in CRF determine structural and functional brain changes that provide mechanistic understanding of the relationships between cardiovascular and cognitive and mental health in middle aged adults.

8. Future lines of research

Studies presented in this thesis have added to the literature regarding CRF and brain health in healthy middle-aged adults, but significant work is still to be done in order to consolidate the clinical value of modifying and maintaining CRF for preserving and promoting brain health during lifespan. Even though association studies are critical to better understand biological constructs, longitudinal and interventional approaches should be taken to better characterize and extend these correlations across the lifespan. In addition, considerations about biological sex interactions are critical in this work given reported differences in CRF (131–133), CVR (134), trajectories of cognitive performance (135) and functional connectivity patterns (136,137) between men and women. What's more, biological differences or similarities between the sexes, and the translation of information on sex differences into preventive diagnostic and therapeutic practices is crucial to improve healthcare and patient outcomes. Therefore, sex-specific research would be essential to contribute and progress on better understand these lifestyles behaviors and brain health relationship across lifespan.

9. Bibliography

1. Crimmins EM. Lifespan and Healthspan: Past, Present, and Promise. Gerontologist. 2015 Dec;55(6):901–11.

2. Passarino G, De Rango F, Montesanto A. Human longevity: Genetics or Lifestyle? It takes two to tango. Immun Ageing. 2016;13:12.

3. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006 Nov;3(11):e442.

4. Gorelick PB, Furie KL, Iadecola C, Smith EE, Waddy SP, Lloyd-Jones DM, et al. Defining Optimal Brain Health in Adults: A Presidential Advisory From the American Heart Association/American Stroke Association. Stroke. 2017;48(10):e284–303.

5. Pascual-Leone A, Freitas C, Oberman L, Horvath JC, Halko M, Eldaief M, et al. Characterizing Brain Cortical Plasticity and Network Dynamics Across the Age-Span in Health and Disease with TMS-EEG and TMS-fMRI. Brain Topogr. 2011 Oct;24(3–4):302–15.

6. McHugh D, Gil J. Senescence and aging: Causes, consequences, and therapeutic avenues. J Cell Biol. 2018 Jan 2;217(1):65–77.

7. Nyberg L, Lövdén M, Riklund K, Lindenberger U, Bäckman L. Memory aging and brain maintenance. Trends Cogn Sci. 2012 May;16(5):292–305.

8. Di Marco LY, Marzo A, Muñoz-Ruiz M, Ikram MA, Kivipelto M, Ruefenacht D, et al. Modifiable lifestyle factors in dementia: a systematic review of longitudinal observational cohort studies. J Alzheimers Dis. 2014;42(1):119–35.

9. Frankish H, Horton R. Prevention and management of dementia: a priority for public health. Lancet. 2017 Dec 16;390(10113):2614–5.

10. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet. 2020 Aug 8;396(10248):413–46.

11. Livingston G, Sommerlad A, Orgeta V, Costafreda SG, Huntley J, Ames D, et al. Dementia prevention, intervention, and care. Lancet. 2017 Dec 16;390(10113):2673–734.

12. Shatenstein B, Ferland G, Belleville S, Gray-Donald K, Kergoat MJ, Morais J, et al. Diet quality and cognition among older adults from the NuAge study. Exp Gerontol. 2012 May;47(5):353–60.

13. Guasch-Ferré M, Willett WC. The Mediterranean diet and health: a comprehensive overview. J Intern Med. 2021 Sep;290(3):549–66.

14. Loughrey DG, Lavecchia S, Brennan S, Lawlor BA, Kelly ME. The Impact of the Mediterranean Diet on the Cognitive Functioning of Healthy Older Adults: A Systematic Review and Meta-Analysis. Adv Nutr. 2017 Jul;8(4):571–86.

15. Pistollato F, Iglesias RC, Ruiz R, Aparicio S, Crespo J, Lopez LD, et al. Nutritional patterns associated with the maintenance of neurocognitive functions and the risk of dementia and Alzheimer's disease: A focus on human studies. Pharmacol Res. 2018 May;131:32–43.

