UNIVERSITAT DE BARCELONA

Active Aging, Healthy Aging?
A Molecular, Brain Volume and Behavioral Approach

Alba Castells Sánchez

Rene sean Amba 2021

PhD Thesis by
ALBA CASTELLS SÁNCHEZ

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Barcelona, September 2021

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ACTIVE AGING, HEALTHY AGING?

A Molecular, Brain Volume and Behavioral Approach

Projecte Moviment Research Project

Thesis presented by

ALBA CASTELLS SÁNCHEZ

to obtain the degree of Doctor by the University of Barcelona
in accordance with the requirements of the international PhD diploma

Supervised by

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Clinical and Health Psychology Doctoral Program
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To the Universe

and those who enlighten me through it

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In the Universe there are multiple pieces that interact and collide producing chains of events and, sometimes, significant transformations of the environment. This thesis is the result of the interaction and energy of multiple people and translated into words and plots by us, producing a deep personal and professional transformation of myself. With the permission of the Reader, I would want to highlight and thank those who made it possible in our shared language.

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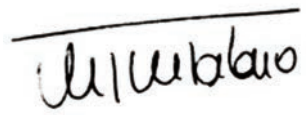
Dr. MARIA MATARÓ SERRAT,

CERTIFY that I have guided and supervised the PhD thesis entitled *Active Aging, Healthy Aging? A Molecular, Brain Volume and Behavioral Approach* presented by Alba Castells Sánchez. I hereby assert that this thesis fulfills the requirements to present her defense to be awarded the title of doctor.

Signature,



Barcelona, September 2021

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Walk, learn, be active, do!

Yes, your lifestyle may be predictive of future cognitive decline.

Luckily, most of us are going to age,

Let’s move your body, let’s rock your cells, brain and self!

Being active might be your best health ally.

Alba and Francesca

Projecte Moviment, 2016-2021

Prologue

Normal aging is related to a widespread physiological deterioration from molecules to main organs, with significant repercussions on brain and cognitive health. In previous studies, Dr. Maria Mataró team contributed to the research field describing the association between individual differences in aging trajectories and biological and behavioral factors. Based on those results, nowadays she leads a research project that raises the possibility to modify the behavioral factors in order to promote healthy aging: the Projecte Moviment.

Projecte Moviment is a research project that addresses the role of lifestyle behaviors in the promotion of brain and cognitive health in aging assessing their effects at molecular, brain and behavioral levels. This research project was born at the Grup de Neuropsicologia of the Departament de Psicologia Clínica i Psicobiologia (Institut de Neurociències) at the Universitat de Barcelona and involved other centers of reference: Unitat de Suport a la Rercerca Metropolitana Nord of the Institut Universitari d'Investigació en Atenció Primària Jordi Gol, Hospital Universitari Germans Trias i Pujol and Institut Guttmann. We internationally collaborated with the Department of Psychology of the University of Pittsburgh in Pittsburgh, United States of America, for the development of the project and the publication of the papers and with Department of Medicine, University of Montréal, Montréal, Canada, in one of the published papers. Projecte Moviment results are discussed in five different papers until now in which I have contributed (see *Annex 1*).

This thesis is presented for the Degree of Doctor by the University of Barcelona (International doctor mention) and is the result of the work carried out at Projecte Moviment Research Project in collaboration with the previously described institutions and the international collaboration from March 2021 until July 2021 with Dr. Chelsea

Stillman at the Department of Psychology of the University of Pittsburgh in Pittsburgh, United States of America. This thesis follows the published papers format and includes three studies. Two of these studies have been published and the third one is currently accepted under review in international scientific journals.

Study 1:

Castells-Sánchez, A., Roig-Coll, F., Lamonja-Vicente, N., Torán-Monserrat, P., Pera, G., Montero, P., Dacosta-Aguayo, R., Bermudo-Gallaguet, A., Bherer, L., Erickson, K. I., & Mataró, M. (2021). **Sex Matters in the Association between Physical Activity and Fitness with Cognition**. *Medicine and science in sports and exercise, 53*(6), 1252–1259. https://doi.org/10.1249/MSS.0000000000002570 (IF: 5.411)

Study 2:

Castells-Sánchez, A., Roig-Coll, F., Dacosta-Aguayo, R., Lamonja-Vicente, N., Sawicka, A. K., Torán-Monserrat, P., Pera, G., Montero-Alía, P., Heras-Tebar, A., Domènech, S., Via, M., Erickson, K. I., & Mataró, M. (2021). **Exercise and Fitness Neuroprotective Effects: Molecular, Brain Volume and Psychological Correlates and Their Mediating Role in Healthy Late-Middle-Aged Women and Men**. *Frontiers in aging neuroscience, 13*, 615247. https://doi.org/10.3389/fnagi.2021.615247 (IF: 5.750)

Study 3:

Castells-Sánchez, A., Roig-Coll, F., Dacosta-Aguayo, R., Lamonja-Vicente, Torán-Monserrat, P., Pera, G., ... & Mataró, M. **Molecular And Brain Volumen Changes Following Aerobic Exercise, Cognitive And Combined Training: The Projecte Moviment**. (Accepted under review in *Psychophysiology*, IF: 4.016).

These three studies will be presented and discussed in six general chapters in the present work. The post-print manuscripts accepted in the journal are included in a chapter of the thesis.

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GLOSSARY OF ABREVIATIONS

AE	Aerobic Exercise
BDNF	Brain-Derived Neurotrophic Factor
BMI	Body Mass Index
BRPES	Borg Rating Of Perceived Exertion Scale
CCT	Computerized Cognitive Training
CI	Confidence Interval
COMB	Combined Training
CORE-OM	Short Informant Questionnaire In Routine Evaluation Outcome Measure
CRF	Cardiorespiratory Fitness
CRP	C-Reactive Protein
CSF	Cerebrospinal fluid
FITT-VP	Frequency, Intensity, Time, Type, Volume And Progression
GDS	Geriatric Depression Scale
GM	Gray Matter
GNPT	Guttmann Neuropersonal Trainer
HDL	High-Density Lipoproteins
HGF	Hepatocyte Growth Factor
HR	Heart Rate
ICAM-1	Intercellular Adhesion Molecule 1
ICV	Intracranial Volume
IGF	Insulin Growth Factor
IL-1	Interleukin-1
IL-6	Interleukin-6
ITT	Intention-To-Treat Sample
MET	Metabolic Equivalent Of Task
MMSE	Mini Mental State Examination
MoCA 5-min	Montreal Cognitive Assessment 5-Min
MRI	Magnetic Resonance Imaging
NS-PA	Non-Sportive Physical Activity
PA	Physical Activity
PP	Per Protocol Sample
PSQI	Pittsburgh Sleep Quality Index
RCT	Randomized Controlled Trial
REGICOR	Registre Gironí Del Cor
RONS	Reactive Oxygen And Nitrogen Species Levels
S-IQCODE	Spanish Version Of The Informant Questionnaire On Cognitive Decline In The Elderly
S-PA	Sportive Physical Activity
SDF1-α	Stromal Cell-Derived Factor-1 Alpha Protein
TNF-α	Tumor Necrosis Factor Alpha
VAMS	Modified Version Of Visual Analog Mood Scale
VEGF	Vascular Endothelial Growth Factor
VO_{2max}	Maximal Aerobic Capacity
VREM	Minnesota Leisure Time PA Questionnaire
WAIS-III	Wechsler Adult Intelligence Scale III
WM	White Matter

Additional abreviations included only in tables and figures:

AMPK	Adenosine Monophosphate-Activated Protein Kinase
AP-1	Activator Protein 1
APOE	Apolipoprotein E
Att	Attention
ATT-SP	Attention-Speed
BBB	Blood-Brain Barrier
BNT	Boston Naming Test
CD14+CD16+	Blood Monocytes
CD4CD25	Regulatory T Cells
CREB	Camp Response Element-Binding Protein

DMN	Default-Mode Network
DV	Dependent Variable
EF	Executive Function
ERK1/2	Extracellular Signal-Regulated Kinases
Fl	Fluency
Flex	Flexibility
GPx	Glutathione Peroxidase
HPC	Hippocampus
HSP	Heat Shock Proteins
IL-10	Interleukin-10
IL-15	Interleukin-15
IL-1α	Interleukin-1alpha
IL-4	Interleukin-4
IL-8	Interleukin-8
IL-ra	Interleukin-1 Receptor Antagonist
INFy	Interferon Gamma
Inh	Inhibition
IV	Independent Variable
LDL	Low-Density Lipoproteins
LNG	Language
LRM	Linear Regression Models
LTP	Long-Term Potentiation
M1: M2	Macrophages 1 To 2 Ratio
M2: M1	Macrophages 2 To 1 Ratio
MAPKp38	P38 Mitogen-Activated Protein Kinases
MCI	Mild Cognitive Impairment
MCP-1	Monocyte Chemoattractant Protein 1
MMR	Memory
mTFA	Mitochondrial Transcription Factor A
NF-kB	Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells
NRF-1	Nuclear Respiratory Factor 1
PFC	Prefrontal Cortex
PGC-1	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1
RAVLT	Rey Auditory Verbal Learning Test
ROCF	Rey-Osterrieth Complex Figure
rPCG	Right Precentral Gyrus
Sig.	Significant
sIL-6R	Soluble Interleukin-6 Receptor
SlQ	Sleep Quality
SOD	Superoxide Dismutase
Sp	Speed
sTNFR2	Soluble Tumour Necrosis Factor Receptor-2
TBI	Traumatic Brain Injury
Th2: Th1	T Helper 2 (Or CD4+) To T Helper 1 Ratio
TLR4	Toll-Like Receptor 4
TMT	Trail Making Test
Vb-M	Verbal Memory
VCAM-1	Vascular Cell Adhesion Protein
VF	Visuospatial Function
Vs-M	Visual Memory
WM	Working Memory

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Abstract

Aging is a normal biological process characterized by a decline at molecular, brain and behavioral level with consequences on cognition and daily functioning. Lifestyle behaviors such as physical activity or stimulating brain activities might enhance neuroprotective benefits when applied single or in combination in healthy adults. Current literature also focuses on understanding the role of physical activity and cardiorespiratory fitness in those benefits as well as better describing the cascade of changes related to exercise.

Therefore, the main aims of this dissertation are: 1) To examine the role of physical activity, grouped as exercise and non-exercise, in the promotion of molecular, brain, psychological health and cognitive benefits; 2) To examine the role of cardiorespiratory fitness in the promotion of these neuroprotective benefits as well as in the exercise-cognition relationship; 3) To identify potential sex-differences in the role of physical activity and cardiorespiratory fitness; 4) To examine the effect of 12-weeks 5 days per week of aerobic exercise, computerized cognitive training and their combination on molecular and brain health and assess their role in the intervention-related cognitive benefits.

Projecte Moviment consists of a 12 weeks 5 days randomized controlled trial, which allowed us to identify intervention-related changes in physically inactive healthy older adults as well as the role of other variables of interest. We recruited 109 participants and assessed them within 2 weeks before and after the intervention; 82 showed >80% adherence out of the 92 who finished the intervention. Moreover, we designed a cross-sectional strategy to describe the physical activity-cognition relationship in a sample that included 115 low to high fit participants. The assessment protocol included a battery of neuropsychogical and psychological health tests, physical activity questionnaries and fitness test, antropometric measures and cardiovascular risk factors, blood test, and neuroimaging.

Results were reported in 3 different papers and the main findings are: 1) We specifically related sportive physical activity, and not non-sportive physical activity, to benefits on cognitive performance as well as markers of inflammation, brain volume and psychological health. 2) We determined that cardiovascular health might be a key factor in the exercise-related benefits, specifically in men. 3) We identified that sex matters in the understanding of exercise and cardiorespiratory fitness benefits. 4) Cardiorespiratory fitness-related benefits in brain volume, executive function and sleep quality in men might be mediated by inflammatory markers. 5) Although after 12-weeks of aerobic exercise and combined training we did not detect significant changes in the targeted molecular and brain volume outcomes that might explain the detected cognitive benefits, significant increases in physical activity outcomes were negatively related to changes in SDF1-α and ICAM-1 levels. 6) We identified that 12-weeks, 5 days per week of home-based multimodal computerized cognitive training enhanced the volume of the precuneus, despite the lack of transfer to our cognitive assessment. Projecte Moviment adds crucial evidence for research and clinical practice to better understand how lifestyle behaviors enhance neuroprotective benefits at late-life.

Resum

L'envelliment és un procés biològic normal caracteritzat per un deteriorament a nivell molecular, cerebral i conductual amb conseqüències en la cognició i el funcionament quotidià. Els comportaments relacionats amb l'estil de vida com l'activitat física o activitats d'estimulació cognitiva podrien tenir beneficis neuroprotectors quan s'apliquen individualment o en combinació en adults sans. La literatura actual també se centra en entendre el paper de l'activitat física i la capacitat cardiorespiratòria en aquests beneficis, així com en descriure millor la cascada de canvis relacionats amb l'exercici.

Per tant, els principals objectius d'aquesta tesi són: 1) Examinar el paper de l'activitat física, agrupada com a exercici i no exercici, en la promoció de beneficis cognitius i de salut molecular, cerebral i psicològica; 2) Examinar el paper del fitness en la promoció d'aquests beneficis neuroprotectors, així com en la relació exercici-cognició; 3) Identificar les possibles diferències de sexe en el paper de l'activitat física i el fitness; 4) Examinar l'efecte de 12 setmanes, 5 dies per setmana d'exercisi aeròbic, entrenament cognitiu computeritzat i la seva combinació en els nivells de factor de creixement cerebral, marcadors inflamació i volum cerebral i avaluar el seu paper en els beneficis cognitius relacionats amb la intervenció.

El Projecte Moviment consisteix en un assaig controlat aleatoritzat de 12 setmanes i 5 dies per setmana que ens va permetre identificar els canvis relacionats amb aquestes intervencions en adults sans físicament inactius. Vam reclutar 109 participants i els vam avaluar durant les dues setmanes d'abans i després de la intervenció; dels 92 que van acabar la intervenció, 82 van mostrar >80% d'adherència. A més, vam dissenyar una estratègia transversal per descriure la relació activitat física-cognició en una mostra que incloïa 115 participants de baix i alt estat físic. El protocol d'avaluació incloïa una bateria de proves neuropsicològiques i de salut psicològica, qüestionaris d'activitat física i proves de condició física, mesures antropomètriques i factors de risc cardiovascular, anàlisi de sang i exploracions de neuroimatge.

Els resultats es van informar en 3 articles diferents i les principals troballes són: 1) Vam relacionar específicament l'activitat física esportiva, i no la no esportiva, amb beneficis en el rendiment cognitiu, així com amb marcadors d'inflamació, volum cerebral i salut psicològica. 2) Vam identificar que la salut cardiovascular podria ser un factor clau en els beneficis relacionats amb l'exercici, específicament en els homes. 3) Vam identificar que el sexe és important en la comprensió dels beneficis relacionats amb l'exercici i la capacitat cardiorespiratòria. 4) Els beneficis relacionats amb la salut cardiorespiratòria en el volum del cervell, la funció executiva i la qualitat del son en homes podrien estar mediats per marcadors inflamatoris. 5) Tot i que després de 12 setmanes d'exercici aeròbic i d'entrenament combinat no vam detectar canvis significatius en les variables moleculars i cerebrals analitzades

que podrien explicar els beneficis cognitius detectats, els augments significatius en les variables d'activitat física es van relacionar negativament amb els canvis en nivells de SDF1-α i ICAM-1. 6) Es va identificar que 12 setmanes, 5 dies a la setmana d'entrenament cognitiu computerizat multimodal des de casa van millorar el volum del precuneus, tot i la manca de transferència dels beneficis cognitius. El Projecte Moviment aporta evidència rellevant per a la investigació i la pràctica clínica en relació als beneficis neuroprotectors que comportaments relacionats amb l'estil de vida poden tenir en l'envelliment.

INTRODUCTION

1. AGING, NORMAL AGING

Luckily, most of us are going to age; that was the eye-catching message we came across when writing one of our papers in 2019 (Roig-Coll et al., 2020). The current global health situation made this statement a personal, institutional and scientific challenge. If you were lucky to age, the following section would be of your interest and will set the fundamentals of Projecte Moviment and this thesis.

Aging, popularly known as the process of becoming older, is defined as the set of progressive physiological changes in an organism that lead to senescence, or a decline of biological function of the organism's ability to adapt to metabolic stress; a multi-level process that takes place in a cell, an organ, and in the holistic function of the organism across the entire adult life span of any living thing (Rogers et al., 2020). In humans, aging is related to major risk of cardiovascular diseases, metabolic syndrome, mitochondrial dysfunction, obesity, sarcopenia, and consequent higher inflammation, oxidative stress, brain and cognitive impairment and loss of quality of life (Murman, 2015; Sallam & Laher, 2016). Yes; *normal aging*, characterized by the lack of overt pathology, is related to larger life experience but also to normal changes in cognitive performance (Dumas, 2015) as a consequence of multi-level and multi-system physiological changes. It is our interest the effect of aging on cognitive and brain health of human beings and how to enhance healthy aging. However, to better understand these changes and the holistic context in which they take place, we should make a trip through the molecular and cellular, brain and behavioral changes - discussed as Level 1, 2 and 3 mechanisms -, based on the model proposed by Stillman et al. (2016) (See Figure 1). Hopefully, you are joining.

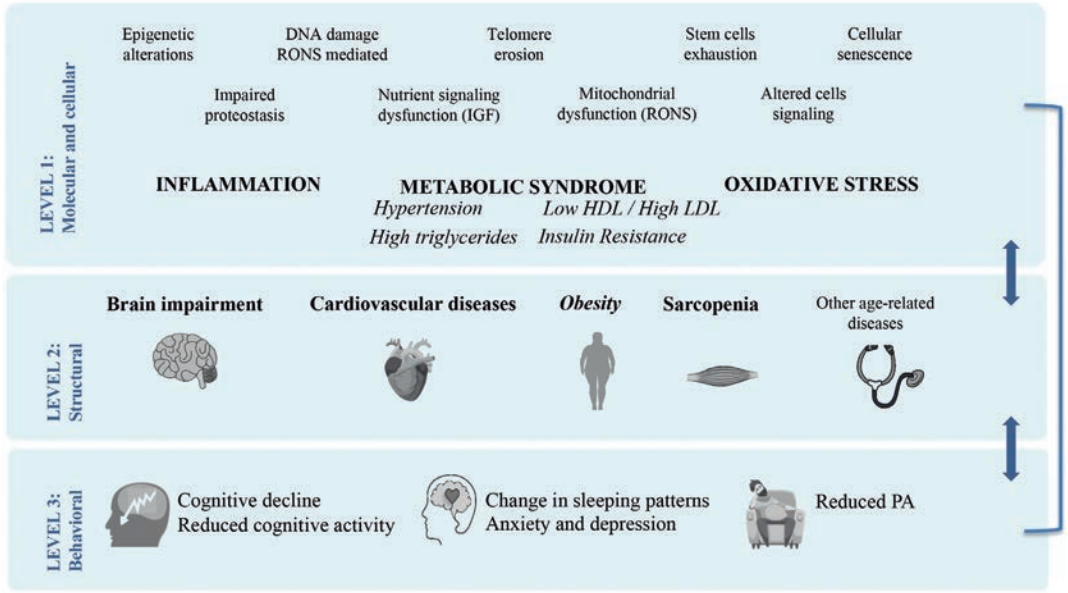


Figure 1. Age-related changes at multiple levels of analysis, created accordingly to the cited bibliography in the section by A. Castells-Sánchez. *In lyrics, those concepts included in the metabolic syndrome. HDL: high-density lipoproteins; IGF: insulin growth factor; LDL: low-density lipoproteins; PA: physical activity; RONS: reactive oxygen and nitrogen species.*

1.1. Level 1: Molecular and cellular changes in aging

At Level 1, aging is related to a cascade of molecular and cellular changes with effects in multiple systems and increased risk of disease. Genomic instability, telomere attrition, epigenetic alterations, loss of protein homeostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication were highlighted as the nine most relevant cellular hallmarks of aging by López-Otín et al. (2013). As a consequence of these changes, it is common to find altered levels of inflammatory, oxidative stress, metabolic, neuronal, and cell growth markers in aging (Garatachea et al., 2015).

The interplay between the inflammatory and oxidative stress processes is key in several physiological aging changes. While lower protein synthesis reduces antioxidant mechanisms, mitochondrial dysfunction elevates reactive oxygen and

nitrogen species levels (RONS), leading to a vicious cycle of oxidative damage (Cotman et al., 2007; Sallam & Laher, 2016). Oxidative stress damages macromolecules (lipids, proteins, and nucleic acids) in the brain, heart, kidney, muscles, etc. and promotes increased pro-inflammatory processes leading to a state of low-grade chronic inflammation, also called inflammaging (Franceschi et al., 2017; Monteiro-Junior et al., 2018; Woods et al., 2012). Therefore, aging is related to increased inflammatory activity in the blood including high levels of tumor necrosis alpha (TNF-α), interleukin-6 (IL-6), interleukin-1 (IL-1), cytokine antagonists and systemic inflammatory biomarkers such as C-reactive protein (CRP) and neopterin, as well as other pro-inflammatory mediators (Cotman et al., 2007; Monteiro-Junior et al., 2018; Sallam & Laher, 2016). In this context, for example, in order to enhance smooth muscle grow, TNF-α promotes the expression of intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1. Those molecules increase adherence of leucocytes to endothelial cells, and are involved in the production of chemokines, such as stromal cell-derived factor-1 alpha protein (SDF1-α), also known as CXCL12 (Bruunsgaard et al., 2000). An activated immune system generates RONS leading to oxidative stress promoting the deleterious circle of inflammation-oxidative stress.

Another deleterious circle is the interaction between inflammaging and deficits in nutrient signaling such us altered levels of insulin growth factor (IGF), which can lead to insulin resistance and cardiovascular risk factors. A pro-inflammatory environment induces a lipid profile coherent with a metabolic syndrome characterized by increased levels of triglycerides, cholesterol, and low-density lipoproteins, decreased concentrations of high-density lipoproteins (HDL) as well as cardiovascular risk diseases such as hypertension, dyslipidemia, obesity and diabetes (Bruunsgaard et al., 2000; Sallam & Laher, 2016). At the same time, these conditions maintain the systemic inflammation. The fact is that IGF is crucial for vascular health and brain maintenance and plasticity given its role in the promotion of other key growth factors, such as vascular endothelial growth factor (VEGF) and brain-derived growth factor (BDNF) (Cotman et al., 2007). On one hand, IGF is associated with increased VEGF

and greater angiogenesis and cardiovascular health, which enhances neuroplasticity in the brain (Cotman et al., 2007). On the other, evidence suggests that IGF facilitates BDNF expression (and its receptor TrkB) in the brain, which in turn is related to neural plasticity and cognitive performance (Ding et al., 2006). Therefore, age-related changes in IGF levels might be related to lower levels of BDNF with age (Erickson et al., 2010a), brain damage, neuroinflammation and cognitive decline in aging.

Sex-Related Box 1

There are sex differences in the response of in the immune system to external stimuli. While women show stronger responses which contributes to better pathogen clearance but also increased inflammatory profile and auto-immune diseases, genomics changes facilitate a greater pro-inflammatory environment in men after age 65 (Márquez et al., 2020). Moreover, recent reviews (Chan & Ye, 2017; Wei et al., 2017) suggested that sex-differences in BDNF signaling might be related to sexually dimorphic neural circuits as well as sex-differences in cognitive and psychological health. In particular, literature reports that BDNF gene is modulated by an estrogen receptor-responsive element. Therefore, not only sex-differences in steroid hormones, but also differences in estradiol levels across the lifespan of women (e.g. estrous cycle or menopause), might modify BDNF levels in brain tissue, and in turn, BDNF role (Chan & Ye, 2017; Wei et al., 2017). Sex-differences in estrogen and testosterone levels across the lifespan, which are pro-inflammatory and anti-inflammatory respectively (Furman et al., 2014), might induce sex-differences in immune and growth factors markers. Therefore, sex should be addressed when exploring molecular changes in aging.

1.2. Level 2: Brain changes in aging

As a result of changes described before, aging has diverse structural effects on the body such as sarcopenia, tendency to obesity, osteoporosis or atherosclerosis that impact several body functions. Our interest in this thesis focuses on age-related structural brain impairment. The effects of aging on the brain are widespread, not uniform, sex-related and result of multiple factors. Changes at Level 1, molecular and cellular level, such as cytokines, hormones and neurotransmitters as well as the influence of genetic factors convey to structural decreases in gray matter (GM) and

white matter (WM) volume as well as WM lesions. Moreover, even in those healthy older adults in which brain volume changes are not detectable, there are declines in WM fractional anisotropy (Sullivan et al., 2006). Another general finding is reduced functional connectivity in older adults in terms of decreased within-network connectivity in the default mode network, salience network, fronto-parietal network and dorsal-attention network, increased connectivity in between networks, less segregated network structure and local efficiency and higher participation coefficient using different types of connectivity analyses (Andrews-Hanna et al., 2007; Betzel et al., 2014; Damoiseaux et al., 2006, 2008; Geerligs et al., 2015; Onoda et al., 2012; Sala-Llonch et al., 2014; Tomasi & Volkow, 2012). Aging also brings increased experience and a baggage of lifestyle factors that might enhance or diminish brain health (Lockhart & DeCarli, 2014; Peters, 2006).

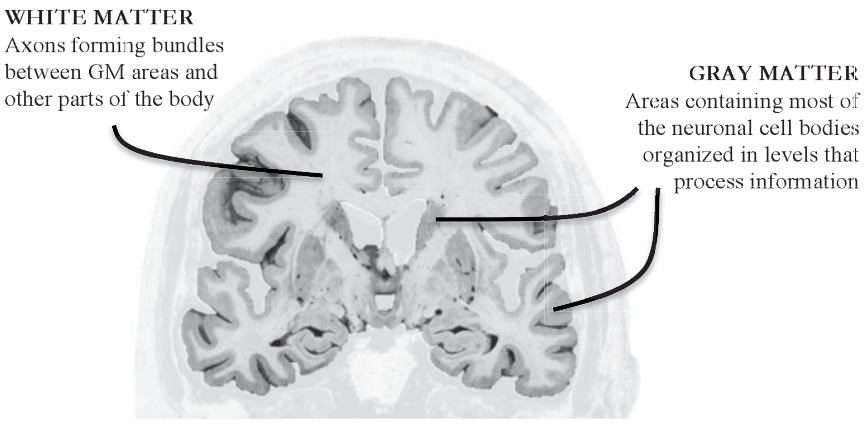
Structural brain age-related changes

It might not be good news but evidence suggests that the volume and weight of the brain shrinks after age 40 at a rate of 5% every 10 years, and at a greater rate after age 70 (Scahill et al., 2003; Svennerholm et al., 1997). The fact that the decrease is not uniform leads to a widely known pattern of GM deterioration in which the prefrontal cortex is the most affected in T1-weigthed magnetic resonance imaging (MRI) scans, coherent with the principle *the last, the first*. Based on a review of cross-sectional studies, the following most affected structures are the striatum, the temporal lobe, the cerebellum and hippocampus; being the occipital lobe the last to be affected (Raz et al., 2005). Other studies highlight the hippocampus as the most affected structure by age (Anderton, 2002). There is also a decline in WM volume and integrity that can be observed specially in the frontal lobe and with the increased volume of the ventricles (Scahill et al., 2003). Beyond mild discrepancies for the title *the most*, these findings are widely accepted and in accordance with age-related changes in cognition.

Box 1. Brain volume and T1-weighted scans

A T1-weighted image is one of the basic pulse sequences in MRI and depicts differences in signal based upon the longitudinal relaxation of the magnetization vector of each tissue. First, an external magnetic field aligns the spins of the tissues, then, a transverse radio pulse impacts and the spins move; finally, spins come back to their original equilibrium. Tissues show differences in the time taken to recover the original position. T1 scans use this principle to obtain the probability of one voxel - a cube-shaped part of the brain - of belonging to a type of tissue and draw a great structural image of the brain. T1 scans are used to calculate brain volume from GM structures, WM, cerebrospinal fluid as well as cortical thickness (Chen et al., 2018).

Image: A. Castells-Sánchez.



Despite the highlighted patterns in age-related decline of the brain structure, there are individual differences in the degree of severity from pathological deterioration to healthy/scarce effects (Hultsch & Macdonald, 2004) (See Figure 2). Some of these differences were linked to lifestyle factors and led to the possibility of modifying the course of the alteration with the right prevention and intervention (Clare et al., 2017).

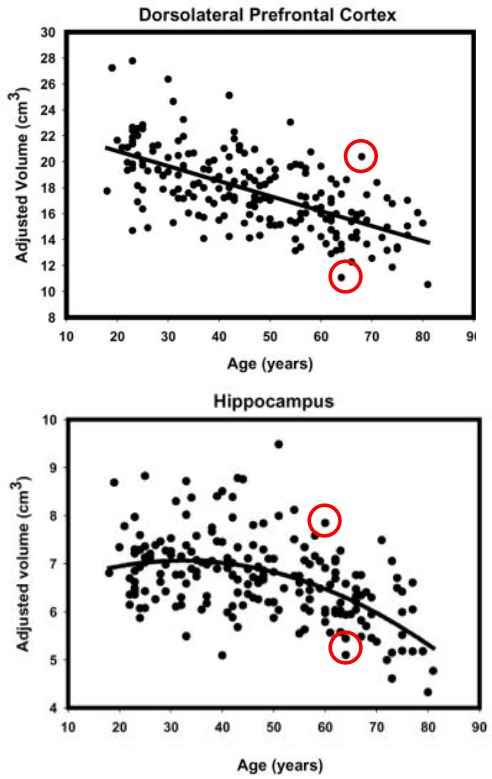


Figure 2. Individual differences in brain volume decline, adapted from Kennedy et al. (2009) by M. Mataró. *Red circles show extreme brain volume difference between individuals at a similar age.*

Sex-Related Box 2

Age-related structural changes might be sex-dependent. Evidence suggest that men tend to show greater volume loss in the hippocampus (Li et al,, 2014; Pruessner et al., 2001; Raz et al., 2004) and frontal lobe (Raz et al., 2004) whereas the parietal lobes are the most affected in women (Murphy et al., 1996). Literature suggests that age-related reductions in brain volume might be related to the role of gonadal hormones on growing factors and cardiovascular parameters across the lifespan (Yoo & Fu, 2020). Therefore, sex should be addressed when exploring brain volume changes in aging.

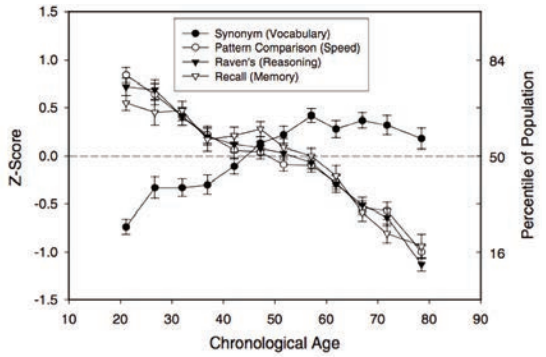
1.3. Level 3: Psychological, physical and cognitive changes in aging

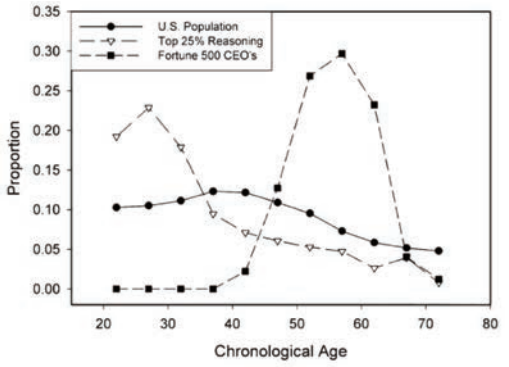
Molecular and structural age-related changes have behavioral consequences that impact daily function. Older adults experience changes in sleeping patterns such as advanced sleep timing, shorter sleep duration and frequent awakenings at night, and more naps during the day (Li et al., 2018). Normal aging is also related with more psychological problems such as depression or anxiety symptoms usually related to physiological changes, life stressors, loss of sense of purpose in life, loss of good health and functional status (Clarke et al., 2000; World Health Organization [WHO], 2017). Being active might play an important role in the maintenance of health and functionality. However, aging is commonly associated with increased sedentarism (Copeland et al., 2015; Harvey et al., 2015), lower resting metabolic rate and total energy expenditure (Johannsen et al., 2008), not only because of social trends (Harridge & Lazarus, 2017), but also as a result of physiological changes such as sarcopenia and obesity. Lower cardiorespiratory fitness (CRF) level is associated with lower cardiovascular health, physical function and autonomy in older adults (Gomez-Bruton et al., 2020). Therefore, physical inactivity contributes to age-related changes at Level 1 and 2. Those maintaining an active lifestyle might delay age-related changes including emotional and cognitive well-being (Coelho et al., 2020).

Late-adulthood is known for being the finest moment for crystallized cognition**;** older adults maintain or even increase vocabulary and expertise-based abilities. However, even healthy normal aging, characterized by the lack of pathology, is related to progressive decline in fluid cognitive abilities, which is associated with reductions in executive function, memory, attention and processing speed and deficits in social and independent functioning (Craik & Bialystok, 2006; Harada et al., 2013; Hausman et al., 2020; Murman, 2015; Salthouse, 1994; Salthouse, 2012). Although age could be considered the greatest risk for cognitive decline, several factors, from genetics to life experiences, might impact cognitive and brain health, generating a great individual diversity in cognitive trajectories.

Box 2. Salthouse's studies

Timothy Salthouse assessed more than 8.000 people in the University of Virgina, where he spent years researching cognitive aging. The plot he published showing cognitive changes in different domains across the lifespan (Salthouse et al., 2004) - see on the right - became one of the main foundations for aging neuropsychology and neuroscience.





Salthouse's research proliferated in the field with several papers highlighting the impact of age on cognition. Despite the evidence of a progressive decline, he also identified significant individual differences. In an easy-reading design, he reported that despite cognitive functions might peak at late 20's for general population, CEOs of Fortune 500 companies showed that peak just before age 60 (Salthouse, 2012). This finding raised the interest for individual differences and the possibility of increased cognition in late-life.

Images: Extracted from Salthouse et al. (2004) and Salthouse (2012), respectively.

In the field of individual differences in cognitive aging, researchers came across with a concept to refer the cognitive resilience to age-related effects; *cognitive reserve.* Stern (2009) described the cognitive reserve as individual differences in neural processes underlying cognitive performance that lead to cope with brain damage in different ways and allows to account for the disjunction between the degree of brain damage and its performance. The model highlights the idea of a cognitive reserve as an active model in which the *reserve* might be modulated by life experiences such as education, leisure/cultural activities and lifestyle factors. Therefore, it holds out the possibility of prevention and intervention in order to promote cognition and healthy aging.

Sex-Related Box 3

There is a growing interest about the effect of sex on trajectories of cognitive aging and discrepancies are rising the opportunity of research. McCarrey et al. (2016) found that, once adjusted for age, education and race, women had better performance in most of the cognitive tests and had no significant decline, whereas men outperformed them on visuospatial tasks but had steeper rates of decline for speed, integration and visuospatial ability. Another study reported that women performed better than men on tests of psychomotor speed and verbal learning and memory, and men had better scores on tests of visuoconstruction and visual perception (Munro et al., 2012). Similar results were reported in the Seattle Longitudinal Study in relation to women (Gerstorf et al., 2011). However, other studies such as the Berlin Study of Aging reported no sex differences (Gerstorf et al., 2006). In accordance with these discrepancies, Rentz et al. (2017) found that women had better scores in all memory measures, but sex differences were attenuated for postmenopausal women. Those results were interpreted in relation to sex differences in physiological parameters such as the role of estradiol in midlife in the shaping of memory. Therefore, sex should be addressed when exploring cognitive changes in aging.

2. Healthy aging & lifestyle interventions

In the previous section we broadly discussed molecular, neural and cognitive changes that occur when aging. Although we brought up the strengths of this process, most of the highlighted points were about a progressive physiological and functional decline. However, as we also pointed before, causes of aging are multifactorial and a great variability emerges in between people when they age. High levels of cognitive performance are identified despite the remarkable brain decline, too. An integrative approach, the Scaffolding Theory of Aging and Cognition (Park & Reuter-Lorenz, 2009), explained this fact as the result of the recruitment of neural compensatory processes that scaffold and sustain the function when the former circuits are becoming inefficient (See Figure 3). The most interesting part of this model is the idea that an active lifestyle might contribute to the brain scaffolding.

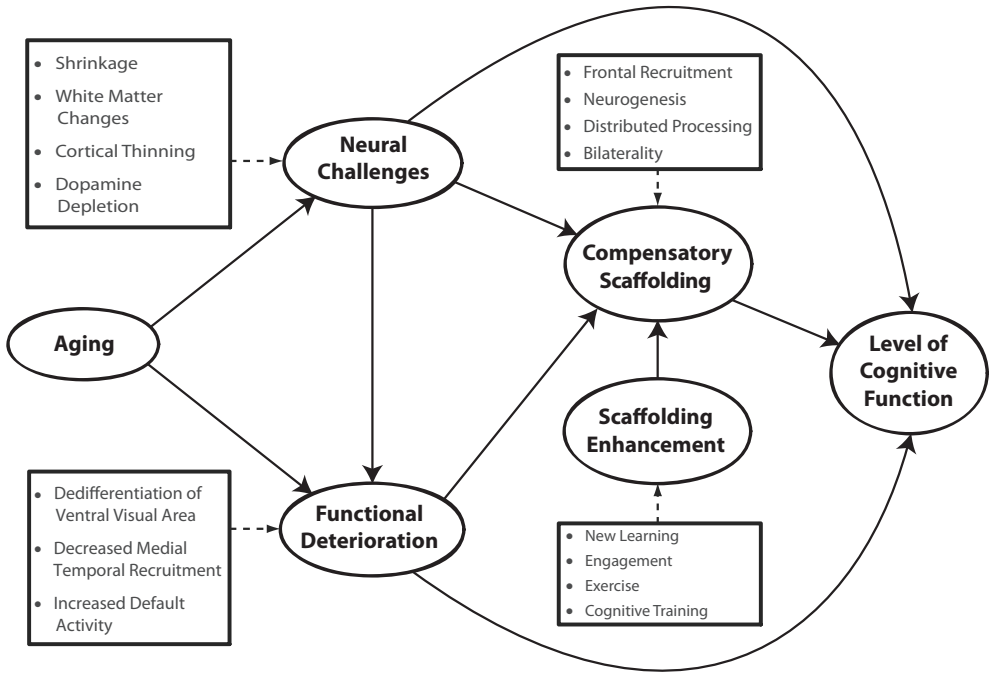


Figure 3. The scaffolding theory of aging and cognition model, extracted from Park & Reuter-Lorenz (2009).

If we consider aging as an interaction between deleterious and protective factors, we might not only focus on the decline, but to identify how we can buffer these protective factors. Actually, successful healthy aging as defined by Rapp et al. (2020) consists on buffering, protecting or compensating the aged brain. Therefore, it is our interest the inclusion of behavioral interventions, such as physical and cognitive training, as potential mechanisms to preserve cognitive and brain health and fortunately, add evidence to the emerging literature indicating that age-related decline may not be inevitable (Stillman et al., 2019).

Box 3. Healthy aging is a must in an aged society

Increased life expectancy and decreased levels of fertility produce a continued change in the age structure of the world population with increased levels of old people. The global population aged 65 is estimated to increase from 9,3% in 2020 to 16% by 2050, which in Europe might represent 40 million people (He et al., 2016; United Nations Department Of Economic and Social Affairs, 2020). Therefore, it is a global responsibility to promote *healthy aging,* conceptualized as "the process of developing and maintaining the functional ability that enables well-being in older age". We need policies and strategies that enhance independence and quality of life while considering economic, social, environmental, and personal determinants as well as health and social services.

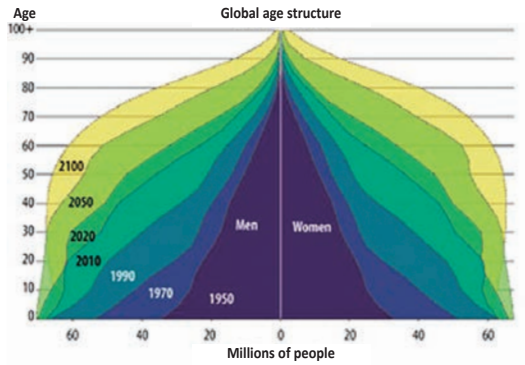


Image: Extracted from United Nations Department of Economic and Social Affairs, World Population Division (2017).

3. Physical activity and exercise

Epidemiological studies have indicated that an active lifestyle including exercise positively impacts several major hallmarks of aging and has neuroprotective benefits (Garatachea et al., 2015). Physical activity (PA) and exercise are known to promote general health, cognitive and psychological benefits in clinical and non-clinical populations (Barha et al., 2017; DiLorenzo et al., 1999; Northey et al., 2018; Penedo & Dahn, 2005); and those benefits have been described when using a measure of self-reported PA and when assessing physiological correlates of habitual PA such as CRF.

Box 4. PA and exercise-related concepts

PA is any body movement produced by skeletal muscles that results in energy expenditure (Caspersen et al., 1985).

Exercise is considered a planned, structured and repetitive subtype of PA that aims to improve physical fitness (Caspersen et al., 1985).

Aerobic exercise (AE) is the type of exercise that involves oxygen consumption and movement of large groups of skeletal muscles during a sustained period of time (Chodzko-Zajko et al., 2009).

CRF is the ability of the cardiovascular system to supply oxygen to the organism during sustained PA. **Maximal aerobic capacity (VO_{2max})** is the gold standard measure to assess CRF (Kline et al., 1987).

Beyond the well-known message "PA has neuroprotective effects" (Stillman et al., 2016) there is still room in the current state of art to discuss whether any type of PA might enhance cognitive effects or, on the contrary, it is specifically exercise, as a subtype of PA activity with specific characteristics, the one facilitating those benefits. Actually, exercise specifically produces an acute body reaction that includes increased energy expenditure, repetitive muscle contractions and an inflammatory and oxidative acute response (Sallam & Laher, 2016; van Praag et al., 2014). As a consequence of a regular practice, exercise, particularly AE, has direct effects on our body: higher oxygen and glucose consumption related to increased energy expenditure, reduction of body fat and increased muscle strength, which have been hypothesized as specific pathways for the physiological relationship between exercise and cognitive function (Cotman et al., 2007; Sallam & Laher, 2016; Stimpson et al., 2018; van Praag et al., 2014). Previous literature has identified potential mechanisms of the exercise-cognition association at multiple levels: molecular, brain, and behavioral. Stillman et al. (2016) included a multi-level model describing the potential pathways by which exercise might exert the cognitive benefits (See Figure 4). Identifying these pathways is a challenge not only due to the multi-level mechanisms but also because of the role of factors that may moderate the association such as sex (Barha et al., 2019), age (Bherer

et al., 2021) and other factors beyond the scope of the current thesis such as genetics - Apolipoprotein E (Colovati et al., 2020; Smith et al., 2011) and BDNF (Canivet et al., 2017) genes -. In this thesis, as we did in the previous section and the published papers, we will discuss about these markers as Level 1, 2, and 3 mechanisms, respectively, based on this same model.

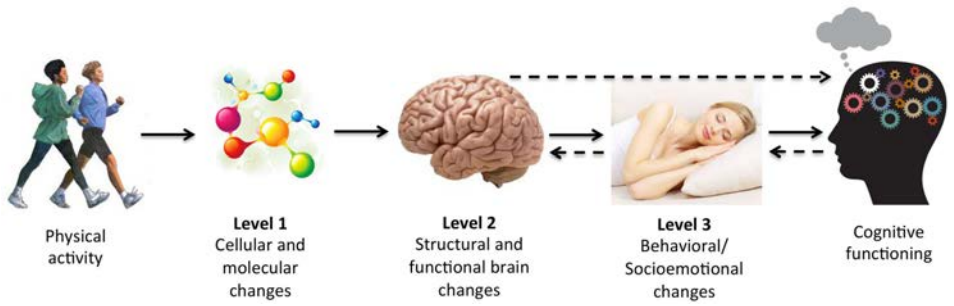


Figure 4. Conceptual model of mechanisms of PA at multiple levels of analysis, extracted from Stillman et al. (2016). *Arrows and broken arrows represent potential pathways by which PA might enhance cognition.*

3.1. PA-related changes at Level 1

Molecular and cellular exercise-related changes are described at Level 1 and include key factors involved in inflammation, oxidative stress, metabolism, angiogenesis and neuroplasticity (See Figure 5).

In particular, regular exercise is known to induce anti-inflammatory and antioxidant actions by modulating cytokines and oxidative stress factors on adipose tissue, body muscles and immune system (Sallam & Laher, 2016). Evidence suggests that exercise might reduce levels of systemic inflammation modulating markers such as CRP, IL-6, IL-1, and TNF-α (Cotman et al., 2007; Sallam & Laher, 2016) through multiple processes, such as the reduction of fat due to increased energy expenditure. In relation to cardiovascular health, Woods et al. (2012) indicated that evidence for a relationship between CRF and TNF-α levels in humans was still insufficient for making definitive conclusions. Regarding the effect of exercise interventions, recent

meta-analyses reported significant benefits on the pro-inflammatory markers in healthy populations after AE interventions (Monteiro-Junior et al., 2018; Zheng et al., 2019), but only Zheng et al. (2019) found significant effects for decreased TNF-α in their meta-analysis. Another theoretical review suggested that the activity in the muscles might induce interleukin-10 and heat shock proteins, reducing the inflammatory environment while suppressing IL-1 and TNF-α and upregulating interluekin-15 and promoting the reparation of the vessels to facilitate blood flow and, as a consequence, facilitating oxygen and nutrient circulation (Sallam & Laher, 2016). The anti-oxidative response is mediated by redox-sensitive transcription factors, which reduce RONS and promotes mitochondrial biogenesis (Sallam & Laher, 2016).

