Immediate effects of a single session of physical exercise on cognition and cerebral blood flow: A randomized controlled study of older adults

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\textbf{A R T I C L E  I N F O}

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\textbf{A B S T R A C T}

\textbf{Background:} Regular physical activity is beneficial for cognitive performance in older age. A single bout of aerobic physical exercise can transiently improve cognitive performance. Researchers have advanced improvements in cerebral circulation as a mediator of long-term effects of aerobic physical exercise on cognition, but the immediate effects of exercise on cognition and cerebral perfusion are not well characterized and the effects in older adults are largely unknown.

\textbf{Methods:} Forty-nine older adults were randomized to a 30-min aerobic exercise at moderate intensity or relaxation. Groups were matched on age and cardiovascular fitness (VO\textsubscript{2} max). Average Grey Matter Blood Flow (GMBF), measured by a pulsed arterial-spin labeling (pASL) magnetic resonance imaging (MRI) acquisition, and working memory performance, measured by figurative n-back tasks with increasing loads were assessed before and 7 min after exercising/resting.

\textbf{Results:} Accuracy on the n-back task increased from before to after exercising/resting regardless of the type of activity. GMBF decreased after exercise, relative to the control (resting) group. In the exercise group, higher n-back performance after exercise was associated with lower GMBF in the right hippocampus, left medial frontal cortex and right orbitofrontal cortex, and higher cardiovascular fitness was associated with lower GMBF.

\textbf{Conclusion:} The decrease of GMBF reported in younger adults shortly after exercise also occurs in older adults and relates to cardiovascular fitness, potentially supporting the link between cardiovascular fitness and cerebrovascular reactivity in older age.

1. Introduction

Physical activity is associated with better cognitive performance (Costa et al., 2019; Liu-Ambrose et al., 2018; Moreau and Chou, 2019) and reduced risk for dementia (Liu-Ambrose et al., 2018), depression (Kandola et al., 2019) and stroke (Kramer et al., 2019). A single bout of physical exercise at moderate intensity (3–6 metabolic equivalents (METs; i.e. requiring three to six times as much energy per minute as resting) can improve cognitive performance immediately after and at least up until 30 min after exercising (Chang et al., 2012). These acute effects of physical exercise are particularly pronounced on working memory (WM) performance (Weng et al., 2015; Pontifex et al., 2009; Voss et al., 2020; Wheeler et al., 2019) and executive functions (Wheeler et al., 2019), as indicated by intervention studies in younger adults and randomized controlled trials in older adults. The immediate exercise-induced effect on cerebral blood flow might play a preponderant role in boosting cognitive performance after a single bout of physical activity (Davenport et al., 2012), contributing to augmenting the supply of excitatory neurotransmitters in key brain regions shortly after exercise (McMorris and Hale, 2012; Vecchio et al., 2018; Szalewska et al., 2017), that may then enhance cognition by influencing cortical arousal (Vecchio et al., 2018; Szalewska et al., 2017). The relationship between exercise and cognition follows an inverted U-shape, with moderate-intensity exercise being the most effective in inducing short- and long-term positive outcomes on cognition (Chang et al., 2012). Aerobic exercise has been indicated as the most efficient, possibly exerting benefi-

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cial effects on executive functions and WM already after interventions of less than 16 weeks (Cabral et al., 2019), with small to moderate effects (Sanders et al., 2019).

Although the positive effects exerted on cognition are observed across the entire lifespan, exercise seems to be more efficient in boosting cognitive performance in children and older adults, when executive functions are respectively developing or declining (Liu-Ambrose et al., 2018; Audiffren and Andre, 2019). A recent meta-analysis of randomized controlled trials in adults has highlighted the importance of age in moderating the effect of regular physical activity interventions, stressing that positive effects on cognition are particularly pronounced in older adults over 65 years (Rathore and Lom, 2017). Fronto-parietal brain regions, key contributors to executive functions, are in fact particularly vulnerable to aging, and might therefore be more sensitive to the impact of moderators such as exercise (Audiffren and Andre, 2019). Moreover, association areas such as the medial frontal regions and the limbic cortices, which are also involved in executive functions, attention, learning and memory, are the most affected by the decline in resting cerebral blood flow occurring with aging (Bentourkia et al., 2000; Chen et al., 2011).