16. Fung CH, Vitiello MV, Alessi CA, Kuchel GA, AGS/NIA Sleep Conference Planning Committee and Faculty. Report and Research Agenda of the American Geriatrics Society and National Institute on Aging Bedside-to-Bench Conference on Sleep, Circadian Rhythms, and Aging: New Avenues for Improving Brain Health, Physical Health, and Functioning. J Am Geriatr Soc. 2016 Dec;64(12):e238–47.

17. Sexton CE, Storsve AB, Walhovd KB, Johansen-Berg H, Fjell AM. Poor sleep quality is associated with increased cortical atrophy in community-dwelling adults. Neurology. 2014 Sep 9;83(11):967–73.

18. Ryff CD, Heller AS, Schaefer SM, van Reekum C, Davidson RJ. Purposeful Engagement, Healthy Aging, and the Brain. Curr Behav Neurosci Rep. 2016 Dec;3(4):318–27.

19. Wilson RS, Bennett DA. How Does Psychosocial Behavior Contribute to Cognitive Health in Old Age? Brain Sci. 2017 May 23;7(6):56.

20. Stern Y, Arenaza-Urquijo EM, Bartrés-Faz D, Belleville S, Cantilon M, Chetelat G, et al. Whitepaper: Defining and investigating cognitive reserve, brain reserve, and brain maintenance. Alzheimers Dement. 2020 Sep;16(9):1305–11.

21. Stern Y. Cognitive Reserve. Neuropsychologia. 2009 Aug;47(10):2015–28.

22. Stern Y. Cognitive reserve in ageing and Alzheimer's disease. The Lancet Neurology. 2012 Nov 1;11(11):1006–12.

23. Arenaza-Urquijo EM, Wirth M, Chételat G. Cognitive reserve and lifestyle: moving towards preclinical Alzheimer's disease. Front Aging Neurosci. 2015 Aug 10;7:134.

24. Elman JA, Oh H, Madison CM, Baker SL, Vogel JW, Marks SM, et al. Neural compensation in older people with brain amyloid-β deposition. Nat Neurosci. 2014 Oct;17(10):1316–8.

25. Reuter-Lorenz PA, Park DC. How does it STAC up? Revisiting the scaffolding theory of aging and cognition. Neuropsychol Rev. 2014 Sep;24(3):355–70.

26. Gelfo F, Mandolesi L, Serra L, Sorrentino G, Caltagirone C. The Neuroprotective Effects of Experience on Cognitive Functions: Evidence from Animal Studies on the Neurobiological Bases of Brain Reserve. Neuroscience. 2018 Feb 1;370:218–35.

27. Claassen JAHR. New cardiovascular targets to prevent late onset Alzheimer disease. European Journal of Pharmacology. 2015 Sep 15;763:131–4.

28. Maasakkers CM, Weijs RWJ, Dekkers C, Gardiner PA, Ottens R, Olde Rikkert MGM, et al. Sedentary behaviour and brain health in middle-aged and older adults: A systematic review. Neuroscience & Biobehavioral Reviews. 2022 Sep 1;140:104802.

29. Kramer AF, Hahn S, Cohen NJ, Banich MT, McAuley E, Harrison CR, et al. Ageing, fitness and neurocognitive function. Nature. 1999 Jul 29;400(6743):418–9.

30. Gomes-Osman J, Cabral DF, Morris TP, McInerney K, Cahalin LP, Rundek T, et al. Exercise for cognitive brain health in aging. Neurol Clin Pract. 2018 Jun;8(3):257–65.

31. Hillman CH, Erickson KI, Kramer AF. Be smart, exercise your heart: exercise effects on brain and cognition. Nat Rev Neurosci. 2008 Jan;9(1):58–65.

32. Northey JM, Cherbuin N, Pumpa KL, Smee DJ, Rattray B. Exercise interventions for cognitive function in adults older than 50: a systematic review with meta-analysis. Br J Sports Med. 2018 Feb;52(3):154–60.