In relation to metabolism, exercise increases energy expenditure. Therefore, it might be also related to higher glucose consumption which may be related to better energy metabolism and insulin sensitivity as well as reduced resistance to leptin and insulin (van Praag et al., 2014). Exercise increases circulating HDL and reverses cholesterol transport, reducing cholesterol levels in blood (Mann et al., 2014). The activity in the cardiovascular system produces laminar shear stress on vascular endothelial cells, which may be related to the down-regulation of oxidative processes, and activates the hypothalamic-pituitary-adrenal triggering the release of glucocorticoids that may help to inhibit the inflammatory system (Cotman et al., 2007; Sallam & Laher, 2016; Stimpson et al., 2018). Shear stress and mechanical load related to exercise induce changes in other anabolic and metabolic growth factors that affect muscles and bones such as hepatocyte growth factor (HGF), VEGF, and IGF. HGF is an hepatocyte with an anti-inflammatory role in adipose tissue, which is primarily linked to liver regeneration, obesity, insulin resistance and diabetes, is capable of modulating the inflammatory response, and promotes angiogenesis and neuroprotection (Kiliaan et al., 2014). However, the effect of exercise on HGF levels has been scarcely addressed in healthy older adults. Yasuda et al. (2004) found increased HGF levels after acute exercise which were negatively related to CRF in patients after acute myocardial infarction.

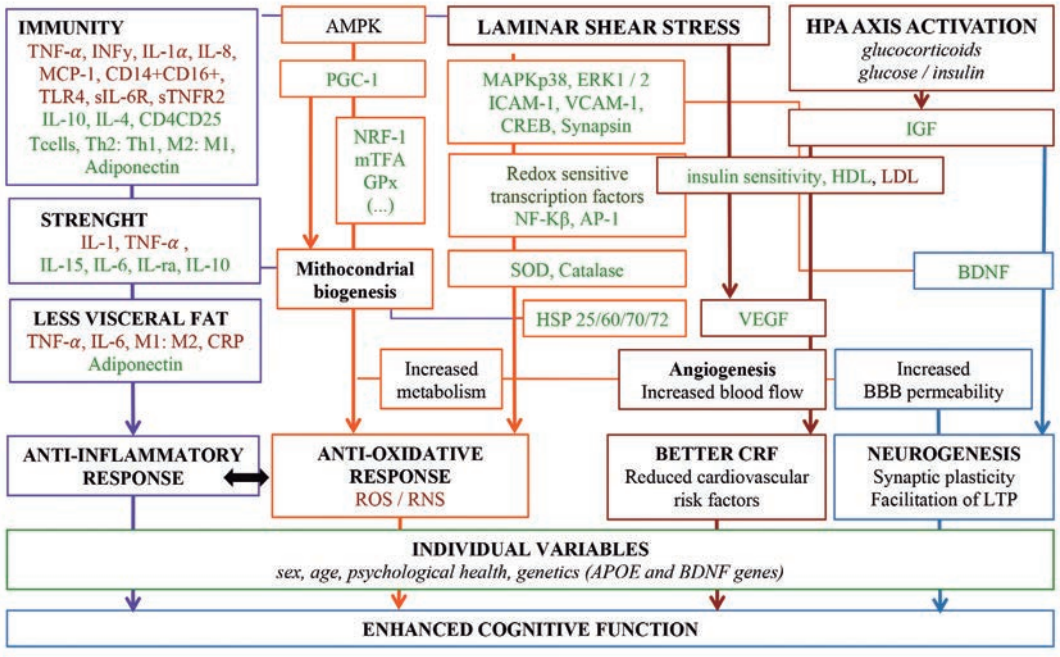


Figure 5. Conceptual model of mechanisms of PA at Level 1, created based on data included in Cotman et al. (2007), Sallam and Laher (2016), Stimpson et al. (2018) by A. Castells-Sánchez. *Green means increase, red means decrease; in bold: related processes; in lyrics: secondary-related processes or variables; black arrow means interaction. AMPK: adenosine monophosphate-activated protein kinase; AP-1: activator protein 1; APOE: apolipoprotein E; BBB: blood-brain barrier; BDNF: brain-derived neurotrophic factor; CD14+CD16+: blood monocyte; CD4CD25: regulatory T cells; CREB: cAMP response element-binding protein; CRF: cardiorespiratory fitness; CRP: c-reactive protein; ERK1/2: extracellular signal-regulated kinases; GPx: glutathione peroxidase; HDL: high-density lipoproteins; HSP: heat shock proteins; ICAM-1: intercellular adhesion molecules 1; IGF: insulin growth factor; IL-1: interleukin-1; IL-10: interleukin-10; IL-15: interleukin-15; IL-1α: interleukin-1alpha; IL-4: interleukin-4; IL-6: interleukin-6; IL-8: interleukin-8; IL-ra: interleukin-1 receptor antagonist; INFy: interferon gamma; LDL: low-density lipoproteins; LTP: long-term potentiation; M1: M2: macrophages 1 to 2 ratio; M2: M1: macrophages 2 to 1 ratio; MAPKp38: p38 mitogen-activated protein kinases; MCP-1: monocyte chemoattractant protein 1; mTFA: mitochondrial transcription factor A; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; NRF-1: nuclear respiratory factor 1; PGC-1: peroxisome proliferator-activated receptor gamma co-activator 1; RNS: reactive nitrogen species; ROS: reactive oxygen species; sIL-6R: soluble interleukin-6 receptor; SOD: superoxide dismutase; sTNFR2: soluble tumor necrosis factor receptor-2; Th2: Th1: T helper 2 (or CD4+) to T helper 1 ratio; TLR4: toll-like receptor 4; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein; VEGF: vascular endothelial growth factor.*

On the subject of angiogenesis, IGF and VEGF have consistently been related to endothelial cell proliferation and vessel growth as well as enhanced neurogenesis in the hippocampus after exercise due to their interaction with BDNF (Cotman et al., 2007; Stimpson et al., 2018). Molecular markers modulated by IGF and VEGF, such as SDF1-α or ICAM-1 were suggested to be involved in the promotion of angiogenesis in the brain after exercise based on animal studies (Stimpson et al., 2018). Significantly decreased levels of ICAM-1 related to PA were found in patients with cardiovascular risk factors (Palmefors et al., 2014) but evidence in healthy samples is still inconclusive with some (El-Kader et al., 2019) showing significant and others (Mohammadi et al., 2018) non-significant changes in post-test vs pre-test comparisons. In healthy young men, SDF1-α levels increased in response to acute exercise with an increase of endothelial cells in a 24h follow-up (Chang et al., 2015). However, the effect of exercise programs on these markers in humans is still insufficient for drawing clear conclusions (Palmefors et al., 2014).

Regarding the effect of exercise on enhanced neurogenesis, IGF might also be involved since it promotes the release of BDNF in the brain, which has been identified as one of the principal factors mediating the effect of exercise on cognition (Sallam & Laher, 2016). BDNF may support newborn cells, regulate synaptic changes and facilitate long-term potentiation, which may be related to the identified brain changes and cognitive benefits (Cabral et al., 2019; Stimpson et al., 2018). While available evidence consistently reported increased BDNF levels after an acute bout of exercise, the effect of regular exercise on resting BDNF remains unclear (Walsh & Tschakovsky, 2018). Initial systematic reviews and meta-analyses suggested that exercise training led to elevated peripheral BDNF (Coelho et al., 2013; Dinoff et al., 2016; Knaepen et al., 2010; Szuhany et al., 2015), but these results were driven by trials including clinical populations (Walsh & Tschakovsky, 2018). Recent reviews including healthy samples have reported non-significant effects of low to moderate AE on BDNF levels (Marinus et al., 2019) and highlight the need of more human studies to elucidate the role of BDNF in the exercise-related enhanced neuroplasticity (Loprinzi, 2019). A systematic review (Huang et al., 2014) reported a negative association between long-term regular PA and CRF with peripheral BDNF in observational studies. Those results may reflect a more efficient uptake mechanism of circulating BDNF into the brain in active subjects (Currie et al., 2009).

In this thesis we focused on the questions raised by the previously described literature since studies reporting molecular changes on inflammatory markers and growth factors in humans and, specifically, in healthy older adults are still scarce and the discussion remains opened to more evidence. Nevertheless, we should acknowledge that there are other factors at molecular level involved in the cascade of changes related to exercise that moderate the cognitive benefits, such as genetics, hormones and neurotransmitters, which Projecte Moviment research project addresses in other studies.

3.2. PA-related changes at Level 2

Exercise-related changes at molecular level have a positive impact in the structure and function of multiple systems in our body (See Figure 6). However, it is our interest and the focus of this thesis, the structural brain benefits related to exercise included at Level 2.

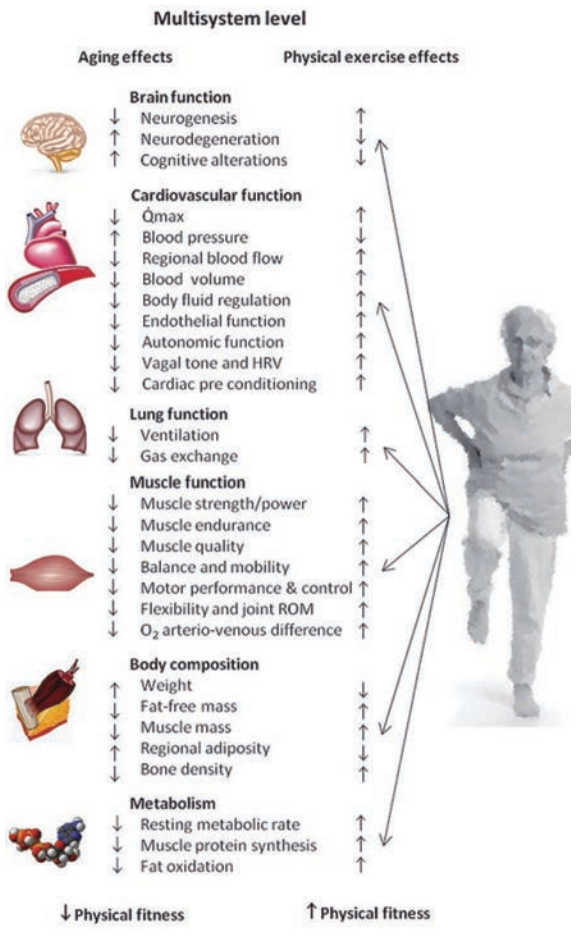


Figure 6. PA effects on multiple systems of the body in aging, extracted from Garatachea et al. (2015).

Brain volume

Reviews and systematic reviews reported beneficial effects of exercise on brain volume in healthy older adults, specifically for areas more related to normal brain aging (Bherer, 2015; Erickson et al., 2014; Sexton et al., 2016; Stillman et al., 2020). Cross-sectional studies showed that higher amounts of PA were associated with greater total brain volume (Benedict et al., 2013; Spartano et al., 2016) and GM volume in the frontal lobe (Bugg & Head, 2011; Erickson et al., 2010b; Eyme et al., 2019; Flöel et al., 2010), hippocampus (Erickson et al., 2010b; Raichlen et al., 2020; Yamamoto et al., 2017), cingulate cortex (Flöel et al., 2010), precuneus (Benedict et al., 2013; Eyme et al., 2019), and nucleus accumbens (Yamamoto et al., 2017). CRF levels have also been positively correlated with overall GM (Raichlen et al., 2020), multiple areas of the frontal lobe (Gordon et al., 2008; Weinstein et al., 2012; Wittfeld et al., 2020), medial-temporal lobe (Gordon et al., 2008; Wittfeld et al., 2020), hippocampus (Erickson et al., 2009; Szabo et al., 2011; Wittfeld et al., 2020), and cingulate cortex (Wittfeld et al., 2020) and caudate and nucleus accumbens (Verstynen et al., 2012) volume in healthy older adults. Findings related to WM indicated that engaging more frequently in PA could increase WM global volume (Arnardottir et al., 2016; Benedict et al., 2013; Gow et al., 2012). Only a few studies have examined the relationship between CRF and WM volume and did not find significant results (Burns et al., 2008; Gordon et al., 2008). However, literature suggests that these associations might be moderated by other variables since not significant results have also been found for the PA-GM volume relationship (Rosano et al., 2010). Sexton et al. (2016) systematically reviewed the association between exercise and WM volume – global, local, lesions, and microstructure –, and found cautious support for this link.

Systematic reviews including exercise interventions reported that AE training could be effective to prevent brain volume loss, increase GM volume (Erickson et al., 2014) and have a positive but small impact on global WM volume (Sexton et al., 2016). Most of the research on this topic has focused on the impact of exercise on the hippocampus showing significant positive effects specifically on left hippocampal

volume (Firth et al., 2018), specifically in older populations (>65 years) and in interventions that lasted over 24 weeks (Wilckens et al., 2021). Increased local GM volumes in prefrontal (Colcombe et al., 2006; Ruscheweyh et al., 2011), cingulate (Ruscheweyh et al., 2011) and temporal cortices (Colcombe et al., 2006) and hippocampus (Erickson et al., 2011; Rosano et al., 2017) have been observed after long-term AE interventions, whereas non-significant changes in these same areas have been observed in trials lasting only 12 weeks (Maass et al., 2015; Matura et al., 2017; Sexton et al., 2020). This fact brings the opportunity to go further in this research topic and address how frequency, intensity, time, type, volume and progression (FITT-VP) of exercise programs might be modulating the effect of the intervention on biomarkers at either at Level 1 or 2 (Cabral et al., 2019; Chen et al., 2020). At this point, we wondered whether we might find significant changes in a short term and high intensity intervention. Moreover, current literature suggests that individual characteristics of participants such as age, sex or genetics (Barha et al., 2019; Stillman et al., 2020) interplay with the intervention modulating the cognitive benefits related to exercise.

3.3. PA-related changes at Level 3

As we let you glimpse at the very first of this section, the cascade of exercise-related changes at Level 1 and 2 might convey into behavioral and functional benefits in older adults in terms of improved psychological health, sleep quality, and cognition (Stillman et al., 2016). An active aging is related to better physical and mental health (Bertheussen et al., 2011), quality of life (Fox et al., 2007), and wellbeing (Black et al., 2015; Lee & Hung, 2011). Moreover, PA (Strawbridge et al., 2002) and CRF (Sui et al., 2009; Willis et al., 2018) might be also protective factors for prevalent and incident depression. In relation to sleep changes, PA is also associated with better sleep quality (Kline et al., 2013; Tan et al., 2018) and efficiency (Kline et al., 2013; Wilckens et al., 2018) and total sleep time (Murray et al., 2017). Nevertheless, literature regarding the impact of CRF levels on sleep quality patterns in healthy older

adults remains underexplored. We consider that this is an opportunity to explore whether this physiological parameter is related to quality of sleep in a similar way that PA does to better understand not only the nature of this parameter but also its relation to psychological benefits. Scarce studies have focused on testing these cross-sectional results on AE interventions including outcomes such as psychological health and daily activities.

AE interventions raise PA levels by definition, at least during the program. As a consequence, it is common for interventional studies to find significantly increased CRF in those participants who finished the exercise condition, which stands for better cardiovascular health (Kramer et al., 1999). The fact is that current literature is interested on better understanding whether CRF intervention-related changes are significantly related to cognitive improvements (Etnier et al., 2006; Young et al., 2015). Therefore, one major issue is to explore the mediating effect of CRF in the relationship between PA and cognition in cross-sectional and interventional studies to better understand its role as a mechanism of cognitive benefits related to PA.

Actually, different types of exercise –aerobic, resistance, stretching-, applied in a regular manner, may produce cognitive benefits (Barha et al., 2017; Cabral et al., 2019) and higher levels of PA, as a general outcome, are known to protect against cognitive decline and reduce the risk for dementia (Sofi et al., 2011). In cross-sectional studies, PA is significantly related to better processing speed and executive function (Frederiksen et al., 2015) but there are mixed results about the relationship between PA and episodic verbal memory (Flöel et al., 2010; Frederiksen et al., 2015). Discrepancies raise further questions and the possibility to better understand results. For example, PA measures (e.g., self-reported questionnaires or actimeters) differ across studies and may explain heterogeneous results. The fact is that several years and studies after the first evidence reporting “PA-related cognitive benefits” (Colcombe & Kramer, 2003; Etnier et al., 1997) there is still need to better describe which type of PA and which type of cognitive benefits. Therefore, it would be interesting to go beyond the general “PA” outcome and study differences in the

relationship between exercise and other types of daily PA with cognition in cross-sectional and longitudinal studies. In cross-sectional studies, higher CRF, directly estimated through progressive exercise tests, has been related to better scores in global cognitive function, executive function (Freudenberger et al., 2016; Netz et al., 2011), attention (Netz et al., 2011) and verbal (Freudenberger et al., 2016) and spatial memory (Erickson et al., 2009). Moreover, CRF is being suggested to mediate the relationship between PA levels and brain volume cognition based in a triple positive correlation between CRF – hippocampus volume – spatial memory found in a cross-sectional study by Erickson et al. (2009). Significant and non-significant results on this issue set one of the current lines of research in the field; to better describe the mediating role of CRF in the PA-cognition.

However, there is an important difference between identifying a significant association (PA or CRF with cognition) and proving a significant cognitive change after a designed intervention; a difference that had a huge repercussion. Colcombe and Kramer (2003) reported in a meta-analysis that AE interventions improved cognitive performance especially for executive function in healthy older adults. Current systematic reviews have replicated those results reporting modest effect sizes of AE interventions on executive function, attention, processing speed, and memory (Barha et al., 2017; Northey et al., 2018; Smith et al., 2010). However, other reviews reported that the evidence was too limited to draw firm conclusions (Brasure et al., 2018; Cox et al., 2016; Sáez de Asteasu et al., 2017; Snowden et al., 2011) or reported no significant effects of exercise on cognition (Angevaren et al., 2008; Kelly et al., 2014; Young et al., 2015). Authors refer to significant variation in FITT-VP to explain heterogeneity across the field. Gomes-Osman et al. (2018) reviewed the relationship between these parameters and positive changes in cognition and concluded that at least 52 h of exercise is required to observe beneficial effects. It is also known that the cognitive effects of AE may be moderated by individual factors such as age and sex (Barha et al., 2017; Colcombe & Kramer, 2003) that are sometimes understudied in randomized controlled trials (RCT).

Sex-Related Box 4

Current literature suggests that sex matters when it is about the neuroprotective effect of PA.

There are sex differences in the cascade of PA-related changes that might mediate the described benefits on cognition. For example, greater PA was related to reduced TNF-α only in men (Elosua et al., 2005), while daily walking has been associated with greater hippocampal (Varma et al., 2015) and dorsolateral prefrontal volume (Barha et al., 2020) and larger surfaces of the subiculum (Varma et al., 2016) in women, but not in men. Moreover, Dimech et al. (2019) found that greater local efficiency was more robustly associated with CRF in males than females and global efficiency was associated with CRF but only in males. Therefore, and given the described sex differences in CRF patterns across the lifespan (Al-Mallah et al., 2016) as well as in the physiological mechanisms related to this parameter (Barha & Liu-Ambrose, 2018) (for example, in the musculoskeletal and respiratory systems), the inconsistencies found in the mediating role of CRF in the relationship between PA and cognition - as well as with its mechanisms at multiple levels - could be better described if assessed by sex.

Moreover, cross-sectional and longitudinal literature about sex differences in the PA-cognition relationship is scarce but results have already shown that higher levels of PA were related to greater reduction in the risk for dementia (Hogervorst, 2012) and less decline in executive function and processing speed in women (Barha et al., 2020). Discrepant results positively related self-reported PA to better performance in Mini-Mental State Examination (MMSE) and executive function in men but not in women (Lindwall et al., 2008). Sex differences in RCTs have been reported in samples including participants with mild cognitive impairment and vascular cognitive impairment, which showed greater improvements in executive function in women (Barha et al., 2017; Van Uffelen et al., 2008). Moreover, samples including >50% of females in the whole sample showed greater effect sizes on executive function after AE programs in meta-analytic studies (Barha et al., 2017; Colcombe & Kramer, 2003). These results support the potential greater cognitive effect of AE programs in women and the need to address this issue in RCTs including healthy older adults. Therefore, addressing the PA-related benefits and the mechanisms that might mediate them stratified by sex might be interesting to better understand exercise as a personalized approach to enhance cognitive health.

4. Computerized cognitive training

Cognitive training is a classical neuropsychological intervention that aims to train, stimulate and rehabilitate brain and cognitive function using repetitive cognitive tasks (Belleville & Bherer, 2012; Ten Brinke et al., 2017). Cognitive training is based on the idea of neuroplasticity or synaptic plasticity, which states that brain connections can be modified, eliminated or created even at late-life depending on stimulation (Belleville & Bherer, 2012). Actually, there is extended evidence and an opened dialogue about its efficacy on clinical and non-clinical populations (Lampit et al., 2014a, 2014b). In particular, computerized cognitive training (CCT) is an emerging subtype of cognitive training that facilitates the administration of cognitive tasks on easy-user and visual engaging online platforms or software. CCT allows investigators to adapt the content and demand of the challenges (Lampit et al., 2014b; Shao et al., 2015). In accordance with the technological and Internet revolution, multiple platforms of CCT appeared bringing the opportunity to design standardized or individualized programs and creating a huge market full of possibilities. Significant and non-significant changes in cognition related to CCT have been reported in studies applying different platforms and designs. This fact brought the opportunity to dig deeper on the role of the parameters of the activity and individual characteristics of the sample to better understand the neuroprotective effects of this type of intervention. Therefore, meta-analyses started going further and included variables such the FITT-VP parameters, trained domains or home-based/professional-guided interventions to better understand CCT-related cognitive benefits (Lampit et al., 2014b; Webb et al., 2018). And yet, the biological pathways, either at Level 1 or 2, by which CCT produces these effects remain poorly understood in humans. While literature on functional brain changes has proliferated, most of the molecular outcomes are still studied in mice under the hypothesis of enhanced CCT-related plasticity and the Hebb principle (Shao et al., 2015).

Box 5. Cognitive training-related concepts

Enriched environment is a research paradigm used in animal studies in which researchers create an environment with high sensory, cognitive or motor stimulation to assess the effects of these circumstances (van Praag et al., 2000).

Cognitive rehabilitation involves an individualized intervention to target disability related to disease in which patients, families and professionals work together to improve daily functioning and well-being (Bahar-Fuchs et al., 2013).

Cognitive training is an intervention consisting of repeated practice of standardized exercises targeting a specific cognitive domain (single-domain) or domains (multi-domain) (Gates & Valenzuela, 2010). Cognitive training might be offered through individual or group sessions to healthy or clinical subjects and might improve or maintain performance in the trained domain and potentially benefit untrained domains (Bahar-Fuchs et al., 2013).

CCT emerged as new tool to apply cognitive training on an individual electronic device requiring a single physical response; for example, pressing a button of the keyboard (Lampit et al., 2014b).

Transfer of training refers to the fact that the improvement in a trained task is applied to a different but similar task or situation (Gates & Valenzuela, 2010).

4.1. CCT-related changes at Level 1

The theoretical background at Level 1 is based on the Hebb principle (Hebb, 1949), which, transferred to the CCT framework, states that the regular practice of a cognitive task may influence cognition by simultaneously activating a group of neurons and, in turn, promoting the strength of synaptic connections (Patterson et al., 1996; Taya et al., 2015). Actually, there is evidence about increased long-term potentiation and enhanced BDNF levels in the hippocampus in animal studies using the “enriched paradigm”, which could be related to greater neurogenesis and synaptogenesis (Valenzuela et al., 2007; Valenzuela & Sachdev, 2009). Therefore, Valenzuela and Sachdev (2009), based on these animal models, suggested that BDNF and nerve growth factor might be the molecules promoting cell survival and proliferation after cognitive stimulation in humans. Findings of significant increased

BDNF in patients with risk of dementia after cognitive training (Damirchi et al., 2018) supported this idea. In relation to healthy populations, the discussion is still ongoing since significant increases of BDNF have been reported after cognitive training programs lasting 5 (Ledreux et al., 2019) and 7 weeks (Rahe et al., 2015a) while other longer trials lasting 10 (Küster et al., 2017) and 12 weeks (Tarassova et al., 2020) did not. This type of discrepancies may rely not only on the quality of the trial but also on the characteristics of the participants and the design.

To our knowledge, other molecular and cellular mechanisms related to cognitive training effects have been scarcely addressed in healthy or clinical human populations. Based on molecular interactions described in previous sections, it could be hypothesized that the interplay between BDNF, IGF and VEGF as well as the negative impact of pro-inflammatory cytokines on these growth factors and the synthesis of other proteins in the brain, are key players in the CCT goal; the enhancement of brain and cognitive health.

4.2. CCT-related changes at Level 2

Although molecular and cellular CCT-related changes remain understudied in humans, there has been a great interest on the neural effect of CCT. While most of the literature has focused on how CCT might enhance the function of the brain, fewer studies have addressed brain volume changes associated with cognitive interventions in healthy older adults (Belleville & Bherer, 2012).

Brain volume

A recent systematic review about CCT-related brain volume changes in healthy older adults included only three trials and reported mixed results (Ten Brinke et al., 2017). On one side Lampit et al. (2015) found an increase in GM density in the right post-central gyrus in the cognitive training group compared to an active control group whereas Heinzel et al. (2014) and Antonenko et al. (2016) found no significant

changes in GM and hippocampal volume, respectively. Further literature reported increases in GM volume default-mode network areas (De Marco et al., 2016). The heterogeneity of the findings could be related to parameters of the training program and sample characteristics (Gates et al., 2020; Taya et al., 2015). However, brain volume changes after CCT remain understudied, which stands for a clear need of RCTs addressing this issue.

4.3. CCT-related changes at Level 3

Up to now we have just described changes at Level 1 and 2 related to cognitive training. However, the final goal of an intervention like CCT is the enhancement of cognitive function and so, the transfer of the trained tasks to the untrained ones. Therefore, the question is whether cognitive training has been associated with cognitive gains in trained and untrained domains. Evidence from animal studies showed that those animals in an enriched condition got better spatial learning in the Morris Water Maze displaying better targeted behaviors (Gelfo et al., 2018). In healthy human older samples, the answer to that question is yes, too (Ball et al., 2002). In particular, CCT interventions have been related to memory, processing speed, and visual spatial ability benefits (Lampit et al., 2014b; Shao et al., 2015). Evidence have also reported fewer efficacy for attention and executive function (Lampit et al., 2014b; Shao et al., 2015) and have showed that gains in memory and processing speed could be detected one year after the end of the intervention (Lampit et al., 2014b). Literature suggests that single-domain CCT tends to specifically benefit the trained task, whereas multi-domain cognitive CCT improve global cognitive function (Gates et al., 2011; Gates & Valenzuela, 2010). Multi-domain training retained the benefits better and showed more potential transfer to other domains compared to single-domain programs (Cheng et al., 2012). Although the described results stand for significant cognitive effects related to CCT, results from recent meta-analyses also report non-significant effects on memory or executive function (Edwards et al., 2018; Kueider et al., 2012; Lampit et al., 2014b).

The ongoing discussion about discrepancies in the efficacy of CCT includes several dimensions. On one side, we must highlight that assessing the degree of transfer between the trained task and untrained tasks is still a challenge and a source of variability in this field (Nguyen et al., 2019), which has to be acknowledged when describing or generalizing about the efficacy of a CCT program. Moreover, in accordance to other type of interventions, inconsistencies might also be related to FITT-VP parameters of the program. For example, Lampit et al. (2014b) found no significant effects when sessions lasted less than 30 min and frequency was more than three sessions of training per week, whereas Chiu et al. (2017) showed greater effectiveness with 3 sessions per week in programs of 8 or more weeks and 24 total training sessions. Individual characteristics such as age could also moderate the CCT-related cognitive changes (Nguyen et al., 2019). Evidence addressing changes in markers at Level 1 and 2 might help to understand the discrepant CCT effects on cognitive outcomes and better describe the efficacy of a specific program.

Sex-Related Box 5

To our knowledge, sex differences after computerized or non-computerized cognitive trainings have been rarely addressed in healthy older adults. We found a single study addressing this issue in subjects with amnestic mild cognitive impairment after a multi-domain cognitive training that took place during 6 weeks (90 minutes, twice a week). Results showed greater effects for immediate and delayed verbal episodic memory and working memory in women, whereas there were not significant changes when analyzing the total sample (Rahe et al., 2015b). This is an example of the urgency to address this issue given the potential similar results in healthy samples and the fact that potential discrepancies obtained in whole samples could be better understood if sex was included in the analysis.

5. Combined training

The idea of "individually, we are one drop; together, we are an ocean" quoted by Ryunosuke Satoro might be also applied in the field of lifestyle interventions. The fact is that reviews suggested that combining interventions might induce greater changes than each intervention separately (Kraft, 2012; Lauenroth et al., 2016). Thus, *combined training* (COMB) became a common concept to refer the interventions combining two or more types of intervention; in this case AE and CCT. The background hypothesis is that increased BDNF and arousal related to exercise will facilitate and broaden the brain activation when performing the CCT (Nilsson et al., 2020), which should translate into greater transfer of cognitive benefits (Dahlin et al., 2008). This approach opened a whole new field of research and possibilities, including questions about the role of the molecular and brain changes as well as the influence of the design of the combined intervention. To our knowledge, results of high-quality RCTs have started to sprout on 2010 and there is still need of evidence to promote a lively discussion about it. Moreover, the combined approach is a source of variability due to all the possibilities when designing the parameters of the interventions, which becomes a challenge when comparing and integrating results. Most of the studies have focused on designs in which AE is applied just immediately before the CCT (Ten Brinke et al., 2020), simultaneously in dual-task designs (Eggenberger et al., 2016) or as a part of a guided but non-scheduled multicomponent program that usually includes diet and health education programs (Ngandu et al., 2015). Findings are unconclusive. Understanding the COMB-related changes at molecular and brain level in relation to each design might help to elucidate discrepancies and guide future research and clinical practice.

5.1. COMB-related changes at Level 1

At a molecular level, current research has focused on hypotheses based on model animals (Fabel et al., 2009; Olson et al., 2006), which suggested that neuroplasticity may be facilitated by exercise and guided by cognitive training. The suggested

mechanism includes potential positive impact of exercise on growth factors and inflammatory profile, which facilitates changes in the structure and function of the brain and, at the same time, might facilitate the neuronal survival and differentiation and plasticity related to cognitive training when they are combined (Joubert & Chainay, 2018). Therefore, the low inflammatory and oxidative stress environment related to regular PA would promote cardiovascular and neural repairing responses as well as enhanced cell proliferation through BDNF, while cognitive stimulation may facilitate the survival of newborn cells and regulate synaptic changes following the Hebb theory of learning and memory (Hebb, 1949). While animal studies have shown promising results in the enriched paradigm, studies in humans are still scarce.

The effects of a COMB program on markers of inflammation have been studied in mice showing decreased TNF-α in the hippocampus. However, research on this topic in human samples is scarce. In relation with BDNF, 3 months of cybercycling that included a cognitive component induced greater changes in BDNF levels compared to traditional cycling (Anderson-Hanley et al., 2012) but a 7-week program of cognitive training, applied alone or in combination with physical training, was related to increased peripheral BDNF from pre to post-testing but not greater compared to single intervention (Rahe et al., 2015a). Greater CRF changes were related to greater increases in BDNF and IGF levels in those high responders following 6 weeks of exercise, single and combined (Heisz et al., 2017) and increased glucose metabolism have been reported in healthy older adults after a 16-weeks COMB program (Shah et al., 2014). Discrepancies in these findings suggests that adding evidence is necessary to elucidate COMB-related changes at Level 1 for each desing and in relation to participant characteristics.

5.2. COMB-related changes at Level 2

Literature addressing changes at Level 2 has addressed the potential greater effect on brain structure and function of a COMB program. The background hypothesis states that in order to facilitate the transfer of cognitive benefits to untrained domains, brain

areas that are activated must overlap. Therefore, increased arousal and BDNF after exercise would induce activation and plasticity in multiple brain areas (Nilsson et al., 2020), which might be helpful to broaden the effects of multi-modal CCT and facilitate the transfer of cognitive benefits (Dahlin et al., 2008). Current reviews claim for evidence to support this statement, either from structural or functional MRI studies.

Brain volume

Literature about changes in the brain structure following a COMB program is scarce and designs are rarely similar to allow firm conclusions. For example, 4 months of spatial navigation dual-task (navigation task + walking) led to stable hippocampal volumes in healthy older men compared to a control group which showed volume decrements consistent with age-related decline (Lövdén et al., 2012). However, a longer program, including 7 months of combined cognitive did not find significant changes GM loss in a sample with mild cognitive impairment (Train the Brain Consortium, 2017). Again, parameters related to the design such as order of the inteventions as well as participants characteristics seems to be involved in discrepant results, which highlight the need of more evidence to better describe the hypothesis of the potential additive effects of both interventions on neurobiological measures.

5.3. COMB-related changes at Level 3

A bunch of evidence suggested the idea that the combination of PA and cognitive stimulation may induce greater cognitive benefits compared to each intervention separately (Bamidis et al., 2014; Curlik & Shors, 2013; Fissler et al., 2013; Kraft, 2012; Lauenroth et al., 2016; Law et al., 2014). General cognitive function (Oswald et al., 2006; Shatil, 2013), executive function (Anderson-Hanley et al., 2012; Barcelos et al., 2015; Eggenberger et al., 2016; Theill et al., 2013) processing speed (León et al., 2015), memory (Fabre et al., 2002) and vocabulary (Schmidt-Kassow et al., 2013) performance may tend to benefit from a COMB. For example, Ten Brinke et al.

(2020) concluded that the combination of 15 min of brisk walking immediately before CCT for 8 weeks provided greater benefits for executive functions, specifically for set shifting. However, evidence is not consistent across trials depending on the type of comparative group and FITT-VP parameters of the intervention (Fabre et al., 2002; León et al., 2015; Oswald et al., 2006). For example, a systematic review of twenty studies concluded that COMB may have a small positive effect only when compared to a control and PA group but not to a cognitive intervention (Zhu et al., 2016). Another paper tested the role of the order of the intervention in four conditions (AE+CCT / CCT+AE / AE / CCT), reporting that average benefit did not differ depending on the order (Nilsson et al., 2020). To our knowledge, evidence is still insufficient to draw clear conclusions about which mechanisms are enhancing these undefined "greater cognitive effects" and how the design, in sequence, dual task or multicomponent induces greater benefits than single interventions. Therefore, addressing changes in markers at Level 1 and 2 following COMB might be informative of how an specific design of COMB might be enhancing cognitive health.

Sex-Related Box 6

Although we did not find any published paper addressing sex differences in the effect of a COMB program until the edition of this thesis, evidence related to AE programs (see *Sex-Related Box 4*) suggests that sex might also influence cognitive changes when AE is combined with CCT.

Box 6. Limitations in research addressing lifestyle interventions

We have broadly discussed how AE, CCT and COMB as lifestyle interventions might enhance cognition due to a cascade of changes that might take place at different levels of analysis. At the same time, the current state of research includes discrepancies between trials for all three interventions. Far from clear consensus, literature challenges new projects to embrace the controversy to better design interventions promoting healthy aging. In order to face this, it is important to be aware of the intrinsic limitations of this type of research. The first source of variability is the intervention itself; all the variables related to the design that were previously summarized under the FITT-VP parameters and other environmental aspects such as the weather seasons, too. The second most important source of variability is the fact that we research on humans. Humans are unique and carry such a tremendous amount of variability that might be difficult to control even with a randomized design. Not only individual variables including sex, age, years of education and the vast number of genes that we know that might influence cognition in the last-term but also those regarding expectations, personal events or previous experiences. Finally, there are multiple variables related to the methodology when assessing, sampling, randomizing, analyzing or even reporting that might bias our experience with the subject of research. After this "finally", there are all those variables that we are not aware, that are beyond our eye and might confound us. So, RCTs might not be enough. We need honest reporting, specific conclusions and lots of "might" when we dare to write general and home-taking conclusions.

SUMMARY OF EVIDENCE

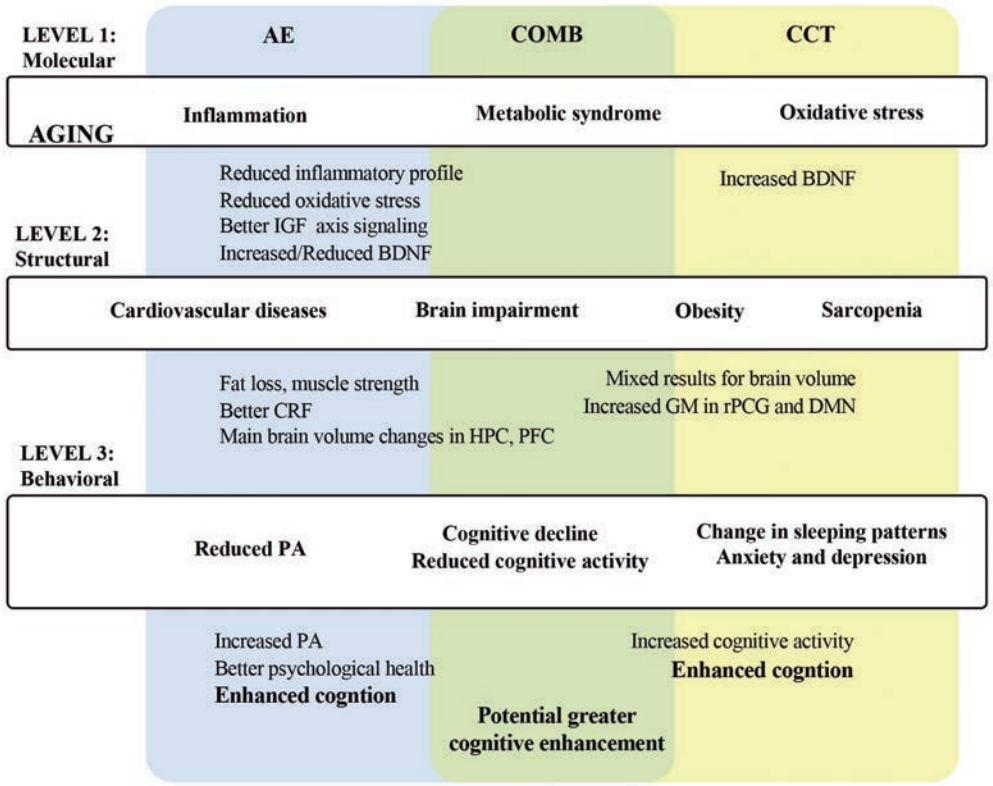


Figure 7. Summary of the content in the introduction section regarding aging and lifestyle interventions at multiple levels of analysis, created by A. Castells-Sánchez. *AE: aerobic exercise; BDNF: brain-derived neurotrophic factor; CCT: computerized cognitive training; COMB: combined training; CRF: cardiorespiratory fitness; DMN: default-mode network; GM: gray matter; HPC: hippocampus; IGF: insulin growth factor; PA: physical activity; PFC: prefrontal cortex; rPCG: right precentral gyrus.*

AIMS & HYPOTHESES

SCOPE: PROJECTE MOVIMENT RESEARCH PROJECT

The multi-level perspective of aging facilitates the understanding that it is a complex natural biological multi-system process with major consequences in brain health; but, more importantly, in the cognitive performance, psychological wellbeing and daily functioning of older adults. Marked individual differences in this deleterious process were related to lifestyle, which suggested the possibility of applying those behaviors with positive impact as clinical interventions that might delay age-related decline. AE and CCT emerged as potential low-cost, high impact strategies with neuroprotective effects at late-life. Evidence from RCTs supported this fact with positive results and suggested that the COMB would provide greater effects. Nevertheless, discrepant results highlighted the need to study intervention and individual variables that might be moderating the effects. Sex-differences have been scarcely considered up to now, despite the growing evidence of their crucial role. Moreover, current literature focuses on the cascade of molecular and brain changes that convey into cognitive benefits as well as on better understanding the role of parameters such as CRF. Therefore, we need cross-sectional and interventional evidence in order to increase the understanding of their mechanisms and design personalized interventions.

Projecte Moviment research project emerged with this purpose; to sum evidence, potentially clarify discrepancies and support the ongoing discussion on these topics. It conveys the interest in lifestyle strategies to promote brain and cognitive health into clinical studies that embrace the role of markers in multiple pathways at different level of analyses. This thesis emerges from Projecte Moviment research project and includes part of its aims and hypotheses. The specific aims and hypotheses addressed in this thesis were reported in 3 papers (see chapter *Articles*) and are described below.

STUDY 1

Aims

1. To explore the relationship between PA, grouped as exercise and non-exercise, and CRF with cognition in the total sample.

    1.1. To assess sex-differences in these relationships stratifying the results by sex.

2. To identify the mediating effects of CRF in the association between PA and cognition in women and men, separately.

Hypotheses

1. In the total sample, only exercise and CRF would be positively related with cognition. We might obtain mild relationships due to potential sex-differences.

    1.1. Exercise would be positively related to cognitive performance in both women and men, with greater associations in women based on evidence showing that women benefit more after AE interventions (Barha et al., 2017). Based on sex-differences in the musculoskeletal and respiratory adaptations after exercise and CRF levels across the lifespan, we might also find sex-differences in the CRF-cognition relationship.

2. Based on previous literature, we might expect that CRF would mediate the exercise-cognition relationship. Accordingly to expected sex-differences in CRF patterns and exercise-related adaptations, we might expect sex-differences in the mediating role of CRF.

STUDY 2

Aims

1. To study the relationship between exercise and CRF with key markers at molecular (Level 1), brain volume (Level 2), and behavioral (Level 3) levels of analysis stratifying the results by sex.

2. To study the potential mediating role of each of these markers in the exercise-cognition and CRF-cognition relationship.

3. To perform exploratory analyses to address the potential pathways by which these markers might influence each other.

Hypotheses

1. Although evidence is scarce and mixed, we expect exercise and CRF to be related (a) to lower levels of inflammatory markers and increased BDNF; (b) to greater brain volume in those areas that first decline when aging; (c) to better psychological health. We expect similar patterns between women and men for exercise relationships. However, CRF might show different patterns for women and men.

2. Markers at Level 1, 2 and 3 would partially mediate the exercise-cognition and CRF-cognition relationship.

3. We might identify significant mediating effects between markers at Level 1, 2 and 3 in accordance with the described theoretical pathways by which exercise enhance cognition.

STUDY 3

Aims

1. To examine the effect of 12-weeks 5 days per week RCT of AE, CCT and COMB on BDNF levels, markers of inflammation and volume changes in relevant brain areas compared to healthy controls.

2. To test whether significant changes in PA outcomes are related to changes in molecular markers and brain volume.

3. To assess the moderating role of sex and age on molecular and brain volume changes

4. To test whether changes in molecular and brain volume outcomes mediate the relationship between the intervention and cognitive benefits.

Hypotheses

1. AE, either single or combined, would be related to lower levels of inflammatory markers, change in BDNF and greater brain volume. CCT, either single or combined, would be related to increased BDNF levels and greater brain volume. Literature about BNDF levels is mixed, which make us aware of potential non-significant, positive or negative changes related to potential greater brain recruitment of BDNF.

2. Significant changes in PA outcomes would be related to lower levels of inflammatory markers, increased BDNF and greater brain volume.

3. Sex and age would have a significant moderating role in the effects of the intervention on markers at Level 1 and 2.

4. Significant changes at Level 1 and 2 would mediate significant intervention- related changes on cognition.

SUMMARY OF AIMS

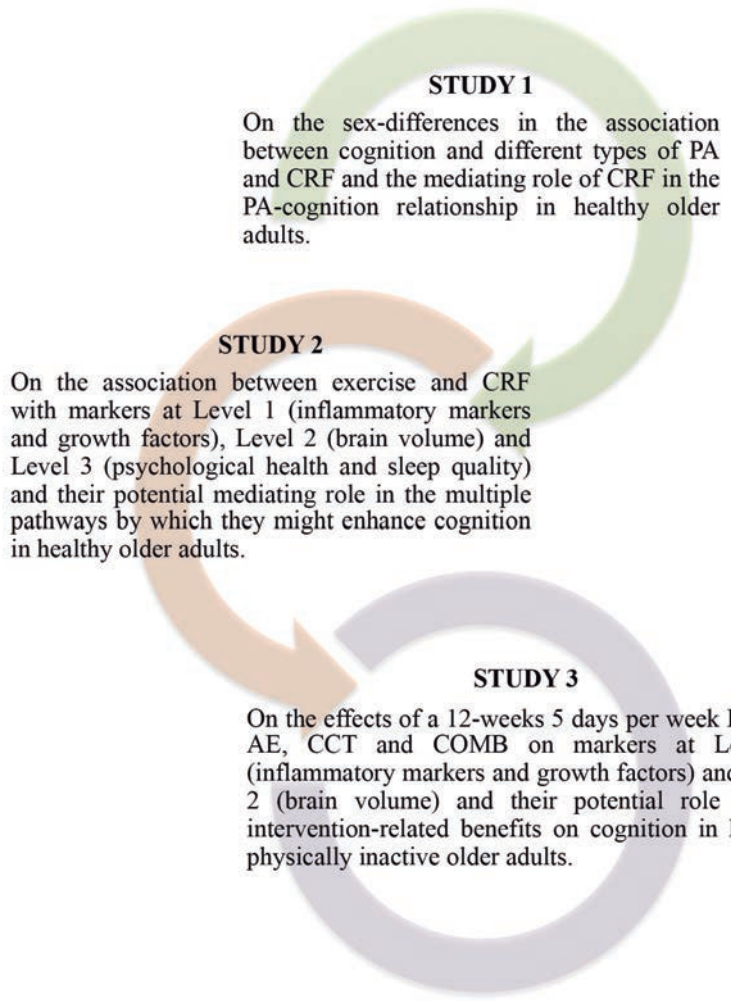


Figure 8. Summary of the aims included in each study, created by A. Castells-Sánchez. *AE: aerobic exercise; CCT: computerized cognitive training; COMB: combined training; CRF: cardiorespiratory fitness; PA: physical activity; RCT: randomized controlled trial.*

METHODS

Overview

In order to address Projecte Moviment aims we had to apply two different approaches. On one side, we designed a RCT, which allowed us to identify intervention-related changes in physically inactive healthy older adults as well as the role of other variables of interest in the observed effects in a controlled experimental environment. On the other side, we designed a cross-sectional strategy to describe the PA-cognition relationship and explore the role of other variables of interest in this association in a sample that included low to high fit participants. The combination of these two strategies brought the opportunity to compare the benefits related to regular exercise described in two out of the three studies of the present thesis and the intervention-related changes and mechanisms of a specific intervention in the following paper.

Figure 9. Studies included in this thesis accordingly to research designs included in Projecte Moviment research project, created by A. Castells-Sánchez.

The studies were carried out by the University of Barcelona in collaboration with Institut Universitari d'Investigació en Atenció Primària Jordi Gol, Hospital Germans Trias i Pujol and Institut Guttmann. They were approved by Bioethics Commission of the University of Barcelona -IRB00003099- and Clinical Research Ethics Committee of IDIAP Jordi Gol -P16/181- following the Declaration of Helsinki.