Researchers have suggested that angiogenesis and improvements in cerebral circulation may be crucial for the long-term beneficial effects of physical exercise on cognition (Vecchio et al., 2018; Tnydall et al., 2018). Cerebral blood flow declines with age, as consequence of arterial stiffness, lower brain engagement and activity at rest and possibly synaptic loss (Bentourkia et al., 2000). Cerebrovascular reserve, defined as the ability of the cerebral blood vessels to dilate in response to a change in metabolic demand, is also impaired in aging (Tnydall et al., 2018). Regular exercise may thus help counteract the detrimental impact of aging on brain health and circulation (Barnes and Corkery, 2018), by increasing global cerebral blood flow, particularly by enhancing blood flow velocity through arteries supplying the neocortex, and/or by improving cardiovascular reactivity (Wheeler et al., 2019; Tarumi et al., 2015; Brugniaux et al., 2014). However, the relationship of cerebral and peripheral vascular function with cognitive function is still debated (Tnydall et al., 2018; Tarumi et al., 2015; Lucas et al., 2012; Young et al., 2015). Importantly, such relationship seems to be mediated by several factors, not least the cognitive health status of the individual (Stillman et al., 2020). Positive effects of long-term physical exercise on cognition have been reported in healthy older adults as well as in older adults with mild cognitive impairment (MCI), though with different underlying modifications in the cerebral blood flow (Alfini et al., 2019). Indeed, adults with MCI had a higher blood flow in the insula compared with healthy adults, that decreased to control levels after 12 weeks of exercise (Alfini et al., 2019). Healthy older adults showed instead increased blood flow in the right anterior cingulate cortex after the exercise program (Alfini et al., 2019). On the other hand, older adults with already developed mild-to-moderate Alzheimer disease showed no impact of physical activity on cerebral blood flow after a 16-week moderate-to-high intensity exercise program (van der Kleij et al., 2018).

Long-term interventions featuring repeated sessions of physical activity are however likely to engage a variety of compensatory and plasticity-based brain mechanisms and are not directly informative of the pathways behind the effects of physical exercise (Basso and Suzuki, 2017). Thus, the need for a more in-depth investigation of the effects of single sessions of physical exercise, particularly if aided by the comparison with a resting control group matched for baseline cardiovascular fitness, has been highlighted (Stimpson et al., 2018).

However, the immediate effects of single sessions of physical exercise on cerebrovascular and cognitive function are not well described so far, and the effects in the healthy older population are largely unknown (Rathore and Lom, 2017; Basso and Suzuki, 2017). The paucity of studies on the immediate effects of exercise in older age might have contributed to the limited possibility for meta-analytic evidence concerning the cognitive effects (or lack thereof) of single sessions of physical activity so far (Rathore and Lom, 2017; Basso and Suzuki, 2017). Moreover, little is known about the immediate effect of physical exercise on cerebrovascular responses and their relation to cognition. Similar to cognition, the cerebrovascular response to physical exercise also depends on the intensity of exercise. The increase in cerebral blood flow in fact mirrors the intensity of the physical exertion up to 60% of maximal oxygen uptake (Ogoh and Ainslie, 2009; Querido and Sheel, 2007). Beyond that point, exercise-induced hyperventilation and sympathetically-mediated protective mechanisms trigger cerebral vasoconstriction, causing a plateau or even a reduction in cerebral flow (Ogoh and Ainslie, 2009; Querido and Sheel, 2007). Independent of the intensity of exertion, the post-exercise hypotension and consequential drop in cardiac output lead to a quick decrease in cerebral blood flow, detectable a few seconds after the cessation of the exercise bout (Querido and Sheel, 2007). At least in young adults, up to forty minutes might be necessary for the grey matter blood flow to resume its baseline levels (Maclntosh et al., 2014), following a physiological re-boost and reactive vasodilation induced by cerebral hypoxia and by the normalization of the cardiac output (Querido and Sheel, 2007). However, findings are still conflicting. Increases of approximately 20% in global cerebral blood flow compared with baseline after moderate intensity exercise have also been reported in young adults, lasting up to 30 min after exercise (Smith et al., 2010). Local increases by 10–12% in blood flow have also been reported to occur in the hippocampus of young and middle-aged adults at 15, 40 and 60 min after a single bout of moderate intensity physical exercise (Steventon et al., 2020). Worth noting, all these studies have measured cerebral blood flow of individuals in a supine position in the scanner, indicating that perfusion changes can be detected even when laying down, when the impact of cardiac output and vascular pressure on the brain is not as critical as in the upright position. Moreover, the exercise-related regulation of cerebral blood flow in older age is still unclear. Older adults have elevated hypocapnic responses (Stefanidis et al., 2019) (i.e. they are more prone to reacting with cerebral vasoconstriction in response to hyperventilation) and lower partial pressure of carbon dioxide (PaCO2) compared with young adults (Braz and Fisher, 2016). Cerebrovascular reactivity also seems to impact cognition differently in younger and older age (Catchlove et al., 2018).