33. Colcombe SJ, Erickson KI, Scalf PE, Kim JS, Prakash R, McAuley E, et al. Aerobic exercise training increases brain volume in aging humans. Journals of Gerontology - Series A Biological Sciences and Medical Sciences. 2006;61(11):1166–70.

34. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. Proceedings of the National Academy of Sciences of the United States of America. 2011;108(7):3017–22.

35. Weinstein AM, Voss MW, Prakash RS, Chaddock L, Szabo A, White SM, et al. The association between aerobic fitness and executive function is mediated by prefrontal cortex volume. Brain Behav Immun. 2012 Jul;26(5):811–9.

36. Christie BR, Eadie BD, Kannangara TS, Robillard JM, Shin J, Titterness AK. Exercising our brains: how physical activity impacts synaptic plasticity in the dentate gyrus. Neuromolecular Med. 2008;10(2):47–58.

37. Knaepen K, Goekint M, Heyman EM, Meeusen R. Neuroplasticity - exercise-induced response of peripheral brain-derived neurotrophic factor: a systematic review of experimental studies in human subjects. Sports Med. 2010 Sep 1;40(9):765–801.

38. Leckie RL, Oberlin LE, Voss MW, Prakash RS, Szabo-Reed A, Chaddock-Heyman L, et al. BDNF mediates improvements in executive function following a 1-year exercise intervention. Front Hum Neurosci. 2014;8:985.

39. Maass A, Düzel S, Brigadski T, Goerke M, Becke A, Sobieray U, et al. Relationships of peripheral IGF-1, VEGF and BDNF levels to exercise-related changes in memory, hippocampal perfusion and volumes in older adults. Neuroimage. 2016 May 1;131:142–54.

40. Rasmussen P, Brassard P, Adser H, Pedersen MV, Leick L, Hart E, et al. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. Exp Physiol. 2009 Oct;94(10):1062–9.

41. Cabral DF, Rice J, Morris TP, Rundek T, Pascual-Leone A, Gomes-Osman J. Exercise for Brain Health: An Investigation into the Underlying Mechanisms Guided by Dose. Neurotherapeutics. 2019 Jul;16(3):580–99.

42. Klevjer M, Nordeidet AN, Bye A. The genetic basis of exercise and cardiorespiratory fitness – relation to cardiovascular disease. Current Opinion in Physiology. 2023 Jun 1;33:100649.

43. Raghuveer G, Hartz J, Lubans DR, Takken T, Wiltz JL, Mietus-Snyder M, et al. Cardiorespiratory Fitness in Youth: An Important Marker of Health: A Scientific Statement From the American Heart Association. Circulation. 2020 Aug 18;142(7):e101–18.

44. Blumenburg WT, Frederick JM, Cross BL, Culver MN, McMillan NK, Montoye AH, et al. Physical fitness, but not physical activity, is associated with mental health in apparently healthy young adults. J Sports Med Phys Fitness. 2021 Dec 21;

45. Lindegård A, Wastensson G, Hadzibajramovic E, Grimby-Ekman A. Longitudinal associations between cardiorespiratory fitness and stress-related exhaustion, depression, anxiety and sleep disturbances. BMC Public Health. 2019 Diciembre;19(1):1726.

46. Pozuelo-Carrascosa DP, Martínez-Vizcaíno V, Sánchez-López M, Bartolomé-Gutiérrez R, Rodríguez-Martín B, Notario-Pacheco B. Resilience as a mediator between cardiorespiratory fitness and mental health-related quality of life: A cross-sectional study. Nurs Health Sci. 2017 Sep;19(3):316–21.

47. Colcombe S, Kramer AF. Fitness effects on the cognitive function of older adults: a meta-analytic study. Psychol Sci. 2003 Mar;14(2):125–30.

48. Newson RS, Kemps EB. Cardiorespiratory fitness as a predictor of successful cognitive ageing. J Clin Exp Neuropsychol. 2006 Aug;28(6):949–67.

49. Pentikainen H, Savonen K, Ngandu T, Solomon A, Komulainen P, Paajanen T, et al. Cardiorespiratory Fitness and Cognition: Longitudinal Associations in the FINGER Study. Journal of Alzheimer's Disease. 2019;68(3):961–8.