1. THE RANDOMIZED CONTROLLED DESIGN

Projecte Moviment trial is a multi-center, single-blind, proof-of-concept RCT which took place between November 2015 and April 2018 with the aim of recruiting 140 participants (three intervention groups, n = 40 each; and one control group, n = 20). It was registered in ClinicalTrials.gov (NCT031123900) and an extended description was published in the study protocol (Castells-Sánchez et al., 2019) including the calculation of the statistic power for the aimed sample. Participants gave their informed consent and were randomized to the four parallel groups. Interventions lasted 12 weeks and there were assessments at baseline and immediately after intervention completion. In the current thesis, Study 3 follows the randomized controlled design. See Figure 10 for an overview of the procedures.

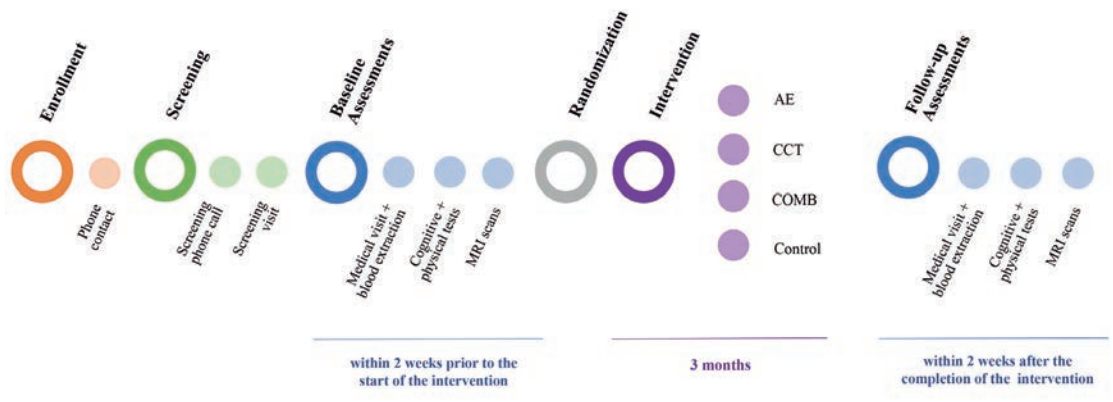


Figure 10. Study design sequence in the RCT study, created by A. Castells-Sánchez and F. Roig-Coll. *AE: aerobic exercise; CCT: computerized cognitive training; COMB: combined training; MRI: magnetic resonance imaging.*

1.1. Participants

We recruited healthy, physically inactive, late-middle aged adults from the Barcelona metropolitan area using multiple strategies: lists of patients of general physicians, volunteers from previous studies, oral presentations in community centers, advertisements and local media (newspapers, radio, and TV). All participants were

50-70 years old, were physically inactive (<2 hours/week over the last 6 months), were not cognitively impaired [MMSE ≥ 24 (Blesa et al., 2001) and Montreal Cognitive Assessment 5-min (MoCA 5-min) ≥ 6 (Wong et al., 2015)], had competency in Catalan or Spanish and had adequate sensory and motor skills. Participants were excluded from the study if they had a neurological diagnosis, psychiatric disease or Geriatric Depression Scale (GDS) score > 9 (Martínez et al., 2002), a history of drug abuse and alcoholism, consumed psychopharmacological drugs, history of chemotherapy and had any contraindication to MRI. Extended details included in Table 1.

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Aged 50-70 years.	Current participation in any cognitive training activity or during last 6 months > 2 hours/week.
Mini-Mental State Examination (MMSE) ≥ 24.	Diagnostic of dementia or mild cognitive impairment.
Montreal Cognitive Assessment 5-min (MoCA 5-min) ≥ 6.	Diagnostic of neurological disorder: stroke, epilepsy, multiple sclerosis, traumatic brain injury, or brain tumor.
Competency in Catalan or Spanish.	Diagnostic of psychiatric illness current or during last 5 years.
Adequate visual, auditory and fine motor skills.	Geriatric Depression Scale 15 (GDS-15) > 9.
Acceptance of participation in the study and signature of the informed consent.	Consumption of psychopharmacological drugs current or during last 5 years; or more than 5 years throughout life.
Low active (<2 hours/week over the last 6 months) [1].	History of drug abuse or alcoholism current or during last 5 years; or more than 5 years throughout life; >28 men and >18 women unit of alcohol/week.
	History of chemotherapy.
	Contraindication to magnetic resonance imaging.

Note: GDS-15 (Martínez et al., 2002); MMSE (Blesa et al., 2001); MoCA 5-min (Wong et al., 2015).
[1] *Only for those participants included in the randomized controlled design.*

1.2. Procedures

We informed and screened those individuals interested over the phone and in an on-site interview. Individuals meeting inclusion and exclusion criteria signed a written informed consent prior to study involvement.

Assessments were conducted in a clinical environment and organized into three appointments that took place at baseline within 2 weeks prior to the start of the intervention, and again at 3 months within 2 weeks after the completion of the intervention:

(1) Medical assessment (30 min): review of medical history and current health status including cardiovascular risk factors and blood extraction between 8 and 9:30 a.m. following an overnight fast.

(2) Cognitive assessment and physical status (2.5 h): administration of a battery of neuropsychological tests, psychological health, subjective performance in daily activities and PA questionnaires and a treadmill CRF test. In order to control the effects of acute exercise, participants were advised not to exercise prior to all appointments and cognitive tests were conducted before the CRF test.

(3) MRI scans (45 min): administration of a neuroimaging protocol to acquire structural and functional data of the brain.

All assessments were carried out in two primary care centers except MRI scans that are performed in the Hospital Germans Trias i Pujol. All subjects received an actimeter the first and last week of intervention to track their daily activity and determine if participants meet the intervention protocol. The same team of psychologists, which administered cognitive tests, and nurses, who collected general health data, followed the protocol in all the centers. Extended details for assessment procedures in Table 1 in *Annex 2*.

In order to prevent medical and personal issues, all the assessments were reviewed by the corresponding health professional before randomization to ensure safety during the intervention. In case any abnormality was identified, it was reported and participants were rerouted to the corresponding healthcare service. Participants were informed about voluntary withdrawal and received clinical reports of all the assessments.

1.3. Randomization

Participants were randomized after the baseline assessments and assigned to intervention and control groups. The allocation sequence was designed by a statistician and consisted of a random combination of sex, age and years of education allowing for balanced groups accounting for these demographic variables. The intervention team was responsible for the allocation and assessors remained blind to group assignment. We determined that blinding would only be broken for medical reasons.

1.4. Interventions

Interventions were home-based and scheduled for 5 days per week for 12 weeks.

AE. Participants randomized to AE group followed a progressive brisk walking program. Week 1: 30 min per day at 9–10 on the Borg Rating of Perceived Exertion Scale (BRPES) (Borg, 1982) perceived as light intensity. Week 2: 45 min per day at 9-10 on BRPES. Week 3 to 12 (10 weeks): 45 min per day at 12-14 on BRPES perceived as moderate-high effort. Subjects were trained to use the BRPES (Borg, 1982) and to record the intensity and frequency of activity in a diary.

CCT. Participants randomized to CCT group performed multimodal cognitive training using the Guttmann Neuropersonal Trainer online platform (GNPT®, Spain) (Solana et al., 2014, 2015) in sessions of 45 minutes targeting executive function, visual and verbal memory and sustained, divided and selective attention. The demand

of the tasks for each participant was adjusted by GNPT platform based on baseline cognitive performance and the ongoing scores of the activities.

COMB. Participants randomized to COMB group performed the brisk walking program and the CCT as described above, separately, in single continuous bouts of 45 minutes for each intervention 5 days per week without order or time-point restrictions.

Control Group. Participants randomized to the control group were on the wait list for 12 weeks and were asked not to alter their regular lifestyle. Once the control condition was finished, they had the option to start one of the interventions (AE, CCT or COMB) but data of this potential additional activity was not included in the trial as they were not considered participants during this period.

Participants monitored their activity in a diary registering the date and duration of the activity and any adverse events occurring as well as the intensity of the walking in BRPES units in case AE was included in the program. Participants had the contact information for the intervention team who followed the intervention (phone calls every 2 weeks and a mid-point visit and a final visit) and ensured participation, solved inconveniences and barriers and obtained adherence based on participants feedback and platform data. Instructions for each intervention included healthy advice to prevent injuries. Participants were sent to a physician in case of medical incident and excluded from the trial based on medical recommendation.

2. The cross-sectional design

The cross-sectional design is based on Projecte Moviment trial and is drawn from the aim to study the benefits related to regular PA. We joined the baseline assessments belonging to the Projecte Moviment trial sample with 20 additional participants with a higher PA profile in order to enlarge the interval of the PA and CRF level and increase the sample size for a cross-sectional analysis. All participants gave their

informed consent, followed the same inclusion and exclusion criteria, except for the amount of self-reported PA, and underwent the same assessment protocol during the same period of time. In the current thesis, Study 1 and 2 follow the cross-sectional design. See Figure 11 for an overview of the procedures.

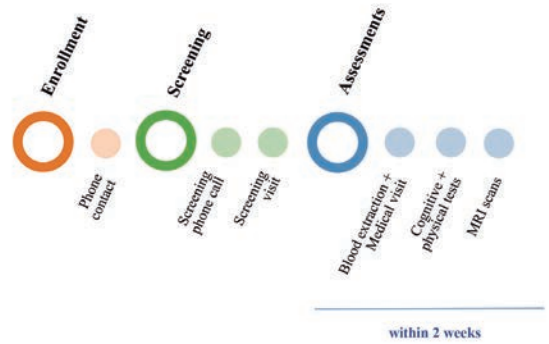


Figure 11. Study design sequence in the cross-sectional studies, created by A. Castells-Sánchez and F. Roig-Coll. *MRI: magnetic resonance imaging.*

2.1. Participants

Participants were community dwelling healthy late-middle-aged adults from the Barcelona metropolitan and were recruited using the same strategies mentioned in *1.1.* of the *Methods* section. Inclusion and exclusion criteria were the same for all the subjects as previously described in Table 1 except for the level of self-reported PA. Projecte Moviment baseline low-active participants were eligible if they did not perform exercise more than 2 hours/week over the last 6 months, whereas the 20 additional participants had a higher PA profile defined as ≥ 5 hours/week moderate PA or 2.5 hours/week intense PA.

2.2. Procedures

All participants were recruited, selected and assessed following the same protocol during the same period of time. They were screened by phone and an on-site interview and signed an informed consent prior to the assessment.

Participants meeting criteria underwent the previously described multimodal assessment organized into three appointments in 2 weeks in the following order: (1) Medical assessment and blood extraction (30 min), (2) Cognitive psychological health and PA assessment (2.5 h), (3) MRI protocol (45 min). In order to control the effects of acute exercise, participants were advised not to exercise 8 hours before all appointments. Assessments were conducted in clinical facilities: medical and cognitive assessments were carried out in two Primary Health Care Centers and MRI scans were performed in the Hospital Germans Trias i Pujol. Participants received clinical reports of all the assessments and were rerouted to the corresponding healthcare service if necessary.

3. Projecte moviment: assessment protocol and outcomes

3.1. Primary outcomes

Cognitive performance

We assessed cognitive function in multiple domains as primary outcomes using an extensive neuropsychological battery, which included standard tests selected for their psychometric qualities and high relevance in the area of study. Tests were performed in a single session of 60-90 min and in the same order for all the participants. These tests provided measures of multiple cognitive functions grouped following a theoretically-driven approach (Lezak et al., 2012; Spreen & Strauss, 1998): Flexibility (Trail Making Test B-A time) (Tombaugh, 2004), Fluency (letter and category fluency) (Peña-Casanova et al., 2009), Inhibition (interference-Stroop Test) (Golden, 2001), Working Memory (backward- Wechsler Adult Intelligence Scale III (WAIS-III)) (Wechsler, 2001), Visuospatial Function (copy accuracy-Rey Osterrieth Complex Figure) (Rey, 2009), Language (Boston Naming Test-15) (Goodglass et al., 2001), Attention (forward span, digit symbol coding and symbol search WAIS-III) (Wechsler, 2001), Speed (Trail Making Test-A) (Wechsler, 2001); copy time-Rey Osterrieth Complex Figure (Rey, 2009), Visual Memory (memory accuracy-Rey Osterrieth Complex Figure) (Rey, 2009)

and Verbal Memory (total learning and recall-II Rey Auditory Verbal Learning Test) (Schmidt, 1996). Five general domains were designed: 1) Executive Function, 2) Visuospatial Function, 3) Language, 4) Attention-Speed, and 5) Memory (extended details Table 2). This comprehensive cognitive assessment was also used to clinically confirm the lack of mild cognitive impairment or dementia. Moreover, an Spanish version of MoCA 5-min (Wong et al., 2015) and MMSE (Blesa et al., 2001) assessed global cognitive function as relevant markers of cognitive decline and were used for screening during the recruitment.

Table 2. Primary outcomes in Projecte Moviment protocol: variables and measures.

Outcome/Variable		
Composites 1st Level	**Composites 2nd Level**	**Tests - Subtest**
EXECUTIVE FUNCTION	Inhibition	Stroop - Interference
	Working Memory	WAIS III - Backward Span
		TMT - B
	Fluency	Letter fluency
		Category fluency
VISUOSPATIAL FUNCTION	Visuospatial	ROCF - Copy Accuracy
MEMORY	Verbal Memory	RAVLT - Total Learning
		RAVLT - Recall II
	Visual Memory	ROCF - Memory Accuracy
LANGUAGE	Language	WAIS III - Vocabulary
		BNT (15 items)
ATTENTION - SPEED	Attention	WAIS III - Forward Span
		WAIS III – Digit Symbol Coding
		WAIS-III - Symbol Search
	Speed	TMT - A
		ROCF - Copy Time

Note: BNT, Boston Naming Test (Goodglass et al., 2001); RAVLT, Rey Auditory Verbal Learning Test (Schmidt, 1996); ROCF, Rey-Osterrieth Complex Figure (Rey, 2009); Stroop Test (Golden, 2001); TMT, Trail Making Test (Tombaugh, 2004); Verbal fluency tests (Peña-Casanova et al., 2009); WAIS-III, Wechsler Adult Intelligence Scale (Wechsler, 2001).

In Study 1 and 2, we obtained z-sample scores and averaged them according to each domain. In Study 3 we calculated change from raw data (follow-up minus baseline) and obtained z-sample scores for each outcome. Then, we averaged z-values according to each domain.

3.2. Secondary outcomes

Demographic data and health status

We registered age, sex and years of education of all participants. A nurse registered anthropometrics measurements and cardiovascular health variables such as blood pressure, related diagnoses and current treatment (see Table 3). From these variables, we obtained the body mass index (BMI) and the Registre Gironí del Cor (REGICOR) score.

Demographic data (age, sex and years of education) and BMI were included in all studies; diagnoses of hypertension and diabetes in Study 1 and 2; and cardiovascular risk factors medication in Study 2 for supplementary analyses.

Self-reported PA

Self-reported PA levels were evaluated with the Minnesota Leisure Time PA Questionnaire (VREM) (Ruiz-Comellas et al., 2012) which asks about frequency and duration of multiple activities -sportive walking, sport/dancing, gardening, climbing stairs, shopping walking and cleaning house- during the last month. We calculated the energy expenditure for each activity transforming hours per month into units of metabolic equivalent of tasks (MET). We added the METs spent in sportive walking and sport/dancing activities to obtain a measure of Sportive Physical Activity (S-PA) and the METs spent in gardening, climbing stairs, shopping walking and cleaning house to obtain a measure of Non-Sportive Physical Activity (NS-PA). The sum of both outcomes resulted in a measure of Total PA. Polar Loop®actimeter (Polar Electro, NY, United States) registered daily PA (hours, steps, km, kcal) and sleeping (hours, %) parameters.

Table 3. Secondary outcomes in Projecte Moviment protocol: variables and measures.

	Variable/Outcome	Outcome measure
PSYCHOLOGICAL HEALTH & DAILY ACTIVITY	Geriatric Depression Scale (GDS-15)	Z score
	Modified version of Visual Analog Mood Scale (VAMS)	Z score
	Short Informant Questionnaire on Cognitive Decline in the Elderly (S-IQCODE)	Z score
	Pittsburg Sleep Quality Index (PSQI)	Z score
	Clinical Outcomes in Routine Evaluation-Outcome Measure (CORE-OM)	Z score
CRF	Cardiorespiratory fitness (Rockport 1-mile walk test)	VO_{2max}
PA	Minnesota leisure time physical activity questionnaire (VREM)	METs
	Actimeter activity parameters (Polar Loop®)	Hours, steps, km, kcal
	Actimeter sleeping parameters (Polar Loop®)	Hours, %
HEALTH STATUS	Weight, height and waist diameter	Kg, cm
	Blood pressure	mm Hg
	Hypertension, diabetes and dyslipidemia	Yes/No
	Tobacco and alcohol use	Yes/No, Units
BLOOD SAMPLE DATA	Hemogram	Conventional units
	Biochemistry in plasma	Conventional units
	Cortisol	ng/mL
	Genetics - Apolipoprotein E (APOE)	E4+/E4-
	Genetics - Brain Derived Neurotrophic Factor (BDNF)	Met+/Met-
	Cytokines and growth factors[1]	ng/mL
NEUROIMAGING	T1-weighted	Volume
	T2- weighted turbo inversion	Volume
	Susceptibility weighted imaging	Volume
	Resting State	Z score
	Diffusion Tensor Imaging	Fractional Anisotropy Index

Note: CORE-OM (Trujillo et al., 2016); CRF: cardiorespiratory fitness; GDS-15 (Martínez et al., 2002); MET: Metabolic Equivalent of Task; PA: physical activity; PSQI (Rico and Fernández, 1997); Rockport 1-mile walk test (Kline et al., 1987); S-IQCODE (Morales González et al., 1992); VAMS (Stern et al., 1997); VREM (Ruiz-Comellas et al., 2012). [1] *The Proteome Profiler Human™ XL Cytokine Array and ELISA immunoassay method to quantitatively analyze specific markers.*

Raw scores of Total PA, S-PA and NS-PA were included in Study 1; and in Study 2 only raw S-PA was included. Change (follow-up minus baseline) in S-PA was used in Study 3. Additional analyses for this thesis were performed with NS-PA to complement Stduy 2.

Cardiorespiratory fitness

We obtained estimated CRF applying the Rockport 1-Mile Walking Test, which is a less invasive and valid method commonly used in healthy elderly population. Participants were instructed to walk one mile on a treadmill (Technogym®, Italy) adjusting their speed in order to be as fast as possible without running. We registered time to complete the mile, heart rate (HR) and average speed during the test once they finished. We estimated VO_{2max} using the linear regression developed by Kline et al. (1987). The equation uses the following variables to estimate VO_{2max}: weight (pounds), age (years), sex (female = 0, male = 1), time to complete the mile (minutes), and HR at the end of the test.

$$VO_{2max} = 132.853 - 0.0769weight - 0.3877age + 6.1350sex - 3.2649time - 0.1565HR$$

Study 1 and 2 included raw scores of estimated CRF; Study 3 included a measure of change (follow-up minus baseline) in CRF.

Blood sample: biomarkers at Level 1

Nurses in the Primary Health Care Centers performed blood extraction between 8:00 and 9:00 am. All participants were instructed to do an overnight fast and to not exercise 8 hours before the blood test. Blood samples were obtained from the antecubital vein and collected in EDTA tubes for plasma analyses. Tubes were immediately transferred to two circuits: (1) the IGTP-HUGTP Biobank integrated in

the Spanish National Biobanks Network of Instituto de Salud Carlos II (PT13/0010/0009) and Tumor Bank Network of Catalonia, and they were processed following standard operating procedures with the appropriate approval of the Ethical and Scientific Committees. Plasma aliquots were stored at -80ºC for posterior analyses. (2) the Dr. Robert Primary Health Center and Laboratori Clinic Metropolitana Nord, Germans Tries i Pujol, Gerència Territorial Metropolitana Nord, Insitut Català de la Salut, processed the samples upon arrival following standard operating procedures to obtain hemogram and lipidic profile.

Samples stored in the IGTP-HUGTP Biobank were processed to obtain several molecular biomarkers. We obtained peripheral BDNF levels using an ELISA kit (Human Free BDNF Quantikine ELISA Kit; R&D Systems, Minnesota, USA). The Proteome Profiler Human™ XL Cytokine Array was used to semi-quantitatively analyze a panel of 105 targeted cytokines (R&D Systems, MN, United States). Based on the results of the array and previously cited literature, TNF-α, ICAM-1, HGF, SDF1-α levels were selected to be quantitatively analyzed using the corresponding ELISA immunoassay method (Human TNF-α Quantikine HS ELISA, Human ICAM-1/CD54 Allele-specific Quantikine ELISA Kit, Human HGF Quantikine ELISA Kit, Human CXCL12/SDF-1 alpha Quantikine ELISA Kit; R&D Systems, Minnesota, USA).

We used raw values of BDNF, TNF-α, ICAM-1, HGF, SDF1-α for Study 2 and change (follow-up minus baseline) in BDNF, TNF-α, ICAM-1, HGF, SDF1-α levels were included in Study 3.

Neuroimaging protocol: biomarkers at Level 2

MRI was obtained in a 3T Siemens Magnetom Verio Symo MR B17 (Siemens Healthineers, Erlangen, Germany). Participants underwent an extensive neuroimaging protocol which included: (1) T1-weighted multi-planar reformat sequence (voxel:

0.9 × 0.9 × 0.9 mm, TR/TE: 1900/2.73 ms, slices: 192; thickness: 0.9 mm); (2) T2-weighted turbo spin-echo sequence (voxel: 0.7 × 0.5 × 3 mm, TR/TE: 6000/74 ms, slices: 35, thickness: 3 mm); (3) T2-weighted turbo inversion recovery magnitude (voxel: 1 × 0.8 × 3 mm, TR/TE: 9000/99 ms, slices: 44, thickness: 3 mm); (4) Susceptibility-weighted imaging with T2 – fl3d sequence (TR/TE: 28/20 ms, slices: 88); (5) Resting state imaging with a gradient echo planar imaging sequence (TR/TE: 2000/25 ms, slices: 39, thickness: 3 mm, volumes: 240); (6) Diffusion tensor imaging, echo planar imaging (voxel: 2 × 2 × 2 mm, TR/TE: 10200/89 ms, 64 directions, 1 acquisition). An expert neuroradiologist visually checked T1-weighted multi-planar reformat sequences for artifacts or clinical brain conditions. All participants received a clinical report of this examination.

We used T1-weighted multi-planar reformat sequences for Study 2 and 3.

Psychological health and daily activities: markers at Level 3

Psychological health and daily activity of participants were assessed using self-reported questionnaires. We applied GDS (Martínez et al., 2002) for depressive symptoms, the Modified Version of Visual Analog Mood Scale (VAMS) (Stern et al., 1997) for mood states and the Short Informant Questionnaire in Routine Evaluation-Outcome Measure (CORE-OM) (Trujillo et al., 2016) to assess psychological distress in the domains of subjective well-being, problems/symptoms, general functioning and risk. We also administered the Pittsburgh Sleep Quality Index (PSQI) (Rico & Fernández, 1997) to evaluate the quality and patterns of sleep and the Short Informant Questionnaire on Cognitive Decline in the Elderly (S-IQCODE) (Morales González et al., 1992) as a measure of subjective cognitive performance in daily activities.

The raw scores of each test were used as Level 3 outcomes in Study 2.

4. Analyses

4.1. Data management

Data was collected without personal identifying information using a code assigned by the assessor and organized in a computerized database. We collected data from all participants regardless of whether the participant withdrew from the intervention or not and revealed the name only in case of incident. Assessments, individual reports and databases were double-checked. Regarding intervention parameters, the intervention staff followed the coherence between personal diaries, phone-call follow-ups and actimeters.

4.2. Neuroimaging analyses

We reconstructed scans from T1 using the tool *dicom2nii* for all the subjects. Images were preprocessed and analyzed accordingly to the aims of Study 2 and 3.

Study 2. For this study, T1-weighted brain images were analyzed using MRICloud (https://mricloud.org/) (Mori et al., 2016) Schmidt-Kassow, which perfoms a fully automated parcellation of 287 volumes based on multiple atlases that fuses different algorithms (Oishi et al., 2009) with a local search algorithm (Coupé et al., 2011). For our sample, we used atlas library version 10A, which includes 30 atlases from cognitively normal individuals and individuals with cognitive impairment or dementia. We selected relevant areas based on previous literature (Erickson et al., 2009; Erickson et al., 2011; Verstynen et al., 2012) to perform analyses: ventricles, total WM and GM of the frontal lobe, dorsolateral prefrontal cortex, cingulate cortex, parietal lobe, precuneus, temporal lobe and hippocampus. In accordance with other volumetric studies, we used the ANCOVA method to regress brain volumes on outcomes of interest. All volumes were normalized by head size by including intracranial volume (ICV) as a covariate. We calculated ICV summing volume of the

brain tissue (Left Hemisphere + Right Hemisphere + Brainstem + Cerebellum) and cerebrospinal fluid (CSF) (Ventricles + Sulci).

Study 3. We used MRICloud (https://mricloud.org/) (Mori et al., 2016) to analyze baseline and follow-up T1-weighted brain images following the same protocol applied in Study 2. We summed volumes of the brain tissue (Left Hemisphere + Right Hemisphere + Brainstem + Cerebellum) and CSF (Ventricles + Sulci) for calculated ICV. Based on previous literature (Erickson et al., 2014; Sexton et al., 2016) and consistent with results in Study 2 (which was previously finished and published), we obtained partial brain volumes of the following relevant areas: ventricles, total WM and GM of the frontal lobe, dorsolateral prefrontal cortex, cingulate cortex, parietal lobe, precuneus, temporal lobe and hippocampus. Then, we obtained post-pre change in each of these outcomes to test our hypotheses.

4.3. Statistical analyses

Data was analyzed using IBM SPSS Statistics for Windows, Version 24.0. We also used other tools or software to obtain specific analyses and plots, which is described when corresponding. First, we ensured data quality by examining the distribution of raw scores (i.e., outliers, skewness). Then, we proceed in each study according to the specific aims. Below, we include the procedures as they are reported in each paper.

Study 1. Demographics and PA variables were analyzed in the total sample and by sex. First, linear regression models were performed to examine the association between CRF and different types of PA with cognitive outcomes in the total sample. Age, years of education, and sex were included as covariates. Then, we analyzed the role of sex in the relationship between physical and cognitive outcomes regressing PA outcomes on cognitive performance for women and men separately, accounting for age and years of education.

Finally, we applied mediation analyses using the PROCESS Macro (Hayes, 2017) in women and men (Figure 12). Path C tested if the independent variable [PA] was associated with the dependent variable [cognitive composites] controlling for age and years of education. Path A was a regression between the independent variable [PA] and the mediator [CRF] accounting for the same covariates. Path B determines if mediator [CRF] changes predict changes in the dependent variable [cognitive composites] accounting for the same covariates. These analyses allowed us to obtain the estimated direct [Path C'] and indirect effect [Path AB]. Path C' reports the association between the independent variable [PA] and cognitive outcomes when the mediator [CRF] and covariates are included in the model. Path AB represents the association with the mediator [CRF] in the model. These analyses were computed with bias-corrected bootstrap 95% confidence intervals (CIs) based on 5,000 bootstrap samples. Significance of mediation was indicated if the CIs in Path AB did not overlap with 0 (Hayes, 2017).

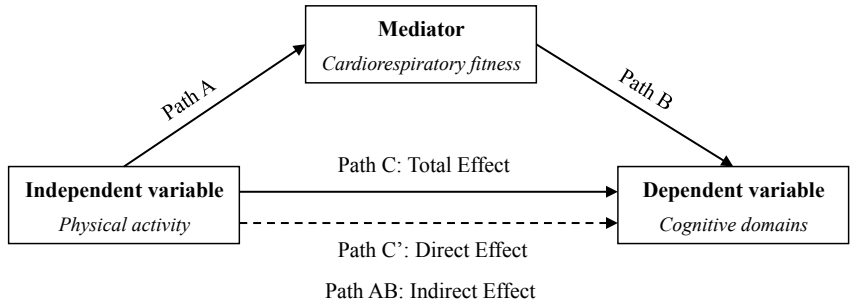


Figure 12. Mediation analysis model for analyses in Study 1, adapted from Hayes (2017) by A. Castells-Sánchez and F. Roig-Coll.

Study 2. Linear regression models were performed to examine the associations between S-PA and CRF with molecular (Level 1), brain volume (Level 2) and psychological (Level 3) outcomes. We stratified analyses by sex and included age and years of education as covariates. We also introduced BMI as a covariate for Level 1 and 2 analyses (Bourassa & Sbarra, 2017; Colbert et al., 2004) and ICV when using Level 2 outcomes. Since molecular outcomes in serum could be influenced by current

cardiovascular risk factors medications, this variable was included as a dichotomous covariate in complementary analyses for Level 1 outcomes.

We analyzed the potential mediating role of these outcomes in the relationship between S-PA and CRF with cognition in women and men separately (see Model 1 in Figure 13). Then, we performed exploratory mediation analyses addressing the potential pathways by which outcomes at Level 1 and 2 might influence markers at Level 2 and 3 (see Model 2, 3 & 4 in Figure 13). We applied mediation analyses using the PROCESS Macro. These analyses were computed with bias-corrected bootstrap or Monte Carlo 95% CIs based on 5,000 bootstrap samples. Significance of mediation was indicated if the CIs in Path AB did not overlap with 0 (Hayes, 2017).

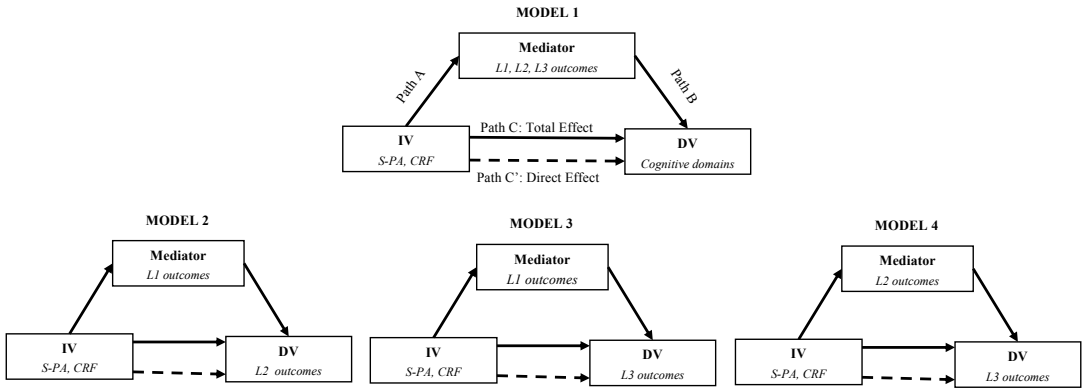


Figure 13. Mediation analysis models for analyses in Study 2, adapted from Hayes (2017) by A. Castells-Sánchez and F. Roig-Coll. *CRF: cardiorespiratory fitness; DV: dependent variable; IV: independent variable; S-PA: sportive physical activity.*

Study 3. In order to address aims in Study 3 we calculated change scores (post-test minus pre-test), compared baseline scores between groups and performed cross-time partial correlations to detect potential confounds and ceiling effects.

Change between baseline and follow-up within group in biomarkers at Level 1 and Level 2 was examined using a t-test of related samples. In order to compare each intervention group to the control group we regressed change in each outcome of

interest on the baseline outcome score, sex, age, years of education, BMI, and the *dummy* treatment variables (AE vs controls, CCT vs controls and COMB vs controls). ICV was included as a covariate for the models including brain volume outcomes.

We addressed whether the significant intervention-related changes in S-PA and CRF observed in the AE and COMB groups previously published in Roig-Coll et al. (2020) were related to change in biomarkers at Level 1 and 2 applying partial correlations adjusted by sex, age, years of education, BMI and, for brain volumes, ICV was added.

We used the PROCESS macro for SPSS (Hayes, 2017) to analyze the moderating effect of age and sex on intervention-related changes for biomarkers at Level 1 and 2. We also used the PROCESS macro to perform mediation analyses to assess whether change in biomarkers at Level 1 or 2 mediated the cognitive benefits observed in the AE and COMB groups (Roig-Coll et al., 2020)(Figure 14). For mediation analyses the independent variable was a treatment variable (condition vs control), the dependent variables were change in cognition for those functions that showed significant intervention-related changes. Extended details of cognitive outcomes and results are published in Roig-Coll et al. (2020). We used as mediators the change in biomarkers at Level 1 and Level 2 controlling for baseline performance score, age, sex, years of education, BMI and, when biomarkers at Level 2 were introduced as mediators, ICV was added. These analyses were computed with bias-corrected bootstrapped 95% CIs based on 5,000 bootstrap samples. Significance of mediation was indicated if the CIs in Path AB did not overlap with 0 (Hayes, 2017).

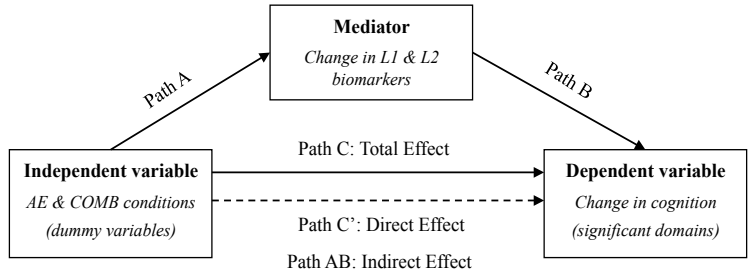


Figure 14. Mediation analysis models for analyses in Study 3, adapted from Hayes (2017) by A. Castells-Sánchez and F. Roig-Coll. *AE: aerobic exercise; COMB: combined training.*

	STUDY 1	STUDY 2	STUDY 3
DESIGN	Cross-sectional design	Cross-sectional design	RCT
OUTCOMES	Cognitive domains Total PA, S-PA, NS-PA CRF *Age, sex and years of education, diagnoses of hypertension and diabetes*	Markers at Level 1, 2 and 3 S-PA CRF Cognitive domains *Age, sex and years of education, BMI, diagnoses of hypertension and diabetes, cardiovascular risk medication*	Change in markers at Level 1 and 2 Change in S-PA and CRF Change in cognitive domains *Age, sex, years of educations, BMI, dummy treatment variables, ICV*
ANALYSES	Demographics **LRM:** CRF & PA, S-PA, NS-PA w/ cognition **LRM:** CRF & PA, S-PA, NS-PA w/ cognition by sex **Mediation:** S-PA – CRF – cognition by sex	Demographics **LRM:** CRF & S-PA w/ L1 **LRM:** CRF & S-PA w/ L2 **LRM:** CRF & S-PA w/ L3 **Mediation:** S-PA, CRF – L1, L2, L3 – cognition S-PA, CRF – L1, L2 – L3 S-PA, CRF – L1 – L2	Demographics **t-test:** follow-up/baseline **LRM:** change condition vs control **Partial correlations**: change in CRF and S-PA with change in L1 and L2 markers **Moderation**: sex and age in L1 and L2 changes **Mediation** group – L1, L2 – cognition

Figure 15. Summary of methods in each study, created by A. Castells-Sánchez. *In lyrics those variables included as covariates. BMI: body mass index; CRF: cardiorespiratory fitness; ICV: intracranial volume; LRM: linear regression models; NS-PA: non-sportive physical activity; PA: physical activity; RCT: randomized controlled trial; S-PA: sportive physical activity.*

RESULTS

STUDY 1

Sex matters in the association between physical activity and fitness with cognition

"Yes, your lifestyle may be predictive of future cognitive decline"

Published as:

Castells-Sánchez, A., Roig-Coll, F., Lamonja-Vicente, N., Torán-Monserrat, P., Pera, G., Montero, P., Dacosta-Aguayo, R., Bermudo-Gallaguet, A., Bherer, L., Erickson, K. I., & Mataró, M. (2021). Sex Matters in the Association between Physical Activity and Fitness with Cognition. *Medicine and science in sports and exercise*, *53*(6), 1252–1259. https://doi.org/10.1249/MSS.0000000000002570

See Study 1 in chapter *Articles*.

This is the first study of Projecte Moviment. In this proof-of-concept study we addressed fundamental questions about the PA-cognition relationship as well as the role of CRF and sex.

1.1. Participants

One hundred fifteen adults were recruited and assessed. The CRF measure could not be estimated for eleven participants, so all analyses were conducted on 104 participants (age = 57.44 ± 5.36; 63% female; years of education = 13.35 ± 5.29; MMSE = 28.22 ± 1.45; BMI = 27.52 ± 4.91). The 65 women and 39 men included in the sample showed no significant differences in the demographic data, except in years of education that is used as a covariate (see Table 4). There was a similar and significant correlation between S-PA and CRF in women ($r = 0.551$, $p < .001$) and men ($r = .631$, $p < .001$) despite sex differences in S-PA and CRF (see Table 4). There were no significant differences in the demographic variables between participants of Projecte Moviment RCTs and the 20 additional participants (see Tables 2 and 3 in *Annex 2*).

Table 4. Demographic and PA variables for the cross-sectional sample.

Demographic variables	**WOMEN** Mean (SD)	**MEN** Mean (SD)	t-Test / χ^2 (p value)
n	65	39	-
Age (years)	56.75 (4.96)	58.59 (5.86)	1.64 (.106)
Education (years)	12.46 (4.97)	14.82 (5.55)	2.18 (.032)
MMSE (/30)	28.15 (1.41)	28.33 (1.53)	0.60 (.552)
BMI	26.99 (4.53)	28.40 (5.44)	-1.43 (.157)
Hypertension (n)	12	10	3.46 (.063)
Diabetes (n)	7	4	3.59 (.058)
PA variables	Mean (SD) [Min – Max]	Mean (SD) [Min – Max]	t-Test (p value)
S-PA	2055.35 (4395.04) [0 - 16512]	6011.35 (9939.24) [0 - 36952]	-2.35 (.023)
NS-PA	9495.88 (6438.50) [1260- 29232]	6173.16 (6780.93) [600 - 39120]	2.50 (.014)
CRF	25.07 (12.31) [0.07 - 49.68]	33.86 (12.92) [3.09- 58.64]	-3.46 (.001)

Note: BMI: body mass index; CRF: cardiorespiratory fitness; MMSE: Mini-Mental State Examination (Blesa et al., 2001); NS-PA: non-sportive physical activity; PA: physical activity; S-PA: sportive physical activity.

1.2. Association between PA, CRF and cognition

Linear regression models examining the association between CRF and different types of PA with cognitive domains in the total sample are in Table 5. Higher CRF was related with better Executive Function, specifically Working Memory, Flexibility and Fluency subdomains; Memory, especially Verbal Memory; and Attention-Speed, both subdomains: Attention and Speed. Greater S-PA was related to better Executive Function, specifically to Working Memory and Fluency; and to Attention-Speed, including Attention and Speed. However, NS-PA was not associated with any cognitive measure.

Table 5. Association between PA outcomes and cognition; lineal regression models for the total cross-sectional sample.

Cognitive Domains	**CRF** R^2; β (p value)	**S-PA** R^2; β (p value)	**NS-PA** R^2; β (p value)
EXECUTIVE FUNCTION	0.11; 0.29 (.012)*	0.15; 0.34 (.001)**	0.05; -0.00 (.990)
Inhibition	0.03; 0.08 (.490)	0.04; 0.12 (.256)	0.03; 0.05 (.614)
Working Memory	0.14; 0.26 (.017)*	0.16; 0.28 (.004)**	0.09; -0.08 (.426)
Flexibility	0.28; 0.21 (.038)*	0.26; 0.14 (.121)	0.26; -0.10 (.256)
Fluency	0.24; 0.37 (<.001)***	0.24; 0.34 (<.001)***	0.13; -0.01 (.890)
VISUOSPATIAL FUNCTION	0.29; 0.13 (.178)	0.28; 0.10 (.260)	0.27; -0.05 (.596)
MEMORY	0.31; 0.33 (.001)**	0.25; 0.16 (.089)	0.23; 0.07 (.472)
Verbal Memory	0.27; 0.36 (<.001)***	0.20; 0.15 (.108)	0.18; 0.11 (.269)
Visual Memory	0.29; 0.11 (.255)	0.29; 0.09 (.320)	0.28; -0.04 (.676)
LANGUAGE	0.28; 0.10 (.316)	0.30; 0.17 (.061)	0.27; 0.04 (.650)
ATTENTION-SPEED	0.43; 0.26 (.004)**	0.43; 0.24 (.004)**	0.38; -0.03 (.765)
Attention	0.39; 0.24 (.012)*	0.39; 0.23 (.006)**	0.35; -0.06 (.465)
Speed	0.35; 0.26 (.006)**	0.34; 0.20 (.019)*	0.30; 0.04 (.640)

Note: CRF: cardiorespiratory fitness; NS-PA: non-sportive physical activity; PA: physical activity; S-PA: sportive physical activity.
Covariates: age, years of education and sex.
** p <.05; ** p<.010; *** p<.001*

1.3. Sex differences in the relationship between PA, CRF and cognition

Table 6 provides the linear regression models stratified by sex. For women, CRF was not significantly associated with cognitive performance in any domain. For men, CRF was positively related to Executive Function, including Inhibition, Working Memory and Fluency; Memory, notably Verbal Memory; Language and Attention-Speed, including both subdomains: Attention and Speed. S-PA was significantly associated with Executive Function especially with Fluency, and Attention-Speed in women and men respectively (see Figure 16; see Figure 1 in *Annex 3* for extended details on data used to build the figure).

Table 6. Association between PA outcomes and cognition; linear regression models for women and men in the cross-sectional sample.

Cognitive Domains	WOMEN		MEN	
	CRF R^2; β (*p* value)	**S-PA** R^2; β (*p* value)	**CRF** R^2; β (*p* value)	**S-PA** R^2; β (*p* value)
EXECUTIVE FUNCTION	0.01; 0.03 (.848)	0.10; 0.31 (.016)*	0.41; 0.63 (<.001)***	0.23; 0.40 (.014)*
Inhibition	0.07; -0.13 (.369)	0.11; 0.22 (.087)	0.14; 0.39 (.022)*	0.01; 0.08 (.638)
Working Memory	0.04; 0.09 (.532)	0.06; 0.17 (.181)	0.22; 0.46 (.005)**	0.18; 0.39 (.015)*
Flexibility	0.32; 0.15 (.216)	0.33; 0.19 (.076)	0.21; 0.27 (.110)	0.16; 0.11 (.477)
Fluency	0.13; 0.15 (.273)	0.20; 0.31 (.011)*	0.51; 0.62 (<.001)***	0.32; 0.39 (.010)*
VISUOSPATIAL FUNCTION	0.31; 0.04 (.775)	0.32; 0.11 (.331)	0.28; 0.22 (.146)	0.24; 0.08 (.575)
MEMORY	0.25; 0.20 (.115)	0.22; 0.04 (.708)	0.41; 0.44 (.003)**	0.29; 0.22 (.135)
Verbal Memory	0.16; 0.25 (.063)	0.11; 0.03 (.790)	0.37; 0.47 (.002)**	0.22; 0.23 (.142)
Visual Memory	0.25; 0.04 (.773)	0.25; 0.04 (.715)	0.30; 0.17 (.253)	0.29; 0.12 (.417)
LANGUAGE	0.18; -0.05 (.729)	0.20; 0.15 (.202)	0.43; 0.34 (.016)*	0.38; 0.23 (.099)
ATTENTION-SPEED	0.36; 0.12 (.301)	0.40; 0.22 (.034)*	0.52; 0.47 (.001)**	0.40; 0.29 (.036)*
Attention	0.37; 0.09 (.462)	0.40; 0.21 (.042)*	0.40; 0.43 (.005)**	0.31; 0.27 (.067)
Speed	0.25; 0.15 (.233)	0.27; 0.20 (.077)	0.46; 0.44 (.002)**	0.35; 0.26 (.069)

Note: CRF: cardiorespiratory fitness; PA: physical activity; S-PA: sportive physical activity.
Covariates: age and years of education.
** p <.05; ** p <.01;*** p <.001*

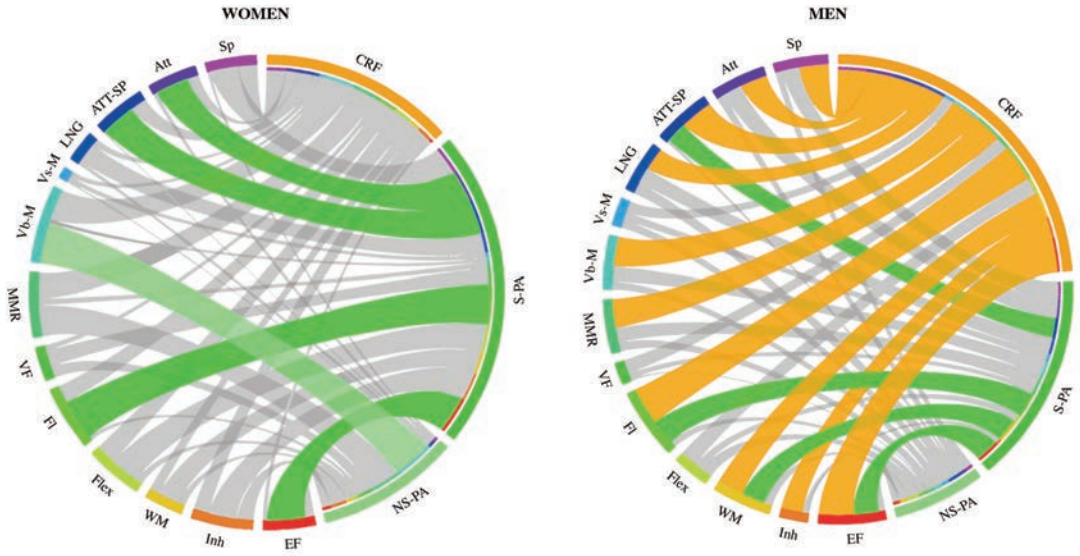


Figure 16. Circos plot of partial correlations between PA outcomes and cognitive domains adjusting for age and years of education for women and men, created by A. Castells-Sánchez and F. Roig-Coll. *Ribbon width indicates percentage of explained variance. The color of the ribbon corresponds to the color of the original segment for p <.05 correlations. Grey ribbons refer to non-significant correlations. Att: attention; ATT-SP: attention-speed; CRF: cardiorespiratory fitness; EF: executive function; Fl: fluency; Flex: flexibility; Inh: inhibition; LNG: language; MMR: memory; NS-PA: non-sportive physical activity; Sp: speed; S-PA: sportive physical activity; Vb-M: verbal memory; VF: visuospatial function; Vs-M: visual memory; WM: working memory.*

1.4. Sex differences in the mediation effects of CRF

We applied mediation analyses for each cognitive outcome using CRF as a mediator for women and men separately (Table 7). We used S-PA as an independent variable given its similar significant association with cognition in women and men.