To our knowledge, no studies have examined immediate exercise-related changes on cerebral blood flow and cognition in older adults. Given the potential applications of exercise as treatment aid for mild cognitive impairment and dementia prevention (Alfini et al., 2019; Geda et al., 2010), it is of great importance to understand its impact on cerebral perfusion in older adults. To bridge this knowledge gap, we therefore investigated the effects of a single session of moderate intensity physical exercise on cerebral blood flow and WM performance in older adults. Participants, matched on age and cardiovascular fitness (VO2 max), were randomized to undergo 30-min of exercising or resting. We hypothesized that exercise would improve WM performance and increase average grey matter blood flow (GMBF) compared with resting, and that the increase in GMBF would positively correlate with the improvement in cognitive performance. We also hypothesized that increases in pulse pressure would be positively associated with increases in GMBF. The study was pre-registered in the Open Science Foundation database (osf.io/ve2zj).

2. Methods

2.1. Ethics statement

The study was approved by the ethics committee of region Stockholm in Sweden (Regionala Etikprövningsnämnden i Stockholm, Dnr: 2018/1340-31/2) and complied with the principles of the Declaration of Helsinki.
Fig. 1. Flow-chart of the experimental protocol. The flow-chart reports the sequential sessions of the experimental protocols. The number of individuals participating in each session and the number of drop-outs are noted.

2.2. Subjects

Participants were recruited via advertisements in local newspapers and flyers in the Stockholm area between September and December 2018, seeking for older volunteers, aged between 65 and 75 years, for a study investigating the effects of exercise and resting on brain function. Two research assistants were responsible for the recruitment. A preliminary check for eligibility according to study criteria was performed via telephone interview of the interested participants. Inclusion criteria were the availability to attend all sessions, adequate hearing, normal or corrected vision, fluent Swedish (required to understand the instructions), adequate mobility in order to ensure they could lie still in the magnetic resonance imaging (MRI) scanner for the whole experimental session, right-handedness, and weight below 120 kg. Exclusion criteria were: previous or current neurological diseases, including Parkinson’s Diseases, dementia and epilepsy; a score below 26 on the Mini-Mental State Examination (MMSE) (Folstein et al., 1975); previous or current cardiovascular diseases, though blood pressure up to 200/100 mmHg was allowed; history of brain damage or stroke; uncontrolled metabolic diseases; type I or pharmacologically-treated type II diabetes (type II diabetes was allowed if treated only with dietary, non-pharmacological interventions); current cancer, unless more than one year had passed since the treatment end; psychiatric illness, except for a history of mild to moderate depression and/or anxiety allowed; history of head trauma resulting in loss of consciousness; previous participation in studies involving cognitive tests; presence of metal in the body; claustrophobia; sound sensitivity (due to MRI noise); neuromotor or musculoskeletal dysfunctions limiting or impeding the fitness test; cardiovascular-active medications potentially affecting maximal and submaximal fitness tests; ongoing infections; chest pain.