50. Wendell CR, Gunstad J, Waldstein SR, Wright JG, Ferrucci L, Zonderman AB. Cardiorespiratory fitness and accelerated cognitive decline with aging. J Gerontol A Biol Sci Med Sci. 2014 Apr;69(4):455–62.

51. Zhu N, Jacobs DR, Schreiner PJ, Yaffe K, Bryan N, Launer LJ, et al. Cardiorespiratory fitness and cognitive function in middle age: the CARDIA study. Neurology. 2014 Apr 15;82(15):1339–46.

52. España-Irla G, Gomes-Osman J, Cattaneo G, Albu S, Cabello-Toscano M, Solana-Sanchéz J, et al. Associations Between Cardiorespiratory Fitness, Cardiovascular Risk, and Cognition Are Mediated by Structural Brain Health in Midlife. J Am Heart Assoc. 2021 Sep 13;10(18):e020688.

53. Kramer AF, Colcombe S. Fitness Effects on the Cognitive Function of Older Adults: A Meta-Analytic Study-Revisited. Perspect Psychol Sci. 2018 Mar;13(2):213–7.

54. Colcombe SJ, Erickson KI, Raz N, Webb AG, Cohen NJ, McAuley E, et al. Aerobic fitness reduces brain tissue loss in aging humans. J Gerontol A Biol Sci Med Sci. 2003 Feb;58(2):176–80.

55. Erickson KI, Prakash RS, Voss MW, Chaddock L, Hu L, Morris KS, et al. Aerobic fitness is associated with hippocampal volume in elderly humans. Hippocampus. 2009;19(10):1030–9.

56. Szabo AN, McAuley E, Erickson KI, Voss M, Prakash RS, Mailey EL, et al. Cardiorespiratory fitness, hippocampal volume, and frequency of forgetting in older adults. Neuropsychology. 2011 Sep;25(5):545–53.

57. Bueno-Antequera J, Munguía-Izquierdo D. Exercise and Depressive Disorder. Adv Exp Med Biol. 2020;1228:271–87.

58. Bueno-Antequera J, Munguía-Izquierdo D. Exercise and Schizophrenia. Adv Exp Med Biol. 2020;1228:317–32.

59. Ruegsegger GN, Booth FW. Health Benefits of Exercise. Cold Spring Harb Perspect Med. 2018 Jul 2;8(7):a029694.

60. Voelcker-Rehage C, Niemann C. Structural and functional brain changes related to different types of physical activity across the life span. Neurosci Biobehav Rev. 2013 Nov;37(9 Pt B):2268–95.

61. Voss MW, Erickson KI, Prakash RS, Chaddock L, Malkowski E, Alves H, et al. Functional connectivity: a source of variance in the association between cardiorespiratory fitness and cognition? Neuropsychologia. 2010 Apr;48(5):1394–406.

62. Buckner C, Fridland E. What is cognition? angsty monism, permissive pluralism(s), and the future of cognitive science. Synthese. 2017 Nov 1;194(11):4191–5.

63. Harada CN, Natelson Love MC, Triebel K. Normal Cognitive Aging. Clin Geriatr Med. 2013 Nov;29(4):737–52.

64. Salthouse TA, Ferrer-Caja E. What needs to be explained to account for age-related effects on multiple cognitive variables? Psychol Aging. 2003 Mar;18(1):91–110.

65. Hedden T, Gabrieli JDE. Insights into the ageing mind: a view from cognitive neuroscience. Nat Rev Neurosci. 2004 Feb;5(2):87–96.

66. Schaie KW. The Seattle Longitudinal Study: a thirty-five-year inquiry of adult intellectual development. Z Gerontol. 1993;26(3):129–37.

67. Schaie KW. Intellectual Development in Adulthood: The Seattle Longitudinal Study. Cambridge University Press; 1996. 446 p.

68. Park DC, Lautenschlager G, Hedden T, Davidson NS, Smith AD, Smith PK. Models of visuospatial and verbal memory across the adult life span. Psychol Aging. 2002 Jun;17(2):299–320.