Women. Path C was equivalent to previous linear regression models for women in Table 6. Path A showed a significant relationship between S-PA and CRF ($\beta = 0.47$, $p < .001$) accounting for age and years of education. Path C' and AB, indicated that CRF did not mediate the association between S-PA and cognitive outcomes in women.

Men. Path C was equivalent to previous linear regression models for men in Table 6. Path A showed a significant relationship between S-PA and CRF ($\beta = 0.64$, $p < .001$) accounting for age and years of education. Path C' and AB showed maximum evidence for mediation of CRF in Executive function especially in Fluency; and Attention-Speed, with Attention as the significant subdomain.

Table 7. Mediation analyses in Study 1.

	Cognitive Domains	Path C' β_2 (SE), p value [95%CI]	Path AB β_{AB} (SE), [95%CI]
WOMEN	EXECUTIVE FUCNTION	0.41 (0.00), .007 [0.00, 0.00]	-0.10 (0.08) [-0.28, 0.06]
	Inhibition	0.38 (0.00), .010 [0.00, 0.00]	-0.16 (0.06) [-0.30, -0.05]
	Working Memory	0.18 (0.00), .239 [0.00, 0.00]	-0.01 (0.09) [-0.18, 0.16]
	Flexibility	0.17 (0.00), .188 [0.00, 0.00]	0.03 (0.07) [-0.11, 0.17]
	Fluency	0.33 (0.00), .021 [0.00, 0.00]	-0.02 (0.08) [-0.18, 0.12]
	VISUOSPATIAL FUNCTION	0.12 (0.00), .339 [0.00, 0.00]	-0.02 (0.08) [-0.17, 0.16]
	MEMORY	-0.07 (0.00), .606 [-0.00, 0.00]	0.11 (0.07) [-0.01, 0.25]
	Verbal Memory	-0.12 (0.00), .412 [-0.00, 0.00]	0.15 (0.07) [0.01, 0.30]
	Visual Memory	0.03 (0.00), .802 [-0.00, 0.00]	0.01 (0.08) [-0.16, 0.17]
	LANGUAGE	0.24 (0.00), .089 [0.00, 0.00]	-0.09 (0.07) [-0.24, 0.04]
	ATTENTION-SPEED	0.23 (0.00), .066 [0.00, 0.00]	-0.00 (0.07) [-0.14, 0.12]
	Attention	0.23 (0.00), .056 [0.00, 0.00]	-0.02 (0.07) [-0.16, 0.11]
	Speed	0.18 (0.00), .181 [0.00, 0.00]	0.02 (0.07) [-0.11, 0.15]
MEN	EXECUTIVE FUNCTION	0.01 (0.00), .951 [0.00, 0.00]	0.39 (0.15) [0.09, 0.69]*
	Inhibition	-0.31 (0.00), .147 [-0.00, 0.00]	0.39 (0.11) [0.21, 0.63]
	Working Memory	0.17 (0.00), .392 [0.00, 0.00]	0.22 (0.16) [-0.08, 0.55]
	Flexibility	-0.10 (0.00), .632 [-0.00, 0.00]	0.21 (0.14) [-0.05, 0.49]
	Fluency	-0.01 (0.00), .974 [0.00, 0.00]	0.39 (0.13) [0.14, 0.64]*
	VISUOSPATIAL FUNCTION	-0.11 (0.00), .587 [0.00, 0.00]	0.19 (0.16) [-0.15, 0.47]
	MEMORY	-0.10 (0.00), .565 [0.00, 0.00]	0.33 (0.16) [-0.04, 0.61]
	Verbal Memory	-0.13 (0.00), .484 [-0.00, 0.00]	0.36 (0.16) [0.00, 0.65]
	Visual Memory	0.01 (0.00), .945 [0.00, 0.00]	0.10 (0.13) [-0.19, 0.33]
	LANGUAGE	0.02 (0.00), .914 [0.00, 0.00]	0.21 (0.15) [-0.15, 0.46]
	ATTENTION-SPEED	-0.02 (0.00), .915 [0.00, 0.00]	0.31 (0.12) [0.09, 0.57]*
	Attention	-0.00 (0.00), .998 [0.00, 0.00]	0.27 (0.13) [0.05, 0.56]*
	Speed	0.04 (0.00), .799 [0.00, 0.00]	0.30 (0.15) [-0.00, 0.59]

*Notes: * significant values (interval not containing zero in path AB)*
Path C' [Direct effects]: Cognitive Domain = β_2S-PA ($\beta_3$cardiorespiratroy fitness) + (covariates) + β_0
Path AB [Indirect effects]: Path C – Path C'

STUDY 2

Exercise and Fitness Neuroprotective Effects: Molecular, Brain Volume and Psychological Correlates and Their Mediating Role in Healthy Late-Middle-Aged Women and Men

"Let's move your body, let's rock your cells, brain and self!"

Published as:

Castells-Sánchez, A., Roig-Coll, F., Dacosta-Aguayo, R., Lamonja-Vicente, N., Sawicka, A.K., Torán-Monserrat, P., Pera, G., Montero-Alía, P., Heras-Tebar, A., Domènech, S., Via, M., Erickson, K.I. & Mataró, M. (2021). Exercise and Fitness Neuroprotective Effects: Molecular, Brain Volume and Psychological Correlates and Their Mediating Role in Healthy Late-Middle-Aged Women and Men. *Frontiers Aging Neuroscience,* 13:615247. doi: 10.3389/fnagi.2021.615247

See Study 2 in see chapter *Articles*.

In this second paper, we went beyond the exercise-cognition relationship. While previous research tends to focus on single levels of analysis when examining possible mechanisms of exercise on cognition, we addressed the relationship between exercise and CRF with key markers at Level 1, 2 and 3 stratifying results by sex. Furthermore, we studied the potential mediating role of each of these markers in the exercise-cognition relationship and perform exploratory analyses to address the potential pathways by which these markers might influence each other.

2.1. Participants

We recruited and assessed 115 healthy adults. The final sample consisted of 104 participants (age = 57.44 ± 5.36; 63% female; years of education = 13.35 ± 5.29; MMSE = 28.22 ± 1.45; BMI = 27.52 ± 4.91) from which we could obtain a valid measure of CRF. There were no significant differences in the demographic data between the 65 women and 39 men included in the sample except in years of education that is used as a covariate (see Table 4). Despite sex differences in S-PA and CRF levels, the correlations between S-PA and CRF in women ($r = .551$, $p < .001$) and men ($r = .631$, $p < .001$) were significant and comparable (see Table 4). There were no significant differences in age, years of education and MMSE scores between participants of Projecte Moviment and the 20 additional participants (see Tables 2 and 3 in *Annex 2*).

2.2. Associations between PA, CRF, and mechanisms at Level 1, 2 and 3

2.2.1. Level 1: PA, CRF, and molecular biomarkers

Linear regression models examining the relationship between S-PA and CRF with Level 1 biomarkers in women and men separately are in Table 8. Engaging in greater amounts of S-PA was significantly associated with reduced levels of TNF-α in women. There were not any significant associations between S-PA and molecular

markers in men. In contrast, higher CRF levels were associated with lower TNF-α and HGF levels in men. There were not significant relationship between CRF and molecular markers in women. Extended details for each model are included in Table 4 in *Annex 2*. Besides, similar results were obtained for these same regression linear models when accounting for the potential influence of cardiovascular risk factors medications as a covariate (see Table 5 in *Annex 2*). Additional results examining these relationships for NS-PA are included in Table 6, *Annex 2*.

Table 8. Linear regression models in women and men: relationship between PA outcomes and molecular biomarkers.

Molecular biomarkers	WOMEN		MEN	
	S-PA β (*p* value)	CRF β (*p* value)	S-PA β (*p* value)	CRF β (*p* value)
BDNF (pg/ml)	-0.04 (.797)	-0.09 (.646)	-0.09 (.642)	-0.32 (.224)
TNF-α (pg/ml)	-0.39 (.007)**	-0.19 (.288)	-0.19 (.304)	-0.65 (.017)*
HGF (pg/ml)	-0.12 (.398)	-0.25 (.143)	-0.23 (.152)	-0.58 (.018)*
ICAM-1 (ng/ml)	-0.12 (.473)	-0.32 (.082)	-0.15 (.423)	-0.07 (.820)
SDF1-α (pg/ml)	0.06 (.695)	0.27 (.132)	0.06 (.781)	-0.07 (.821)

Note: BDNF: brain-derived growth factor; CRF: cardiorespiratory fitness; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; PA: physical activity; SDF1-α: stromal cell-derived factor-1 alpha protein; S-PA: sportive physical activity; TNF-α: tumor necrosis alpha.
Covariates: age, years of education and body mass index.
*S-PA is measured in metabolic equivalent of task units and CRF in ml/kg*min.*
** p <.05; ** p <.01.*

2.2.2. Level 2: PA, CRF, and brain volumes

Linear regression models examining the relationship between S-PA and CRF with brain volumes, Level 2 outcomes, in women and men separately are described in Table 9. Engaging in greater amounts of S-PA were positively correlated with the volume of the dorsolateral prefrontal cortex in women and with temporal lobe volume in both women and men. There was also a relationship between S-PA and precuneus volume in men. Greater levels of CRF were associated with larger precuneus and temporal lobes and with smaller ventricles in men. There was also a correlation

between CRF and parietal lobe volumes in men. In women, there were no significant relationships between CRF and brain volumes. Extended details for each model are included in Table 7 in *Annex 2.* See Table 8 in *Annex 2* for additional results showing the relationship between NS-PA and brain volume outcomes.

Table 9. Linear regression models in women and men: relationship between PA variables and brain volumes.

Brain Volumes (mm^3)	WOMEN		MEN	
	S-PA β (*p* value)	CRF β (*p* value)	S-PA β (*p* value)	CRF β (*p* value)
Ventricles	0.23 (.104)	0.08 (.642)	-0.28 (.113)	-0.69 (.002)**
Total White Matter	-0.02 (.739)	0.06 (.442)	-0.07 (.530)	-0.07 (.616)
Frontal Lobe	-0.03 (.632)	0.02 (.778)	0.11 (.260)	0.23 (.074)
Dorsolateral Prefrontal Cortex	0.22 (.015)*	0.19 (.074)	0.06 (.632)	-0.09 (.618)
Cingulate Cortex	0.12 (.183)	-0.02 (.856)	0.18 (.134)	0.05 (.754)
Parietal Lobe	-0.00 (.965)	-0.07 (.377)	0.09 (.279)	0.20 (.051)
Precuneus	-0.07 (.545)	-0.12 (.404)	0.28 (.054)	0.46 (.014)*
Temporal Lobe	0.14 (.041)*	0.11 (.191)	0.20 (.033)*	0.25 (.048)*
Hippocampus	0.07 (.567)	-0.02 (.899)	-0.07 (.694)	-0.06 (.779)

Note: CRF: cardiorespiratory fitness; PA: physical activity; S-PA; sportive physical activity.
Covariates: age, years of education, intracraneal brain volume and body mass index.
*S-PA is measured in metabolic equivalents units and CRF in ml/kg*min.*
** p <.05; ** p <.01.*

2.2.3. Level 3: PA, CRF, and psychological health and daily activity

Linear regression models examining the relationship between S-PA and CRF with behavioral outcomes at Level 3 in women and men separately are in Table 10. There were no significant associations between S-PA and any of the scores in women and men. However, when analyzing the subscales, we found that in men, higher levels of S-PA were related to better subjective sleep efficiency ($\beta = 0.41$, $p = .008$) and fewer sleep disturbances ($\beta = -0.43$, $p = .008$) in the PSQI and higher scores of well-being ($\beta = -0.31$, $p = .065$) in the corresponding subscale of the CORE-OM test. In addition,

in men, higher CRF was significantly associated with fewer depressive symptoms measured by the GDS. There was also a moderate negative relationship between levels of CRF and VAMS scores in men. Extended details for each model are included in Table 9 in *Annex 2.* See Table 10 in *Annex 2* for additional results showing the relationship between NS-PA and brain volume outcomes.

Table 10. Linear regression models in women and men: relationship between PA variables and behavior outcomes.

Psychological Status & Daily Activity	WOMEN		MEN	
	S-PA β (*p* value)	CRF β (*p* value)	S-PA β (*p* value)	CRF β (*p* value)
GDS	-0.16 (.207)	-0.05 (.729)	-0.23 (.174)	-0.39 (.021)*
VAMS	-0.10 (.446)	-0.26 (.070)	-0.06 (.717)	-0.30 (.055)
S-IQCODE	-0.02 (.875)	0.19 (.193)	-0.14 (.403)	-0.16 (.354)
PSQI	-0.11 (.416)	-0.04 (.784)	-0.13 (.445)	-0.19 (.286)
Total CORE-OM	-0.19 (.138)	-0.17 (.238)	-0.15 (.378)	-0.10 (.547)

Note: CORE-OM: Short Informant Questionnaire in Routine Evaluation-Outcome Measure (Trujillo et al., 2016); CRF: cardiorespiratory fitness; GDS: Geriatric Depression Scale (Martínez et al., 2002); PA: physical activity; PSQI: Pittsburgh Sleep Quality Index (Rico & Fernández, 1997); S-IQCODE: Short Informant Questionnaire on Cognitive Decline in the Elderly (Morales González et al., 1992); S-PA: sportive physical activity; VAMS: Visual Analog Mood Scale (Stern et al., 1997).
Covariates: age and years of education.
*S-PA is measured in metabolic equivalents units and CRF in ml/kg*min.*
** p <.05*

2.3. Mediating effects

2.3.1. Model 1

Model 1 (see Figure 13 in *Methods*) tested the mediating effect of each molecular, brain volume and behavioral outcome in the relationship between S-PA and CRF with the assessed cognitive functions in women and men. In the relationship between S-PA and cognitive domains, none of the outcomes at Level 1, 2 or 3 showed significant indirect effects in the mediation analyses in women or men. When CRF was the

predictor, we found statistically significant indirect effects for HGF in the CRF-executive function (Path AB = β = 0.29, *SE* = 0.17, 95% CI: 0.02, 0.70) and in the CRF-working memory (Path AB = β = 0.25, *SE* = 0.15, 95% CI: 0.02, 0.59) relationships in men. Indirect effects were also significant when TNF-α was the mediator in the association between CRF and inhibition (Path AB = β = 0.31, *SE* = 0.19, 95% CI: 0.04, 0.77) only in men. There were no significant indirect effects for any outcome in the CRF-cognition relationship in women (see Figure 17).

2.3.2. Model 2

Model 2 (see Figure 13 in *Methods*) examined the mediating effects of molecular outcomes in the relationship between S-PA and CRF with brain volumes in women and men. In the association between S-PA and brain volumes, none of the molecular outcomes showed significant mediating effect in women or men. In the mediation analyses with CRF as a predictor, we found statistically significant indirect effects for TNF-α in the relationship between CRF and cingulate cortex volume (Path AB = β = -0.26, *SE* = 0.14, 95% CI: -0.58, -0.03) only in men. We did not find any significant mediating effects for any molecular outcome in the CRF-brain volume association in women (see Figure 17).

2.3.3. Model 3

Model 3 (see Figure 13 in *Methods*) assessed the mediating effects of molecular outcomes in the relationship between SP-A and CRF with psychological health and daily activity. In the relationship between S-PA and behavioral outcomes, none of the molecular outcomes showed significant indirect effects in women or men. When CRF was the predictor, we found statistically significant indirect effects for HGF in the relationship between CRF and the subscale of subjective sleep quality of the PSQI (Path AB = β = -0.21, *SE* = 0.13, 95% CI: -0.53, -0.03) in men. There were no significant mediating effects for any molecular outcome in the CRF-behavioral relationship in women (see Figure 17).

2.3.4 Model 4

Model 4 (see Figure 13 in *Methods*) tested the mediating effects of brain volume outcomes in the relationship between S-PA and CRF with psychological health and daily activity. In the association between S-PA and behavioral outcomes, none of the brain volume outcomes showed significant mediating effects in women or men. In the mediation analyses with CRF as a predictor, we found statistically significant indirect effects of precuneus volume in the relationship between CRF and a subscale of subjective sleep quality from the PSQI (Path AB = β = -0.26, *SE* = 0.17, 95% CI: -0.65, -0.00) in men. We did not find any significant mediating effects for any brain volume outcomes in the CRF-behavioral association in women (see Figure 17).

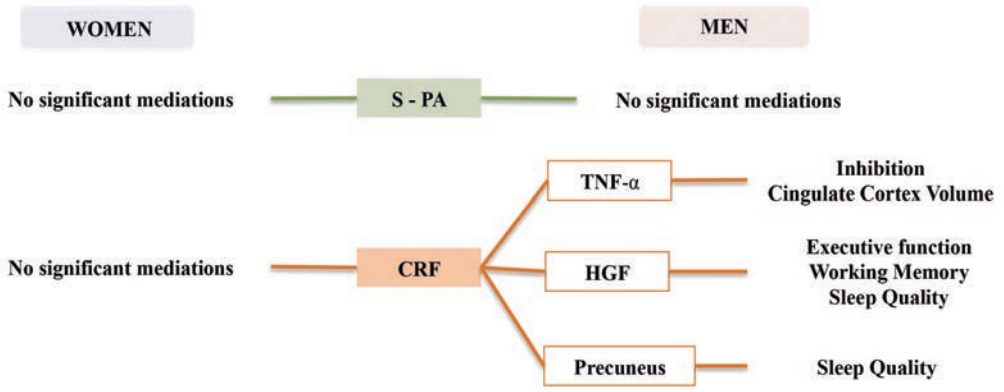


Figure 17. Mediation results of Models 1, 2, 3 and 4 in women and men, created by A. Castells-Sánchez and F. Roig-Coll. *CRF: cardiorespiratory fitness; HGF: hepatocyte growth factor; S-PA: sportive physical activity; TNF-α : tumor necrosis factor alpha.*

STUDY 3

Molecular and Brain Volume Changes Following Aerobic Exercise, Cognitive and Combined Training: the Projecte Moviment

"Being active might be your best health ally"

Accepted under review as:

Castells-Sánchez, A., Roig-Coll, F., Dacosta-Aguayo, R., Lamonja-Vicente, N., Torán-Monserrat, P., Pera, G., García-Molina, A., Tormos, J.M., Montero, P., Heras, Tébar, A., Soriano-Raya, J.J., Cáceres, C., Domènech, S., Via, M., Erickson, K. I., & Mataró, M. (2021). Molecular and Brain Volume Changes Following Aerobic Exercise, Cognitive and Combined Training: the Projecte Moviment. *Psychophysiology.*

See Study 3 in see chapter *Articles*.

Study 3 was conceived after Roig-Coll et al. (2020) reported AE-related benefits in Executive Function and Attention-Speed and COMB-related benefits in Attention-Speed compared to controls in Projecte Moviment RCTs. Moreover, AE and COMB conditions provided significant changes in S-PA and CRF, too. In accordance, in Study 3 we aimed to identify the pathways by which cognition was enhanced in those participants in the AE and COMB conditions including markers at Level 1 and 2. We included inflammatory markers and growth factors as well as brain volume changes in Study 3 in order to maintain a coherent line of research between cross-sectional and RCT analyses.

3.1. Participants

We conducted analyses in the Per Protocol (PP) sample which included 82 subjects with a level of adherence >80% (n = 82, 62% female; age = 58.38 ± 5.47; see Table 11) out of the 109 who completed the baseline assessment and the 92 who completed the intervention (intention to treat, ITT) (see Figure 18; extended details in (Roig-Coll et al., 2020)). There were no significant differences in demographic variables between groups in the ITT sample and between the ITT and PP sample (see Table 11 and 12 in *Annex 2*).

Table 11. Participants characteristics at baseline in the RCT design.

	Total Mean (SD)	**AE** Mean (SD)	**CCT** Mean (SD)	**COMB** Mean (SD)	**Control** Mean (SD)	**Group test**
n total / n females	82 / 51	25 / 13	23 / 16	19 / 14	15 / 8	$X^2(3) = 3.20$, $p = .361$
Age (years)	58.38 (5.47)	58.40 (5.12)	57.91 (5.31)	60.32 (5.54)	56.60 (5.97)	H(3) = 3.53, $p = .317$
Years of education (years)	12.52 (5.57)	12.44 (5.75)	12.04 (4.94)	12.37 (5.43)	13.60 (6.72)	H(3) = 0.28, $p = .963$
Vocabulary subtest (direct score-WAIS-III)	44.14 (8.30)	43.92 (9.53)	44.26 (7.16)	44.53 (8.02)	43.80 (8.98)	F(3,77) = 0.03, $p = .993$
BMI (kg/m^2)	28.63 (4.96)	28.14 (5.53)	28.13 (4.26)	28.37 (4.42)	30.49 (5.70)	H(3)=1.72, $p = .632$

Note: AE: aerobic exercise; BMI: body mass index; CCT: computerized cognitive training; COMB: combined training; WAIS-III: Wechsler Adult Intelligence Scale III.

The PP sample showed no significant baseline differences between groups in demographic, molecular, brain volume, physical and cognitive outcomes except for SDF1-α and NS-PA (see Table 13, 14, 15 and 16 in *Annex 2*). SDF1-α at baseline was included as a covariate.

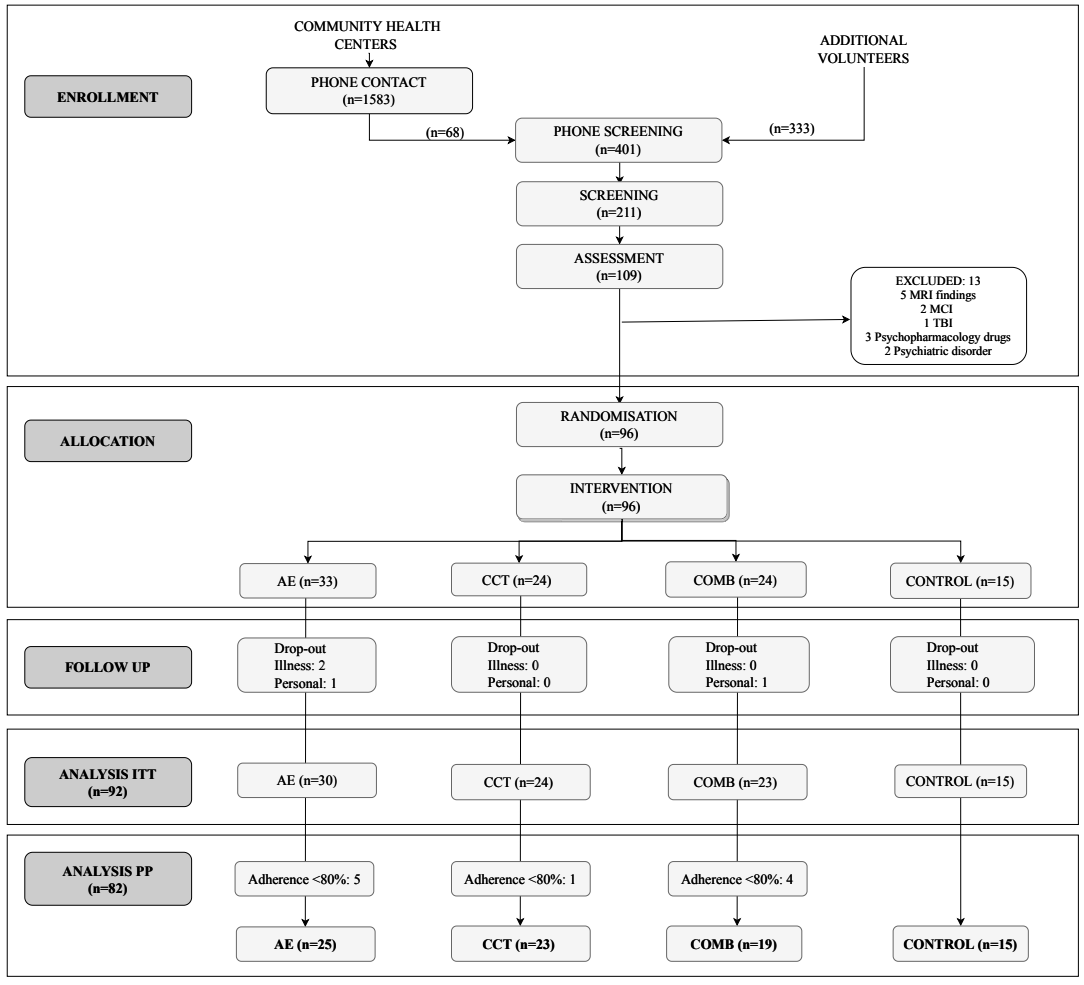


Figure 18. Flow chart of participants in the RCT, created by A. Castells-Sánchez and F. Roig-Coll. *AE: aerobic exercise; CCT: computerized cognitive training; COMB: combined training; ITT: intention-to-treat; MCI: mild cognitive impairment; MRI: magnetic resonance imaging; PP: per protocol; TBI: traumatic brain injury.*

3.2. Intervention-related changes in molecular biomarkers

There were no significant changes in molecular biomarkers between baseline and follow-up in any group. Contrasts between each intervention and control group for molecular outcomes are reported in Table 12. Results for these outcomes showed no significant changes in AE, CCT and COMB groups compared to control for any molecular biomarker outcome.

Table 12. Intervention-related changes in molecular biomarkers (Level 1).

Molecular biomarkers	AE vs Controls B (95%CI), SMD, *p* value	CCT vs Control B (95%CI), SMD, *p* value	COMB vs Control B (95%CI), SMD, *p* value
BDNF (pg/ml)	-1293.54 (-4333.82, 1746.74), SMD = -0.13, *p* = .398	-1081.93 (-3925.15, 1761.30), SMD = -0.11, *p* = .450	-128.39 (-3235.57, 2978.79), SMD = -0.01, *p* = .934
TNF-α (pg/ml)	0.08 (-0.06, 0.21), SMD = 0.16, *p* = .287	<0.01 (-0.14, 0.14), SMD <0.01, *p* = .990	-0.03 (-0.18, 0.12), SMD = -0.07, *p* = .663
HGF (pg/ml)	24.81 (-102.48, 152.10), SMD = 0.06, *p* = .669	2.47 (-123.84, 128.78), SMD = 0.01, *p* = .969	30.48 (-105.27, 166.23), SMD = 0.07, *p* = .656
ICAM-1 (ng/ml)	-4.54 (-22.39, 13.31), SMD = -0.08, *p* = .614	-0.50 (-18.19, 17.20), SMD = -0.01, *p* = .955	-9.04 (-27.94, 9.86), SMD = -0.14, *p* = .343
SDF1-α (pg/ml)	-38.11 (-211.95, 135.73), SMD = -0.06, *p* = .663	-48.23 (-221.07, 124.61), SMD = -0.08, *p* = .580	140.87 (-47.06, 328.79), SMD = 0.22, *p* = .139

Note: AE: aerobic exercise; BDNF: brain-derived growth factor; CCT: computerized cognitive training; COMB: combined training; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; SDF1-α: stromal cell-derived factor-1 alpha protein; TNF-α: tumor necrosis alpha.
Covariates: sex, age, years of education, body mass index and baseline.
SMD= β.

3.3. Intervention-related changes in brain volume

There was a significant change in precuneus volume between baseline and follow-up for the CCT group (t = -2.62, p = .019). There were no other significant changes between baseline and follow-up for the AE, COMB and control groups. Contrasts between each intervention and control group for brain volume outcomes are reported in Table 13. The results for these outcomes showed a significant change in frontal lobe and in precuneus volume in the CCT compared to control group. There were no significant changes in AE and COMB groups compared to control for any outcome.

Table 13. Intervention-related changes in brain volumes (Level 2).

Brain Volumes (mm^3)	**AE vs Controls** B (95%CI), SMD, *p* value	**CCT vs Control** B (95%CI), SMD, *p* value	**COMB vs Control** B (95%CI), SMD, *p* value
Ventricles	-335.42 (-2176.65, 1505.81), SMD = -0.07, *p* = .716	-89.42 (-2020.52, 1841.69), SMD = -0.02, *p* = .926	348.67 (-1581.37, 2278.71), SMD = 0.06, *p* = .719
Total White Matter	-598.23 (-2268.85, 1072.39), SMD = -0.12, *p* = .476	140.20 (-1654.67, 1935.08), SMD = 0.03, *p* = .876	-432.88 (-2216.83, 1351.08), SMD = -0.08, *p* = .629
Frontal Lobe	2462.00 (-1611.56, 6535.56), SMD = 0.21, *p* = .231	5031.79 (717.77, 9345.82), SMD = 0.41, *p* = .023*	3566.03 (-759.51, 7891.57), SMD = 0.29, *p* = .104
Dorsolateral Prefrontal Cortex	119.31 (-757.30, 995.92), SMD = 0.05, *p* = .786	567.92 (-352.66, 1488.50), SMD = 0.23, *p* = .221	220.64 (-691.70, 1132.98), SMD = 0.09, *p* = .630
Cingulate Cortex	240.33 (-339.98, 820.64), SMD = 0.15, *p* = .410	387.42 (-227.20, 1002.05), SMD = 0.23, *p* = .212	281.35 (-332.97, 895.66), SMD = 0.16, *p* = .363
Parietal Lobe	95.67 (-1843.45, 2034.79), SMD = 0.02, *p* = .922	1312.57 (-770.68, 3395.82), SMD = 0.23, *p* = .212	148.65 (-1888.81, 2186.12), SMD = 0.03, *p* = .884
Precuneus	34.64 (-453.43, 522.71), SMD = 0.02, *p* = .887	530.47 (21.65, 1039.28), SMD = 0.35, *p* = .041*	289.19 (-222.30, 800.68), SMD = 0.19, *p* = .262
Temporal Lobe	55.82 (-2084.45, 2196.08), SMD = 0.01, *p* = .958	145.31 (-2100.17, 2390.79), SMD = 0.02, *p* = .897	-1385.20 (-3645.44, 875.04), SMD = -0.22, *p* = .225
Hippocampus	23.91 (-75.07, 122.89), SMD = 0.08, *p* = .630	15.56 (-88.00, 119.13), SMD = 0.05, *p* = .764	44.47 (-60.54, 149.47), SMD = 0.14, *p* = .400

Note: AE: aerobic exercise; CCT: computerized cognitive training; COMB: combined training.
Covariates: sex, age, years of education, body mass index, baseline and intracranial brain volume.
**p < .05*
SMD= β.

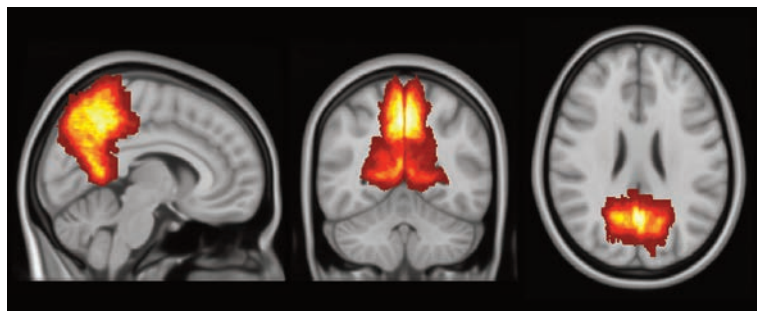


Figure 19. Precuneus volume, extracted from the Harvard-Oxford structural atlas.

3.4. Relation between biomarkers at Level 1 and 2 with changes in PA outcomes

As mentioned above, AE and COMB groups showed significant intervention-related changes in S-PA and CRF levels (Roig-Coll et al., 2020). In the AE group, the increase of S-PA was negatively associated with change in ICAM-1 levels (r = -.55, p = .016). Results were not significant for changes in BDNF, TNF-α, HGF or SDF1-α. Moreover, the increased levels of CRF in the AE group were negatively associated with change in SDF1-α levels (r = -.50, p = .057) and not significantly associated with the other biomarkers. In the COMB group, the significant change in S-PA was not related to changes in any of the measured molecular markers. However, the significant benefits on CRF were negatively related to change in ICAM-1 (r = -.66, p = .020) and SDF1-α (r = -.89, p = <.001) levels. Intervention-related changes in PA in AE and COMB groups were not significantly related to changes in any measure of brain volume.

3.5. Sex and age moderation effects on biomarkers at Level 1 and 2

Moderation analyses showed that age did not significantly moderate the effect of the intervention on biomarkers at Level 1 and Level 2 in any group. Sex was not a significant moderator of the effects of the intervention on biomarkers at Level 1 in any group but showed significant effects on some of the targeted brain volume areas. In the AE group sex (women = 1, men = 0) moderated the effects of the intervention on total WM (β = -3133.51, t(53) = -2.45, p = .018), parietal (β = -3060.29, t(53) = -2.07, p = .043) and temporal lobe (β = -4028.46, t(53) = -2.47, p = .017) and dorsolateral prefrontal cortex volumes (β = -1819.39, t(53) = -2.89, p = .006). In the COMB group sex also moderated the effects of intervention on ventricles (β = -5380.97, t(53) = -3.60, p < .001), temporal lobe (β = 4690.43, t(53) = 2.56, p = .013) and dorsolateral prefrontal cortex (β = 1670.17, t(53) = 2.26, p = .028).

3.6. Mediation effects on intervention-related cognitive benefits

We applied mediation analyses to observe whether changes in biomarkers at Level 1 and Level 2 mediated the association between the intervention and the cognitive domains that demonstrated a significant change as reported in Roig-Coll et al. (2020) including Executive Function (Working Memory) and Attention-Speed (Attention) in the AE group and for Attention-Speed (Attention and Speed) in the COMB group. Mediation analyses showed that changes in BDNF, TNF-α, HGF, SDF1-α and ICAM-1 levels and the targeted brain volume areas did not significantly mediate the observed cognitive benefits for any group.

SUMMARY OF FINDINGS

Study 1

In the total sample, S-PA was positively related to Executive Function and Attention-Speed while NS-PA was unrelated to cognitive domains; CRF was positively associated with Executive Function, Memory and Attention-Speed. Both women and men showed that greater amounts of S-PA were associated with Executive Function and Attention-Speed. Only men showed a significant positive relationship between CRF and Executive Function, Memory, Language and Attention-Speed. Only in men, CRF was a significant mediator of the positive effects of S-PA on Executive Function and Attention-Speed. Those results support evidence suggesting cognitive benefits from greater S-PA. We added evidence of non-significant benefits of NS-PA as well as the male-specific mediating role of CRF in the exercise-cognition benefits.

Study 2

In women, higher amounts of S-PA were related to lower TNF-α levels and greater dorsolateral prefrontal cortex and temporal lobe volumes. In men, higher amounts of S-PA were related to greater temporal lobe volumes. In women, CRF levels were not significantly related to any of the analyzed outcomes. In men, higher CRF was associated with lower TNF-α, HGF and ventricle volumes, greater volume of temporal and parietal lobe and fewer depressive symptoms and better mood. In men, reduced TNF-α and HGF levels mediated brain and cognitive CRF-related benefits for executive function, working memory and inhibition. Moreover, TNF-α mediated the relationship between CRF and volume in cingulate cortex and HGF and precueneus volume had a significant mediating role in the CRF-sleep quality association in men. These results showed that exercise is a promising approach for influencing inflammation and brain volume in women and men. CRF showed a male-specific relationship with molecular, brain volume and psychological health markers contributing to the ongoing discussions about the physiological mediators for the association between CRF and cognition in men.

Study 3

Results from the RCT sample showed there were no significant changes in molecular biomarker concentrations in any intervention group. However, changes in PA outcomes in AE and COMB groups were negatively associated with changes in ICAM-1 and SDF1-α. In relation to Level 2 outcomes, participants in the CCT showed a significant increase in precuneus volume. While AE and COMB groups did not show significant brain volume changes, we found sex moderated the intervention-related effect on brain volume in these two conditions. When addressing the mediating role of markers at Level 1 and 2, changes in molecular biomarkers and brain volumes did not significantly mediate the cognitive-related benefits previously found for any group. In this study we could identify changes in molecular and brain volume markers that might be related to lifestyle interventions at early stages. We highlight the value of examining activity parameters, individual difference characteristics such as sex and the use of a multi-level analysis approach to address these questions.

Figure 2 in Annex 3 contains a summary of Projecte Moviment research project results. In the printed version of the thesis this figure is a flyer that might be used as bookmarker and might accompany the Reader.

DISCUSSION

In the following chapter we discuss our results grouped in short sections that address each one of the main ideas we want to highlight in this thesis: 1) the role of S-PA and NS-PA; 2) the role of CRF; 3) sex-differences in our results; 4) mechanisms at molecular, brain and behavioral levels in the exercise-cognition relationship; 5) AE intervention-related changes; 6) CCT intervention-related changes; and 7) COMB intervention-related changes.

1. PA OR S-PA, THAT IS THE QUESTION

Since Etnier et al. (1997) published, to our knowledge, the very first systematic review stating that PA might have a positive effect on cognition, research has focused on understanding how PA, as a low cost strategy, might have a high impact on brain and cognitive health as well as on quality of life and wellbeing of an aging society. While RCTs have focused on the effect of specific types of exercise and reported related benefits (Barha et al., 2017; Northey et al., 2018), cross-sectional studies still discuss PA and exercise at the same level with scarce distinction. Literature tends to undervalue the difference between PA, defined as any body movement ranging from daily activities to marathons, and exercise, a subtype of structured PA performed in order to improve fitness (Caspersen et al., 1985). Although such a conceptual mix might stand for discrepancies when trying to understand the whole PA-cognition picture, we did not find solid bibliography addressing this issue, which is crucial to better design clinical guides promoting healthy aging (Koblinsky et al., 2021). Therefore, understanding the benefits related to exercise was one of the main goals of Projecte Moviment research project and the current thesis.

Based on previous literature, we expected a positive relationship between exercise and cognition, especially for executive function and processing speed (Frederiksen et al., 2015). Moreover, we anticipated that daily PA belonging to a non-sportive category would be less related to cognition, based on the lack of significant physiological stress that might lead to body adaptations and any significant cascade

of changes. In Study 1 we grouped the common measure of total PA into S-PA and NS-PA and results revealed that **older adults performing greater amounts of S-PA showed better performance for Executive Function, specifically for Working Memory and Fluency; and Attention-Speed, including both Attention and Speed domains**, which tied well with previous findings (Frederiksen et al., 2015). Further analyses showed that non-sportive daily activities were not significantly associated with cognitive benefits in older adults. Our results agree with previous cross-sectional data reporting cognitive benefits of regular exercise in domains that are crucial for daily functioning and healthy aging (Sofi et al., 2011) but we also go beyond specifically highlighting the importance of the "sportive" component of PA when prescribing it to older adults. This "sportive" component might be related to differences in PA parameters such as frequency, dose and intensity between S-PA and NS-PA as well as other psychosocial parameters such as the social interaction and wellbeing enhanced when exercising (Stillman et al., 2016).

Those results were in accordance with findings in Study 2, which addressed how exercise was related to molecular, brain volume, and psychological health outcomes. **We published that exercise might benefit brain volume health. Moreover, performing greater amounts of exercise was related to less inflammatory profile in women and better general wellbeing and subjective sleep quality in men.** We performed additional analyses addressing these same issues for NS-PA in order to state that these benefits might be specifically related to S-PA, which complemented Study 2 with further and consistent novel findings. **Engaging in NS-PA did not benefit women or men in relation to our molecular, brain volume or psychological health outcomes.** Actually, engaging in greater amounts of NS-PA related to higher levels of TNF-α and less volume of the parietal lobe in women and less volume in the hippocampus in men. It is worth to highlight that not only NS-PA might not benefit but also relate to inflammatory markers and decline in relevant brain structures for visuospatial and memory function, respectively. We might hypothesize that the lack of immune, metabolic and CRF benefits related to NS-PA compared to the ones observed for S-PA might explain differences in the observed cognitive benefits related to S-PA

and NS-PA. Nevertheless, a recent study have reported that household activities, and not recreational PA, benefited brain volume but not cognitive performance (Koblinsky et al., 2021). We agree on the fact that household activities might be a source of moderate PA for those old people with restricted mobility. Actually, it might be helpful to reduce in a low-risk manner the deleterious effects of a sedentary lifestyle (Coelho et al., 2020; Koblinsky et al., 2021). However, their sample is much older than ours and has a high percentage of women, which might be a source of bias. To our knowledge, women in this average age tend to perform more household activities than men and also show greater brain volume in specific brain areas due to the role of estradiol on BDNF levels across the lifespan (Luine & Frankfurt, 2013). Therefore, "household activities" might be a confounding variable in the household-brain volume association masking the real effect of the variable *women* on GM volume. Moreover, the fact that they did not report any type of benefit for recreational PA, when it is widely common in literature, suggests that potential methodological issues might bias their conclusions. In this field of research there are other methodological issues such as the bias related to self-reported PA measures, which must be acknowledged when discussing and might have had an impact in our data too.

Our results seem to go further and cast a new light on the take-home message "PA cognitive benefits", which has crucial clinical and research implications. On one side, we added evidence of the importance of exercise when prescribing PA to older adults and the fact that clinical guidelines might insist on the sportive component of the activity. On the other, our data stress the need to address the effects of PA by categories that allow separating exercise (or even subtypes of exercise) from other types of PA in order to be concise in our assumptions.

Like Shakespeare, we might wonder about "to be or not to be". However, in relation to the question regarding PA or S-PA, we would conclude that specifically exercise, and not non-sportive PA, seems to be related to cognitive benefits as well as molecular, brain and psychological health markers that are relevant for daily functioning when aging.

2. CRF, A KEY PLAYER BUT NOT THE ONLY CONTENDER

CRF, which is a physiological index of cardiorespiratory health that reflects the body ability to supply oxygen to the skeletal muscles during sustained PA (Caspersen et al., 1985), seemed to overtake the limitations of a self-reported measure of PA when analyzing the neuroprotective benefits potentially related to PA and exercise. This measure has been related to molecular (Huang et al., 2014), brain volume (Raichlen et al., 2020; Wittfeld et al., 2020) and cognitive outcomes (Freudenberger et al., 2016) indicating that those with higher CRF levels might have a healthier aging. Based on the fact that those performing greater amounts of exercise tend to show greater CRF levels (Halse et al., 2015; Wang et al., 2010), this outcome has been used in a similar way when discussing PA or exercise benefits. For example, Colcombe and Kramer (2003) analyzed the effect on cognition of what they called aerobic fitness interventions included in RCTs. They stated an "unequivocal yes" for the "reliable benefits of fitness training" and results were published under the title "Fitness effects on the cognitive function of older adults: a meta-analytic study" (Colcombe & Kramer, 2003). From our perspective, that publication was a relevant game changer in this field given the possibility to enhance cognition in late-life through CRF. Somehow it seemed that CRF was the rookie player in the exercise-cognition match. Since, exercise and CRF have been exchanged without restrictions when reporting conclusions, which might have led to the idea that CRF effects correspond to exercise effects. Moreover, when addressing the pathways by which exercise might enhance cognition, some studies have suggested that CRF might mediate this relationship. Actually, Erickson et al. (2009) stated this fact when reporting that CRF was positively related to hippocampus volume and memory performance. However, it remains unclear whether the effect of exercise and CRF is similar and which is the role of cardiorespiratory health in the exercise-related neuroprotective effects. Projecte Moviment and this thesis included the aim to better understand how CRF related to outcomes at different levels as we did with S-PA. Moreover, we aimed to better describe the role of CRF in the exercise-cognition relationship addressing its potential mediating role.

In Study 1 we expected that CRF would positively relate with cognition showing a pattern similar to S-PA and have a significant mediating role in the exercise-cognition relationship. **Participants with greater CRF levels showed better performance in Executive Function, Verbal Memory and Attention-Speed,** which is in accordance with S-PA related benefits in our sample and in the same line of previous CRF literature (Freudenberger et al., 2016; Netz et al., 2011). Our data revealed something beyond this well-known fact when we stratified results by sex. We observed **CRF-related benefits for Executive Function, Memory, Language and Attention-Speed only in men**, showing a pattern similar to S-PA. In Study 2, and in accordance with results in Study 1, **higher CRF levels were related to a lower inflammatory profile, in terms of lower TNF-α and HGF levels; better brain volume, meaning lesser ventricles volume and greater volume of temporal and parietal lobes; and positive psychological health, referred as fewer depressive symptoms and better mood, only in men.** Our results agree with previous literature considering CRF a key outcome for healthy cognitive aging when analyzing total samples and extends the current knowledge adding crucial evidence about the different role of CRF in women and men. These findings might account for non-significant results in studies that include samples with higher percentage of women and perform analyses involving CRF in the total sample without addressing sex.

Interestingly, results were in the same line when addressing the potential mediating role of CRF in the exercise-cognition association. We found that **cardiorespiratory health explained the positive associations between S-PA and Executive Function and Attention-Speed only in men.** These findings highlight the fact that CRF and related physiological markers might be key factors involved in the positive effects of exercise, especially in men.

We should acknowledge that although our analyses were performed with a commonly used and validated estimation of VO_{2max}, procedures should be replicated in samples obtaining direct VO_{2max}. Our results could be influenced by sex-differences in PA outcomes, despite a similar correlation between S-PA and CRF was found in women

and men. Other studies have also reported sex-differences in CRF levels despite similar amounts of PA between women and men (Al-Mallah et al., 2016; Dimech et al., 2019). Therefore, it is possible that sex-differences in CRF levels across the life-span would be related to our results (Zeiher et al., 2019). These facts highlight that much more should be done to better understand the role of cardiorespiratory health in the enhancement of cognitive health. Our results might bring new clues for future research suggesting that CRF might explain exercise-related benefits but stratifying results by sex might be helpful to solve discrepant results. From a clinical point of view, it might be interesting the fact that women might be benefiting from exercise independently of their CRF levels.

We would conclude that maintaining higher levels of CRF benefited the inflammatory profile, brain volume, psychological health and cognition of men in our sample. Therefore, cardiorespiratory health might be a key player of the exercise-related neuroprotective benefits. However, it might not be the only contender.

3. Oh yes, sex matters

First evidences appeared suggesting that sex moderated the intervention-related changes on cognition following AE programs (Colcombe & Kramer, 2003) and higher levels of PA were related to greater reduction in the risk for dementia in women (Hogervorst, 2012) but sex-differences in PA-related benefits on cognition were no deeper explored in cross-sectional or RCTs approaches. Other studies reported brain benefits of PA on the volume of the hippocampi (Varma et al., 2015) and dorsolateral prefrontal cortex volume (Barha et al., 2020) and larger surfaces of the subiculum (Varma et al., 2016) in women, but not in men. Later on, a systematic review and meta-analysis (Barha et al., 2017) specifically analyzing the role of sex in exercise interventions reported larger executive benefits in studies with higher percentage of women compared to studies with a lower percentage. The emerging evidence (Barha et al., 2020; Barha & Liu-Ambrose, 2018) of the potentially relevant role of sex set

this variable as a matter of interest at the time we were assessing participants. Hence, understanding the role of sex in the PA-cognition association as well as in the effects of a 12-weeks brisk walking intervention became a must and was included as part of Projecte Moviment and this thesis aims.