Individuals fulfilling the study criteria were invited to attend an introduction meeting at the Aging Research Center (Karolinska Institute, Stockholm). The protocol consisted of three sessions: introductory meeting, fitness characterization session, and experimental session (Fig. 1). Of the 73 individuals who had initially expressed their interest in the study, 50 joined the experimental session (Fig. 1). One was excluded due to MRI signs suggestive of past, undiagnosed stroke.

Sample size was determined by statistical power analyses (G’Power). The meta-analysis of McMorris and Hale (McMorris and Hale, 2012) indicated an effect of moderate exercise on cognitive performance of 0.5 SD, but this is likely an overestimation due to publication bias. Instead, we based our analyses on a conservative estimate of half of this effect (0.25), with smaller effects than this also being of limited interest in this context. Assuming measurement reliability and correlation among repeated measures of 0.8 for our cognitive performance measures (Lebedev et al., 2018) and an alpha level of 0.05, we needed a total sample size of 54 (27 per group) to detect this effect with a power of 0.8. The final sample size was 51 individuals. At a post-hoc power analysis with the same above-stated parameters, the power actual power resulted to be 0.79. More details can be found in the preregistration.

2.3. Behavioral and demographic measures

During the introductory meeting, participants received detailed information concerning the study. Relaxation exercises have also been reported to exert positive effects on cognition (Ma et al., 2017; Ferreira et al., 2015; Tang et al., 2007; Gard et al., 2014; Galvin et al., 2006; Siponkoski et al., 2019), and the participants were not informed about the study hypothesis (i.e. whether moderate intensity physical exercise was expected to be superior to resting in improving cognition and brain markers), with the aim of minimizing the influence of expectations on the cognitive performance measurements. Next they signed the informed consent form. All participants filled in questionnaires focused on demographic information, as well as the Edinburgh Handedness Inventory to confirm right-handedness and the MMSE. Habitual physical activity was assessed with the International Physical Activity Questionnaire (IPAQ) (Craig et al., 2003). Participants were also asked to rate their memory, to rate their memory compared to when they were 20 years old, and to rate their agreement with the following sentence: “I think we have a certain mental ability that cannot be changed to any greater degree”. Blood pressure was also measured. Instructions on how to perform the n-back task to be done in the main experimental session were also handed out, and participants could try it out for 5 min.

2.4. Fitness characterization

Prior to the fitness characterization, a second health screening was performed via telephone interview by a physician at Åstrand Laboratory at the Swedish School of Sport and Health Sciences, GIH. The fitness characterization was performed at GIH. Participants performed a submaximal incremental test on a cycle ergometer and a maximal incremental running test on a treadmill to assess their VO2max and maximal heart rate (Nilsson et al., 2020). The heart rate, O2 and CO2-data acquired during the submaximal cycling were used to identify a work rate and heart rate at which the VO2 reached around 60% of that recorded during the maximal running test. This work rate is expected to result in a perceived level of exertion from 13 to 15 on the Borg RPE scale and a heart rate of around 60–70% of maximal individual heart rate. The submaximal test on the bike was not used to estimate VO2max, but to identify the individual work rate and heart rate during biking at which the VO2 reached around 60% of the VO2max recorded during the maximal running test. This individual work rate and heart rate was then used during biking in the exercise intervention of the experimental session. This was necessary since we did not record VO2 during the experimental sessions.

The submaximal test was performed on a cycle ergometer (model 828E, Monark, Varberg, Sweden). The protocol started with 4 minutes cycling at a standard work rate (resistance of 0.5 kp, 60 rpm), after which the resistance was increased in steps of 0.5–1 kp, until reaching
60–80% of individual maximal capacity and a rate of perceived exertion (RPE) of around 16 (and not above 16).