69. Spencer BE, Banks SJ, Dale AM, Brewer JB, Makowski-Woidan B, Weintraub S, et al. Alzheimer's polygenic hazard score in SuperAgers: SuperGenes or SuperResilience? Alzheimers Dement (N Y). 2022;8(1):e12321.

70. Di Marco LY, Marzo A, Muñoz-Ruiz M, Ikram MA, Kivipelto M, Ruefenacht D, et al. Modifiable lifestyle factors in dementia: a systematic review of longitudinal observational cohort studies. J Alzheimers Dis. 2014;42(1):119–35.

71. Dominguez LJ, Veronese N, Vernuccio L, Catanese G, Inzerillo F, Salemi G, et al. Nutrition, Physical Activity, and Other Lifestyle Factors in the Prevention of Cognitive Decline and Dementia. Nutrients. 2021 Nov 15;13(11):4080.

72. Jia J, Zhao T, Liu Z, Liang Y, Li F, Li Y, et al. Association between healthy lifestyle and memory decline in older adults: 10 year, population based, prospective cohort study. BMJ. 2023 Jan 25;380:e072691.

73. Kivipelto M, Mangialasche F, Ngandu T. Lifestyle interventions to prevent cognitive impairment, dementia and Alzheimer disease. Nat Rev Neurol. 2018 Nov;14(11):653–66.

74. Rosenberg A, Ngandu T, Rusanen M, Antikainen R, Bäckman L, Havulinna S, et al. Multidomain lifestyle intervention benefits a large elderly population at risk for cognitive decline and dementia regardless of baseline characteristics: The FINGER trial. Alzheimers Dement. 2018 Mar;14(3):263–70.

75. Stern Y. What is cognitive reserve? Theory and research application of the reserve concept. J Int Neuropsychol Soc. 2002 Mar;8(3):448–60.

76. Stern Y, Barnes CA, Grady C, Jones RN, Raz N. Brain Reserve, Cognitive Reserve, Compensation, and Maintenance: Operationalization, Validity, and Mechanisms of Cognitive Resilience. Neurobiol Aging. 2019 Nov;83:124–9.

77. World Health Organization. Mental health: strengthening our response [Internet]. 2023 [cited 2023 Jan 27]. Available from: https://www.who.int/news-room/fact-sheets/detail/mental-health-strengthening-our-response

78. Shapero BG, Chai XJ, Vangel M, Biederman J, Hoover CS, Whitfield-Gabrieli S, et al. Neural markers of depression risk predict the onset of depression. Psychiatry Research: Neuroimaging. 2019 Mar 30;285:31–9.

79. Lazarus RSL, Folkman S. Stress, Appraisal, and Coping. Springer Publishing Company; 1984. 460 p.

80. Hellhammer DH, Wüst S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology. 2009 Feb;34(2):163–71.

81. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. The impact of stress on body function: A review. EXCLI J. 2017 Jul 21;16:1057–72.

82. Hermans EJ, Henckens MJAG, Joëls M, Fernández G. Dynamic adaptation of large-scale brain networks in response to acute stressors. Trends in Neurosciences. 2014 Jun 1;37(6):304–14.

83. Menon V. Large-scale brain networks and psychopathology: a unifying triple network model. Trends Cogn Sci. 2011 Oct;15(10):483–506.

84. Dias-Ferreira E, Sousa JC, Melo I, Morgado P, Mesquita AR, Cerqueira JJ, et al. Chronic stress causes frontostriatal reorganization and affects decision-making. Science. 2009 Jul 31;325(5940):621–5.

85. Marin MF, Lord C, Andrews J, Juster RP, Sindi S, Arsenault-Lapierre G, et al. Chronic stress, cognitive functioning and mental health. Neurobiol Learn Mem. 2011 Nov;96(4):583–95.

86. Fox MD, Snyder AZ, Vincent JL, Corbetta M, Van Essen DC, Raichle ME. The human brain is intrinsically organized into dynamic, anticorrelated functional networks. Proc Natl Acad Sci U S A. 2005 Jul 5;102(27):9673–8.