The starting point was a mixed literature, which challenged us when establishing hypotheses. Nevertheless, we expected sex-differences based on evidence reporting sex-differences in the amount of PA performed, in steroids hormones, in the respiratory and musculoskeletal systems and hence, in the CRF patterns across the lifespan and adaptations after exercise (Al-Mallah et al., 2016; Wang et al., 2010; Zeiher et al., 2019). Once we run analyses we found that yes, **sex mattered in Study 1, 2 and 3**. Sex-differences in our results have been spotted in the two previous sections when discussing the role of S-PA and CRF, respectively. Although both **women and men showed brain and cognitive benefits for Executive Function and Attention-Speed related to exercise** in Study 1, **only men showed greater Executive Function, Verbal Memory, Language and Attention-Speed in relation to higher CRF levels**. Moreover, **only in men CRF significantly explained the association between exercise and the detected cognitive benefits.** According to these findings, in Study 2 **only men showed that higher CRF might be related to less inflammatory markers and greater brain volume and better mood.** Again, we found **significant mediation of these markers in the CRF-related benefits only in men.** And yet, our data revealed that **women benefited the inflammatory profile, brain volume and cognition from exercise independently of CRF.**

From our point of view, the male-specific role of CRF in our findings could be related to the fact that CRF is a parameter highly related to exercise but also influenced by other variables (Zeiher et al., 2019). Sex differences in CRF across the lifespan have already been described reporting lower levels in females and related to physiological, social and behavioral parameters (Al-Mallah et al., 2016). In relation to the mentioned physiological differences, our results could be related to structural and functional sex-differences in the musculoskeletal and cardiovascular systems and, thus, in their

adaptations in response to exercise (Ansdell et al., 2020; Barha & Liu-Ambrose, 2018). Current literature suggests that exercise benefits cardiovascular health more in men than in women. Women experience less metabolic stress for the same amount of exercise and, in turn, lesser adaptive response (e.g., less increase in CRF levels) whereas studies report lower blood pressure, increased blood flow and less endothelial dysfunction in males for the same amount of exercise (Ansdell et al., 2020; Barha & Liu-Ambrose, 2018; DeSouza et al., 2000). Concerning the social and behavioral differences, and in accordance with our results, previously published literature reported that men and women differ in the amount of exercise performed, being men more physically active (Al-Mallah et al., 2016). Thus, another potential explanation could be a dose-effect bias related to the described differences in the amount of exercise performed by women and men, despite a similar and significant correlation between S-PA and CRF. This fact could be a source of variance but there is evidence reporting no significant differences between PA levels but showing sex differences in CRF levels and significant associations between CRF and functional brain outcomes only in men (Dimech et al., 2019). Although we acknowledge the need to reproduce these findings with a direct measure of CRF and exercise-balanced groups, we consider that Projecte Moviment research project is adding evidence about sex-differences in CRF-related benefits and contributing to the discussion of the potential sex-dependent role of CRF, which is a crucial matter for clinical practice and research.

When publishing Study 1, one reviewer sent us a very interesting question: *if not CRF, what is mediating the exercise-related cognitive benefits in women?* At that point, we considered that the question was beyond the data we included in that paper and it should be addressed in Study 2. However, we could not find significant findings that might answer this question further than stating that none of the molecular, brain volume or psychological health outcomes analyzed in Study 2 seemed to explain those benefits in the women of our sample. The fact is that sex-differences throughout each one of these levels may lead to different interactions and adaptations after exercise in women and men and, in turn, they may affect cognition in a different manner (Barha & Liu-Ambrose, 2018). Moreover, evidence suggests that the integrative response to exercise might be mediated by an estrogenic effect in females. Differences in sex hormones that act as neurosteroids and interact with molecular growth factors, brain structures and cognition in a different manner for women and men (Ansdell et al., 2020) could explain sex differences in the association of exercise with brain and cognitive outcomes (Barha et al., 2019; Dimech et al., 2019; Lindwall et al., 2008; Varma et al., 2015; Varma et al., 2016). Thus, it is also possible that women maintain brain and cognitive health independently of CRF due to the role of parameters such estradiol, which might have a protective effect enhancing BDNF and, hence, neuroplasticity (Luine & Frankfurt, 2013). Future research should study the role of hormones and menopause as well as sex-differences in other physiological correlates when addressing exercise and CRF-related benefits.

In accordance with these findings, we assessed whether sex moderated intervention-related changes on the targeted molecular and brain volume outcomes in Study 3. **We did not detect any significant moderating effects of sex on intervention-related changes in molecular biomarkers** (Level 1) in any group despite previous evidence suggesting that sex moderates the effect of exercise on BDNF levels (Szuhany et al., 2015). Interestingly, the variable **sex moderated the effect of the AE in the brain volume of total WM, parietal, temporal and dorsolateral prefrontal cortex and the effect of the COMB on ventricles, temporal and dorsolateral prefrontal cortex.** Results suggest that exercise, single or in combination with CCT, benefited brain volume more in men than in women contrary to the larger effects on executive functions after AE interventions, which were attributed to women participants in a previous systematic review (Barha et al., 2017). Nevertheless, it might be in accordance with the greater increase of CRF levels and the related neuroprotective effects in men as well as the larger physiological adaptations described in the literature that men might experience after exercise (Al-Mallah et al., 2016). However, results must be interpreted in a cautious way since group samples were small and the number of male participants in the AE group was balanced but not in the COMB group. Future larger studies should stratify results by sex since it might be more informative than just accounting for sex as a covariate.

We would conclude that sex matters in the understanding of exercise and CRF neuroprotective benefits and our results define sex as a *must* in future research and clinical guidelines. Moreover, it highlights the need to better discover other pathways by which women benefit from exercise.

4. There are no simple pathways to Rome

Exercise involves a metabolic stress that initiates a physiological cascade of changes and body adaptations, which might be detected through global parameters such as CRF (Cotman et al., 2007; Stimpson et al., 2018). As aforementioned, we detected a potential male-specific role of CRF, which suggests global indexes are not highways in the link between exercise and cognition and that other parameters might explain part of the variability in a complex map of interactions. Several reviews have published results on the significant relationship between exercise and specific markers in the immune system, oxidative stress, cardiovascular health/HPA axis, neural and behavioral pathways (Cotman et al., 2007; Sallam & Laher, 2016; Stillman et al., 2020; Stimpson et al., 2018). Just recently, systematic reviews have discussed these pathways in terms of markers at Level 1, 2 and 3 corresponding to molecular and cellular, structural and functional brain and behavioral outcomes, respectively (Stillman et al., 2016, 2020). Therefore, our goal was to explore a part of this complex map and relate these outcomes to cognition based on what previous literature had suggested. In this thesis we included a part of the whole set of parameters included in Projecte Moviment protocol to discuss about the relationship between PA outcomes and inflammatory, growing factors, structural brain and psychological health data in our sample.

At a molecular level, Level 1, literature was quite mixed in humans to be able to draw clear hypotheses. Regarding BDNF, we expected that PA outcomes would relate to lower levels of BDNF in blood, meaning better cerebral use of it, or to higher levels of peripheral BDNF, representing higher production (Currie et al., 2009; Huang et al., 2014). **Our data did not reveal any significant association between PA outcomes and BDNF in women or men.** Accordingly to previous reviews, it might be related to the fact that significant changes in BDNF might be easy to detect after an acute bout of exercise and in clinical populations (Marinus et al., 2019). Moreover we would grant that peripheral BDNF is highly influenced by multiple variables (Miranda et al., 2019). In relation to inflammatory markers, we expected that higher levels of regular exercise and CRF would be related to lower levels of inflammatory markers, as a general prediction. In accordance with previous reviews (Sallam & Laher, 2016), our results in Study 2 suggest that **exercise might be related to lower levels of inflammation**. In particular, we found that **reduced TNF-α levels were related to greater S-PA and CRF levels in women and men, respectively.** Those **men with higher CRF also showed reduced levels of HGF**. Interestingly these results were significant even though we controlled for BMI and cardiovascular treatments. Both TNF-α and HGF are related to pro-inflammatory processes, obesity, and insulin resistance and, therefore, reduced levels might be informative of a better physiological environment, which might facilitate a neuroprotective effect (Kiliaan et al., 2014). Nevertheless, we could not discard that these results could be related to other processes besides inflammation given the pleiotropic nature of these markers. Interestingly, we found that reduced levels of these inflammation-related markers might be an important mediator of the brain and cognitive CRF-related benefits, specifically in men. **HGF was a significant mediator in the association between CRF and executive function, particularly working memory; and TNF-α was a significant mediator in the relationship between CRF and inhibition in men.** Moreover, those **men with higher CRF also showed that TNF-α was a significant mediator of the cingulate cortex volume**, which is functionally involved in executive function processes such as inhibition (Huang et al., 2020). This fact highlights the potential neuroprotective role of reduced neuroinflammation as a result of exercise that might benefit both women and men and the positive role of enhanced CRF in the maintenance of brain volume and better executive functions in men. These findings establish that exercise-related reduced levels of inflammation in women and men might be a crucial point in our map of pathways. These findings might raise the interest on understanding how the inflammatory markers might be modulated in a

similar or different way in women and men. Actually, one of the first hypotheses is that steroids hormones might modulate inflammatory processes, and thus, be a source of sex-differences in the exercise-cognition association and a potential explanation for the lack of significant results for CRF in women.

Regarding markers at Level 2, we addressed how PA outcomes related to brain volume in women and men. One of our relevant results is that both **women and men showed that exercise was related with greater temporal lobe volume**, in accordance with PA being consistently associated with the maintenance of brain volume and with less atrophy across the lifespan (Erickson et al., 2014; Wilckens et al., 2021). This finding highlights the benefits of exercise on the volume of a brain structure key for memory and daily functioning (Voss et al., 2017). Moreover, consistent with Barha et al. (2020), expending more energy in **S-PA was related to greater dorsolateral prefrontal cortex volume in women,** which is involved in supporting executive functions, especially working memory. Results are in accordance with greater executive performance related to S-PA in the women of our sample.

In relation to CRF-related brain benefits, previous evidence reported greater brain volumes in total samples (Raichlen et al., 2020; Wittfeld et al., 2020). Interestingly, in our study higher **CRF levels were associated with less ventricular volume and higher volumes of temporal and parietal lobes, specifically of the precuneus, in men.** Although the role of the precuneus has been underestimated for a long time, it is known to be involved in visuospatial processes and also in other complex processes such as voluntary attention shifts, episodic memory retrieval and the self-representation (Cavanna & Trimble, 2006). Despite previous papers hypothesized about the mediating effects of brain volume in the relationship between PA outcomes and cognition, such as the mediating role of the hippocampus in the association between CRF and spatial memory (Erickson et al., 2009), we found that **exercise and CRF were associated with larger brain volumes, as well as cognition, but the mediation was unexpectedly non-significant.** From our point of view, these findings only stress the

fact that there no highways in the understanding of the exercise-related neuroprotective effects and that discrepant results should be explored in order to better delineate maps instead of running the same highway again and again.

Apropos of psychological health and daily functioning - behavioral outcomes at Level 3 - we expected that exercise would be related with better mood (Fox et al., 2007; Willis et al., 2018) and sleep quality (Kline et al., 2013) in both healthy older women and men. Curiously, exercise shocked our expectations again; **there were not significant associations between exercise and the global scores of behavioral outcomes in women and men.** These findings were in accordance with the non-significant effects on these outcomes following the 12-weeks AE intervention in Projecte Moviment RCT, published by Roig-Coll et al. (2020). Interestingly, we found that those **men performing more exercise showed greater wellbeing and sleep efficiency, and lesser sleep disturbances**, when further analyzing the subscales of the tests in the cross-sectional design. We hypothesize that sex-differences in these benefits might relate to the well-known effects that hormones have on the mood (Wharton et al. 2012) and sleep (Baker et al., 2020) patterns in women. Moreover, we must acknowledge that we have not collected personal events that might be interesting to account for when performing analyses and our assessment of psychological health was performed with clinical scales, which might no detect mild fluctuations in the mood of healthy samples.

In relation to **CRF, we found a positive relationship between CRF and psychological health in men, in terms of lower GDS score**, meaning that those men with higher CRF reported better mood. Moreover, and in accordance with previous papers relating diminished precuneus with insomnia (Grau-Rivera et al., 2020) and sleep restriction (Long et al., 2020), we found that **greater volume of the precuneus in men mediated the higher CRF-better subjective sleep quality association.** As aforementioned, CRF was also related to lower HGF levels in men and further mediating analyses revealed that the lower HGF levels significantly explained the CRF-sleep quality association too. From our point of view, these findings highlight

how exercise-related changes at a molecular level might be promoting a better environment, which might lead to greater wellbeing at a behavioral level in terms of better sleep quality. Therefore, these findings might add evidence about the importance to understand the body as whole where the behavioral and molecular levels are highly connected, and thus, research and clinical professionals addressing molecular and behavioral issues should be too.

We should acknowledge that our trip in these pathways had an exploratory mode as it used to happen when discovering new worlds. We encourage future researchers to explore these findings in samples with exercise-balanced levels between women and men and direct measures of CRF in order to add evidence that might redefine our findings. Moreover, it would be interesting to go through the steroid hormones pathway to better understand how they might influence inflammation and define the exercise-cognition map.

To sum, we would conclude that there are no highways in the exercise-cognition relationship. CRF, reduced inflammation, brain volume and behavioral outcomes in interaction with sex were significant locations in the map of exercise-related neuroprotective effects.

5. 'Cause *walking* is worth it

Findings about the positive impact of specific lifestyle behaviors changed the rules of how the impairment related to age should be addressed and the possibility to have low cost clinical interventions that might prevent it beyond the potential pharmacological treatments (Schott & Krull, 2019). Therefore, these findings established a new paradigm: is it possible to enhance the same exercise-related benefits observed in cross-sectional designs applying clinical exercise interventions in older adults? Systematic reviews and meta-analyses suggested that exercise clinical programs, and particularly AE interventions, might have significant modest effect on

executive function, attention, processing speed, and memory (Barha et al., 2017; Northey et al., 2018; Smith et al., 2010). However, the FITT-VP parameters of the activity and individual variables such as age and sex were spotted as potential moderators of these effects. To better describe these findings, the current interest is not only *what* cognitive domains are modified after AE interventions but also *how* this might be happening. Cabral et al. (2019) reviewed molecular and cerebral changes after AE highlighting dose-effect differences. They found increased BDNF levels and diverse significant connectivity and structural changes in WM and GM areas such as hippocampus, cingulate cortex and frontal cortices after medium term AE programs (defined as 24 to 40 weeks) with better results when interventions included at least 150 min per week and progressive increase of exercise. However, after short-term AE interventions (lasting 1 day to 16 weeks) they reported brain connectivity changes in the frontal and temporal cortices but minimum structural changes - only in the volume of the hippocampus when there was significant cerebral blood flow change - and non-significant changes in molecular markers. In Projecte Moviment trial we wondered whether we could find significant changes not only in cognition but also at molecular, brain and behavioral level after a short-term AE intervention (12 weeks) but increasing the frequency of activity to 45 minutes, 5 days per week in order to exceed the 150 min/per week. Moreover, we aimed to compare the effects of an intervention to the benefits related to the regular practice of exercise. Thus, in Study 3, we aimed to describe AE intervention-related changes in those same molecular and structural brain markers that were targeted in a cross-sectional desing in Study 2 in order to be able to compare results.

In Study 3, we would expect that participants in the AE and COMB conditions would significantly reduce levels of inflammatory markers and increase BDNF levels and the volume of specific structures such as the hippocampus since we had increased the frequency of the training to 45 minutes / 5 days per week in a short-term intervention. Our findings agreed with previous evidence (Cabral et al., 2019; Wilckens et al., 2021) revealing **non-significant changes in any of the targeted molecular markers or brain volumes after 12-weeks of a briskly walking**

program, applied single or in combination with CCT, in a follow-up/baseline comparison or compared to controls. In further analyses examining the potential mediating role of changes in molecular or structural brain markers, results informed that **changes in markers at Level 1 and 2 did not significantly mediate the observed cognitive benefits in AE (Executive Functions and Attention-Speed) or COMB (Attention-Speed)**. Further analyses revealed that significant changes in PA outcomes, found in the AE and COMB condition, were related to markers involved in immune, cardiovascular and brain health. Results revealed that **change in ICAM-1 levels were negatively related with increases in the amount of PA and CRF observed in the AE and COMB, respectively**. This finding is interesting since ICAM-1 is a pro-inflammatory molecule promoted by IGF and TNF-α and highly expressed in endothelial cells and related to atherosclerosis (Burke-Gaffney & Hellewell, 1996; Che et al., 2002; Frank & Lisanti, 2008). Moreover, **participants in the AE and COMB showed that increases in CRF levels were also negatively related to change in SDF1-α,** which is the most common variant of SDF1. SDF1 is a chemokine protein involved in angiogenesis and brain tissue repair that contributes to neuroinflammation and in higher concentrations can lead to toxic environments and clinical consequences (Guyon, 2014). As reported in previous reviews (Cabral et al., 2019; Gonçalves et al., 2020), these findings suggest that molecular or structural brain changes might be not significant enough to be detected after a 12-weeks intervention even with a higher frequency but there might be initial changes in molecular markers related to changes in PA outcomes, which might be more significant in case the intervention takes longer. For example, BDNF levels mediated the cognitive benefits in older adults after a 1-year AE intervention (Leckie et al., 2014). Therefore, since molecular and structural brain changes are detected following 6 to 12 months AE interventions (Erickson et al. 2011; Ruscheweyh et al., 2011) the length of the intervention could be an important factor not only due to the persistence of the activity but also for the time needed to observe those changes in healthy subjects. It is also possible we find increased connectivity and brain perfusion in future analyses as they are known to be the first improvements at neural

level, which might enhance angiogenesis and, in turn, metabolism and neurogenesis, accordingly to Cabral et al. (2019). Another potential explanation might be the lack of pathology and relatively young average age of our participants. Erickson et al. (2014) suggested that the positive effects of exercise on GM volume might be significant in older age ranges or clinical populations in which there are greater brain volume losses and, thus, evidence tends to be more consistently significant. However, in accordance to literature suggesting that age might be significantly relevant at older ages (Bherer et al., 2021), **age of our participants did not moderate changes in molecular or brain volume outcomes** accordingly to the previously published non-significant moderating effect of age in the intervention-related cognitive changes detected in our sample (Roig-Coll et al., 2020). As aforementioned and suggested in previous reviews (Barha et al., 2017), AE intervention-related neuroprotective effects might be sex-moderated. Since sex moderated brain volume, but not molecular markers, in the AE and COMB, we hypothesize that the detected intervention-related cognitive benefits in our sample might be mediated by changes in other neuroplastic processes, hormones, neurotransmitter systems and neurotrophic factors. Those markers might interact differently for women and men and in relation to brain outcomes such as increased WM integrity and better functional connectivity.

At this point, the reader might wonder about the title statement of this section *'cause walking is worth it* and probably assume we are almost falling into bad marketing. However, results in Study 1 and Study 2 clearly support that the regular practice of S-PA might be related to cognitive, CRF, anti-inflammatory, brain volume and psychological health benefits in both women and men in domains that are crucial for healthy aging. What happened when we applied AE as a clinical intervention? There were significant cognitive and physical benefits after only 12 weeks, 5 days per week 45 minutes per day of briskly walking, applied single or in combination with CCT in this same sample (Roig-Coll et al., 2020). Those benefits were detected in cognitive domains that tend to be altered when aging and are key for autonomy and wellbeing.

Although we did not find significant intervention related changes in the targeted molecular markers or brain volume areas, we did find that early changes in PA outcomes were related to initial molecular changes that might become a cascade at a medium and long term.

Hence, it seems that exercising regularly is worth it in the promotion of healthy aging and this has a huge value for clinical guidelines. Projecte Moviment continues analyzing other molecular or brain changes such as cardiovascular factors and functional and structural brain connectivity as well as other individual variables that might be related to the detected AE intervention-related cognitive benefits in our sample.

6. Computerized *brain* training

The detection of the cognitive reserve as a mechanism to delay the onset of overt cognitive impairment due to compensating brain mechanisms, which might be enhanced throughout life, was a game changer too (Stern, 2009). Actually, two key factors highlighted the possibility to enhance cognition in late life in this field. On one side, the fact that those people who underwent more years of education or brain stimulating activities as part of their lifestyle behaviors showed better compensatory neural mechanisms. On the other, evidence about the possibility to modify or create new brain connections at late-life if brain was stimulated (Belleville & Bherer, 2012). These findings opened a whole new world of potential benefits related to cognitive training. CCT, which is a current form to apply cognitive training in technological devices that allows to practice in clinical or home-based environments as well as to adapt the demand of the task to the challenge, benefits memory, processing speed, and visual spatial ability in healthy older adults (Lampit et al., 2014b; Shao et al., 2015). The diversity in software, clinical programs and subjects that might benefit from CCT raised the need to better understand which variables might moderate the efficacy of CCT as well as the changes that such intervention might have in markers at Level 1, 2 and 3. Recent systematic reviews

agreed in the global cognitive effect of multi-domain CCT and the challenge of significant transfer of the benefits but disagree in the role of FITT-VP parameters such as number of sessions or length of the program that might maximize its efficacy (Belleville & Bherer, 2012; Lampit et al., 2014b; Shao et al., 2015). Projecte Moviment research project aimed to add evidence about CCT as a behavioral strategy that might enhance healthy aging while promoting the maintenance of brain and cognitive function. We used a program that could be applied in daily life and might add evidence on the pathways by which a specific CCT program might be enhancing cognition. We previously published that, unexpectedly, those participants in the CCT did not show any significant transfer of the training to the cognitive tests we applied (Roig-Coll et al., 2020). However, in Study 3 we aimed to identify if the CCT would have had any effect in molecular or brain outcomes as we did with AE.

Literature was highly mixed in relation to what we would anticipate about BDNF levels and brain volume CCT-related changes, whereas we did not expect significant changes in inflammatory markers as we did for AE. Our findings showed that those **participants who followed 12 weeks 5 days per week 45 minutes per day of CCT did not show significant changes in BDNF levels** in accordance with other trials lasting 12 weeks (Tarassova et al., 2020). One of the potential explanations for these findings might be the fact that significant changes in BDNF after CCT might be easily detected in clinical and older samples as published by Damirchi et al. (2018) than in younger and healthy samples in which the BDNF might not be significantly altered yet. Moreover, we have described the findings in the framework of this specific training program in which the FITT-VP parameters might impact the effects. In relation to other markers at Level 1, **we did not detect significant changes in any of the inflammatory markers as expected**. However, based in recent bibliography (Szabó et al., 2014), it would be interesting to address in future studies the role of baseline inflammation in the efficacy of CCT to improve brain and cognitive health.

Interestingly, we found CCT-related changes in markers at Level 2. **Participants engaging in a multimodal home-based CCT for 45 min a day, 5 days a week for**

12 weeks showed a significant intervention-related increase in the volume of the precuneus and compared to the change observed in the control group. These findings suggest that CCT might enhance the volume of a structure that is key in complex cognitive processes and highlights this intervention as a promising approach to promote healthy brain aging. As we mentioned in a previous section, the precuneus is highly involved in the integration of tasks, visuo-spatial imagery, episodic memory retrieval as well as self-referential processing (Cavanna & Trimble, 2006). Curiously, we also had found that higher levels of CRF levels were related to higher volume of the precuneus in men, which might be informative of how sensitive this structure might be to external factors. Moreover, our results also raise the discussion on a current research challenge in this field: describing the CCT-related benefits. We could have concluded about the non-significant efficacy of our CCT program given the lack of cognitive transfer. However, we did find significant brain volume change in the precuneus after this same intervention. Thus, we recommend future research to describe the neuroprotective effects of a cognitive training program at molecular, brain and cognitive/behavioral level in order to better understand its efficacy. We also think it would be interesting to include the improvements in the trained tasks recorded in the CCT software and relate them to the molecular, brain and cognitive outcomes in order to better describe transfer effects. Moreover, we wonder whether this brain volume change precedes further cognitive changes that might be detectable after longer interventions. Finally, our results add support for clinical guidelines to include CCT in order to promote brain health. We must acknowledge, as we did in the previous sections, that we describe results in a specific framework, in which the design of the interventions as well as the characteristics of our samples are influencing results.

We would sum that, up to know our CCT program could be also called *computerized brain training*, since 12-weeks, 5 days per week 45 minutes per day might increase the volume of the precuneus in healthy older adults. Future research should describe the efficacy of CCT in the promotion of healthy aging at different outcome levels.

7. COMB, *C*OULDN'T *O*BTAIN *M*ORE *B*ENEFIT

Research is always trying to find approaches that maximize the efficacy of clinical practice; so it was in this field. COMB, which in our case refers to the combination of AE and CCT, meant the possibility of facilitating the neuroprotective effects of cognitive training through exercise (Gheysen et al., 2018; Guo et al., 2020). Recently, multiple RCTs have studied this issue addressing the effect of sequential (Ten Brinke et al., 2020), dual-task (Anderson-Hanley et al., 2012) or multicomponent programs (Rahe et al., 2015a) in clinical and non-clinical populations. In accordance with previous evidence we reported greater Executive function and Attention-Speed following our COMB program compared to controls (Roig-Coll et al., 2020). Moreover, our results were coherent with evidence suggesting that COMB-related effect sizes do not differ from single interventions (Nilsson et al., 2020) and controverted evidence reporting greater benefits for the COMB design (Roig-Coll et al., 2020). Adding evidence might be helpful to understand discrepant results, which might be related to methodological and FITT-VP variables. From our point of view, addressing the effects of COMB at multiple levels of analysis might be also informative of the whole cascade of changes that this intervention might have. The fact is that literature addressing molecular and brain volume COMB-related changes in humans is scarce and mixed. Therefore, the interest of this thesis, belonging to Projecte Moviment too, is to add evidence about the effects of a COMB design in BDNF levels, inflammatory markers and brain volume after COMB.

Although drawing hypotheses on COMB-related changes based on previous literature was a challenge, theoretical models anticipated greater BDNF levels, lower levels of inflammatory markers and positive impact on brain volume compared to controls. Moreover, effects sizes for change in BDNF and brain volume would be bigger compared to single interventions (Nilsson et al., 2020). Results in Study 3, showed **non-significant changes in BDNF, inflammatory markers or brain volume areas in those participants who combined brisk walking and multimodal CCT for**

12 weeks, 5 days per week in bouts of 45 minutes each, analyzed pre to post-test and compared to the control group. We would like to discuss the fact ***we could not obtain more or greater benefits*** **in the targeted molecular and brain volume outcomes for a non-scheduled COMB program than single interventions.** One potential explanation for our non-significant results might be related to the scheduled order of the interventions. Previous short-term dual-tasks programs, in which exercise and cognitive training are performed simultaneously, displayed greater increases in BDNF levels (Anderson-Hanley et al., 2012) and stability of the hippocampus volume (Lövden et al., 2012) compared to single interventions whereas short-term multicomponent programs reported increased BDNF in the combined condition but not greater than single interventions (Rahe et al., 2015a). Moreover, a recent paper highlighted the benefits of increased BDNF following exercise on cognitive training-related gains only when exercise immediately preceded cognitive training (Nilsson et al., 2020). Based on these facts and our results, one one side, we would hypothesize that the acute exercise-related changes such as increased BDNF and arousal as well as the role of specific neurotransmitters might be involved in the greater benefits reported following sequential or simultaneous COMB programs. In this type of programs exercise before or during the CCT might be relevant to boost the effect and transfer of a CCT program even in short interventions. On the other side, we would hypothesize that the regular practice of exercise, which reduces inflammatory profile and improves cardiovascular health (Sallam & Laher, 2016; Stillman et al., 2020; Stimpson et al., 2018) might be involved in the better physiological environment detected following short-medium term non-scheduled COMB programs but not greater than single interventions (Nilsson et al., 2020). Thus, the physiological benefits related to regular exercise such as reduced levels of inflammatory markers might facilitate the effect of a CCT at long-term (Szabó et al, 2014). This fact might explain why results for cognitive performance, inflammatory markers and brain volume following a short-term COMB program in which interventions are not scheduled might be similar to the ones detected in the AE condition as we found. We consider this perspective adds value for future research suggesting different pathways for different COMB strategies. Moreover, it might be interesting for clinical guidelines in order to prescribe the adequate program depending on the targeted population. We should likewise underline that current reviews (Gheysen et al., 2018; Guo et al., 2020) also highlight the rest of FITT-VP parameters as well as individual variables as sources of variance. For example, our sample was much younger than the ones included in the aforementioned trials, which might suggest again that changes in BDNF levels might be more detectable in older samples.

From a clinical point of view, based on the COMB-related benefits in cognition observed in our sample we would conclude that combining walking and cognitive training is an efficacious tool to promote cognition at late-life. Nevertheless, we *could not obtain more benefits* in the targeted molecular or brain volume markers than the single interventions separately following a non-scheduled short-term COMB program.

8. STRENGHTS, LIMITATIONS AND FUTURE DIRECTIONS

Projecte Moviment research project is one more piece building our knowledge on how lifestyle behaviors might be promoting healthy aging. The reader who has gone through these pages and the genuine papers has already picked the strengths and limitations of our designs, samples and procedures. We would like to discuss the key points in the following section in order to inspire future research.

Addressing the effect of lifestyle interventions at multiple levels of analysis is one of the major contributions of our studies. To our knowledge, we are the first ones integrating molecular, brain, psychological health, PA as well as cognitive data in single studies of this field. The assessment protocol included in Projecte Moviment allowed us to contribute to the understanding of the individual as a continuous interaction between biological and behavioral aspects at multiple levels of analysis. The inclusion of well-known molecular markers such as BDNF or TNF-α in combination with other related but novel markers such as HGF brought the

opportunity to test hypotheses based on animal studies. Although these markers have a pleiotropic character and show high sensitivity to life conditions or treatments that might influence our data, we deeply believe that embracing them is the only way to add evidence to better describe the cascade of changes related to these lifestyle behaviors. In a similar way, neuroimaging data and analyses are great sources of variance due to differences in statically decisions and other parameters, which stress the need to clearly describe the procedures in order to replicate or compare results. Moreover, we made an a-priori selection of structural brain areas to be analyzed that seemed an appropriate and straightforward method to compare our results with previous findings. However, this specificity might have also limited the opportunity to detect changes in other areas, which could have been identified by other data-driven methods such as a voxel-based analysis. Regarding outcomes at Level 3, we believe that the comprehensive neuropsychological assessment, which allowed us to determine the extent of the sex differences, gives this work an added value. However, clinical scales for the assessment of psychological health of healthy participants might have undermined the detection of mild changes. In relation to the assessment too, we believe that grouping the total measure of PA into S-PA and NS-PA is an innovative approach that revealed crucial related neuroprotective effects specifically related to exercise. In the same line, the possibility to work with a measure of self-reported PA, recognizing the potential bias when reporting, and an estimation of CRF on the same data set, contributed to the discussion about these two outcomes and identify the potential male-specific role of CRF although both women and men might benefit from exercise. We must acknowledge that we used the Rockport 1-Mile Walking test, which is a less invasive and valid technique to administer to elderly athletes and non-athletes in order to obtain the estimation of CRF level. It would be interesting to address these issues using a direct measure of VO_{2max} and controlling for the history of PA, which may influence current physical and cognitive health. In sum, collecting all these outcomes allowed to draw a wide picture of the situation. However, sex-differences revealed in our analyses, together with recent bibliography (Barha & Liu-Ambrose, 2018), stress the need to stratify results by sex and study the role of the

hormonal profile in the molecular cascade that might explain the benefits of exercise. We highly encourage future studies to engage this challenge in order to better understand, not only the neuroprotective effect of lifestyle interventions, but also the fundamentals of potential sex-differences in metabolic, immune, cardiovascular and neural processes in which steroids hormones might be related and impact in a different way for women and men. There is a clear need to better describe what is encrypted behind the variable sex.

Another innovative point of this thesis is the inclusion of studies with cross-sectional and RCTs designs to address the benefits related to the regular practice of S-PA and NS-PA and the exercise-related changes after specific interventions. Therefore, on one side, we could describe statistical relationships and, on the other, assess the casual effects of an intervention and describe whether results were similar in both designs. As highlighted throughout the discussion, we understand that as reality has a time-space system, our studies have design-sample coordinates framing our conclusions although we controlled multiple factors and we made the effort to discuss results in these terms. We would like to underline the enrollment of our sample as one of our strengths. Recruiting physically inactive healthy participants that were not previously involved in exercise or cognitive training but wanted and could participate in a short but high intense program while keeping a randomized strategy during the selection and allocation of participants became a challenge as big as enlisting clinical participants. In the end, we did not reach the aimed number of participants, which diminish the possibility to run intra-group analyses in the RCTs, but we recruited a high-quality randomized sample that was useful to control several confounding variables. Moreover, the level of adherence was high and the cases of dropping-out were scarce and exclusively for medical or personal reasons. Results from the RCTs would be informative of the effects of the intervention in healthy physically inactive participants in the same range of age whereas we highlight the possibility to generalize results from the cross-sectional design to healthy adults aged 50 to 70 since we added 20 additional more physically active participants. Moreover, the random selection process led to sex-differences in PA outcomes, which seemed to be coherent with previous literature

reporting sex-differences in CRF levels (Dimech et al., 2019). However, further studies should examine these results in samples with different PA and CRF profiles as well as in samples in which S-PA is balanced between women and men.

Concerning the interventions, we would like to celebrate the success of a home-based intervention that might be prescribed to be performed individually based on the observed cognitive benefits, the qualitative feedback of our participants, the scarce number of dropouts and the high adherence. Nevertheless, we acknowledge potential desirability bias in our sample since we obtained self-reported adherence and participants were highly motivated. It would be interesting to perform the same intervention in clinical or social centers. We hypothesize that a group-based approach might enhance benefits emerging from social interaction. At the same time, based on qualitative feedback, the home-based approach might be a good strategy for those adults in the younger range of our sample that still have professional responsibilities. In relation to the AE program we believe that choosing brisk walking might be a low cost and high impact strategy that all our participants could perform without additional material costs. We must acknowledge that even though we trained them in order to keep the intensity based on the BRPES and report it on the dairy, we only have objective measures of the intensity for the first and last week of the intervention as well as in the follow-up diary, which were used by the intervention staff to follow participants. In relation to CCT, we would like to mention that all participants, even those with scarce experience with computers, adapted well to the cognitive training software. The possibility to perform the activities in their own devices facilitated the task to younger members of our sample, whereas in other cases they needed support to install it or a computer had to be facilitated. Another common issue of CCT is the assessment of its benefits, which might be done in terms of training engagement (performance on trained task), near transfer (performance on non-trained tasks), far transfer (performance on cognitively demanding tasks) or environmental transfer (daily functioning) (Harvey et al., 2018). Up to now, we included in our papers measures of cognitive performance that challenged trained and non-trained domains and a measure of daily functioning. We consider that adding information stored in the CCT software in relation to the improvements in the trained tasks in future studies might be helpful to better understand the CCT effects in our sample. We would like to add that the possibility to choose the order of the interventions for those participants in the COMB condition facilitated the engagement and adherence but at the same time it might have limited further conclusions. Finally, the inclusion of two different interventions, their combination and a waitlist control group allowed us to compare the mean change not only with the control group, but also compare the effect sizes between interventions.

To conclude, we consider Projecte Moviment is adding crucial evidence for research and clinical guidelines that might be replicated in future studies controlling for those variables that were beyond our scope and adding those variables that our discussion highlighted as relevant.

CONCLUSIONS

The main conclusions of the thesis can be summarized as follows:

I. S-PA, and not NS-PA, seems to be related to cognitive benefits as well as molecular, brain and psychological health markers that are relevant for daily functioning when aging.

II. Cardiorespiratory health is related to benefits in the inflammatory profile, brain volume, psychological health and cognition and significantly explained the relationship between exercise and cognition specifically in men.

III. Sex matters in the understanding of exercise and CRF benefits. The regular amount of exercise benefited both women and men, whereas we identified male-specific CRF-related benefits. Moreover, sex moderated brain volume intervention-related changes following 12 weeks 5 days per week of AE, applied single or in combination with CCT.

IV. At level 1, TNF-α and HGF are involved in the exercise and CRF benefits on the inflammatory profile. At level 2, the temporal lobe in women and men and the dorsolateral prefrontal cortex, specifically in women, are brain structures that benefit from exercise. CRF is related to benefits in the volume of the ventricles, temporal and parietal lobes, specifically of the precuneus, in men. At level 3, sleep quality and wellbeing are involved in exercise and CRF benefits in men. Moreover, the inflammation-related markers might be an important mediator of the brain volume, sleep quality and executive function CRF-related benefits, specifically in men.

V. 12-weeks, 5 days per week 45 minutes per day of AE, applied single or in combination with CCT did not provide significant changes in BDNF levels and the targeted inflammatory markers and brain volume areas. These markers did not mediate the intervention-related cognitive benefits previously identified in our sample. Significant AE intervention-related changes in PA outcomes were related to early molecular changes such as SDF1-α and ICAM-1 values. Length of the intervention and the amount of regular exercise seem to be key factors in order to observe the whole cascade of neuroprotective benefits of exercise.

VI. CCT might be a good clinical approach in the promotion of brain health in older adults. 12-weeks, 5 days per week 45 minutes of multi-modal home-based CCT benefited the volume of the precuneus, despite the lack of detected transfer of cognitive benefits.

VII. Combining walking and cognitive training might be an efficacious tool to promote cognition based on previous evidence of cognitive-related benefits in our sample, but 12-weeks, 5 days per week 45 minutes per day did not provide greater benefits than individual interventions, neither in the targeted molecular or brain volume outcomes.

ARTICLES

STUDY 1

Sex matters in the association between physical activity and fitness with cognition

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ABSTRACT

Purpose: The benefits from physical activity (PA) and cardiorespiratory fitness (CRF) on normal age-related cognitive decline might be sex-dependent. Our aim was to explore the relationship between different types of PA, CRF and cognition and to identify the mediating effects of CRF in the association between PA and cognition in women and men.

Methods: We recruited 115 healthy adults aged 50-70 years. We obtained demographic, cognitive and PA status data based on Projecte Moviment Protocol. We calculated cognitive domains by grouping z-sample scores. We obtained self-reported total energy expenditure during the last month and grouped it into sportive PA (S-PA) and non-sportive PA (NS-PA). CRF was estimated using the 1-mile Rockport Walk Test. We applied regression models and mediation analyses in a final sample of 104 individuals (65 women and 39 men).

Results: In the total sample, CRF was positively associated with Executive Function, Verbal Memory and Attention-Speed. S-PA was positively related to Executive Function and Attention-Speed while NS-PA was unrelated to cognitive domains. Greater amounts of S-PA were associated with Executive Function and Attention-Speed for both women and men. Higher CRF was associated with Executive Function, Memory, Language and Attention-Speed only in men. Mediation analyses showed that CRF was a significant mediator of the positive effects of S-PA on Executive Function and Attention-Speed in men but not in women.

Conclusions: Both women and men show cognitive benefits from greater S-PA, but not from NS-PA. However, there were sex differences in the mediating effects of CRF in this relationship showing that CRF was mediating these benefits only in men.

Keywords: cognitive performance, sex-differences, exercise, cardiorespiratory fitness, healthy adults.

INTRODUCTION

Yes, your lifestyle may be predictive of future cognitive decline. Aging is related to normal late-life cognitive decline even for those who do not experience dementia or other neurodegenerative pathologies (1). Executive function, processing speed, memory and psychomotor ability deteriorate over the lifespan and may alter daily function and well-being (2). However, an active lifestyle may benefit cognitive health (3) and improve or maintain specific cognitive domains when applied as interventions (4).

Physical activity (PA), defined as daily movements involving skeletal muscles that result in energy expenditure (5), is an essential factor of an active lifestyle for brain health (3). Higher levels of PA protect against cognitive decline and reduce the risk for dementia (6). In cross-sectional studies, PA is significantly related to better processing speed and executive function (7). There are mixed results about the relationship between PA and episodic verbal memory (7,8). PA measures (e.g. questionnaires and actimeters) differ across studies and may explain heterogeneous results. One remaining question is whether any type of PA is sufficient for improving cognition or whether exercise, defined as a structured and repetitive subtype of PA that aims to improve physical fitness (5), through moderate to vigorous intensity levels, is necessary (9). Reviews of exercise intervention studies report that exercise has significant but modest positive effects on cognitive performance, especially on executive function (10-12), when interventions consist of aerobic exercise programs (10,11) or last at least 52 hours (13). However, there is a lack of literature clearly reporting differences in the relationship between exercise (sportive PA), other types of daily PA (non-sportive PA) and cognition in cross-sectional and longitudinal studies.

Cardiorespiratory fitness (CRF) is a widely used measure to describe the relationship between cognition and cardiovascular health related to exercise. Maximal aerobic capacity (VO_{2max}) is the gold standard measure to assess CRF. In cross-sectional studies, higher CRF, directly estimated through progressive exercise tests, has been related to better scores in global cognitive function, executive function (14,15), attention (14) and verbal (15) and spatial memory (16). Interventional studies showed that participants in aerobic exercise programs significantly increased CRF and improved cognitive functions (17). However, evidence is still insufficient to prove that those CRF changes are significantly related to cognitive improvements (18). Relevant reviews in this field (9,12,18) highlight methodological, PA variables and individual-related factors to explain these discrepancies. One major issue is to explore the mediating effect of CRF in the relationship between PA and cognition in cross-sectional studies to better understand its role as a mechanism of cognitive benefits related to PA.

Current trends focus on understanding individual difference variables that explain variation in the effects of PA in order to improve personalized interventions for enhancing cognitive function. Meta-analytic studies examining the effects of aerobic training interventions in healthy older adults reported greater effect sizes on executive function in the whole group when samples had >50% of females (10,11). Sex differences in randomized controlled trials (RCTs) have not been studied with healthy populations yet. RCTs including participants with mild cognitive impairment and vascular cognitive impairment found greater improvements in executive function in women (19,20). Sex differences related to PA and cognition have been scarcely assessed in cross-sectional and longitudinal studies. Hogervorst and colleagues (21) reported an association between greater amounts of PA and a greater reduction in the risk for dementia in women. Similarly, Barha and colleagues (22) reported that greater amounts of PA over 10-years was associated with less decline in executive functions and processing speed in women. Other discrepant results showed that self-reported PA was related to better performance in Mini-Mental State Examination (MMSE) and executive functions in men but not in women (23). Sex has become a relevant matter since it is considered an important moderator of the effects of PA on cognition (11,24). Current reviews underline the fact that studies should address potential sex differences and describe potential physiological mechanisms such as sex steroids hormones and differences in the musculoskeletal and cardiorespiratory adaptations after exercise (24). Given the fact that women and men show different patterns of CRF across the lifespan (25), the mediating effects of CRF in the relationship between PA and cognition should be assessed in women and men separately.

In this proof-of-concept study we addressed previously described gaps that exist in the literature assessing sex differences in relation to PA, CRF, and cognition. First, our aim was to explore the relationship between different types of PA, CRF and cognition in the total sample, then assess the moderating effect of sex in these relationships by stratifying the results to examine effects in women and men. Second, we aim to identify the mediating effects of CRF in the association between PA and cognition in women and men. To our knowledge, there is not a previous literature describing the role of sex in the relationship between PA and cognitive performance including CRF as a mediator.

METHODS

Study Design

This is a cross-sectional study based on Projecte Moviment (26) which is a RCT that aims to study the effect of aerobic exercise, cognitive training and the combination on cognition. For this study, we used the baseline assessments belonging to the Projecte Moviment sample and 20 additional participants with a higher physical activity profile in order to enlarge the interval of the PA and CRF level and increase the sample size for a cross-sectional analysis. All participants were recruited and selected following the same procedures described in the cited protocol (26) and during the same period of time. The University of Barcelona led this research and the corresponding ethics committee following the Declaration of Helsinki approved it.

Participants

One hundred and fifteen community-dwelling healthy adults were recruited. Participants were eligible for this study if they were 50-70 years old, were not cognitively impaired as defined by the MMSE≥24 (27) and the Montreal Cognitive Assessment 5-min (MoCA-5min≥6) (28), had competency in Catalan or Spanish and had adequate sensory and motor skills. Participants from the Projecte Moviment trial were eligible if they did not perform structured sportive PA more than 2 hours/week over the last 6 months, whereas additional participants had to do at least 5 hours/week of moderate PA or 2.5 hours/week of intensive PA. We excluded individuals who had a neurological diagnosis, psychiatric disease and Geriatric Depression Scale>9 (29), a history of drug abuse and alcoholism or consumed psychopharmacological drugs (see Table, SDC 1, which contains extended details).

Assessment

Participants meeting criteria went through a multimodal assessment described elsewhere (26). We selected only the demographic data and baseline cognitive and physical activity outcomes needed to address our specific aims. We instructed participants not to exercise before the appointment and cognitive tests were administered before the CRF test to control for the effect of acute exercise.

Demographic data and Cognitive assessment

We registered age, sex and years of education of all participants. As cardiovascular health variables, we obtained the body mass index (BMI) -using weight and height- and collected data about diagnoses of hypertension and diabetes.

We assessed cognitive function using an extensive neuropsychological battery which included: Stroop Test (30), Wechsler Adult Intelligence Scale III (WAIS-III; subtests: forward and backward digit span, vocabulary, digit symbol coding, symbol search) (31) Trail Making Test A & B (TMT A & B) (32), Verbal Fluency Test (33), Rey-Osterrieth Complex Figure (ROCF) (34), Rey Auditory Verbal Learning Test (RAVLT) (35) and Boston Naming Test (BNT) (36). This comprehensive cognitive assessment was also used to clinically confirm the lack of MCI or dementia.

Self-reported PA

We applied the reduced Minnesota Leisure Time Physical Activity Questionnaire (37) to obtain self-reported PA. This questionnaire asked participants the number of hours performed during the last month in the following categories: sportive walking, sports, gardening, climbing stairs, shopping walking and cleaning house. Moreover, for the sports category, participants had to specify how many and which sports were they performing and for the climbing stairs, participants had to estimate the amount of stairs climbed per day.