The maximal incremental running test on a treadmill was used to directly measure VO2 max. Participants warmed up for 5–10 min before starting the test. The treadmill was initially set to an incline of 1° and a comfortable speed (around RPE 12–13). Incline degree and/or speed were increased every minute until volitional exhaustion. Participants wore a safety harness to prevent falling and increase their confidence in reaching exhaustion. VO2 max was measured using a computerized metabolic system (Jaeger Oxycor Pro, Hoechberg, Germany). According to previous studies (Bjorkman et al., 2016; Ekblom-Bak et al., 2014), the fulfilment of three out of five of the following criteria was required to accept the validity of the VO2max measurement: (a) VO2 was leveling off despite an increase in speed or decline, (b) RPE exceeded 16, (c) a respiratory exchange ratio exceeded 1.1, (d) maximal HR within ±15 beats per minute from age-predicted maximal HR and (e) a work with time above 6 minutes was performed. The highest 30 seconds of registered values of VO2 were referred to as VO2 max.

2.5. Randomization and blinding

Before the experimental session, participants were randomized (1:1) to two groups: resting and exercising condition. Age and VO2max were used as stratifiers. The randomization was conducted using label shuffling with post-hoc non-parametric tests for the stratifiers, as described by Lebedev et al. (2020). The code for the randomization procedure was written by AL and used by AL and MVH (senior lab manager; see acknowledgments) to allocate the participants to the exercise or resting condition. The subjects were not aware of which of the paradigms represented control and active conditions. No blinding was required for the staff collecting the data for this study.

2.6. Experimental session protocol

No blinding was required for this study. The experimental session was performed at the Siemens Prisma 3-Tesla facility at the Karolinska University Hospital in Huddinge. Prior to the scanning, participants were allowed to practice the WM task for 10 min. MRI acquisition was carried out at baseline, and after 30 min of exercise or resting. Subjects randomized to the exercise group cycled on a stationary bike for 30 min, starting at the intensity estimated from the fitness characterization. Subjects randomly allocated to the resting condition were asked to lay down and relax for 30 min, while listening to relaxing music with water-sounds in the background. Heart rate was measured with a chest-strap heart rate monitor throughout the period between the scanning.

The post-test MRI scanning started seven minutes after the end of the; this time was necessary for placing, in a relaxed manner, the subject back in the scanner. Systolic and diastolic blood pressure was measured at four different time-points: before pre-test scanning; after pre-test scanning (before exercising/resting for 30 min); after physical exercise/resting (before the post-test scanning); and after post-test scanning. The imaging protocol consisted of grey matter blood flow assessment via a whole-brain 3D pulsed arterial-spin labeling (pASL) sequence, task-fMRI acquired during the performance of the n-back task; anatomical assessment via T1-weighted magnetization prepared gradient-echo sequence (MPRAGE). A routine clinical T2-weighted structural MRI was also acquired at pre-test for the neuroradiological assessment of the participants. For the current study and according to preregistration, only the pASL and the MPRAGE sequences were included in the analyses.

2.7. Adverse events

One subject exhibited unstable heart-beat after the maximal fitness test, and was brought to the hospital to seek medical attention. This subject was excluded from the randomization process and thus from the study. No other adverse events occurred during the physical activity procedures.

2.8. N-back task

N-back performance was measured before and after the exercise or resting session. The task consisted of a figural n-back task to assess WM performance. Three blocks with increasing cognitive load (1-back, 2-back, 3-back) were administered in an alternate fashion for three times, with a 4-s fixation screen between blocks indicating the rule for the forthcoming level. Each block lasted one minute. The performance on the task was expressed in terms of accuracy, calculated as:

\[(\text{correct hits} + \text{correct rejections})/\text{total stimuli};\]

Accuracy for the 1-back, 2-back, and 3-back was calculated, along with average accuracy across loads. Reaction time was also recorded. Prior to pre-test imaging, a short practice of the n-back task was performed when the subject was in the scanner. Due to a malfunctioning of the keyboard, the n-back data of nine participants could not be recorded.