87. Launer LJ. The epidemiologic study of dementia: A life-long quest? Neurobiology of Aging. 2005;26(3):335–40.

88. Sperling RA, Aisen PS, Beckett LA, Bennett DA, Craft S, Fagan AM, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. Alzheimers Dement. 2011 May;7(3):280–92.

89. Yousaf T, Dervenoulas G, Politis M. Advances in MRI Methodology. Int Rev Neurobiol. 2018;141:31–76.

90. Pascual-Leone A. Disrupting the brain to guide plasticity and improve behavior. Prog Brain Res. 2006;157:315–29.

91. Pascual-Leone A, Amedi A, Fregni F, Merabet LB. The plastic human brain cortex. Annu Rev Neurosci. 2005;28:377–401.

92. Friston KJ. Functional and effective connectivity in neuroimaging: A synthesis. Human Brain Mapping. 1994;2(1–2):56–78.

93. Hillman EMC. Coupling Mechanism and Significance of the BOLD Signal: A Status Report. Annu Rev Neurosci. 2014 Jul 8;37:161–81.

94. Raichle ME. The restless brain: how intrinsic activity organizes brain function. Philos Trans R Soc Lond B Biol Sci. 2015 May 19;370(1668):20140172.

95. Greicius MD, Krasnow B, Reiss AL, Menon V. Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. Proc Natl Acad Sci U S A. 2003 Jan 7;100(1):253–8.

96. Raichle ME. The Brain's Default Mode Network. Annu Rev Neurosci. 2015 Jul 8;38(1):433–47.

97. Seeley WW. The Salience Network: A Neural System for Perceiving and Responding to Homeostatic Demands. J Neurosci. 2019 Dec 11;39(50):9878–82.

98. Dragomir A, Omurtag A. Brain's Networks and Their Functional Significance in Cognition. In: Thakor NV, editor. Handbook of Neuroengineering [Internet]. Singapore: Springer; 2020 [cited 2023 Nov 2]. p. 1–30. Available from: https://doi.org/10.1007/978-981-15-2848-4_76-2

99. Mattay VS, Goldberg TE, Sambataro F, Weinberger DR. Neurobiology of Cognitive Aging: Insights from Imaging Genetics. Biol Psychol. 2008 Sep;79(1):9–22.

100. Raz N, Rodrigue KM, Kennedy KM, Head D, Gunning-Dixon F, Acker JD. Differential aging of the human striatum: longitudinal evidence. AJNR Am J Neuroradiol. 2003 Oct;24(9):1849–56.

101. Andrews-Hanna JR, Snyder AZ, Vincent JL, Lustig C, Head D, Raichle ME, et al. Disruption of Large-Scale Brain Systems in Advanced Aging. Neuron. 2007 Dec 6;56(5):924–35.

102. Jia J, Zhao T, Liu Z, Liang Y, Li F, Li Y, et al. Association between healthy lifestyle and memory decline in older adults: 10 year, population based, prospective cohort study. BMJ. 2023 Jan 25;380:e072691.

103. Beason-Held LL, Goh JO, An Y, Kraut MA, O'Brien RJ, Ferrucci L, et al. Changes in brain function occur years before the onset of cognitive impairment. J Neurosci. 2013 Nov 13;33(46):18008–14.

104. Falck RS, Landry GJ, Best JR, Davis JC, Chiu BK, Liu-Ambrose T. Cross-Sectional Relationships of Physical Activity and Sedentary Behavior With Cognitive Function in Older Adults With Probable Mild Cognitive Impairment. Phys Ther. 2017 Oct 1;97(10):975–84.

105. Kooistra M, Boss HM, van der Graaf Y, Kappelle LJ, Biessels GJ, Geerlings MI, et al. Physical activity, structural brain changes and cognitive decline. The SMART-MR study. Atherosclerosis. 2014 May;234(1):47–53.