Cardiorespiratory fitness

We assessed CRF using the Rockport 1-Mile Walking Test which is useful for accurately estimating VO_{2max} in healthy older adults (38). We instructed participants to walk one mile on a treadmill (Technogym®, Italy) adjusting their speed in order to be as fast as possible without running. Maximal aerobic capacity (VO_{2max}) was estimated with the standard equation developed by Kline and colleagues (39) using the following variables: weight, age, sex, time to complete the mile and heart rate at the end of the test.

Statistical analyses

The data were analyzed using IBM SPSS Statistics for Windows, Version 24.0. Demographics and physical activity variables were analyzed in the total sample and by sex. We calculated z-sample scores for all neuropsychological tests and grouped them following a theoretical-driven approach based on the classification of tests in previous literature (40,41). First, we calculated nine domains: Inhibition (interference-Stroop Test), Working Memory (backward-WAIS-III), Flexibility (TMT B-A), Fluency (letter and category fluency), Visuospatial Function (copy accuracy-ROCF), Verbal Memory (total learning and recall-II RAVLT), Visual Memory (memory accuracy-ROCF), Language (BNT-15), Attention (forward span, digit symbol coding and symbol search WAIS-III) and Speed (TMT-A, copy time-ROCF). Then, we designed five general domains: Executive Function, Visuospatial Function, Memory, Language and Attention-Speed.

We transformed hours per month expended in each category from the Minnesota Questionnaire into units of the metabolic equivalent tasks (METs). Based on the previous definition of exercise (5), we summed METs grouped into the following categories: Sportive PA (S-PA) –sportive walking and sports activities- and Non-Sportive PA (NS-PA) -gardening, climbing stairs, shopping walking and cleaning house- and calculated METs values separately for both categories.

Linear regression models were performed to examine the association between CRF and different types of PA with cognitive outcomes in the total sample. Age, years of education and sex were included as covariates. We analyzed the role of sex in the relationship between physical and cognitive outcomes regressing physical activity outcomes on cognitive performance for women and men, accounting for age and years of education.

Finally, we applied mediation analyses using the PROCESS Macro (42) in women and men (Figure 1). Path C tested if the independent variable (PA) was associated with the dependent variable (cognitive composites) controlling for age and years of education. Path A was a regression between the independent variable (PA) and the mediator (CRF) accounting for the same covariates. Path B determines if mediator (CRF) changes predict changes in the dependent variable (cognitive composites) accounting for the same covariates. These analyses allowed us to obtain the estimated direct (Path C') and the indirect effect (Path AB). Path C' reports the association between the independent variable (PA) and cognitive outcomes when the mediator (CRF) and covariates are included in the model. Path AB represents the association with the mediator (CRF) in the model. These analyses were computed with bias-corrected bootstrap 95% confidence intervals (CIs) based on 5,000 bootstrap samples. Significance of mediation was indicated if the CIs in Path AB did not overlap with 0 (42).

RESULTS

Participants

One hundred fifteen adults were recruited and assessed. The CRF measure could not be estimated for eleven participants, so all analyses were conducted on 104 participants (age=57.44 ± 5.36; 63% female; years of education=13.35 ± 5.29; MMSE=28.22 ± 1.45; BMI=27.52 ± 4.91). The 65 women and 39 men included in the sample showed no significant differences in the demographic data, except in years of educations that is used as a covariate (see Table 1). There was a similar and significant correlation between S-PA and CRF in women (r=.551, p<.001) and men (r=.631, p<.001) despite sex differences in S-PA and CRF (see Table 1). There were no significant differences in the demographic variables between participants of Projecte Moviment and the 20 additional participants (see Tables, SDC 2, extended details).

Association between PA, CRF and cognition

Linear regression models examining the association between CRF and different types of PA with cognitive domains in the total sample are in Table 2. Higher CRF was related with better Executive Function, specifically Working Memory, Flexibility and Fluency subdomains; Memory, especially Verbal Memory; and Attention-Speed, both subdomains: Attention and Speed. Greater S-PA was related to better Executive Function, specifically to Working Memory and Fluency; and to Attention-Speed, including Attention and Speed. However, NS-PA was not associated with any cognitive measure.

Sex differences in the relationship between PA, CRF and cognition

Table 3 provides the linear regression models stratified by sex. For women**,** CRF was not significantly associated with cognitive performance in any domain. For men, CRF was positively related to Executive Function, including Inhibition, Working Memory and Fluency; Memory, notably Verbal Memory; Language and Attention-Speed, including both subdomains: Attention and Speed. S-PA was significantly associated with Executive Function especially with Fluency, and Attention-Speed in women and men respectively (see Figure 2).

Sex differences in the mediation effects of CRF

We applied mediation analyses for each cognitive outcome using CRF as a mediator for women and men separately (Table 4). We used S-PA as an independent variable given its similar significant association with cognition in women and men.

Women. Path C was equivalent to previous linear regression models for women in Table 3. Path A showed a significant relationship between S-PA and CRF (β=.47, p<.001) accounting for age and years of education. Path C' and AB, indicated that CRF did not mediate the association between S-PA and cognitive outcomes in women.

Men. Path C was equivalent to previous linear regression models for men in Table 3. Path A showed a significant relationship between S-PA and CRF (β=.64, p<.001.) accounting for age and years of education. Path C' and AB showed maximum evidence for mediation of CRF in Executive function especially in Fluency; and Attention-Speed, with Attention as the significant subdomain.

DISCUSSION

In this paper, we first aimed to explore the relationship between different types of PA, CRF and cognition in the total sample. CRF emerged as a key factor of cognitive health as results showed that higher estimated CRF was significantly associated with better performance on Executive Function, Verbal Memory and Attention-Speed in the whole sample. Those results support previous literature stating CRF is related to better performance in executive functions, attention and memory (14,15). We found interesting results when we grouped PA into S-PA and NS-PA suggesting that only the sportive subtype was related to cognitive performance. S-PA had a positive relationship with Executive Function and Attention-Speed while NS-PA had not. Our results clearly relate cognitive benefits specifically to S-PA and not to NS-PA adding support to the fact that PA should not be mistaken for exercise (43). This fact highlights the importance of the "sportive" component of the PA when prescribing PA to people. This "sportive" component might be related to differences in PA parameters such as frequency, dose and intensity between S-PA and NS-PA as well as other psychosocial parameters such as the social interaction and well-being enhanced when exercising (44,45).

In our study, results suggested that sex mattered. Sex moderated the association between CRF and cognition, but not for S-PA. S-PA was significantly associated with Executive Function and Attention-Speed for both women and men. Interestingly, CRF was not significantly related to cognitive performance in women but it was positively associated with Executive Function, Verbal Memory, Language and Attention-Speed in men. Thus, our results not only add support to the cognitive benefits related to regular exercise in both women and men but also opens the debate of the potential sex-dependent role of CRF in these benefits. CRF is a parameter highly related to exercise but also influenced by other variables such as sex (25,46) which might lead to differences in the exercise-related benefits.

Sex mattered again when analyzing the mediating effects of CRF in the association between PA and cognition in women and men. We introduced S-PA in the model given the fact that our previous results suggested that benefits are related specifically to exercise. In men, CRF was a significant mediator of the positive relationship between S-PA and Executive Functions and Attention-Speed but CRF was not a significant mediator in women. These results add support to sex-dependent role of CRF in the exercise-related benefits in cognition. Sex differences in CRF across the lifespan have already been described reporting lower levels in females and related to physiological, social and behavioral parameters (25). On one side, they have been related to structural and functional differences in the respiratory, musculoskeletal and cardiovascular system. Thus, women and men differ in the physiological responses of these systems after exercise (24). Exercise benefits cardiovascular health more in men than in women: studies report lower blood pressure, increased blood flow and less endothelial dysfunction in males (24,47). On the other side, and in accordance with our results, previously published literature reported that men and women differ in the amount of exercise performed, being men more physically active (25). However, we found a similar and significant correlation between S-PA and CRF in women and men. Therefore, our results suggest sex differences in the mediating effects of CRF in the relationship between cognition and exercise in healthy adults which might be related to physiological and behavioral causes.

However, it remains an interesting question: if not CRF, what is mediating the exercise-related cognitive benefits in women? This requires an answer that goes beyond the scope of this paper.

Previous literature suggest that regular exercise is known to initiate a cascade of changes at a molecular, cellular, brain and psychological level (45) which might be different for women and men (48). Sex differences throughout each one of these levels may lead to different adaptations after exercise in women and men and, in turn, they may affect cognition in a different manner (24). For example, there may be sex differences in the BDNF response to exercise that are related to the differential relationship between BDNF and sex steroid hormones in males and females (11). Therefore, sex-differences in physiological correlates mediating the relationship between PA and cognition must be addressed.

Strengths and limitations

We included a comprehensive neuropsychological assessment allowing us to determine the extent of these sex differences, an objective estimation of CRF and measurements of self-reported PA commonly used in previous studies which we categorize into different subtypes in order to observe discrepancies as was suggested in a previous review (9,11). However, although Rockport 1-Mile Walking test is a less invasive and valid technique to administer to elderly athletes and non-athletes (38), it is not a direct physiological measurement of VO_{2max}. Further studies should examine those preliminary results in samples with different PA and CRF profiles to assess if this could be a potential confound. It could be interesting to explore those results in different age groups, using a direct measure of VO_{2max} and controlling for the history of PA, which may influence current physical and cognitive health.

Conclusions and future recommendations

To our knowledge, our study is one of the first to relate cognitive benefits specifically to the sportive component of PA and to report sex differences in the mediating effects of CRF in the relationship between S-PA and cognitive performance. We hypothesize that the mediating effects of CRF might be different in women and men given sex-differences in the physiological correlates that shape CRF and we suggest other potentially involved mechanisms.

Our results highlight the fact that sex must be included as a moderator in cross-sectional and RCTs of this field studying the effect of an intervention and its relationship with change in

PA activity outcomes. These results add support to the importance of individual factors to understand the effects of exercise on cognitive health and its underlying physiological mechanisms as well as to improve the design of personalized interventions. Determining the causes of the observed difference between women and men goes beyond the scope of this paper but will be included in our future aims.

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Author Contributions. ACS and FRC were involved in the study concept and design, acquisition, analyses and interpretation of data as well as in elaboration of the manuscript. NLV and AB collaborated in the acquisition of the data. NLV, PT, GP, PM, AB, RDA, LB, KE critically reviewed the content of the article. MM conceptualized the study, contributed to the study design and the implementation as Principal Investigator and supervised all procedures and the elaboration of the manuscript.

Conflict of Interest. None. The results of the study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by ACSM.

Patient Consent. Obtained.

Ethics Approval. Bioethics Commission of the University of Barcelona (IRB00003099) and Clinical Research Ethics Committee of IDIAP Jordi Gol (P16/181).

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Figures

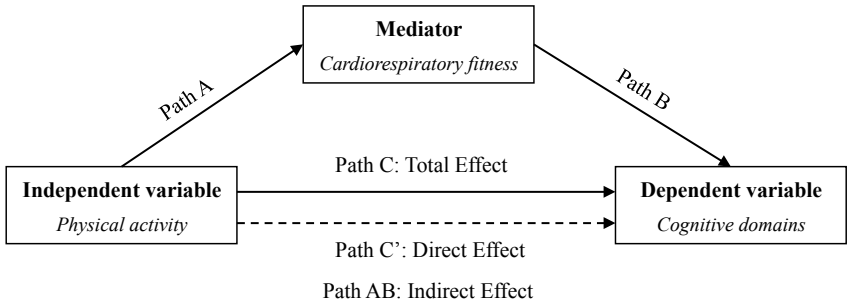

Figure 1. Mediation analyses template.

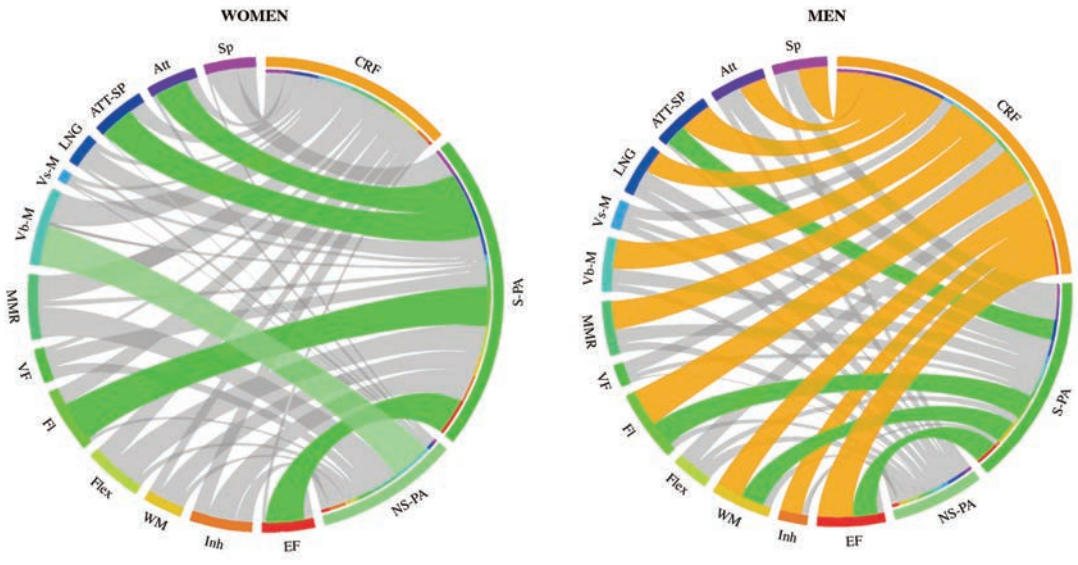

Figure 2. Circos plot of partial correlations between physical activity outcomes and cognitive domains adjusting for age and years of education for men and women. Ribbon width indicates percentage of explained variance. The color of the ribbon corresponds to the color of the original segment for p<.05 correlations. Grey ribbons refer to non-significant correlations. (See Figure, SDC 3, which contains values for partial correlations) (49).

Note: Att: attention, Att-Sp: attention-speed; CRF: cardiorespiratory fitness; EF: executive function; Fl: fluency; Flex: flexibility; Inh: inhibition; LNG: language; MMR: memory; NS-PA: non-sportive physical activity; Sp: speed; S-PA: sportive physical activity; Vb-M: verbal memory; VF: visuospatial function; Vs-M: visual memory; WM: working memory.

Tables

Table 1. Demographic and PA variables for the cross-sectional sample.

Demographic variables	**WOMEN** Mean (SD)	**MEN** Mean (SD)	t-Test / χ^2 (*p* value)
n	65	39	-
Age (years)	56.75 (4.96)	58.59 (5.86)	1.64 (.106)
Education (years)	12.46 (4.97)	14.82 (5.55)	2.18 (.032)
MMSE (/30)	28.15 (1.41)	28.33 (1.53)	0.60 (.552)
BMI	26.99 (4.53)	28.40 (5.44)	-1.43 (.157)
Hypertension (n)	12	10	3.46 (.063)
Diabetes (n)	7	4	3.59 (.058)
PA variables	Mean (SD) [Min – Max]	Mean (SD) [Min – Max]	t-Test (*p* value)
S-PA	2055.35 (4395.04) [0 - 16512]	6011.35 (9939.24) [0 - 36952]	-2.35 (.023)
NS-PA	9495.88 (6438.50) [1260- 29232]	6173.16 (6780.93) [600 - 39120]	2.50 (.014)
CRF	25.07 (12.31) [0.07 - 49.68]	33.86 (12.92) [3.09- 58.64]	-3.46 (.001)

Note: BMI: body mass index; CRF: cardiorespiratory fitness; MMSE: Mini-Mental State Examination (Blesa et al., 2001); NS-PA: non-sportive physical activity; PA: physical activity; S-PA: sportive physical activity.

Table 2. Association between PA outcomes and cognition; lineal regression models for the total cross-sectional sample.

Cognitive Domains	CRF R^2; β (*p* value)	S-PA R^2; β (*p* value)	NS-PA R^2; β (*p* value)
EXECUTIVE FUNCTION	0.11; 0.29 (.012)*	0.15; 0.34 (.001)**	0.05; -0.00 (.990)
Inhibition	0.03; 0.08 (.490)	0.04; 0.12 (.256)	0.03; 0.05 (.614)
Working Memory	0.14; 0.26 (.017)*	0.16; 0.28 (.004)**	0.09; -0.08 (.426)
Flexibility	0.28; 0.21 (.038)*	0.26; 0.14 (.121)	0.26; -0.10 (.256)
Fluency	0.24; 0.37 (<.001)***	0.24; 0.34 (<.001)***	0.13; -0.01 (.890)
VISUOSPATIAL FUNCTION	0.29; 0.13 (.178)	0.28; 0.10 (.260)	0.27; -0.05 (.596)
MEMORY	0.31; 0.33 (.001)**	0.25; 0.16 (.089)	0.23; 0.07 (.472)
Verbal Memory	0.27; 0.36 (<.001)***	0.20; 0.15 (.108)	0.18; 0.11 (.269)
Visual Memory	0.29; 0.11 (.255)	0.29; 0.09 (.320)	0.28; -0.04 (.676)
LANGUAGE	0.28; 0.10 (.316)	0.30; 0.17 (.061)	0.27; 0.04 (.650)
ATTENTION-SPEED	0.43; 0.26 (.004)**	0.43; 0.24 (.004)**	0.38; -0.03 (.765)
Attention	0.39; 0.24 (.012)*	0.39; 0.23 (.006)**	0.35; -0.06 (.465)
Speed	0.35; 0.26 (.006)**	0.34; 0.20 (.019)*	0.30; 0.04 (.640)

Note: CRF: cardiorespiratory fitness; NS-PA: non-sportive physical activity; PA: physical activity; S-PA: sportive physical activity.
Covariates: age, years of education and sex.
** p <.05; ** p<.010; *** p<.001*

Table 3. Association between PA outcomes and cognition; linear regression models for women and men in the cross-sectional sample.

Cognitive Domains	WOMEN		MEN	
	CRF R^2; β (*p* value)	S-PA R^2; β (*p* value)	CRF R^2; β (*p* value)	S-PA R^2; β (*p* value)
EXECUTIVE FUNCTION	0.01; 0.03 (.848)	0.10; 0.31 (.016)*	0.41; 0.63 (<.001)***	0.23; 0.40 (.014)*
Inhibition	0.07; -0.13 (.369)	0.11; 0.22 (.087)	0.14; 0.39 (.022)*	0.01; 0.08 (.638)
Working Memory	0.04; 0.09 (.532)	0.06; 0.17 (.181)	0.22; 0.46 (.005)**	0.18; 0.39 (.015)*
Flexibility	0.32; 0.15 (.216)	0.33; 0.19 (.076)	0.21; 0.27 (.110)	0.16; 0.11 (.477)
Fluency	0.13; 0.15 (.273)	0.20; 0.31 (.011)*	0.51; 0.62 (<.001)***	0.32; 0.39 (.010)*
VISUOSPATIAL FUNCTION	0.31; 0.04 (.775)	0.32; 0.11 (.331)	0.28; 0.22 (.146)	0.24; 0.08 (.575)
MEMORY	0.25; 0.20 (.115)	0.22; 0.04 (.708)	0.41; 0.44 (.003)**	0.29; 0.22 (.135)
Verbal Memory	0.16; 0.25 (.063)	0.11; 0.03 (.790)	0.37; 0.47 (.002)**	0.22; 0.23 (.142)
Visual Memory	0.25; 0.04 (.773)	0.25; 0.04 (.715)	0.30; 0.17 (.253)	0.29; 0.12 (.417)
LANGUAGE	0.18; -0.05 (.729)	0.20; 0.15 (.202)	0.43; 0.34 (.016)*	0.38; 0.23 (.099)
ATTENTION-SPEED	0.36; 0.12 (.301)	0.40; 0.22 (.034)*	0.52; 0.47 (.001)**	0.40; 0.29 (.036)*
Attention	0.37; 0.09 (.462)	0.40; 0.21 (.042)*	0.40; 0.43 (.005)**	0.31; 0.27 (.067)
Speed	0.25; 0.15 (.233)	0.27; 0.20 (.077)	0.46; 0.44 (.002)**	0.35; 0.26 (.069)

Note: CRF: cardiorespiratory fitness; PA: physical activity; S-PA: sportive physical activity.
Covariates: age and years of education.
** p <.05; ** p <.01;*** p <.001*

Table 4. Mediation analyses in Study 1.

	Cognitive Domains	Path C' β_2 (SE), *p* value [95%CI]	Path AB β_{AB} (SE), [95%CI]
WOMEN	EXECUTIVE FUCNTION	0.41 (0.00), .007 [0.00, 0.00]	-0.10 (0.08) [-0.28, 0.06]
	Inhibition	0.38 (0.00), .010 [0.00, 0.00]	-0.16 (0.06) [-0.30, -0.05]
	Working Memory	0.18 (0.00), .239 [0.00, 0.00]	-0.01 (0.09) [-0.18, 0.16]
	Flexibility	0.17 (0.00), .188 [0.00, 0.00]	0.03 (0.07) [-0.11, 0.17]
	Fluency	0.33 (0.00), .021 [0.00, 0.00]	-0.02 (0.08) [-0.18, 0.12]
	VISUOSPATIAL FUNCTION	0.12 (0.00), .339 [0.00, 0.00]	-0.02 (0.08) [-0.17, 0.16]
	MEMORY	-0.07 (0.00), .606 [-0.00, 0.00]	0.11 (0.07) [-0.01, 0.25]
	Verbal Memory	-0.12 (0.00), .412 [-0.00, 0.00]	0.15 (0.07) [0.01, 0.30]
	Visual Memory	0.03 (0.00), .802 [-0.00, 0.00]	0.01 (0.08) [-0.16, 0.17]
	LANGUAGE	0.24 (0.00), .089 [0.00, 0.00]	-0.09 (0.07) [-0.24, 0.04]
	ATTENTION-SPEED	0.23 (0.00), .066 [0.00, 0.00]	-0.00 (0.07) [-0.14, 0.12]
	Attention	0.23 (0.00), .056 [0.00, 0.00]	-0.02 (0.07) [-0.16, 0.11]
	Speed	0.18 (0.00), .181 [0.00, 0.00]	0.02 (0.07) [-0.11, 0.15]
MEN	EXECUTIVE FUNCTION	0.01 (0.00), .951 [0.00, 0.00]	0.39 (0.15) [0.09, 0.69]*
	Inhibition	-0.31 (0.00), .147 [-0.00, 0.00]	0.39 (0.11) [0.21, 0.63]
	Working Memory	0.17 (0.00), .392 [0.00, 0.00]	0.22 (0.16) [-0.08, 0.55]
	Flexibility	-0.10 (0.00), .632 [-0.00, 0.00]	0.21 (0.14) [-0.05, 0.49]
	Fluency	-0.01 (0.00), .974 [0.00, 0.00]	0.39 (0.13) [0.14, 0.64]*
	VISUOSPATIAL FUNCTION	-0.11 (0.00), .587 [0.00, 0.00]	0.19 (0.16) [-0.15, 0.47]
	MEMORY	-0.10 (0.00), .565 [0.00, 0.00]	0.33 (0.16) [-0.04, 0.61]
	Verbal Memory	-0.13 (0.00), .484 [-0.00, 0.00]	0.36 (0.16) [0.00, 0.65]
	Visual Memory	0.01 (0.00), .945 [0.00, 0.00]	0.10 (0.13) [-0.19, 0.33]
	LANGUAGE	0.02 (0.00), .914 [0.00, 0.00]	0.21 (0.15) [-0.15, 0.46]
	ATTENTION-SPEED	-0.02 (0.00), .915 [0.00, 0.00]	0.31 (0.12) [0.09, 0.57]*
	Attention	-0.00 (0.00), .998 [0.00, 0.00]	0.27 (0.13) [0.05, 0.56]*
	Speed	0.04 (0.00), .799 [0.00, 0.00]	0.30 (0.15) [-0.00, 0.59]

*Notes: * significant values (interval not containing zero in path AB)*
Path C' [Direct effects]: Cognitive Domain = β_2S-PA ($\beta_3$cardiorespiratroy fitness) + (covariates) + β_0
Path AB [Indirect effects]: Path C – Path C'

STUDY 2

Exercise and fitness neuroprotective effects: molecular, brain volume and psychological correlates and their mediating role in healthy late-middle-aged women and men

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Keywords: exercise, fitness, molecular biomarkers, brain volume, psychological health, cognition

ABSTRACT

Background: Although exercise is known to have a neuroprotective effect in aging, the mediators underlying the exercise-cognition association remain poorly understood. In this paper we aimed to study the molecular, brain and behavioral changes related to physical activity and their potential role as mediators.

Methods: We obtained demographic, physical activity outcomes (sportive physical activity and cardiorespiratory fitness (CRF)), plasma biomarkers (TNF-α, ICAM-1, HGF, SDF1-α and BDNF), structural-MRI (brain volume areas), psychological and sleep health (mood, depressive and distress symptoms and sleep quality) and multi-domain cognitive data from 115 adults aged 50-70 years. We conducted linear regression models and mediation analyses stratifying results by sex in a final sample of 104 individuals (65 women (age=56.75 ± 4.96) and 39 men (age=58.59 ± 5.86)).

Results: Women engaging in greater amounts of exercising showed lower TNF-α levels and greater dorsolateral prefrontal cortex and temporal lobe volumes. Men engaging in greater amounts of exercise showed greater temporal lobe volumes. CRF levels were not related to any of the analyzed outcomes in women but in men higher CRF was associated with lower TNF-α, HGF and ventricle volumes, greater volume of temporal and parietal lobes and fewer depressive symptoms and better mood. In men, reduced TNF-α and HGF levels mediated brain and cognitive CRF-related benefits.

Conclusion: Our results show that exercise is a promising approach for influencing inflammation and brain volume and also contributes to ongoing discussions about the physiological mediators for the association between CRF and cognition in men.

INTRODUCTION

"Let's move your body; let's rock your cells, brain and self!" Epidemiological studies have indicated that an active lifestyle including exercise positively impacts several major hallmarks of aging and has neuroprotective benefits (Garatachea et al., 2015). Exercise, a subtype of physical activity (PA) applied in a regular manner in order to improve physical fitness, is related to reduced risk of dementia and better cognitive health in clinical and non-clinical populations, with greatest effects for measures of executive function (Barha et al., 2017, Northey et al., 2018). Those benefits have been described when using a measure of self-reported exercise habits and when assessing physiological correlates of habitual PA such as cardiorespiratory fitness (CRF). CRF is the ability of the cardiovascular system to supply oxygen to the organism during sustained PA. Previous literature has identified potential mechanisms of the exercise-cognition association at multiple levels: molecular, brain and behavioral. In this paper we will discuss them as Level 1, 2 and 3 mechanisms, respectively, based on Stillman et al. (2016).

At the molecular level, Level 1, aging is related to altered levels of inflammatory, oxidative stress, metabolic and neuronal and cell growth markers (Garatachea et al., 2015). Exercise reduces levels of systemic inflammation modulating markers such as C-reactive protein (CRP), Interleukin 6 (IL-6), Interleukin 1 (IL-1) and Tumor Necrosis Factor alpha (TNF-α) (Sallam & Laher, 2016). However, Woods et al. (2012) indicated that the evidence for a relationship between CRF and TNF-α levels in humans was still insufficient for making definitive conclusions. Studies with mice have demonstrated that exercise also produces an increased shear stress on endothelial cells that modulates other markers of the inflammatory process such as Intercellular Adhesion Molecule 1(ICAM-1), Nuclear factor-κB (NF-κB), Mitogen-activated protein kinase (MAPK) and Cyclooxygenases (COX-2) (Sallam & Laher, 2016). Shear stress and mechanical load related to exercise induce changes in other anabolic and metabolic growth factors that affect muscles and bones such as Hepatocyte growth factor (HGF), Vascular endothelial grow factor (VEGF) and Insulin-like growth factor-1 (IGF1). HGF, which is commonly related to obesity and insulin resistance and is capable of modulating the inflammatory response, promotes angiogenesis and neuroprotection in the brain (Kiliaan et al., 2014). Exercise-induced VEGF is also related to angiogenesis and improved circulation in humans. It upregulates the chemokine Stromal cell-derived factor 1 (SDF1-α), also known CXCL12 (Stimpson et al., 2018), which promotes both endothelial progenitor cells and the endothelial nitric oxide synthase enzyme in mice (Gertz et al. 2006). Exercise also influences brain derived neurotrophic factor (BDNF), a neuronal growth factor involved in neurogenesis. A systematic review (Huang et al., 2014) reported a negative association between long-term

regular PA and CRF with peripheral BDNF in observational studies. Those results may reflect a more efficient uptake mechanism of circulating BDNF into the brain in active subjects (Currie et al., 2009).

At a more macroscopic level, Level 2, reviews and systematic reviews reported beneficial effects of exercise on brain volume in healthy older adults, specifically for areas more related to normal brain aging (Erickson et al., 2014; Sexton et al., 2016; Stillman et al., 2016). Cross-sectional studies showed that higher amounts of PA were associated with greater total brain volume (Benedict et al., 2013; Spartano et al., 2019) and grey matter (GM) volume in the frontal lobe (Flöel et al., 2010; Erickson et al., 2010; Bugg & Head, 2011; Eyme et al., 2019), hippocampus (Erickson et al. 2010; Yamamoto et al., 2017; Raichlen et al., 2019), cingulate cortex (Flöel et al., 2010), precuneus (Benedict et al., 2013; Eyme et al., 2019) and nucleus accumbens (Yamamoto et al., 2017). CRF levels have also been positively correlated with overall grey matter (Raichlen et al., 2019), multiple areas of the frontal lobe (Gordon et al., 2008; Weinstein et al., 2012; Wittfeld et al., 2020), medial-temporal lobe (Gordon et al., 2008; Wittfeld et al., 2020), hippocampus (Erickson et al., 2009; Szabo et al., 2011; Wittfeld et al., 2020) and cingulate cortex (Wittfeld et al., 2020) volume in healthy older adults. Findings related to white matter (WM) indicated that engaging more frequently in PA could increase WM global volume (Gow et al., 2012; Bennedict et al., 2013; Arnadottir et al., 2016). Only a few studies have examined the relationship between CRF and WM volume and did not find significant results (Gordon et al., 2008; Burns et al., 2008; Honea et al., 2009).

At a behavioral level, Level 3, aging is commonly associated with increased sedentarism (Copeland et al., 2015), changes in sleeping patterns (Li et al., 2018) and more psychological problems such as depression or anxiety symptoms (WHO, 2017). For older adults, exercising is related with better physical and mental health (Bertheussen et al., 2011), quality of life (Fox et al., 2007) and well-being (Lee & Hung, 2011; Black et al., 2015). Higher levels of PA (Strawbridge et al., 2002) and CRF (Sui et al., 2009; Willis et al., 2018) are also protective for prevalent and incident depression. PA is also associated with better sleep quality (Kline et al., 2013; Tan et al., 2018) and efficiency (Kline et al., 2013; Wilckens et al., 2018) and total sleep time (Murray et al., 2017). However, the relationship between sleep patterns and CRF in healthy older adults is not well established.

Conceiving mechanisms at multiple levels might be helpful to better understand the relationship between exercise and cognition. As the evidence suggests, exercise might initiate a molecular cascade that promotes macroscopic changes in the brain and/or behaviors that in turn enhance cognition For example, PA has been related to reduced inflammatory profile and greater brain volume (Stillman et al., 2016). However, there is a poor understanding of the mediating role of these variables in the PA-cognition relationship and the multiple pathways by which microscopic and macroscopic biomarkers might influence each other to promote cognition are in current research. Identifying these pathways by which the benefits of exercise are realized is a challenge not only due to the multi-level mechanisms but also because of the role of factors that may moderate the association such as sex (Barha et al., 2019). Previous evidence suggests that both women and men might positively benefit cognition from exercise, although the CRF-cognition relationship was only significant in men (Castells-Sánchez et al., in press). Moreover, there are sex differences in the mediators of this relationship described in the ongoing research. For example, greater PA was related to reduced TNF-α only in men (Elosua et al., 2005), while daily walking has been associated with greater hippocampal (Varma et al., 2015) and dorsolateral prefrontal volume (Barha et al., 2020) and larger surfaces of the subiculum (Varma et al., 2016) in women, but not in men. Therefore, addressing the role of these mechanisms stratified by sex might be interesting to better understand exercise as a personalized approach to enhance cognitive health.

To our knowledge, previous research focused on single levels of analyses when examining and describing possible mechanisms of exercise on cognition (Stillman et al., 2016). In this paper, we first aim to study the relationship between exercise and CRF with key markers at molecular, brain volume and behavioral levels of analysis and further stratifying the results by sex because of established sex-related differences in many of these measures. Secondly, we aim to study their potential mediating role of each of these markers in the exercise-cognition relationship and perform exploratory analyses to address the potential pathways by which these markers might influence each other.

MATERIALS AND METHODS

This is a cross-sectional study based on Projecte Moviment (Castells-Sánchez, et al. 2019) and is drawn from our previously published results (Castells-Sánchez et al., in press). The study was carried out by the University of Barcelona in collaboration with Institut Universitari d'Investigació en Atenció Primària Jordi Gol and Hospital Germans Trias i Pujol. It was approved by the responsible ethics committees following the Declaration of Helsinki.

Participants

One hundred and fifteen community dwelling healthy late-middle-aged adults were recruited from the Barcelona metropolitan area using multiple strategies (lists of volunteers from previous studies, advertisements in local media, presentations in local community organizations, etc.). They were 50-70 years old, were not cognitively impaired (Mini Mental State Examination, MMSE ≥ 24 (Blesa et al., 2001) and Montreal Cognitive Assessment 5-min, MoCA 5-min ≥6 (Wong et al., 2015)), had competency in Catalan or Spanish and had adequate sensory and motor skills. Participants were excluded from the study if they had a neurological diagnosis, psychiatric disease or Geriatric Depression Scale score >9 (Martínez et al., 2002), a history of drug abuse and alcoholism, consumed psychopharmacological drugs, history of chemotherapy and had any contraindication to magnetic resonance imaging (MRI). As previously published (Castells-Sánchez et al., in press), the sample consisted of Projecte Moviment baseline low-active participants (<2 hours/week over the last 6 months) and 20 additional participants with a higher physical activity profile (≥5 hours/week moderate PA or 2.5 hours/week intense PA) in order to enlarge the interval of the PA and CRF levels and increase the sample size for a cross-sectional analysis. All participants were recruited, selected and assessed following the same protocol during the same period of time. They were screened by phone and an on-site interview and signed an informed consent prior to the assessment.

Assessments and Outcomes

All participants underwent a multimodal assessment organized into three appointments in 2 weeks in the following order: (1) Medical assessment and blood extraction (30 min), (2) Cognitive psychological health and physical activity assessment (2,5 h), (3) MRI protocol (45 min). In order to control the effects of acute exercise, participants were advised not to exercise 8 hours before all appointments. Assessments were conducted in clinical facilities: medical and cognitive assessments were carried out in two Primary Health Care Centers and MRI scans were performed in the Hospital Germans Trias i Pujol.

Physical activity and cardiorespiratory fitness

Self-reported PA was evaluated by the Validated Spanish short version of Minnesota Leisure Time Physical Activity Questionnaire (VREM) (Ruiz et al., 2012). Participants reported frequency and duration of the following activities during the last month: sportive walking (walking in order to exercise), sport/dancing, gardening, climbing stairs, shopping, walking, and cleaning the house. We transformed hours per month expended in each category into units of metabolic equivalent of tasks (METs). This allowed us to estimate energy expenditure in Sportive PA (S-PA) adding up the METs spent in sportive walking and sportive/dancing activities.

We obtained estimated CRF applying the Rockport 1-Mile Walking Test which is a less invasive and valid method commonly used in healthy elderly population. Participants walked one mile on a treadmill (Technogym®, Italy) adjusting their speed in order to be as fast as possible without running. We registered time to complete the mile, heart rate and average speed during the test once they finished. We estimated maximal aerobic capacity (VO_{2max}) using the linear regression developed by Kline et al. (1987).

Biomarkers

Blood extraction was performed between 8:00 and 9:00 a.m. following an overnight fast by nurses in the Primary Health Care Centers. All participants were instructed to not exercise 8 hours before the blood test. Blood samples were obtained from the antecubital vein and collected in EDTA tubes for plasma analyses. Tubes were immediately transferred to the IGTP-HUGTP Biobank integrated in the Spanish National Biobanks Network of Instituto de Salud Carlos II (PT13/0010/0009) and Tumor Bank Network of Catalonia, and they were processed following standard operating procedures with the appropriate approval of the Ethical and Scientific Committees. Plasma aliquots were stored at -80ºC.

As Level 1 outcomes, we obtained peripheral BDNF levels using an ELISA kit (Human Free BDNF Quantikine ELISA Kit; R&D Systems, Minnesota, USA). The rest of the molecular markers were selected according to the Projecte Moviment trial (Castells-Sánchez et al., 2019). TNF-α, ICAM-1, HGF and SDF1-α levels were analyzed quantitatively using the corresponding ELISA immunoassay method (Human TNF-α Quantikine HS ELISA, Human ICAM-1/CD54 Allele-specific Quantikine ELISA Kit, Human HGF Quantikine ELISA Kit, Human CXCL12/SDF-1 alpha Quantikine ELISA Kit; R&D Systems, Minnesota, USA).

Neuroimaging

Structural MRI data was collected in a 3T Siemens Magnetom Verio Symo MR B17 (Siemens 243 Healthineers, Erlangen, Germany) for all participants (Castells-Sánchez et al., 2019). We acquired T1-weighted multi-planar reformat sequences (acquisition time: 5:26 min, voxel: 0.9x0.9x0.9mm, TR/TE/TI: 1900/2.73/900ms, flip angle: 9º, slices: 192; thickness: 0.9 mm) and an expert neuroradiologist visually checked them for artifacts or clinical brain conditions. All participants received a clinical report of the MRI.

Brain images were analyzed using MRICloud (https://mricloud.org/) (Mori et al., 2016), an online cloud-computing platform that includes a function to calculate grey and white matter brain volumes (Wu et al. 2016). It performs a fully automated parcellation of 287 volumes based on multiple atlases and fuses different algorithms (transformation algorithm, Large Deformation Diffeomorphic Metric Mapping (LDDMM) and the atlas label-fusion algorithm) (Christensen et al., 1997; Oishi et al., 2009; Wang et al., 2012) with a local search algorithm (Coupé et al., 2011). For our sample, we used atlas library version 10A, which includes 30 atlases from cognitively-normal individuals and individuals with cognitive impairment or dementia. For Level 2 outcomes, we selected relevant areas based on previous literature (Erickson et al., 2009; Erickson et al., 2011; Verstynen et al., 2012) to perform analyses: ventricles, total WM and GM of the frontal lobe, dorsolateral prefrontal cortex, cingulate cortex, parietal lobe, precuneus, temporal lobe and hippocampus. In accordance with other volumetric studies, we used the ANCOVA method to regress brain volumes on outcomes of interest. All volumes were normalized by head size by including intracranial volume (ICV) as a covariate. We calculated ICV summing volume of the brain tissue (Left Hemisphere + Right Hemisphere + Brainstem + Cerebellum) and CSF (Ventricles + Sulci).

Psychological health and daily activity

Psychological health and daily activity of participants were assessed using self-reported questionnaires and the raw scores of each test were used as Level 3 outcomes. We applied GDS-15 (Martínez et al., 2002) for depressive symptoms, the Modified Version of Visual Analog Mood Scale (VAMS, Stern et al., 1997) for mood states and the Short Informant Questionnaire in Routine Evaluation-Outcome Measure (CORE-OM, Trujillo et al., 2016) to assess psychological distress in the domains of subjective well-being, problems/symptoms, general functioning and risk. We also administered the Pittsburgh Sleep Quality Index (PSQI, Rico & Fernández, 1997) to evaluate the quality and patterns of sleep and the Short Informant Questionnaire on Cognitive Decline in the Elderly (S-IQCODE, Morales et al., 1992) as a measure of subjective cognitive performance in daily activities. We used the raw scores of each test as Level 3 outcomes.

Cognition and Demographic data

We assessed cognitive function using an extensive neuropsychological battery which included standard tests selected for their psychometric qualities and high relevance in the area of study. These tests provided measures of multiple cognitive functions grouped following a theoretically-driven approach (Strauss & Spreen, 1998; Lezak et al., 2012) into five domains: 1) Executive (Inhibition, Flexibility, Fluency, Working Memory); 2) Visuospatial Function, 3) Language, 4) Memory (Verbal Memory, Visual Memory), 5) Attention-Speed (Attention, Speed). Extended details of the assessment are provided in the Supplementary Material Table 1. We obtained age, sex, and years of education, height and weight to calculate BMI, diagnoses of hypertension and diabetes as cardiovascular health variables and current medication for cardiovascular risk factors such as dyslipidemia, hypertension and diabetes.

Statistical analyses

We used IBM SPSS Statistics for Windows, Version 24.0, for statistical analyses. Linear regression models were performed to examine the associations between S-PA and CRF with molecular (Level 1), brain volume (Level 2) and psychological (Level 3) outcomes. We

stratified analyses by sex and included age and years of education as covariates. We also introduced BMI as a covariate for Level 1 and 2 analyses (Colbert et al., 2004; Bourassa & Sbarra, 2017) and ICV when using Level 2 outcomes. Since molecular outcomes in serum could be influenced by current cardiovascular risk factors medications, this variable was included as a dichotomous covariate in complementary analyses for Level 1 outcomes.

We analyzed the potential mediating role of these outcomes in the relationship between S-PA and CRF with cognition in women and men separately (see Model 1 in Figure 1). Then, we performed exploratory mediation analyses addressing the potential pathways by which outcomes at Level 1 and 2 might influence markers at Level 2 and 3 (see Model 2, 3 & 4 in Figure 1). We applied mediation analyses using the PROCESS Macro (Figure 1). These analyses were computed with bias-corrected bootstrap or Monte Carlo 95% confidence intervals (CIs) based on 5,000 bootstrap samples. Significance of mediation was indicated if the confidence intervals (CIs) in Path AB did not overlap with 0 (Hayes, 2017).

RESULTS

Participants

We recruited and assessed 115 healthy adults. The final sample consisted of 104 participants (age=57.44 ± 5.36; 63% female; years of education=13.35 ± 5.29; MMSE=28.22 ± 1.45; BMI= 27.52 ± 4.91) from which we could obtain a valid measure of CRF. There were no significant differences in the demographic data between the 65 women and 39 men included in the sample except in years of education that is used as a covariate (see Table 1). Despite sex differences in S-PA and CRF levels, the correlations between S-PA and CRF in women ($r=.551$, $p<.001$) and men ($r=.631$, $p<.001$) were significant and comparable (see Table 1). There were no significant differences in age, years of education and MMSE scores between participants of Projecte Moviment and the 20 additional participants (see Supplementary Material, Table 2 & 3).

Associations between Physical Activity, CRF, and Mechanisms at Level 1, 2 and 3

Level 1: Physical Activity, CRF, and Molecular biomarkers

Linear regression models examining the relationship between S-PA and CRF with Level 1 biomarkers in women and men separately are in Table 2. Engaging in greater amounts of S-PA was significantly associated with reduced levels of TNF-α in women. There were not any significant associations between S-PA and molecular markers in men. In contrast, higher CRF levels were associated with lower TNF-α and HGF levels in men. There were not significant relationship between CRF and molecular markers in women. Extended details for each model are included in Supplementary Material Table 4.1. Besides, similar results were obtained for these same regression linear models when accounting for the potential influence of cardiovascular risk factors medications as a covariate (see Supplementary Material Table 5).

Level 2: Physical activity, CRF, and brain volumes

Linear regression models examining the relationship between S-PA and CRF with brain volumes, Level 2 outcomes, in women and men separately are described in Table 3. Engaging in greater amounts of S-PA were positively correlated with the volume of the dorsolateral prefrontal cortex in women and with temporal lobe volume in both women and men. There was also a relationship between S-PA and precuneus volume in men. Greater levels of CRF were associated with larger precuneus and temporal lobes and with smaller ventricles in men. There was also a correlation between CRF and frontal and parietal lobe volumes in men. In women, there were no significant relationships between CRF and brain volumes. Extended details for each model are included in Supplementary Material Table 4.2.

Level 3: Physical activity, CRF, and psychological health and daily activity

Linear regression models examining the relationship between S-PA and CRF with behavioral outcomes at Level 3 in women and men separately are in Table 4. There were no significant associations between S-PA and any of the scores in women and men. However, when analyzing the subscales, we found that in men, higher levels of S-PA were related to better subjective sleep efficiency ($\beta=.41$, $p=.008$) and fewer sleep disturbances ($\beta=-.43$, $p=.008$) in

the Pittsburgh Sleep Quality Index and higher scores of well-being (β=-.31, p=.065) in the corresponding subscale of the CORE-OM test. In addition, in men, higher CRF was significantly associated with fewer depressive symptoms measured by the GDS. There was also a moderate negative relationship between levels of CRF and VAMS scores in men. Extended details for each model are included in Supplementary Material Table 4.3.

Mediating effects

Model 1

Model 1 (see Figure 1) tested the mediating effect of each molecular, brain volume and behavioral outcome in the relationship between S-PA and CRF with the assessed cognitive functions in women and men. In the relationship between S-PA and cognitive domains, none of the outcomes at Level 1, 2 or 3 showed significant indirect effects in the mediation analyses in women or men. When CRF was the predictor, we found statistically significant indirect effects for HGF in the CRF- executive function (Path AB= β =0.29, SE=0.17, 95% CI: 0.02, 0.70) and in the CRF – working memory (Path AB= β =0.25, SE=0.15, 95% CI: 0.02, 0.59) relationships in men. Indirect effects were also significant when TNF-α was the mediator in the association between CRF and inhibition (Path AB= β =0.31, SE=0.19, 95% CI: 0.04, 0.77) only in men. There were no significant indirect effects for any outcome in the CRF-cognition relationship in women (see Figure 2).

Model 2

Model 2 (see Figure 1) examined the mediating effects of molecular outcomes in the relationship between S-PA and CRF with brain volumes in women and men. In the association between S-PA and brain volumes, none of the molecular outcomes showed significant mediating effect in women or men. In the mediation analyses with CRF as a predictor, we found statistically significant indirect effects for TNF-α in the relationship between CRF and cingulate cortex volume (Path AB= β =-0.26, SE=0.14, 95% CI: -0.58, -0.03) only in men. We did not find any significant mediating effects for any molecular outcome in the CRF-brain volume association in women (see Figure 2).