2.9. MRI acquisition

Grey matter blood flow was recorded via a whole-brain 3D pulsed arterial-spin labeling (pASL) sequence (scan duration ≈ 5 min). Arterial spin labeling measures cerebral blood flow by magnetically labeling arterial water and using it as an endogenous tracer. The following parameters were used for the pASL acquisition: repetition time (TR) 4000 ms; time to echo (TE) 12 ms; inversion time (TI) 2000 ms; field of view (FOV) 128 × 128; 1.9 × 1.9 × 4.5 mm³; slice thickness 4.5 mm; 32 slices. T1-weighted magnetization prepared gradient-echo sequence (MPRAGE) was used to assess brain structure (scan duration ≈ 5 min), with the following parameters: TR 2300 ms; TE 2.01 ms; flip angle: 9°; FOV 240 × 256; voxel size: 1 mm³; slice thickness 1 mm; 208 slices.

2.10. ASL images pre-processing

Prior to pre-processing, ASL data were checked for outliers on movement. Root mean square signal change (DVARS) values, defined as the root mean square intensity difference of paired volumes (volume N to volume N + 1) (Power et al., 2012), were used to check for movement. Data-point with DVARS values exceeding the threshold of 75th percentile + 1.5 times the InterQuartile Range (IQR) were excluded. The number of data-points with excessive movement ranged between 0 and 15. Subjects with more than 30% of the data-points exceeding this threshold were removed. This led to the exclusion of three individuals, of which one subject belonged to the exercise group and two subjects belonged to the resting group.

ASL data were pre-processed with FSL (FMRIB Software Library), by using the oxford_asl command included in the BASIL (Bayesian Inference for Arterial Spin labeling MRI) toolbox to process ASL data (Chappell et al., 2009). Structural data were pre-processed according the fslnat pipeline, consisting of the following steps: (1) the images are reoriented to match the standard Montreal Neurological Institute (MNI) orientation; (2) the images are then cropped and (3) bias-field correction is applied, using the FAST (FMRIB’s Automated Segmentation Tool) algorithm (Zhang et al., 2001); (4) the images are linearly (using FLIRT - FMRIB’s Linear Image Registration Tool) (Jenkinson et al., 2002) and non-linearly (using FNIRT - FMRIB’s Non-linear Image Registration Tool) registered to the MNI space; (5) brain extraction is performed with the Bet Extraction Toolbox (BET) (Smith, 2002) and (6) tissue-type segmentation is carried out with the FAST algorithm for cortical structures and with FIRST (FMRIB’s Integrated Registration and Segmentation Tool) for subcortical structures. The pre-processed structural images were fed into the oxford_asl command in order to achieve the registration of the ASL data to the MNI standard space.
The oxfordasl command automatically performs a series of preprocessing steps on ASL data, in an automated pipeline. First, motion correction is performed. A tissue perfusion map is then produced, and registered to the MNI standard space by applying the parameters generated during the registration of the structural image and stored in a transformation matrix. The calibrated perfusion map is then generated using the proton density weighted image and setting the cerebrospinal fluid (CSF) as a reference to calculate the equilibrium magnetization of blood within a CSF mask, automatically generated from the segmentation of the structural image and normalized to the MNI space. A quality check was performed visually to ensure the good quality of the preprocessing. The calibrated images were used for subsequent statistical analyses, as they expressgrey matter blood flow as ml/100 g/min. Prior to statistical analyses, the images were smoothed with an 8 mm FWHM gaussian kernel.

2.11. Statistical analyses

To assess stability of the measurement over time, we calculated pre- to post-test correlations for average n-back accuracy, blood flow, and movement (mean DVARS) separately within each group. The data were not normally distributed, thus Spearman’s rho was used for correlation analyses. The threshold for significance was set at p < .05. Below we describe the preregistered hypotheses (H1) and research questions (RQ1) and the statistical analyses (confirmatory and exploratory, respectively) used to address them.