106. Makizako H, Liu-Ambrose T, Shimada H, Doi T, Park H, Tsutsumimoto K, et al. Moderate-intensity physical activity, hippocampal volume, and memory in older adults with mild cognitive impairment. J Gerontol A Biol Sci Med Sci. 2015 Apr;70(4):480–6.

107. Xu L, Jiang CQ, Lam TH, Zhang WS, Thomas GN, Cheng KK. Dose-response relation between physical activity and cognitive function: guangzhou biobank cohort study. Ann Epidemiol. 2011 Nov;21(11):857–63.

108. Daimiel L, Martínez-González MA, Corella D, Salas-Salvadó J, Schröder H, Vioque J, et al. Physical fitness and physical activity association with cognitive function and quality of life: baseline cross-sectional analysis of the PREDIMED-Plus trial. Sci Rep. 2020 Feb 26;10:3472.

109. Engeroff T, Ingmann T, Banzer W. Physical Activity Throughout the Adult Life Span and Domain-Specific Cognitive Function in Old Age: A Systematic Review of Cross-Sectional and Longitudinal Data. Sports Med. 2018 Jun;48(6):1405–36.

110. Smith PJ, Blumenthal JA, Hoffman BM, Cooper H, Strauman TA, Welsh-Bohmer K, et al. Aerobic exercise and neurocognitive performance: a meta-analytic review of randomized controlled trials. Psychosom Med. 2010 Apr;72(3):239–52.

111. Cattaneo G, Bartrés-Faz D, Morris TP, Sánchez JS, Macià D, Tarrero C, et al. The Barcelona Brain Health Initiative: A Cohort Study to Define and Promote Determinants of Brain Health. Front Aging Neurosci. 2018;10:321.

112. Cattaneo G, Bartrés-Faz D, Morris TP, Solana Sánchez J, Macià D, Tormos JM, et al. The Barcelona Brain Health Initiative: Cohort description and first follow-up. PLoS ONE. 2020;15(2):e0228754.

113. Younes L, Albert M, Moghekar A, Soldan A, Pettigrew C, Miller MI. Identifying Changepoints in Biomarkers During the Preclinical Phase of Alzheimer's Disease. Front Aging Neurosci. 2019;11:74.

114. Erkinjuntti T, Inzitari D, Pantoni L, Wallin A, Scheltens P, Rockwood K, et al. Limitations of clincal criteria for the diagnosis of vascular dementia in clinical trials. Is a focus on subcortical vascular dementia a solution? Annals of the New York Academy of Sciences. 2000;903:262–72.

115. Gorelick PB, Scuteri A, Black SE, Decarli C, Greenberg SM, Iadecola C, et al. Vascular contributions to cognitive impairment and dementia: A statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2011;42(9):2672–713.

116. Launer LJ, Ross GW, Petrovitch H, Masaki K, Foley D, White LR, et al. Midlife blood pressure and dementia: the Honolulu-Asia aging study. Neurobiol Aging. 2000 Feb;21(1):49–55.

117. Mielke MM, Rosenberg PB, Tschanz J, Cook L, Corcoran C, Hayden KM, et al. Vascular factors predict rate of progression in Alzheimer disease. Neurology. 2007 Nov 6;69(19):1850–8.

118. Ott A, Breteler MM, de Bruyne MC, van Harskamp F, Grobbee DE, Hofman A. Atrial fibrillation and dementia in a population-based study. The Rotterdam Study. Stroke. 1997 Feb;28(2):316–21.

119. Skoog I, Lernfelt B, Landahl S, Palmertz B, Andreasson LA, Nilsson L, et al. 15-year longitudinal study of blood pressure and dementia. Lancet. 1996 Apr 27;347(9009):1141–5.

120. Gujral S, Aizenstein H, Reynolds CF, Butters MA, Grove G, Karp JF, et al. Exercise for Depression: A Feasibility Trial Exploring Neural Mechanisms. Am J Geriatr Psychiatry. 2019 Jun;27(6):611–6.