Model 3

Model 3 (see Figure 1) assessed the mediating effects of molecular outcomes in the relationship between SP-A and CRF with psychological health and daily activity. In the relationship between S-PA and behavioral outcomes, none of the molecular outcomes showed significant indirect effects in women or men. When CRF was the predictor, we found statistically significant indirect effects for HGF in the relationship between CRF and the subscale of subjective sleep quality of the Pittsburgh Sleep Quality Index (Path AB= β =-0.21, SE=0.13, 95% CI: -0.53, -0.03) in men. There were no significant mediating effects for any molecular outcome in the CRF-behavioral relationship in women (see Figure 2).

Model 4

Model 4 (see Figure 1) tested the mediating effects of brain volume outcomes in the relationship between S-PA and CRF with psychological health and daily activity. In the association between S-PA and behavioral outcomes, none of the brain volume outcomes showed significant mediating effects in women or men. In the mediation analyses with CRF as a predictor, we found statistically significant indirect effects of precuneus volume in the relationship between CRF and a subscale of subjective sleep quality from the Pittsburgh Sleep Quality Index (Path AB= β =-0.26, SE=0.17, 95% CI: -0.65, -0.00) in men. We did not find any significant mediating effects for any brain volume outcome in the CRF-behavioral association in women (see Figure 2).

DISCUSSION

To our knowledge, we are the first to describe the relationship between physical activity outcomes and three different levels of potential mechanisms - molecular, brain volume and behavioral - in healthy late-middle-aged women and men. Moreover, we analyzed these potential mediators in the relationship between exercise and CRF with cognition and between themselves stratifying results by sex.

At the molecular level, Level 1, our results support previous evidence (Sallam & Laher, 2016) suggesting that physical activity might be related to reduced levels of inflammation. However,

despite that self-reported exercise and CRF measures were highly correlated, we found sex differences when examining these outcomes. In particular, we found that reduced TNF-α levels were related to greater energy expenditure in sportive physical activity only in women and to higher CRF levels only in men. Men with higher CRF also showed reduced levels of HGF, independently of BMI. Both biomarkers are related to pro-inflammatory processes, obesity, and insulin resistance and, therefore, reduced levels might induce a neuroprotective effect (Kiliaan et al., 2014). Nevertheless, we can not discard that these results could be related to other processes besides inflammation given the pleiotropic nature of these markers. Interestingly, when analyzing the mediating role of molecular outcomes in the physical activity-cognition association, we found that the reduced levels of these inflammation-related markers might be an important mediator of the brain and cognitive CRF-related benefits. In men, HGF was a significant mediator in the association between CRF and executive function, working memory, and sleep quality and TNF-α was a significant mediator in the relationship between CRF and inhibition. Interestingly, those men with higher CRF also showed that TNF-α was a significant mediator of the cingulate cortex volume, which is functionally involved in executive function processes such as inhibition (Huang et al., 2020). This fact highlights the potential role of reduced neuroinflammation as a result of exercise and enhanced CRF to promote greater executive functions.

Our findings at brain volume level, Level 2, are consistent with previous findings that physical activity has been consistently associated with the maintenance of brain volume and with less atrophy across the lifespan (Erickson et al., 2014). One of our relevant results is that frequent exercise was related with greater temporal lobe volume in women and men, which is a key structure for memory and it deteriorates with aging. Moreover, consistent with Barha et al. (2019), women that expended more energy in sportive physical activities had greater dorsolateral prefrontal cortex volume, which is involved in supporting executive functions, especially working memory. In other studies greater CRF levels have been linked to greater brain volumes (Raichlen et al., 2019; Wittfeld et al., 2020). Interestingly, in our study, higher CRF levels were associated with less ventricular volume and higher volumes of temporal and parietal lobes, specifically of the precuneus, in men but not in women. Despite previously published evidence for the mediating effects of brain volume in the

relationship between physical activity outcomes and cognition, such as the mediating role of the hippocampus in the association between CRF and spatial memory (Erickson et al., 2009), we found that physical activity and fitness were associated with larger volumes, but that this was unrelated to cognition.

Previous literature about Level 3 outcomes reported that active lifestyles such as exercise are also related with mood (Fox et al. 2007; Willis et al., 2018) and sleep patterns (Kline et al., 2013) in older adults. Curiously, we found similar results, but this was significant only in men. Men performing more exercise reported higher sleep efficiency and fewer sleep disturbances and those with higher levels of CRF presented fewer depressive symptoms and better general mood. In mediation analyses, and in accordance with previous papers relating diminished precuneus with insomnia (Grau-Rivera et al., 2020) and sleep restriction (Long et al., 2020), we found that greater volume of the precuneus in men mediated the higher CRF- better subjective sleep quality association. Surprisingly, in our sample, physical activity was not related with these behavioral outcomes in women. Based on previous papers, we hypothesize that mood (Wharton et al., 2012) and sleep (Baker et al., 2020) patterns in women could be influenced by other parameters, such as hormonal changes.

Our results are consistent with previous findings about exercise on health outcomes as we described above. Nevertheless, we not only add support to the multi-level benefits of regular exercise but also highlight the sex differences in the role of each physical activity outcome as we stratified results by sex. CRF was an outcome highly related to cognition and to the molecular, brain and psychological cascade of changes in men but not in women. However, benefits of exercise at the molecular level including reduced TNF-α levels and brain volumes –dorsolateral prefrontal cortex and temporal lobe, were also observed in women but were linked to the amount of self-reported energy expended in sportive activities during last month and not to CRF. These results could be related to sex differences in the musculoskeletal and cardiovascular systems and their adaptations in response to exercise (Barha & Liu Ambrose, 2018; Ansdell et al., 2020). Current literature suggests that women experience less metabolic stress for the same amount of exercise and, in turn, lesser adaptative response (e.g. less increase in CRF levels). Moreover, evidence suggests that the integrative response to exercise might be mediated by an oestrogenic effect in females. Therefore, differences in sex hormones that act as neurosteroids and interact with molecular growth factors, brain structures and

cognition in a different manner for women and men could explain part of this variability (Ansdell et al., 2020). Besides, this is in accordance with recent bibliography stating sex differences in the association of exercise with brain and cognitive outcomes (Lindwall et al. 2008, Varma et al., 2015, Varma et al. 2016, Barha et al., 2019, Dimech et al., 2019). Another potential explanation could be a dose-effect bias related to the described differences in the amount of physical activity performed by women and men. Although this fact could be a source of variance, sex differences in the distribution of SPA and CRF observed in our sample are consistent with previously published literature reporting that men are more physically active (Al-Mallah et al., 2016). Additionally, there is evidence reporting no significant differences between PA levels but showing sex differences in CRF levels and significant associations between CRF and functional brain outcomes only in men (Dimech et al., 2019). Therefore, future studies should address the influence of dose by sex in sex-balanced samples.

It must be acknowledged that our results are based on a cross-sectional design which allows us to describe statistical relationships but not causal conclusions about the neuroprotective effects of exercise. Moreover, further studies should address these aims in samples with different physical activity profiles, age groups, sex-balanced groups and a non-estimated measure of CRF. Including Heart Rate Reserve to estimate intensity/exertion during the test could provide further information about the physical activity status of participants and inform about its role in relation with physiological mechanisms". We stress the need to stratify results by sex and study the role of the hormonal profile in the molecular cascade that might explain the benefits of exercise to brain health.

CONCLUSION

From a clinical perspective, we showed that exercise might be related to reduced levels of inflammatory markers and increased brain volume in areas commonly deteriorating in aging. Regular exercise is also associated to better psychological and sleep health in men.

Our results contribute to the field of research adding evidence about the mediating effects of molecular biomarkers capable to modulate the immune system in the relationship between CRF and cognition in men. Moreover, our results suggest sex differences in the association between physical activity outcomes and molecular, brain and psychological outcomes.

This might be related to sex differences in the musculoskeletal and cardiovascular adaptations after exercise as well as sex differences in the hormonal profile. Future studies should address the interaction of the hormonal profile with the molecular and brain measures commonly studied in cross-sectional designs and RCTs.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

ACS and FRC participated in the study concept and design, acquisition, analyses and interpretation of data as well as in the elaboration of the manuscript. RDA contributed processing the neuroimaging data. NLV collaborated in the acquisition of the data. RDA, NLV, AS, PT, GP, PM, AH, SD, MV and KE critically reviewed the content of the article. MM conceptualized the study, contributed to the study design and the implementation as Principal Investigator and supervised all procedures and the elaboration of the manuscript.

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Ethics Approval

Bioethics Commission of the University of Barcelona (IRB00003099) and Clinical Research Ethics Committee of IDIAP Jordi Gol (P16/181).

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Figures

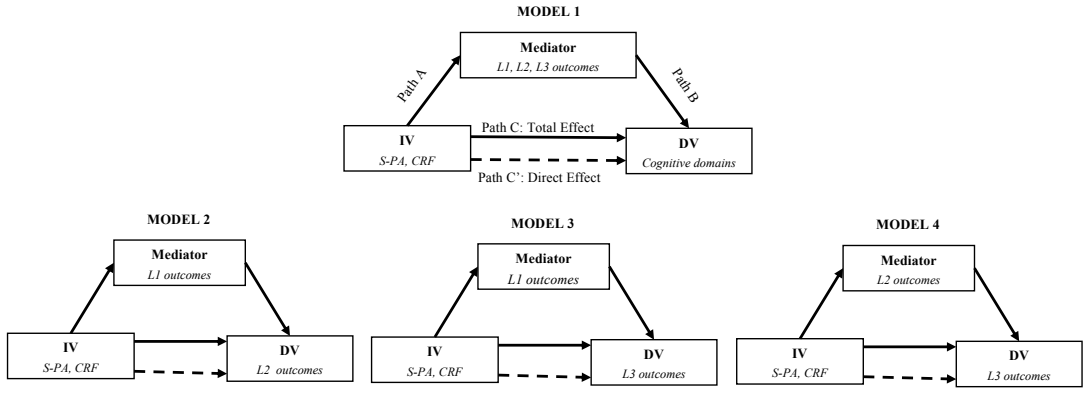

Figure 1. Mediating models

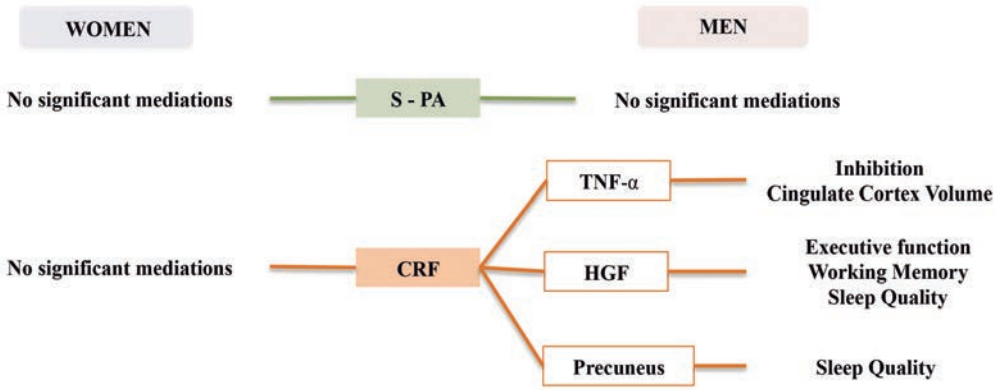

Figure 2. Mediation results of Models 1, 2, 3 and 4 in women and men.

Tables

Table 1. Demographic and PA variables for the cross-sectional sample.

Demographic variables	**WOMEN** Mean (SD)	**MEN** Mean (SD)	t-Test / χ^2 (*p* value)
n	65	39	-
Age (years)	56.75 (4.96)	58.59 (5.86)	1.64 (.106)
Education (years)	12.46 (4.97)	14.82 (5.55)	2.18 (.032)
MMSE (/30)	28.15 (1.41)	28.33 (1.53)	0.60 (.552)
BMI	26.99 (4.53)	28.40 (5.44)	-1.43 (.157)
Hypertension (n)	12	10	3.46 (.063)
Diabetes (n)	7	4	3.59 (.058)
PA variables	Mean (SD) [Min – Max]	Mean (SD) [Min – Max]	t-Test (*p* value)
S-PA	2055.35 (4395.04) [0 - 16512]	6011.35 (9939.24) [0 - 36952]	-2.35 (.023)
NS-PA	9495.88 (6438.50) [1260- 29232]	6173.16 (6780.93) [600 - 39120]	2.50 (.014)
CRF	25.07 (12.31) [0.07 - 49.68]	33.86 (12.92) [3.09- 58.64]	-3.46 (.001)

Note: BMI: body mass index; CRF: cardiorespiratory fitness; MMSE: Mini-Mental State Examination (Blesa et al., 2001); NS-PA: non-sportive physical activity; PA: physical activity; S-PA: sportive physical activity.

Table 2. Linear regression models in women and men: relationship between PA outcomes and molecular biomarkers.

Molecular biomarkers	**WOMEN**		**MEN**	
	S-PA β (*p* value)	**CRF** β (*p* value)	**S-PA** β (*p* value)	**CRF** β (*p* value)
BDNF (pg/ml)	-0.04 (.797)	-0.09 (.646)	-0.09 (.642)	-0.32 (.224)
TNF-α (pg/ml)	-0.39 (.007)**	-0.19 (.288)	-0.19 (.304)	-0.65 (.017)*
HGF (pg/ml)	-0.12 (.398)	-0.25 (.143)	-0.23 (.152)	-0.58 (.018)*
ICAM-1 (ng/ml)	-0.12 (.473)	-0.32 (.082)	-0.15 (.423)	-0.07 (.820)
SDF1-α (pg/ml)	0.06 (.695)	0.27 (.132)	0.06 (.781)	-0.07 (.821)

Note: BDNF: brain-derived growth factor; CRF: cardiorespiratory fitness; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; PA: physical activity; SDF1-α: stromal cell-derived factor-1 alpha protein; S-PA: sportive physical activity; TNF-α: tumor necrosis alpha.
Covariates: age, years of education and body mass index.
*S-PA is measured in metabolic equivalent of task units and CRF in ml/kg*min.*
** p <.05; ** p <.01.*

Table 3. Linear regression models in women and men: relationship between PA variables and brain volumes.

Brain Volumes (mm^3)	WOMEN		MEN	
	S-PA β (*p* value)	**CRF** β (*p* value)	**S-PA** β (*p* value)	**CRF** β (*p* value)
Ventricles	0.23 (.104)	0.08 (.642)	-0.28 (.113)	-0.69 (.002)**
Total White Matter	-0.02 (.739)	0.06 (.442)	-0.07 (.530)	-0.07 (.616)
Frontal Lobe	-0.03 (.632)	0.02 (.778)	0.11 (.260)	0.23 (.074)
Dorsolateral Prefrontal Cortex	0.22 (.015)*	0.19 (.074)	0.06 (.632)	-0.09 (.618)
Cingulate Cortex	0.12 (.183)	-0.02 (.856)	0.18 (.134)	0.05 (.754)
Parietal Lobe	-0.00 (.965)	-0.07 (.377)	0.09 (.279)	0.20 (.051)
Precuneus	-0.07 (.545)	-0.12 (.404)	0.28 (.054)	0.46 (.014)*
Temporal Lobe	0.14 (.041)*	0.11 (.191)	0.20 (.033)*	0.25 (.048)*
Hippocampus	0.07 (.567)	-0.02 (.899)	-0.07 (.694)	-0.06 (.779)

Note: CRF: cardiorespiratory fitness; PA: physical activity; S-PA; sportive physical activity.
Covariates: age, years of education, intracraneal brain volume and body mass index.
*S-PA is measured in metabolic equivalents units and CRF in ml/kg*min.*
** p <.05; ** p <.01.*

Table 4. Linear regression models in women and men: relationship between PA variables and behavior outcomes.

Psychological Status & Daily Activity	WOMEN		MEN	
	S-PA β (*p* value)	**CRF** β (*p* value)	**S-PA** β (*p* value)	**CRF** β (*p* value)
GDS	-0.16 (.207)	-0.05 (.729)	-0.23 (.174)	-0.39 (.021)*
VAMS	-0.10 (.446)	-0.26 (.070)	-0.06 (.717)	-0.30 (.055)
S-IQCODE	-0.02 (.875)	0.19 (.193)	-0.14 (.403)	-0.16 (.354)
PSQI	-0.11 (.416)	-0.04 (.784)	-0.13 (.445)	-0.19 (.286)
Total CORE-OM	-0.19 (.138)	-0.17 (.238)	-0.15 (.378)	-0.10 (.547)

Note: CORE-OM: Short Informant Questionnaire in Routine Evaluation-Outcome Measure (Trujillo et al., 2016); CRF: cardiorespiratory fitness; GDS: Geriatric Depression Scale (Martínez et al., 2002); PA: physical activity; PSQI: Pittsburgh Sleep Quality Index (Rico & Fernández, 1997); S-IQCODE: Short Informant Questionnaire on Cognitive Decline in the Elderly (Morales González et al., 1992); S-PA: sportive physical activity; VAMS: Visual Analog Mood Scale (Stern et al., 1997).
Covariates: age and years of education.
*S-PA is measured in metabolic equivalents units and CRF in ml/kg*min.*
** p <.05*

STUDY 3

Molecular and Brain Volume Changes Following Aerobic Exercise, Cognitive and Combined Training: the Projecte Moviment

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Running heading: Molecular & Brain Volume Changes after AE, CCT and COMB: Projecte Moviment

ABSTRACT

Background: Behavioral interventions have shown promising neuroprotective effects, but the cascade of molecular, brain and behavioral changes involved in these benefits remains poorly understood.

Methods: Projecte Moviment is a 12-week (5 days per week – 45 min per day) multi-domain, single-blind, proof-of-concept randomized controlled trial examining the cognitive effect and underlying mechanisms of an aerobic exercise (AE), computerized cognitive training (CCT) and a combined (COMB) groups compared to a waitlist control group. Adherence was >80% for 82/109 participants recruited (62% female; age = 58.38 ± 5.47). In this study we report intervention-related changes in plasma biomarkers (BDNF, TNF-α, HGF, ICAM-1, SDF1-α) and structural-MRI (brain volume) and how they related to changes in physical activity and individual difference variables (age and sex) and their potential role as mediators in the cognitive changes.

Results: Although there were no significant changes in molecular biomarker concentrations in any intervention group, changes in ICAM-1 and SDF1-α were negatively associated with changes in physical activity outcomes in AE and COMB groups. Brain volume changes were only found in the CCT showing a significant increase in precuneus volume. Sex moderated the brain volume change in the AE and COMB groups. Changes in molecular biomarkers and brain volumes did not significantly mediate the cognitive-related benefits found previously for any group.

Conclusions: There are molecular and brain volume changes related to lifestyle interventions at early stages. We highlight the value of examining activity parameters, individual difference characteristics and using a multi-level analysis approach to address these questions.

Clinical Trial Registration: NCT03123900, April 21st, 2017 in www.ClinicalTrials.gov.

Keywords: aerobic exercise, computerized cognitive training, combined training, brain volume, BDNF, neuroinflammation.

INTRODUCTION

Being active might be your best health ally. Current scientific literature states that an active lifestyle might have a positive impact on most hallmarks of aging, especially for brain and cognitive health [1]. Behavioral interventions such as aerobic exercise (AE) and cognitive training programs are promising approaches to maintain and enhance cognition [2, 3]. Systematic reviews generally agree on the small-to-moderate effect size and consistent effects of AE on executive function, attention and speed [4-7] and the benefits of cognitive training in the trained domains but limited transfer to non-trained domains [3]. Combining both interventions showed promising results about the potential for greater effects on cognition [8] yet recent systematic reviews reported that the extent of the combined effects were not significantly different from the individual programs [9, 10]. Studies including these approaches are examining biological mechanisms that could inform associations in prior studies. Identifying the molecular/cellular and brain changes related to exercise and cognitive training benefits, applied individually or in combination, is key to better understanding and prescribing these interventions as well as elucidating which combination of strategies would be more successful.

AE-related cognitive benefits are the result of multiple biological and psychosocial changes which were organized in a three-level model by Stillman et al. [11]. Molecular and cellular changes are described at Level 1 and include key factors involved in neuroplasticity, angiogenesis, metabolism and inflammation. For example, brain derived neurotrophic factor (BDNF) seems sensitive to AE [12]. While available evidence consistently reported increased BDNF levels after an acute bout of exercise, the effect of regular exercise on resting BDNF remains unclear [13]. Initial systematic reviews and meta-analyses suggested that exercise training led to elevated peripheral BDNF [12, 14-16], but these results were driven by trials including clinical populations [13]. Recent reviews including healthy samples have reported non-significant effects of low to moderate AE on BDNF levels [17] and conclude that evidence in human studies is still insufficient for making strong conclusions [18]. Other growth factors such as insulin growth factor (IGF) and vascular endothelial growth factor (VEGF) have consistently been related to endothelial cell proliferation and vessel growth as well as enhanced neurogenesis in the hippocampus after exercise due to their interaction with BDNF

[19, 20]. Molecular markers modulated by IGF and VEGF, such as stromal cell derived factor 1 (SDF1) or intercellular adhesion molecule 1 (ICAM-1) were suggested to be involved in the promotion of angiogenesis in the brain after exercise based on animal studies [20]. Significantly decreased levels of ICAM-1 related to physical activity were found in patients with cardiovascular risk factors [21] but evidence in healthy samples is still inconclusive with some [22] showing significant and others [23] non-significant changes in post-test vs pre-test comparisons. In healthy young men, SDF1 levels increased in response to acute exercise with an increase of endothelial cells in a 24h follow-up [24]. However, the effect of exercise programs on these markers in humans is still insufficient for drawing clear conclusions [21]. Another growth factor potentially involved in this cascade of changes is the hepatocyte growth factor (HGF) which is a hepatokine primarily linked to liver regeneration. HGF has an anti-inflammatory role in adipose tissue and is linked to insulin resistance and diabetes [25]. However, the effect of exercise on HGF levels has been scarcely addressed. Yasuda et al. [26] found increased HGF levels after acute exercise which were negatively related to cardiovascular fitness (CRF) in patients after acute myocardial infarction. Exercise is known to also induce antioxidant and anti-inflammatory actions by modulating cytokines and oxidative stress factors on adipose tissue, body muscles and immune system [27]. Recent meta-analyses reported significant benefits on the pro-inflammatory markers in healthy populations after AE interventions [28, 29]. However, evidence is still unclear about how exercise might influence each one of these markers. For example, Zheng et al. [29] and Monteiro-Junior et al. [28] agree on reduced levels of C-reactive protein and interleukin 6, but only Zheng et al. [29] found significant effects for decreased TNF-α.

Changes at the molecular level are involved in the structural and functional brain benefits related to exercise as described at Level 2 by Stillman et al. [11]. In relation to brain volume, systematic reviews reported that AE training could be effective to prevent brain volume loss, increase grey matter volume [30] and have a positive but small impact on global white matter volume [31]. Most of the research on this topic has focused on the impact of exercise on the hippocampus showing significant positive effects specifically on left hippocampal volume [32], specifically in older populations (>65 years) and in interventions that lasted over 24 weeks [33]. However, there are still inconsistencies among trials including healthy samples.

For example, increased local gray matter volumes in prefrontal [34, 35], cingulate [35] and temporal cortices [34] and hippocampus [36, 37] have been observed after long-term AE interventions, whereas non-significant changes in these same areas have been observed in trials lasting only 12 weeks [38-40]. This fact is in accordance with evidence highlighting how FITT-VP (Frequency, Intensity, Time, Type, Volume and Progression) parameters of exercise programs might be modulating the effect of the intervention on biomarkers at either at Level 1 or 2 [41, 42]. Moreover, current literature suggests that individual characteristics of participants such as age, sex or genetics [7, 43] interplay with the intervention modulating the cognitive benefits related to exercise.

The Hebb principle [44] has been used to generate hypotheses about the mechanisms involved in cognitive benefits related to cognitive training. Many authors have suggested that the regular practice of a cognitive task would repeatedly and simultaneously activate a group of neurons and, in turn, strengthen synaptic connections [45-47]. Animal studies using an “enriched environment” revealed increased long-term potentiation and enhanced BDNF levels in the hippocampus which led many authors to hypothesize a similar pattern in humans [48]. While cognitive training induces a significant increase of BDNF in patients with risk of dementia [49], evidence is still unclear if this effect occurs in healthy populations. There is evidence showing significant increases of BDNF after cognitive training programs lasting 5 [50] and 7 weeks [51] and, at the same time, other trials did not find significant changes in BDNF after 10 [52] and 12 weeks [53]. To our knowledge, other molecular and cellular mechanisms related to cognitive training effects have been scarcely addressed in healthy or clinical human populations. However, based on the overlapping effects of BDNF, IGF and VEGF in the promotion of neuronal growth in the brain [19], it could be suggested that these growth factors and other related molecular factors such SDF1 might also mediate cognitive training effects. For example, given the negative effect of pro-inflammatory cytokines on IGF signaling, which may alter protein synthesis in the brain [19], we might expect a complex interaction between multiple factors at this level. Szabó et al. [54] found that the number of stressful events and nuclear factor-jB, which is related to pro-inflammatory cytokines, predicted the increase of the right hippocampus after a videogame-based cognitive training approach. Therefore, understanding cognitive training related changes occurring at the molecular and cellular level

could help to interpret any observed benefits in the structure and function of the brain. Few studies have published structural brain changes associated with cognitive interventions in healthy older adults [55]. A systematic review [46] reported mixed results from only three trials reporting changes in brain volume in healthy older adults. Lampit et al. [56] found an increase in grey matter density in the right post-central gyrus in the cognitive training group compared to an active control group. However, Heinzel et al. [57] and Antonneko et al. [58] found no significant changes in grey matter and hippocampal volume respectively. As suggested above, the heterogeneity of the findings at a microscopic and macroscopic level could be related to parameters of the training program and sample characteristics [45, 47]. The cascade of molecular mechanisms of cognitive training-induced neuroplasticity in healthy older adults remains understudied and the research on structural brain changes is still limited [46, 47].

Current research has focused on whether the positive impact of exercise on growth factors, inflammatory profile and brain structure might facilitate the neuroprotective effect of cognitive training when they are combined [59]. In relation with BDNF, Rahe et al. [51] found increased peripheral BDNF from pre to post-testing when cognitive training was applied alone or in combination with physical training in a 7-week program. Anderson-Hanley et al. [60] reported that 3 months of cybercycling that included a cognitive component induced greater changes in BDNF levels compared to traditional cycling. The effects of a combined training (COMB) program on markers of inflammation have been studied in mice showing decreased TNF-α in the hippocampus. Related to effects on brain structure, Lövden et al. [61] reported that healthy older men engaging in a spatial navigation task (navigation task + walking) for 4 months maintained stable hippocampal volumes after the intervention compared to a control group which showed volume decrements consistent with age-related decline. However, a 7 month program of combined cognitive and AE training in a sample with MCI found no effect on grey matter loss [62]. Therefore, despite the hypothesis of the potential additive effects of both interventions on neurobiological measures, there is a great degree of mixed evidence and more research in humans is needed.

Projecte Moviment is a randomized controlled trial about the effect of a high-frequency (5 days per week) short-term (12 weeks) program of AE, computerized cognitive training (CCT) and their combination in healthy physically inactive older adults [63]. The observed changes on cognition, psychological status and physical activity outcomes have been published in Roig-Coll et al. [64]. In this study, we aim to examine the effect of the interventions on BDNF levels, markers of inflammation and volume changes in relevant brain areas compared to healthy controls. Secondly, we aim to test whether significant changes in physical activity outcomes are related to changes in molecular markers and brain volume. Finally, we aim to assess the moderating role of sex and age on molecular and brain volume changes and the possibility that changes in molecular and brain volume outcomes mediate the relationship between the intervention and cognitive benefits.

MATERIALS AND METHODS

Study Design

Projecte Moviment is a multi-center, single-blind, proof-of-concept RCT which took place between November 2015 and April 2018 (ClinicalTrials.gov; NCT031123900). Participants were assigned to four parallel groups: an AE group, a CCT group, a COMB group and a waitlist control group. Interventions lasted 12 weeks and there were assessments at baseline and trial completion. The study was developed by the University of Barcelona in collaboration with Institut Universitari d'Investigació en Atenció Primària Jordi Gol, Hospital Germans Trias i Pujol and Institut Guttmann, and approved by the responsible ethics committees (Bioethics Commission of the University of Barcelona –IRB00003099- and Clinical Research Ethics Committee of IDIAP Jordi Gol -P16/181-) following the Declaration of Helsinki.

This research paper follows the previously published protocol [63] and results on the primary hypothesis [64].

Participants

We recruited healthy adults aged 50 to 70 years old from the Barcelona metropolitan area using multiple strategies (lists of patients of general physicians, volunteers from previous studies, oral presentations in community centers, advertisements and local media).

We informed and screened those interested over the phone and in an on-site interview. Individuals meeting inclusion and exclusion criteria (see Table 1) signed a written informed consent prior to study involvement.

Participants were randomized after the baseline assessments and assigned to AE, CCT, COMB and control groups. The allocation sequence was designed by a statistician and consisted of a random combination of sex, age and years of education allowing for balanced groups accounting for these demographic variables. The intervention team was responsible for the allocation and assessors remained blind to group assignment.

Interventions

Interventions were home-based and scheduled for 5 days per week for 12 weeks. Participants randomized to AE group followed a progressive brisk walking program (Week 1: 30 min per day at 9–10 on the Borg Rating of Perceived Exertion Scale (BRPES) [68] perceived as light intensity. Week 2: 45 min per day at 9-10 on BRPES. Week 3 to 12 (10 weeks): 45 min per day at 12-14 on BRPES perceived as moderate-high effort). Participants randomized to CCT group performed multimodal cognitive training using the Guttmann Neuropersonal Trainer online platform (GNPT®, Spain) [69, 70] in sessions of 45 minutes targeting executive function, visual and verbal memory and sustained, divided and selective attention. The demand of the tasks for each participant was adjusted by GNPT platform based on baseline cognitive performance and the ongoing scores of the activities. Participants randomized to COMB group performed the brisk walking program and the CCT as described above, separately, in single continuous bouts of 45 minutes for each intervention 5 days per week without order or time-point restrictions. Participants randomized to the control group were on the wait list for 12 weeks and were asked not to alter their regular lifestyle.

Participants monitored their activity in a diary registering the date and duration of the activity and any adverse events occurring as well as the intensity of the walking in BRPES units. Participants had the contact information for the intervention team who followed the intervention (phone calls every 2 weeks and a mid-point visit and a final visit) and ensured participation, solved inconveniences and barriers and obtained adherence based on participants feedback and platform data.

The protocol for each intervention condition is explained in more detail elsewhere [63].

Assessment

All assessments were conducted in clinical environments at baseline within 2 weeks prior to the start of the intervention and, again, within 2 weeks after the completion of the program [63].

Blood sample: biomarkers at Level 1

Nurses in the Primary Health Care Centers obtained blood samples from the antecubital vein in EDTA tubes for plasma analyses between 8:00 and 9:00 a.m. All participants were instructed to fast overnight and not exercise 8 hours before the blood test. Tubes were immediately transferred to the IGTP-HUGTP Biobank integrated in the Spanish National Biobanks Network of Instituto de Salud Carlos II (PT13/0010/0009) and Tumor Bank Network of Catalonia. They were processed following standard operating procedures with the appropriate approval of the Ethical and Scientific Committees and plasma aliquots were stored at -80ºC.

We obtained peripheral BDNF levels using an ELISA kit (Human Free BDNF Quantikine ELISA Kit; R&D Systems, Minnesota, USA). The Proteome Profiler HumanTM XL Cytokine Array was used to semi-quantitatively analyze a panel of 105 targeted cytokines (R&D Systems, MN, United States). Based on the results of the array and previously cited literature, TNF-α, ICAM-1, HGF, SDF1-α levels were selected to be quantitatively analyzed using the corresponding ELISA immunoassay method (Human TNF-α Quantikine HS ELISA, Human ICAM-1/CD54 Allele-specific Quantikine ELISA Kit, Human HGF Quantikine ELISA Kit, Human CXCL12/SDF-1 alpha Quantikine ELISA Kit; R&D Systems, Minnesota, USA).

Neuroimaging: biomarkers at Level 2

Structural MRI data was collected at the Hospital Germans Trias i Pujol using a 3T Siemens Magnetom Verio Symo MR B17 (Siemens 243 Healthineers, Erlangen, Germany). We acquired T1-weighted multi-planar reformat sequences (acquisition time: 5:26 min, voxel: 0.9x0.9x0.9mm, TR/TE/TI: 1900/2.73/900ms, flip angle: 9º, slices: 192; thickness: 0.9 mm) and visually checked by an expert neuroradiologist. We used MRICloud (https://mricloud.org/) [71] to analyze brain images performing a fully automated parcellation of 287 volumes based on multiple atlases that fuses different algorithms [72] with a local search algorithm [73]. For our sample, we used atlas library version 10A, which includes 30 atlases from cognitively-

normal individuals and individuals with cognitive impairment or dementia. We summed volumes of the brain tissue (Left Hemisphere + Right Hemisphere + Brainstem + Cerebellum) and CSF (Ventricles + Sulci) for calculated intracranial volume (ICV). Based on previous literature [30, 31] and consistent with our recently published results [64], we obtained partial brain volumes of the following relevant areas: ventricles, total WM and GM of the frontal lobe, dorsolateral prefrontal cortex, cingulate cortex, parietal lobe, precuneus, temporal lobe and hippocampus.

Physical Activity

Physical activity levels were evaluated with the Minnesota Leisure Time Physical Activity Questionnaire (VREM) [74] which asks about frequency and duration of multiple activities -sportive walking, sport/dancing, gardening, climbing stairs, shopping walking and cleaning house- during the last month. We calculated the energy expenditure for each activity transforming hours per month into units of metabolic equivalent of tasks (METs). We added the METs spent in sportive walking and sport/dancing activities to obtain a measure of Sportive Physical Activity (S-PA) and the METs spent in gardening, climbing stairs, shopping walking and cleaning house to obtain a measure of Non Sportive Physical Activity (NS-PA).

Cardiorespiratory Fitness

CRF was assessed using the Rockport 1-Mile Test in which participants were instructed to walk one mile on a treadmill adjusting their speed in order to be as fast as possible without running. We used the standard equation reported by Kline et al. [75] to estimate the maximal aerobic capacity (VO_{2max}). The equation uses the following variables to estimate VO_{2max}: weight, age, sex, time to complete the mile, and heart rate at the end of the test.

Cognitive Performance

An extensive cognitive assessment was conducted before the CRF test or any type of exercise in order to control for the effect of acute exercise on cognitive performance. The neuropsychological battery was constructed using a theoretically-driven [76, 77] selection of tests addressing multiple cognitive functions: Flexibility (Trail Making Test B-A time) [78],

Fluency (letter and category fluency) [79], Inhibition (interference-Stroop Test) [80], Working Memory (backward-WAIS-III) [81], Visuospatial Function (copy accuracy-Rey Osterrieth Complex Figure) [82], Language (Boston Naming Test-15) [83], Attention (forward span, digit symbol coding and symbol search WAIS-III) [81], Speed (Trail Making Test-A) [78]; copy time-Rey Osterrieth Complex Figure [82], Visual Memory (memory accuracy-Rey Osterrieth Complex Figure) [82] and Verbal Memory (total learning and recall-II Rey Auditory Verbal Learning Test) [84]. Six general domains were designed: 1) Executive Function, 2) Visuospatial Function, 3) Language, 4) Attention-Speed, 5) Memory and 6) Global Cognitive Function. Extended details in Supplementary Material Table 1.

Statistical analysis

We conducted statistical analyses using Statistical IBM SPSS Statistics 24. First, we ensured data quality by examining the distribution of raw scores (i.e., outliers, skewness). Then, we calculated change scores (post-test minus pre-test), compared baseline scores between groups and performed cross-time partial correlations to detect potential confounds and ceiling effects. Change between baseline and follow-up within group was examined using a t-test of related samples. In order to compare each intervention group to the control group we regressed change in each outcome of interest on the baseline outcome score, sex, age, years of education, BMI, and the *dummy* treatment variables (AE vs controls, CCT vs controls and COMB vs controls). ICV was included as a covariate for the models including brain volume outcomes.

We addressed whether the significant intervention-related changes in S-PA and CRF observed in the AE and COMB groups previously published in Roig-Coll et al. [64] were related to change in biomarkers at Level 1 and 2 applying partial correlations adjusted by sex, age, years of education, BMI and, for brain volumes, ICV was added.

We used the PROCESS macro for SPSS [85] to analyze the moderating effect of age and sex on intervention-related changes for biomarkers at Level 1 and 2. We also used the PROCESS macro to perform mediation analyses to assess whether change in biomarkers at Level 1 or 2 mediated the cognitive benefits observed in the AE and COMB groups [64]. For mediation analyses the independent variable was a treatment variable (condition vs control), the dependent variables

were change in cognition for those functions that showed significant intervention-related changes. We used as mediators the change in biomarkers at Level 1 and Level 2 controlling for baseline performance score, age, sex, years of education, BMI and, when biomarkers at Level 2 were introduced as mediators, ICV was added. These analyses were computed with bias-corrected bootstrapped 95% confidence intervals (CIs) based on 5,000 bootstrap samples. Significance of mediation was indicated if the CIs in Path AB did not overlap with 0 [85].

RESULTS

Participants

We conducted analyses in the Per Protocol (PP) sample which included 82 subjects with a level of adherence >80% (n = 82, 62% female; age = 58.38 ± 5.47; see Table 2) out of the 109 who completed the baseline assessment and the 92 who completed the intervention (intention to treat, ITT) (see Figure 1; extended details on Roig-Coll et al., [64]). There were no significant differences in demographic variables between groups in the ITT sample and between the ITT and PP sample (see Supplementary Material Table 2 & 3).

The PP sample showed no significant baseline differences between groups in demographic, molecular, brain volume, physical and cognitive outcomes except for SDF1-α and NS-PA (extended details in Supplementary Material Tables 4). SDF1-α at baseline was included as a covariate.

Intervention-related changes in molecular biomarkers

There were no significant changes in molecular biomarkers between baseline and follow-up in any group. Contrasts between each intervention and control group for molecular biomarker outcomes are reported in Table 3. The results for these outcomes showed no significant changes in AE, CCT and COMB groups compared to control for any molecular biomarker outcome.

Intervention-related changes in brain volume

There was a significant change in precuneus volume between baseline and follow-up for the CCT group (t = -2.62, p = .019). There were no significant changes between baseline and follow-up for the AE, COMB and control groups. Contrasts between each intervention and control group for brain volume outcomes are reported in Table 4. The results for these outcomes showed a significant change in frontal lobe and in precuneus volume in the CCT compared to control group. There were no significant changes in AE and COMB groups compared to control for any outcome.

Relation between biomarkers at Level 1 and 2 with changes in physical activity outcomes

As mentioned above, AE and COMB groups showed significant intervention-related changes in S-PA and CRF levels. In the AE group, the increase of S-PA was negatively associated with change in ICAM-1 levels (r = -0.55, p = .016). Results were not significant for changes in BDNF, TNF-α, HGF or SDF1-α. Moreover, the increased levels of CRF in the AE group was negatively associated with change in SDF1-α levels (r = -0.50, p = .057) and not significantly associated with the other biomarkers. In the COMB group, the significant change in S-PA was not related to changes in any of the measured molecular markers. However, the significant benefits on CRF were negatively related to change in ICAM-1 (r = -0.66, p = .020) and SDF1-α (r = -0.89, p = <.001) levels. Intervention-related changes in physical activity in AE and COMB groups were not significantly related to changes in any measure of brain volume.

Sex and age moderation effects on biomarkers at Level 1 and 2

Moderation analyses showed that age did not significantly moderate the effect of the intervention on biomarkers at Level 1 and Level 2 in any group. Sex was not a significant moderator of the effects of the intervention on biomarkers at Level 1 in any group but showed significant effects on some of the targeted brain volume areas. In the AE group sex (women=1, men= 0) moderated the effects of the intervention on total WM (β = -3133.51, t(53) = -2.45, p = .018), parietal (β = -3060.29, t(53) = -2.07, p = .043) and temporal lobe (β = -4028.46, t(53) = -2.47, p = .017) and dorsolateral prefrontal cortex volumes (β = -1819.39, t(53) = -2.89, p = .006). In the COMB group sex also moderated the effects of intervention on ventricles (β = -5380.97, t(53) = -3.60, p = <.001), temporal lobe (β = 4690.43, t(53) = 2.56, p = .013) and dorsolateral prefrontal cortex (β = 1670.17, t(53) = 2.26, p =.028).

Mediation effects on intervention-related cognitive benefits

We applied mediation analyses to observe whether changes in biomarkers at Level 1 and Level 2 mediated the association between the intervention and the cognitive domains that demonstrated a significant change as reported in Roig-Coll et al. [64] including Executive Function (Working Memory) and Attention-Speed (Attention) in the AE group and for Attention-Speed (Attention and Speed) in the COMB group. Mediation analyses showed that changes in BDNF, TNF-α, HGF, SDF1-α and ICAM-1 levels and the targeted brain volume areas did not significantly mediate the observed cognitive benefits for any group.

DISCUSSION

In this paper we report molecular and brain volume changes in the Projecte Moviment trial, which studied the effects of AE, CCT and the combination of the interventions in healthy inactive late-middle-aged adults compared to a waitlist control group. Cognitive changes in Executive Function and Attention-Speed in the AE group and in Attention-Speed in the COMB group were previously published [64] and led us to address other micro and macroscopic correlates that might be involved in these interventions.

We previously found that regular exercise was related to the inflammatory profile, brain volume and cognition in a cross-sectional sample [86, 87]. In the present trial, participants in the AE group did not show significant within-group pre to post-test changes nor significant changes in comparison with the control group for any of the molecular correlates or targeted brain volume areas after 12 weeks in a 5 days per week, 45 min a day brisk walking program. The intervention-related results are in accordance with recent evidence stating that short-term trials typically do not report significant changes in neurotrophic factors or structural brain changes although they do in brain connectivity markers [33, 41]. These results suggest that despite an increased frequency of activity during a 12-week program, the duration of the program might be more relevant for these molecular and structural brain changes since training programs lasting on the order of 6-12 months reported structural brain benefits [34-36]. Therefore, our results suggest that the parameters of the activity, such as duration and intensity, are critical aspects of an exercise intervention. Another issue that might explain our results is that the sample was healthy and aged 50 to 70 years. As suggested by Erickson et al. [30], the positive effects of exercise on gray matter volume might be significant in older age ranges in which there are greater brain volume losses. This suggestion could be extended to samples with pathology in which evidence tends to be more consistently significant [33].

Participants in the CCT group did not show significant changes on any of the targeted biomarkers at Level 1 which might be due to several factors. For example, the magnitude of the changes might not be detectable yet over this duration and might require more prolonged exposure to cognitive training to induce significant changes in these markers. In addition, the parameters of the CCT program, the lack of pathology and age of our participants might also be playing a role as described for the AE interventions. However, the CCT group showed a significant intervention-related increase in the volume of the precuneus and compared to the change observed in the control group. These findings suggest that engaging in a multimodal CCT for 45 min a day, 5 days a week for 12 weeks might increase the volume of the precuneus which has a fundamental role in cognition: it is highly involved in the integration of tasks, visuo-spatial imagery, episodic memory retrieval as well as self-referential processing [88]. This result highlights current research challenges in this field since participants in the CCT group did not show significant changes in the cognitive assessments compared to the control group [64] in accordance with other trials of cognitive training [89]. The possible neuroprotective effects of a cognitive training program should be addressed by examining molecular, brain and cognitive markers given the challenge of detecting far transfer effects. We also found that change in the frontal lobe was also significant in the CCT group compared to the control group although neither participants in the CCT group nor the control group showed significant increase or loss in this area.

Our study also shows that combining brisk walking and multimodal CCT for 12 weeks, 5 days per week in bouts of 45 minutes for each did not lead to greater benefits in the molecular biomarkers and brain volume areas analyzed pre to post-test and compared to the control group. As suggested for each intervention individually, parameters of the activity or characteristics of the sample might be responsible, though the scarcity of studies and high heterogeneity in the intervention characteristics [51, 60, 61] make a challenge to draw firm conclusions. Moreover, determining whether a sequential or simultaneous strategy is best for

cognitive benefits is still a controversy [10]. Our findings might relate to the importance of the scheduled order of the interventions, specifically when interventions are applied simultaneously [10, 90]. Enhanced effects have been reported when exercising before the cognitive training suggesting that exercise facilitates an environment that enhances the effect of cognitive training.

We also found that significant changes in physical activity outcomes (amount of PA and CRF), found in the AE and COMB groups [64], were related to significant changes in immune, cardiovascular and brain health biomarkers. Increases in the amount of PA observed in the AE group and higher CRF in the COMB group were related to reduced levels of ICAM-1, which is a pro-inflammatory molecule promoted by IGF and TNF-α and highly expressed in endothelial cells and related to atherosclerosis [91, 92, 93]. Increase in CRF in the AE and COMB groups was also related to reduced levels of SDF1-α which is the most common variant of SDF, a chemokine protein involved in angiogenesis and brain tissue repair, that contributes to neuroinflammation which in higher concentrations can lead to toxic environments and clinical consequences [94]. These results suggest that a 12-week program of brisk walking, in combination with cognitive training or alone, might enhance markers associated with cardiovascular and inflammatory health.

Further analyses examined the potential moderating role of individual characteristics on the interventions given their important role when designing personalized programs. We did not find a significant moderation effect of age on intervention-related changes in biomarkers at Level 1 and Level 2 in any group. Despite evidence is scarce for CCT programs, previous papers reported interactions of age with the neuroprotective effect of exercise [4, 30] suggesting that the effect could be more apparent at older ages due to greater brain volume losses. Moreover, the mediating role of CRF in exercise-related cognitive benefits has been found in participants aged 70 or older but not in younger [95]. Therefore, the lack of a significant moderating effect of age in our study could be related to the relatively younger age of our participants as well as the limited age range of our participants. In relation to sex, we did not detect any significant moderation effects of sex on intervention-related changes in molecular biomarkers (Level 1) in any group despite previous evidence suggesting that sex moderates the effect of exercise on BDNF levels [15]. However, we found that sex moderated the effects of the intervention on brain volumes on AE (total WM, parietal, temporal and dorsolateral prefrontal cortex) and COMB groups (ventricles, temporal and dorsolateral prefrontal cortex). These results are consistent with recent reviews [43, 96] and with our previously published results [86, 87] which proposed that biological sex is a relevant moderator of the relationship between exercise and brain health. Sex differences in the physical activity-induced cognitive and brain changes may be associated with specific physiological adaptations in women and men, such as changes in neuroplastic processes, hormones, neurotransmitter systems and neurotrophic factors [43]. Projecte Moviment adds support to the recent and growing evidence suggesting that sex matters. However, larger samples are necessary to run more highly powered intra-group analyses and further research should address the mechanisms underlying cognitive and physical training effects stratified by sex.