121. Thoits PA. Stress and health: major findings and policy implications. J Health Soc Behav. 2010;51 Suppl:S41-53.

122. Ades PA, Toth MJ. Accelerated Decline of Aerobic Fitness With Healthy Aging. Circulation. 2005 Aug 2;112(5):624–6.

123. Northoff G. Anxiety Disorders and the Brain's Resting State Networks: From Altered Spatiotemporal Synchronization to Psychopathological Symptoms. Adv Exp Med Biol. 2020;1191:71–90.

124. Whitfield-Gabrieli S, Ford JM. Default mode network activity and connectivity in psychopathology. Annu Rev Clin Psychol. 2012;8:49–76.

125. Cabello-Toscano M, Vaqué-Alcázar L, Cattaneo G, Solana-Sánchez J, Bayes-Marin I, Abellaneda-Pérez K, et al. Functional brain connectivity prior to the COVID-19 outbreak moderates the effects of coping and perceived stress on mental health changes. A first year of COVID-19 pandemic follow-up study. Biol Psychiatry Cogn Neurosci Neuroimaging. 2022 Aug 20;S2451-9022(22)00188-4.

126. Stillman CM, Donofry SD, Erickson KI. Exercise, Fitness and the Aging Brain: A Review of Functional Connectivity in Aging. Archives of Psychology [Internet]. 2019 Jun 27 [cited 2023 Jan 2];3(4). Available from: https://archivesofpsychology.org/index.php/aop/article/view/98

127. Cheval B, Orsholits D, Sieber S, Courvoisier D, Cullati S, Boisgontier MP. Relationship between decline in cognitive resources and physical activity. Health Psychol. 2020 Jun;39(6):519–28.

128. Avila C, Holloway AC, Hahn MK, Morrison KM, Restivo M, Anglin R, et al. An Overview of Links Between Obesity and Mental Health. Curr Obes Rep. 2015 Sep;4(3):303–10.

129. Rajan T, Menon V. Psychiatric disorders and obesity: A review of association studies. J Postgrad Med. 2017;63(3):182–90.

130. Donofry SD, Stillman CM, Erickson KI. A review of the relationship between eating behavior, obesity and functional brain network organization. Social Cognitive and Affective Neuroscience. 2020 Nov 10;15(10):1157–81.

131. Al-Mallah MH, Juraschek SP, Whelton S, Dardari ZA, Ehrman JK, Michos ED, et al. Sex Differences in Cardiorespiratory Fitness and All-Cause Mortality: The Henry Ford ExercIse Testing (FIT) Project. Mayo Clin Proc. 2016 Jun;91(6):755–62.

132. Wang CY, Haskell WL, Farrell SW, Lamonte MJ, Blair SN, Curtin LR, et al. Cardiorespiratory fitness levels among US adults 20-49 years of age: findings from the 1999-2004 National Health and Nutrition Examination Survey. Am J Epidemiol. 2010 Feb 15;171(4):426–35.

133. Weltman A, Weltman JY, Hartman ML, Abbott RD, Rogol AD, Evans WS, et al. Relationship between age, percentage body fat, fitness, and 24-hour growth hormone release in healthy young adults: effects of gender. J Clin Endocrinol Metab. 1994 Mar;78(3):543–8.

134. Mosca L, Barrett-Connor E, Wenger NK. Sex/Gender Differences in Cardiovascular Disease Prevention What a Difference a Decade Makes. Circulation. 2011 Nov 8;124(19):2145–54.

135. McCarrey AC, An Y, Kitner-Triolo MH, Ferrucci L, Resnick SM. Sex differences in cognitive trajectories in clinically normal older adults. Psychol Aging. 2016 Mar;31(2):166–75.

136. Ficek-Tani B, Horien C, Ju S, Xu W, Li N, Lacadie C, et al. Sex differences in default mode network connectivity in healthy aging adults. Cereb Cortex. 2023 May 9;33(10):6139–51.

137. Yang CC, Totzek JF, Lepage M, Lavigne KM. Sex differences in cognition and structural covariance-based morphometric connectivity: evidence from 28,000+ UK Biobank participants. Cereb Cortex. 2023 Sep 26;33(19):10341–54.