Finally, we examined the potential mediation effect of changes in molecular biomarkers and brain volume of targeted areas in the intervention-cognitive benefits through testable models in order to add evidence about the mechanisms underlying the neuroprotective effect of behavioral interventions [7]. The results of these mediation analyses showed that changes in biomarkers at Level 1 and Level 2 did not significantly mediate the observed cognitive benefits in AE (Executive Functions and Attention-Speed) and in COMB groups (Attention-Speed). Previous evidence including a 1-year AE intervention reported that change in BDNF levels mediated the effect of intervention-related cognitive (task-switch) benefits in older adults (>71 years) [97] and that intervention-related increases in hippocampal volume were significantly correlated to improvements in memory performance [36]. Since our intervention programs lasted 12 weeks, the duration of the activity as well as other potential methodological issues might be key parameters to find consistent effects of these interventions on these molecular and structural brain outcomes and their effect on cognition. The length of the intervention could be an important factor not only due to the persistence of the activity but also for the time needed to observe molecular and structural changes in healthy subjects. We might also hypothesize that early intervention-related cognitive benefits might be mediated by changes in other molecular markers such as other growth factor, cytokines or hormones which might interact differently for women and men and in relation to brain outcomes such as increased white matter integrity and better functional connectivity [41]. The sample size is also an important issue for trials examining the mechanisms of interventions since large samples are needed to detect mediation effects when the outcome measure is highly variable

(neuroimaging data, molecular biomarkers) [11]. Those questions should be addressed for COMB interventions including AE and CCT in order to better understand the role of each single intervention. New omics technology might shed light on the complexity of biological networks underlying the cognitive benefits related to behavioral interventions [98].

The cascade of changes initiated by behavioral interventions that lead to cognitive enhancements is complex and requires a combined multi-level approach that addresses moderators such as sex, age, pathology and genetics. Further research should overcome our limited intra-group sample and include a wider range of participants with age and sex balanced across groups in order to be able to make specific intra-group analyses. We also acknowledge potential desirability bias in our sample since we obtained self-reported adherence and participants were highly motivated, the interventions were individual and home-based, and the use of a waitlist control group.

In conclusion, our results highlight the role of AE in the promotion of a molecular cascade involving immunity and cardiovascular related markers that might enhance a neuroprotective effect in the AE and COMB groups, as well as the importance of sex as a relevant variable for the brain volume changes related to these interventions. CCT showed promising results enhancing the brain volume of the precuneus, despite previously published results that did not show significant changes in cognitive assessments. These results highlight the importance of a multi-level approach when assessing and describing the neuroprotective effect of behavioral interventions. Moreover, parameters of the activity such as duration or intensity might be relevant to detect changes in the targeted molecular and structural brain outcomes. Other molecular and brain mechanisms should be addressed in order to better understand the cognitive benefits observed at early stages.

Declarations

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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.

Availability of data and material. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Code availability. Not applicable

Ethics approval. This study was carried out in accordance with the recommendations of CONSORT Statement with written informed consent from all subjects. The protocol was approved by the Bioethics Commission of the University of Barcelona (IRB00003099) and Clinical Research Ethics Committee of IDIAP Jordi Gol (P16/181).

Consent to participate. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Consent for publication. Not applicable (No identifying information included in this article.)

Author contributions. MM conceptualized the study and contributed to the study design and implementation as Principal Investigator. MM also guided and supervised all the statistical analysis and writing of this paper. PT and KE made substantial contributions to the design and implementation of the trial. ACS and FRC contributed to the design and implementation of this trial, they collaborated with recruitment and evaluated participants before and after interventions. ACS and FRC also analyzed the data and wrote this paper. NLV collaborated with recruitment and did the follow-up of the intervention groups. AGM and JMT assisted in the use of GNPT program for computerized cognitive training. GP guided and supervised the statistical analysis. RDA contributed processing the neuroimaging data and MV with the management of molecular markers. PMA, AHT, JSR, CC and SD contributed to the implementation of the trial from their area of expertise. All authors reviewed the manuscript and provided final approval for publication of the content.

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Figure

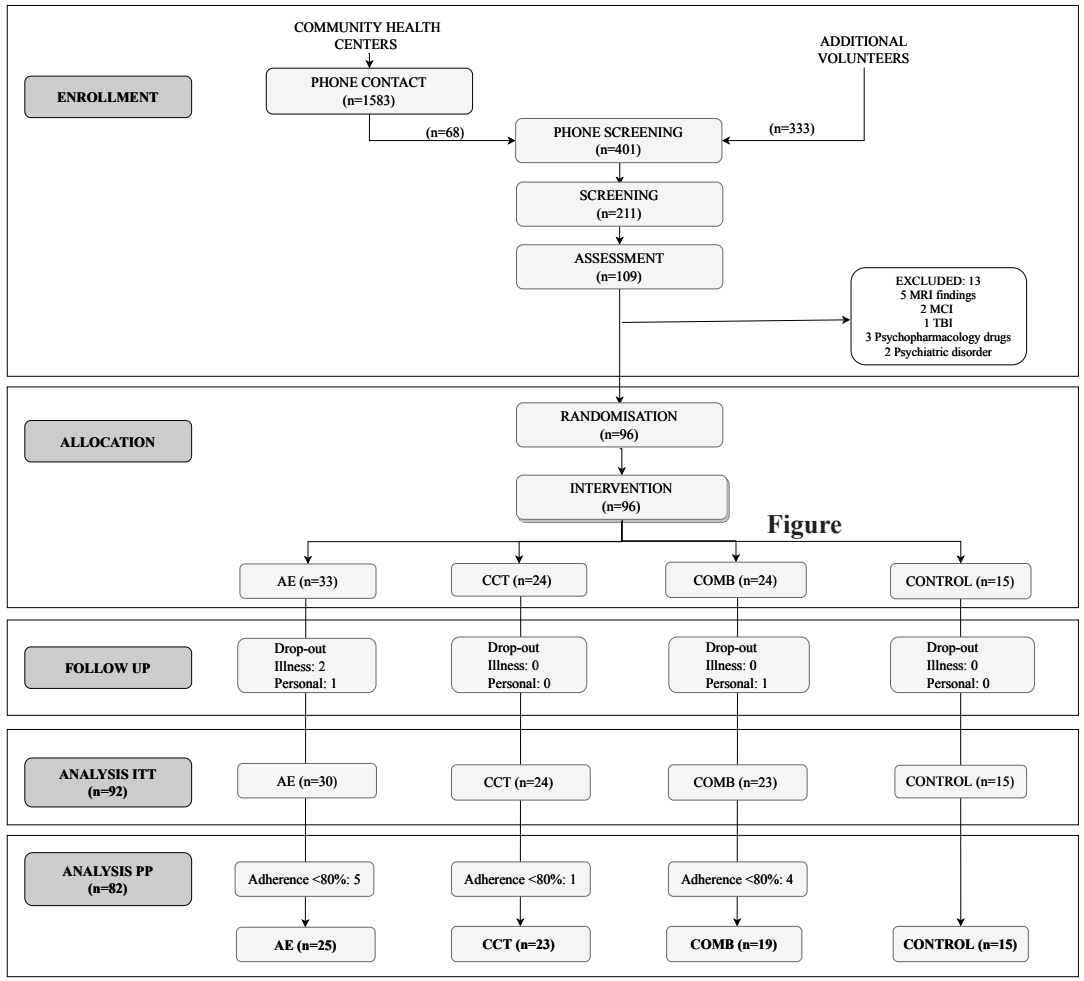

Figure 1. Flow chart of participants in the RCT

Tables

Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
Aged 50-70 years.	Current participation in any cognitive training activity or during last 6 months > 2 hours/week.
Mini-Mental State Examination (MMSE) ≥ 24.	Diagnostic of dementia or mild cognitive impairment.
Montreal Cognitive Assessment 5-min (MoCA 5-min) ≥ 6.	Diagnostic of neurological disorder: stroke, epilepsy, multiple sclerosis, traumatic brain injury, or brain tumor.
Competency in Catalan or Spanish.	Diagnostic of psychiatric illness current or during last 5 years.
Adequate visual, auditory and fine motor skills.	Geriatric Depression Scale 15 (GDS-15) > 9.
Acceptance of participation in the study and signature of the informed consent.	Consumption of psychopharmacological drugs current or during last 5 years; or more than 5 years throughout life.
Low active (<2 hours/week over the last 6 months) [1].	History of drug abuse or alcoholism current or during last 5 years; or more than 5 years throughout life; >28 men and >18 women unit of alcohol/week.
	History of chemotherapy.
	Contraindication to magnetic resonance imaging.

Note: GDS-15 (Martínez et al., 2002); MMSE (Blesa et al., 2001); MoCA 5-min (Wong et al., 2015).
[1] Only for those participants included in the randomized controlled design.

Table 2. Participants characteristics at baseline in the RCT design.

	Total Mean (SD)	AE Mean (SD)	CCT Mean (SD)	COMB Mean (SD)	Control Mean (SD)	Group test
n total / n females	82 / 51	25 / 13	23 / 16	19 / 14	15 / 8	$X^2(3) = 3.20$, $p = .361$
Age (years)	58.38 (5.47)	58.40 (5.12)	57.91 (5.31)	60.32 (5.54)	56.60 (5.97)	H(3) = 3.53, $p = .317$
Years of education (years)	12.52 (5.57)	12.44 (5.75)	12.04 (4.94)	12.37 (5.43)	13.60 (6.72)	H(3) = 0.28, $p = .963$
Vocabulary subtest (direct score-WAIS-III)	44.14 (8.30)	43.92 (9.53)	44.26 (7.16)	44.53 (8.02)	43.80 (8.98)	F(3,77) = 0.03, $p = .993$
BMI (kg/m^2)	28.63 (4.96)	28.14 (5.53)	28.13 (4.26)	28.37 (4.42)	30.49 (5.70)	H(3)=1.72, $p = .632$

Note: AE: aerobic exercise; BMI: body mass index; CCT: computerized cognitive training; COMB: combined training; WAIS-III: Wechsler Adult Intelligence Scale III.

Table 3. Intervention-related changes in molecular biomarkers (Level 1).

Molecular biomarkers	AE vs Controls B (95%CI), SMD, *p* value	CCT vs Control B (95%CI), SMD, *p* value	COMB vs Control B (95%CI), SMD, *p* value
BDNF (pg/ml)	-1293.54 (-4333.82, 1746.74), SMD = -0.13, $p = .398$	-1081.93 (-3925.15, 1761.30), SMD = -0.11, $p = .450$	-128.39 (-3235.57, 2978.79), SMD = -0.01, $p = .934$
TNF-α (pg/ml)	0.08 (-0.06, 0.21), SMD = 0.16, $p = .287$	<0.01 (-0.14, 0.14), SMD <0.01, $p = .990$	-0.03 (-0.18, 0.12), SMD = -0.07, $p = .663$
HGF (pg/ml)	24.81 (-102.48, 152.10), SMD = 0.06, $p = .669$	2.47 (-123.84, 128.78), SMD = 0.01, $p = .969$	30.48 (-105.27, 166.23), SMD = 0.07, $p = .656$
ICAM-1 (ng/ml)	-4.54 (-22.39, 13.31), SMD = -0.08, $p = .614$	-0.50 (-18.19, 17.20), SMD = -0.01, $p = .955$	-9.04 (-27.94, 9.86), SMD = -0.14, $p = .343$
SDF1-α (pg/ml)	-38.11 (-211.95, 135.73), SMD = -0.06, $p = .663$	-48.23 (-221.07, 124.61), SMD = -0.08, $p = .580$	140.87 (-47.06, 328.79), SMD = 0.22, $p = .139$

Note: AE: aerobic exercise; BDNF: brain-derived growth factor; CCT: computerized cognitive training; COMB: combined training; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; SDF1-α: stromal cell-derived factor-1 alpha protein; TNF-α: tumor necrosis alpha.
Covariates: sex, age, years of education, body mass index and baseline.
SMD= β.

Table 4. Intervention-related changes in brain volumes (Level 2).

Brain Volumes (mm^3)	AE vs Controls B (95%CI), SMD, *p* value	CCT vs Control B (95%CI), SMD, *p* value	COMB vs Control B (95%CI), SMD, *p* value
Ventricles	-335.42 (-2176.65, 1505.81), SMD = -0.07, $p = .716$	-89.42 (-2020.52, 1841.69), SMD = -0.02, $p = .926$	348.67 (-1581.37, 2278.71), SMD = 0.06, $p = .719$
Total White Matter	-598.23 (-2268.85, 1072.39), SMD = -0.12, $p = .476$	140.20 (-1654.67, 1935.08), SMD = 0.03, $p = .876$	-432.88 (-2216.83, 1351.08), SMD = -0.08, $p = .629$
Frontal Lobe	2462.00 (-1611.56, 6535.56), SMD = 0.21, $p = .231$	5031.79 (717.77, 9345.82), SMD = 0.41, $p = .023$*	3566.03 (-759.51, 7891.57), SMD = 0.29, $p = .104$
Dorsolateral Prefrontal Cortex	119.31 (-757.30, 995.92), SMD = 0.05, $p = .786$	567.92 (-352.66, 1488.50), SMD = 0.23, $p = .221$	220.64 (-691.70, 1132.98), SMD = 0.09, $p = .630$
Cingulate Cortex	240.33 (-339.98, 820.64), SMD = 0.15, $p = .410$	387.42 (-227.20, 1002.05), SMD = 0.23, $p = .212$	281.35 (-332.97, 895.66), SMD = 0.16, $p = .363$
Parietal Lobe	95.67 (-1843.45, 2034.79), SMD = 0.02, $p = .922$	1312.57 (-770.68, 3395.82), SMD = 0.23, $p = .212$	148.65 (-1888.81, 2186.12), SMD = 0.03, $p = .884$
Precuneus	34.64 (-453.43, 522.71), SMD = 0.02, $p = .887$	530.47 (21.65, 1039.28), SMD = 0.35, $p = .041$*	289.19 (-222.30, 800.68), SMD = 0.19, $p = .262$
Temporal Lobe	55.82 (-2084.45, 2196.08), SMD = 0.01, $p = .958$	145.31 (-2100.17, 2390.79), SMD = 0.02, $p = .897$	-1385.20 (-3645.44, 875.04), SMD = -0.22, $p = .225$
Hippocampus	23.91 (-75.07, 122.89), SMD = 0.08, $p = .630$	15.56 (-88.00, 119.13), SMD = 0.05, $p = .764$	44.47 (-60.54, 149.47), SMD = 0.14, $p = .400$

Note: AE: aerobic exercise; CCT: computerized cognitive training; COMB: combined training.
Covariates: sex, age, years of education, body mass index, baseline and intracranial brain volume.
**p < .05*
SMD= β.

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ANNEXES

Annex 1: Projecte Moviment studies

Castells-Sánchez, A., Roig-Coll, F., Lamonja-Vicente, N., Altés-Magret, M., Torán-Monserrat, P., Via, M., García-Molina, A., Tormos, J. M., Heras, A., Alzamora, M. T., Forés, R., Pera, G., Dacosta-Aguayo, R., Soriano-Raya, J. J., Cáceres, C., Montero-Alía, P., Montero-Alía, J. J., Jimenez-Gonzalez, M. M., Hernández-Pérez, M., Perera, A., ... Mataró, M. (2019). **Effects and Mechanisms of Cognitive, Aerobic Exercise, and Combined Training on Cognition, Health, and Brain Outcomes in Physically Inactive Older Adults: The Projecte Moviment Protocol.** *Frontiers in aging neuroscience, 11*, 216. https://doi.org/10.3389/fnagi.2019.00216

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ANNEX 2: SUPPLEMENTARY TABLES

Table 1. Assessments schedule in the RCT design.

	Enrollment & Screening	**Baseline Assessments** (within 2 weeks prior to the start of the intervention)			**Intervention**			**Post-Intervention Assessments** (within 2 weeks after the completion of the intervention)		
	Telephone Screening	**Visit 1: Screening**	**Visit 2: Baseline Assessment**	**Visit 3: Baseline Assessment**	**Visit 4: Baseline Assessment**	**Visit 5: initial Intervention**	**Visit 6: Mid-Intervention**	**Visit 7: Final Intervention**	**Visit 8: Post-Intervention Assessment**	**Visit 9: Post-Intervention Assessment**	**Visit 10: Post-Intervention Assessment**
First review of criteria	X										
Montreal Cognitive Assessment 5-min (MoCA 5-min)	X										
Information of study	X	X									
Exhaustive review of criteria		X									
Informed consent form		X									
Mini-Mental State Examination (MMSE)		X								X	
Geriatric Depression Scale (GDS-15)	X									X	
Medical history		X									
General health status		X							X		
Blood extraction		X							X		
Battery of neuropsychological tests			X							X[1]	
Psychological health and daily activities scales			X							X	
Physical activity questionnaire (VREM)			X							X	
Cardiorespiratory fitness (Rockport 1-mile walk test)			X							X	
Magnetic resonance imaging				X							X
Information about intervention					X						
Follow-up adherence questions[2]						X	X				
Actimeter[3] (Polar Loop®)					X			X			
Intervention follow-up diary[4]					X	X	X				

Notes: GDS-15 (Martínez et al., 2002); MMSE (Blesa et al., 2001); MoCA 5-min (Wong et al., 2015); RCT: randomized controlled trial; Rockport 1-mile walk test (Kline et al., 1987); VREM- Reduced Minnesota leisure time physical activity questionnaire (Ruiz et al., 2012).

[1]All tests except Vocabulary subtest. [2]This information is also collected in phone calls every two weeks between visits. [3]Participants carry the actimeter the first and last week of training. [4]Participants record the diary every day during the intervention.

Table 2. Demographic variables Projecte Moviment sample vs. extra participants.

	Projecte Moviment sample	Extra participants	t-Test, *p* value
n total / n female	84/56	20/9	
Age (years)	57.56 (5.46)	56.95 (5.04)	0.46, .650
Education (years)	12.95 (5.38)	15.00 (4.67)	-1.565, .121
MMSE (/30)	28.12 (1.36)	28.65 (1.73)	-1.482, .141

Note: mean (SD); MMSE: Mini-Mental State Examination (Blesa et al., 2001).

Table 3. PA and CRF outcomes in Projecte Moviment sample and extra participants.

	Projecte Moviment sample		Extra participants	
	mean (SD)	[Min – Max]	mean (SD)	[Min – Max]
S-PA	497.48 (7723.75)	[0 - 3066]	16312.60 (8232.39)	[4524 - 36952]
NS-PA	8288.33 (6903.68)	[600 - 39120]	8088.30 (6125.75)	[1560 - 29232]
CRF	24.42 (11.14)	[0.07 - 44.70]	44.91 (6.67)	[31.16 - 58.64]

Note: CRF: cardiorespiratory fitness; NS-PA: non-sportive physical activity; PA: physical activity; S-PA: sportive physical activity.

Table 4. Data regarding linear regression models for the relationship between PA outcomes and molecular biomarkers in women and men.

Molecular Biomarkers	WOMEN		MEN	
	S-PA	CRF	S-PA	CRF
	R^2,(df)F, *p* value	R^2,(df)F, *p* value	R^2,(df)F, *p* value	R^2,(df)F, *p* value
BDNF (pg/ml)	0.07, (4,51)1.02, .408	0.08, (4,51)1.06, .388	0.13, (4,32)1.16, .348	0.16, (4,32)1.53, .216
TNF-α (pg/ml)	0.27, (4,50)4.65, .003	0.17, (4,50)2.61, .046	0.22, (4,31)2.18, .094	0.33, (4,31)3.81, .012
HGF (pg/ml)	0.19, (4,50)2.98, .028	0.22, (4,50)3.43, .015	0.40, (4.31)5.11, .003	0.46, (4,31)6.70, .001
ICAM-1 (ng/ml)	0.04, (4,50)0.47, .759	0.08, (4,50)1.14, .348	0.16, (4,31)1.47, .234	0.14, (4,31)1.30, .292
SDF1-α (pg/ml)	0.10, (4,50)1.32, .277	0.13, (4,50)1.92, .122	0.09, (4,31)0.78, .550	0.09, (4,31)0.77, .554

Note: BDNF: brain-derived growth factor; CRF: cardiorespiratory fitness; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; PA: physical activity; SDF1-α: stromal cell-derived factor-1 alpha protein; S-PA: Sportive physical activity; TNF-α: tumor necrosis alpha.
Covariates: age, years of education and body mass index.
*S-PA is measured in metabolic equivalent of task units and CRF in ml/kg*min.*

Table 5. Linear regression models in women and men: relationship between PA outcomes and molecular biomarkers.

Molecular Biomarkers	WOMEN		MEN	
	S-PA	CRF	S-PA	CRF
	β (*p* value)	β (*p* value)	β (*p* value)	β (*p* calue)
BDNF (pg/ml)	-0.03 (.844)	-0.07 (.720)	-0.13 (.484)	-0.31 (.245)
TNF-α (pg/ml)	-0.40 (.006)**	-0.17 (.326)	-0.26 (.178)	-0.71 (.009)*
HGF (pg/ml)	-0.15 (.318)	-0.25 (.148)	-0.27 (.118)	-0.61 (.015)*
ICAM-1 (ng/ml)	-0.12 (.464)	-0.33 (.087)	-0.12 (.545)	-0.03 (.917)
SDF1-α (pg/ml)	0.07 (.673)	0.30 (.096)	0.12 (.552)	-0.02 (.956)

Note: BDNF: brain-derived growth factor; CRF: cardiorespiratory fitness; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; PA: physical activity; SDF1-α: stromal cell-derived factor-1 alpha protein; S-PA: sportive physical activity; TNF-α: tumor necrosis alpha.
Covariates: age, years of education, body mass index, cardiovascular risk factors medication.
*S-PA is measured in metabolic equivalent of task units n units and CRF in ml/kg*min.*
** p <.05; ** p <.01.*

Table 6. Linear regression models in women and men: relationship between NS-PA and molecular biomarkers.

Molecular Biomarkers	NS-PA	
	WOMEN	**MEN**
	β (*p* value)	β (*p* value)
BDNF (pg/ml)	-0.18 (.186)	-0.09 (.580)
TNF-α (pg/ml)	0.26 (.052)	0.03 (.841)
HGF (pg/ml)	-0.23 (.062)	-0.08 (.572)
ICAM-1 (ng/ml)	-0.12 (.383)	-0.04 (.822)
SDF1-α (pg/ml)	0.26 (.058)	-0.16 (.321)

Note: BDNF: brain-derived growth factor; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; NS-PA: non-sportive physical activity; SDF1-α: stromal cell-derived factor-1 alpha protein; TNF-α: tumor necrosis alpha.
Covariates: age, years of education and body mass index.
NS-PA is measured in metabolic equivalent of task units.

Table 7. Data regarding linear regression models for the relationship between PA variables and brain volumes in women and men.

Brain Volumes (mm^3)	WOMEN		MEN	
	S-PA	**CRF**	**S-PA**	**CRF**
	R^2,(df)F, *p* value	R^2,(df)F, *p* value	R^2,(df)F, *p* value	R^2,(df)F, *p* value
Ventricles	0.30, (5,52)4.41, .002	0.26, (5,52)3.73, .006	0.49, (5,29)5.48, .001	0.61, (5,29)8.89, <.001
Total White Matter	0.83, (5,52)49.73, <.001	0.82, (5,52)50.30, <.001	0.82, (5,29)25.92, <.001	0.82, (5,29)25.77, <.001
Frontal Lobe	0.86, (5,52)63.06, <.001	0.86, (5,52)62.84, <.001	0.84, (5,29)29.52, <.001	0.85, (5,29)31.99, <.001
Dorsolateral Prefrontal Cortex	0.72, (5,52)26.17, <.001	0.70, (5,52)24.31, <.001	0.71, (5,29)14.10, <.001	0.71, (5,29)14.11, <.001
Cingulate Cortex	0.71, (5,52)25.84, <.001	0.70, (5,52)24.63, <.001	0.76, (5,29)18.74, <.001	0.75, (5,29)16.96, <.001
Parietal Lobe	0.83, (5,52)49.22, <.001	0.83, (5,52)50.13, <.001	0.89, (5,29)48.87, <.001	0.90, (5,29)54.16, <.001
Precuneus	0.48, (5,52)9.43, <.001	0.48, (5,52)9.55, <.001	0.66, (5,29)11.12, <.001	0.69, (5,29)12.59, <.001
Temporal Lobe	0.83, (5,52)51.66, <.001	0.82, (5,52)48.77, <.001	0.86, (5,29)34.56, <.001	0.85, (5,29)33.65, <.001
Hippocampus	0.44, (5,52)8.21, <.001	0.44, (5,52)8.10, <.001	0.47, (5,29)5.21, .002	0.47, (5,29)5.18, .002

Note: CRF: cardiorespiratory fitness; PA: physical activity; S-PA: sportive physical activity.
Covariates: age, years of education, intracranial brain volume and body mass index.
*S-PA is measured in metabolic equivalent of task units and CRF in ml/kg*min.*

Table 8. Data regarding linear regression models for the relationship between NS-PA and brain volumes in women and men.

Brain Volumes (mm^3)	NS-PA	
	WOMEN	**MEN**
	β (*p* value)	β (*p* value)
Ventricles	0.16 (.893)	-0.06 (.689)
Total White Matter	0.03 (.613)	-0.05 (.552)
Frontal Lobe	0.10 (.063)	-0.04 (.546)
Dorsolateral Prefrontal Cortex	0.03 (.685)	-0.08 (.379)
Cingulate Cortex	-0.03 (.703)	-0.03 (.771)
Parietal Lobe	-.18 (.002)**	-0.01 (.872)
Precuneus	0.02 (.880)	-0.03 (.768)
Temporal Lobe	0.03 (.639)	-0.02 (.790)
Hippocampus	<.01 (.999)	-0.24 (.052)

Note: NS-PA: non-sportive physical activity.
Covariates: age, years of education, intracranial brain volume and body mass index.
*NS-PA is measured in metabolic equivalent of task units and CRF in ml/kg*min.*
*** p <.01*

Table 9. Data regarding linear regression models for the relationship between PA variables and behavior outcomes in women and men.

Psychological Status & Daily Activity	WOMEN		MEN	
	S-PA	CRF	S-PA	CRF
	R^2,(df)F, *p* value	R^2,(df)F, *p* value	R^2,(df)F, *p* value	R^2,(df)F, *p* value
GDS	0.09, (3,60)2.00, .123	0.07, (3,60)1.46, .234	0.07, (3,35)0.84, .482	0.16, (3,35)2.15, .111
VAMS	0.02, (3,61)0.39, .761	0.06, (3,61)1.34, .270	0.18, (3,35)2.62, .066	0.26, (3,35)4.16, .013
S-IQCODE	0.00, (3,61)0.05, .986	0.03, (3,61)0.62, .606	0.06, (3,35)0.67, .574	0.06, (3,35)0.73, .541
PSQI	0.01, (3,61)0.25, .683	0.00, (3,61)0.05, .986	0.03, (3,35)0.38, .768	0.05, (3,35)0.57, .636
Total CORE-OM	0.12, (3,61)2.69, .054	0.11, (3,61)2.38, .078	0.06, (3,35)0.72, .545	0.05, (3,35)0.58, .636

Note: CORE-OM: Short Informant Questionnaire in Routine Evaluation-Outcome Measure (Trujillo et al., 2016); CRF: cardiorespiratory fitness; GDS: Geriatric Depression Scale (Martínez et al., 2002); PA: physical activity; PSQI: Pittsburgh Sleep Quality Index (Rico & Fernández, 1997); S-IQCODE: Short Informant Questionnaire on Cognitive Decline in the Elderly (Morales González et al., 1992); S-PA: sportive physical activity; VAMS: Visual Analog Mood Scale (Stern et al., 1997).
Covariates: age, years of education.
*S-PA is measured in metabolic equivalent of task units and CRF in ml/kg*min.*

Table 10. Data regarding linear regression models for the relationship between NS-PA and behavior outcomes in women and men.

Psychological Status & Daily Activity	NS-PA	
	WOMEN	MEN
	β (*p* value)	β (*p* value)
GDS	0.11 (.381)	0.24 (.111)
VAMS	0.04 (.766)	0.04 (.814)
S-IQCODE	0.14 (.263)	0.13 (.414)
PSQI	0.10 (.433)	0.07 (.658)
Total CORE-OM	0.20 (.094)	-0.05 (.756)

Note: CORE-OM: Short Informant Questionnaire in Routine Evaluation-Outcome Measure (Trujillo et al., 2016); GDS: Geriatric Depression Scale (Martínez et al., 2002); NS-PA: non-sportive physical activity; PSQI: Pittsburgh Sleep Quality Index (Rico & Fernández, 1997); S-IQCODE: Short Informant Questionnaire on Cognitive Decline in the Elderly (Morales González et al., 1992); VAMS: Visual Analog Mood Scale (Stern et al., 1997).
Covariates: age, years of education.
NS-PA is measured in metabolic equivalent of task units.

Table 11. Participants characteristics in the ITT sample at baseline.

	Total	AE	CCT	COMB	Control	t-Test,
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	***p* value**
n total / n females	92 / 58	30 / 16	24 / 17	23 / 17	15 / 8	$X^2(3) = 3.61$, $p = .306$
Age (years)	57.91 (5.50)	57.90 (5.22)	57.63 (5.38)	59.09 (5.79)	56.60 (5.97)	H(3) = 1.66, $p = .645$
Years of education (years)	12.76 (5.40)	13.15 (5.56)	12.04 (4.83)	12.43 (5.04)	13.60 (6.72)	H(3) = 1.05, $p = .788$
Vocabulary subtest (direct score-WAIS-III)	44.04 (8.02)	43.59 (8.91)	43.88 (7.26)	44.96 (7.40)	43.80 (8.98)	F(3,87) = 0.14, $p = .939$

Note: AE: Aerobic exercise; CCT: Computerized cognitive training; COMB: Combined training; ITT: intention-to-treat sample; WAIS-III: Wechsler Adult Intelligence Scale III.

Table 12. Comparison of demographic data between ITT and PP sample.

Groups		n total /n females		Age (years)		Years of education		Vocabulary subtest WAIS-III	
Total Sample[1]	ITT	92 / 58	$X^2(1) = 0.01$, $p = .908$	57.91 (5.50)	U = 3582.50, $p = .567$	12.76 (5.40)	U = 3637.50, $p = .648$	44.04 (8.02)	U = 3641.00, $p = .891$
	PP	82 / 51		58.38 (5.47)		12.52 (5.57)		44.14 (8.30)	
AE	ITT	30 / 16	$X^2(1) = 0.01$, $p = .921$	57.90 (5.22)	t(53) = -0.36, $p = .723$	13.15 (5.56)	t(53) = 0.46, $p = .644$	43.59 (8.91)	t(51) = -0.13, $p = .897$
	PP	25 / 13		58.40 (5.12)		12.44 (5.75)		43.92 (9.53)	
CCT	ITT	24 / 17	$X^2(1) = 0.01$, $p = .924$	57.63 (5.38)	t(45) = -0.19, $p = .854$	12.04 (4.83)	t(45) = -0.00, $p = .999$	43.88 (7.26)	t(45) = -0.18, $p = .855$
	PP	23 / 16		57.91 (5.31)		12.04 (4.94)		44.26 (7.16)	
COMB	ITT	23 / 17	$X^2(1) = 0.00$, $p = .987$	59.09 (5.79)	t(40) = -0.70, $p = .489$	12.43 (5.04)	U = 213.00, $p = .888$	44.96 (7.40)	t(40) = 0.18, $p = .858$
	PP	19 / 14		60.32 (5.54)		12.37 (5.43)		44.53 (8.02)	

Note: AE: Aerobic exercise; CCT: Computerized cognitive training; COMB: Combined training; ITT: intention-to-treat sample; PP: per protocol sample; WAIS-III: Wechsler Adult Intelligence III.
[1]Control group is the same in ITT and PP sample.

Table 13. Group comparison at baseline: molecular markers at Level 1.

Variables	Groups	n	Mean	SD	ANOVA / H de Kruskall Wallis
BDNF	AE	20	4102.85	2875.20	H(3) = 1.88, $p = .597$
	CCT	23	5362.52	5648.96	
	COMB	17	3878.65	3521.72	
	Control	14	7036.71	6843.76	
TNF-α	AE	25	0.76	0.22	H(3) = 2.751, $p = .432$
	CCT	23	0.76	0.23	
	COMB	19	0.77	0.21	
	Control	15	0.86	0.23	
HGF	AE	25	1044.17	211.37	H(3) = 1.785, $p = .618$
	CCT	23	1088.67	294.30	
	COMB	19	1012.87	190.02	
	Control	15	1202.16	461.63	
ICAM-1	AE	25	183.65	46.18	H(3) = 3.533, $p = .317$
	CCT	23	199.26	59.23	
	COMB	19	192.52	33.51	
	Control	15	209.43	46.50	
SDF-1-α	AE	25	1690.36	352.13	H(3) = 8.809, $p = .032$
	CCT	23	1573.73	357.46	
	COMB	19	1799.50	249.63	
	Control	15	1528.19	378.00	

Note: AE: Aerobic exercise; BDNF: brain-derived growth factor; CCT: Computerized cognitive training; COMB: Combined training; HGF: hepatocyte growth factor; ICAM-1: intercellular cell adhesion molecule-1; SDF1-α: stromal cell-derived factor-1 alpha protein; TNF-α: tumor necrosis alpha.

Table 14. Group comparison at baseline: brain volume markers at Level 2.

Variables	Groups	n	Mean	SD	ANOVA / H de Kruskall Wallis
Ventricles	AE	23	25685.17	7956.12	H(3) = 1.79, *p* = .618
	CCT	19	24435.68	8863.03	
	COMB	18	31135.22	18069.32	
	Control	14	32700.07	25718.23	
Total White Matter	AE	23	166776.35	15774.44	H(3) = 1.22, *p* = .747
	CCT	19	164346.89	22250.41	
	COMB	18	168286.06	15338.20	
	Control	14	168022.29	17676.56	
Frontal Lobe	AE	23	259245.04	26322.05	F(3,70) = 1.06, *p* = .370
	CCT	19	250909.84	29807.35	
	COMB	18	259359.61	24133.85	
	Control	14	267289.79	2341.23	
Dorsolateral Prefrontal Cortex	AE	23	28906.61	3102.48	F(3,70) = 1.87, *p* = .144
	CCT	19	28273.47	3796.36	
	COMB	18	29472.83	2679.67	
	Control	14	30755.14	2404.55	
Cingulate Cortex	AE	23	37448.04	4553.70	H(3) = 3.38, *p* = .336
	CCT	19	36007.37	2865.67	
	COMB	18	37538.17	4944.15	
	Control	14	38820.86	4972.64	
Parietal Lobe	AE	23	146732.48	13667.88	H(3) = 0.95, *p* =.814
	CCT	19	143949.68	13134.27	
	COMB	18	146130.44	13771.51	
	Control	14	147455.57	12404.42	
Precuneus	AE	23	20884.17	2408.45	H(3) = 3.18, *p* = .364
	CCT	19	19815.32	2633.22	
	COMB	18	20679.00	2208.34	
	Control	14	20683.64	2889.26	
Temporal Lobe	AE	23	170217.52	17892.31	F(3,70) = 0.72, *p* = .545
	CCT	19	165355.53	17157.27	
	COMB	18	168624.50	16474.14	
	Control	14	174026.07	16926.58	
Hippocampus	AE	23	7349.96	899.55	F(3,70) = 1.21, *p* = .313
	CCT	19	7004.68	841.45	
	COMB	18	6947.56	704.79	
	Control	14	7280.57	641.06	

Note: AE: Aerobic exercise; CCT: Computerized cognitive training; COMB: Combined training.

Table 15. Group comparison at baseline: PA outcomes and CRF.

Variables	Groups	n	Mean	SD	ANOVA / H de Kruskall Wallis
CRF	AE	19	25.25	10.16	F(3,67) = 1.08, *p* = .362
	CCT	20	26.11	12.50	
	COMB	17	27.34	8.75	
	Control	15	20.65	12.69	
S-PA	AE	25	451.98	699.40	H(3) = 2.92, *p* = .404
	CCT	23	439.83	713.63	
	COMB	19	778.79	908.77	
	Control	15	366.80	618.17	
NS-PA	AE	25	5595.73	3918.34	H(3) = 7.96, *p* = .047
	CCT	23	9113.74	7104.64	
	COMB	19	10295.68	6159.04	
	Control	15	7038.40	6628.45	

Note: AE: Aerobic exercise; CCT: Computerized cognitive training; COMB: Combined training; CRF: cardiorespiratory fitness; NS-PA: non-sportive physical activity; PA: physical activity; S-PA: sportive physical activity.

Table 16. Group comparison at baseline: z-scores of cognitive domains.

Variables	Groups	n	Mean	SD	ANOVA / H de Kruskall Wallis
Executive Function	AE	24	-0.01	0.72	F(3,75) = 0.81, *p* = .492
	CCT	23	0.16	0.60	
	COMB	19	-0.08	0.50	
	Control	13	-0.15	0.81	
Flexibility	AE	25	0.10	1.05	H(3) = 1.53, *p* = .676
	CCT	23	-0.10	1.06	
	COMB	19	-0.03	0.80	
	Control	14	0.04	1.12	
Fluency	AE	25	0.02	0.91	F(3,77) = 1.19, *p* = .318
	CCT	23	0.22	0.76	
	COMB	19	-0.10	0.82	
	Control	14	-0.28	0.80	
Inhibition	AE	24	-0.06	1.03	F(3,77) = 1.18, *p* = .323
	CCT	23	0.32	0.86	
	COMB	19	-0.16	1.18	
	Control	15	-0.20	0.89	
Working Memory	AE	25	-0.06	1.10	H(3) = 1.29, *p* = .732
	CCT	23	0.14	1.05	
	COMB	19	-0.01	0.69	
	Control	15	-0.11	1.16	
Visuospatial Function	AE	25	0.14	0.93	H(3) = 3.40, *p* = .334
	CCT	23	-0.20	1.04	
	COMB	19	-0.19	1.24	
	Control	15	0.32	0.62	
Language	AE	25	0.01	1.09	H(3) = 0.71, *p* = .870
	CCT	23	-0.04	1.08	
	COMB	19	-0.10	0.91	
	Control	15	0.16	0.89	
Attention-Speed	AE	24	-0.02	1.01	H(3) = 2.34, *p* = .506
	CCT	23	0.13	0.65	
	COMB	19	-0.17	0.68	
	Control	14	0.09	0.44	
Attention	AE	25	0.03	0.95	F(3,77) = 0.70, *p* = .553
	CCT	23	0.15	0.71	
	COMB	19	-0.20	0.76	
	Control	14	0.03	0.62	

Continued

Speed	AE	24	-0.12	1.21	H(3) = 1.64, *p* = .650
	CCT	23	0.11	0.77	
	COMB	19	-0.12	0.74	
	Control	15	0.17	0.32	
Memory	AE	25	0.03	0.70	H(3) = 0.95, *p* = .815
	CCT	23	0.13	0.66	
	COMB	19	-0.14	0.92	
	Control	14	-0.09	0.95	
Visual Memory	AE	25	0.17	1.05	F(3,78) = 1.00, *p* =.398
	CCT	23	0.06	0.98	
	COMB	19	-0.34	1.17	
	Control	15	0.04	0.65	
Verbal Memory	AE	25	-0.04	0.71	H(3) = 0.70, *p* = .874
	CCT	23	0.16	0.79	
	COMB	19	-0.04	1.08	
	Control	14	-0.14	1.29	
Global Cognitive Function	AE	23	0.01	0.73	F(3,74) = 0.51, *p* = .675
	CCT	23	0.11	0.56	
	COMB	19	-0.13	0.57	
	Control	13	-0.03	0.59	

Note: AE: Aerobic exercise; CCT: Computerized cognitive training; COMB: Combined training.

ANNEX 3: SUPPLEMENTARY FIGURES

WOMEN

TABLE DATA USED FOR FIGURE

data	data	1	2	3
data	data	255,165,0	50,205,50	144,238,144
data	data	CRF	S-METS	NS-METS
4	EF	3	30	7
5	Inh	12	22	13
6	W-M	8	17	5
7	Flex	16	23	5
8	Fl	14	32	3
9	VF	4	13	9
10	MMR	20	5	24
11	Vb-M	24	3	32
12	Vs-M	4	4	1
13	LNG	5	16	3
14	ATT-SP	13	27	1
15	Att	9	26	3
16	Sp	15	22	2

MEN

TABLE DATA USED FOR FIGURE

data	data	1	2	3
data	data	255,165,0	50,205,50	144,238,144
data	data	CRF	S-METS	NS-METS
4	EF	60	42	11
5	Inh	37	8	3
6	W-M	45	40	14
7	Flex	29	13	20
8	Fl	64	43	11
9	VF	24	10	3
10	MMR	49	26	15
11	Vb-M	50	26	13
12	Vs-M	19	14	14
13	LNG	39	28	19
14	ATT-SP	56	36	5
15	Att	47	32	13
16	Sp	50	30	11

Figure 1. Partial correlations (%) between physical status outcomes and cognitive domains adjusting for age and years of education for men and women. Screenshot of the tables introduced in Circos platform to obtain Figure 16. *Att: attention; ATT-SP: attention-speed; CRF: cardiorespiratory fitness; EF: executive function; Fl: fluency; Flex: flexibility; Inh: inhibition; LNG: language; MMR: memory; NS-PA: non-sportive physical activity; Sp: speed; S-PA: sportive physical activity; Vb-M: verbal memory; VF: visuospatial function; Vs-M: visual memory; WM: working memory.*

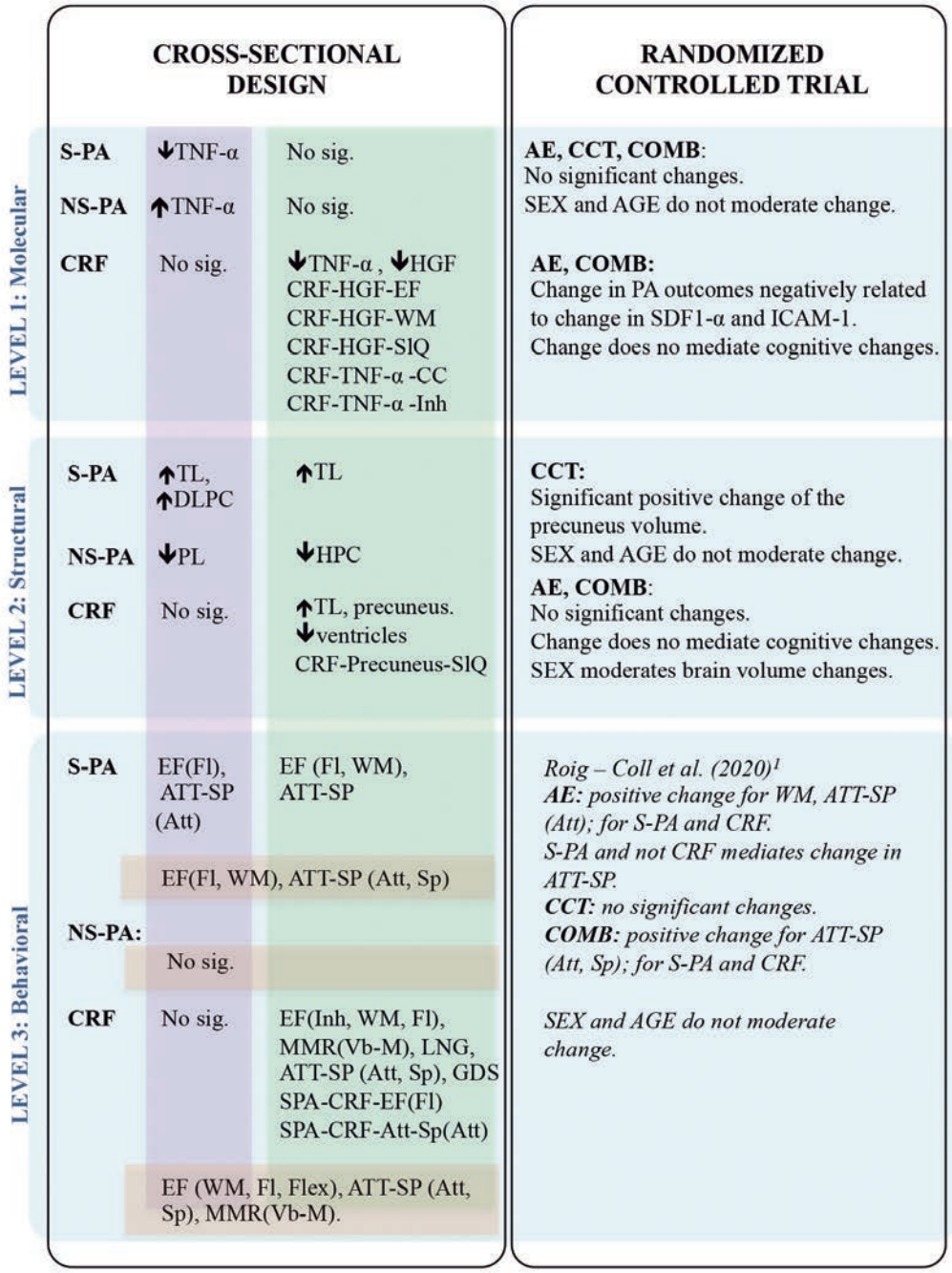

Figure 2. Summary of Projecte Moviment Research Project results, created by A. Castells-Sánchez. *This figure is printed like a flyer in the printed version of the thesis. Purple box indicates women, green box indicates men, orange box indicates total sample. AE: aerobic exercise; Att: attention; ATT-SP: attention-speed; CC: cingulate cortex; CCT: computerized cognitive training; CRF: cardiorespiratory fitness; COMB: combined training; DLPC: dorsolateral prefrontal cortex; EF: executive function; Fl: fluency; Flex: flexibility; GDS: Geriatric Depression Scale; HGF:hepatocyte growth factor; HPC: hippocampus; ICAM-1: intercellular cell adhesion molecule-1; Inh: inhibition; LNG: language; MMR: memory; NS-PA: non-sportive physical activity; PA: physical activity; PL: parietal lobe; SDF1-α: stromal cell-derived factor-1 alpha protein; sig: significant; SlQ: Sleep Quality; Sp: speed; S-PA: sportive physical activity; TL: temporal lobe; TNF-α: Tumor Necrosis Factor Alpha; Vb-M: verbal memory; WM: working memory. [1]Results reported in Roig-Coll et al. (2020), which belongs to Projecte Moviment but it is not included in the current thesis.*