2.12. H1. Working memory performance will improve more after exercise than after resting

Statistical analyses were performed with SPSS (Statistical Package for Social Science), v26 (https://www.ibm.com/analytics/spss-statistics-software). Nine people had missing data on the n-back performance. The n-back subsample thus consisted of 20 people in each group.

Box plots were used to check for the presence of outliers on group×session×load combinations. Outliers were defined as mild for values between 1.5 and 3 IQR, and as extreme if outside 3 IQR. Five data-points reached the cut-off for being extreme outliers, while six additional data-points were categorized as mild outliers. These led to the identification of two extreme outliers and two mild outliers, all belonging to the resting group. For each outlier, corresponding data-points on both sessions were removed, as our interest lay in performance change over time. Extreme outliers were excluded, while mild outliers were retained as they can be reflective of the normal population variability, when the sample is not big enough to be representative of the whole population. Nonetheless, the analyses were run with and without mild outliers to confirm that results were not driven by the presence of outliers. As the presence of mild outliers did not impact the analysis, they were retained for subsequent correlation analyses.

A generalized linear mixed model (GLMM) with a binomial distribution and logit link function was used to analyse the task performance. The binomial distribution function specifies the number of times that an event occurs in a sequence of n independent trials. The number of correct answers (correct hits + correct rejection) over 90 trials (for each load) was entered as dependent variable. Group, time and load were entered as fixed factors; the group represented the between-subject factor, while time and load were within-subject factors. The subjects factor represented the random factor. A random intercept model was preferred over the random coefficient model given the low number of measurements for each individual (two time-points only) (Wright, 2017). A full factorial model was carried out to test for main effects, two-ways and three-ways interactions between the factors: group, time and load. The residual method was used for the denominator degrees of freedom estimation, and the robust estimation was chosen for coefficient estimates. The critical effects to test our hypothesis were the group×time and group×time×load interaction terms. The threshold for significance was set at p < .05. A GLMM, with the same factors and a linear distribution, was carried out to analyse reaction time (Table S4, Supplementary materials). This was an exploratory analysis, not reported in the preregistration of the current study.

Effect sizes were calculated on the statistically significant pairwise comparisons using Cohen’s d for paired observations (\(d=(M_1-M_2)/(\sqrt{(S_1^2+S_2^2)/2})\)), where \(M\) is the mean and \(S\) is the standard deviation. Effect sizes are regarded as (Funder and Ozer, 2019): very small (0.05), very small for the explanation of single events but potentially consequential in the not-very-long run; small (0.1) at the level of single events but potentially more ultimately consequential; medium (0.2) with some explanatory and practical use even in the short run and therefore even more important; large (0.3) and potentially powerful in both the short and the long run.

2.13. H2. GMBF will increase after exercise, compared with resting

Mean DVARS was calculated for each subject, and was tested for main effects of group, session, and group×session, to ensure that movement did not differ between groups and sessions. Mean GMBF was automatically calculated as part of the output of the oxfordasl and was imported in SPSS for further analyses. Mean GMBF was tested for normality of the distribution with Shapiro Wilk’s test. The data were not normally distributed (p = .008), and the distribution was right-skewed. A GLMM with a gamma distribution with log link function was used for the analysis. Group and session were entered as fixed factors; the group represented the between-subject factor, while session was set as within-subject factor. Subject identity was entered as random factor (random intercept model). Main effects of the intervention and session, and the effect of the group×session interaction were tested. The interaction effect was critical to test our hypothesis, as we expected GMBF to increase more in the exercise group than in the resting group. The residual method was used for the denominator degrees of freedom estimation, and the robust estimation was chosen for coefficient estimates. The threshold for significance was set at p < .05. Effect sizes were calculated as described above.

2.14. RQ1. What is the regional distribution of exercise-induced GMBF changes?

Voxel-wise analyses on ASL data were carried out in SPM 12. A flexible factorial design was used to test for the main effects of group (between-group factors) and session (within-group factor), and group×session interaction on GMBF (critical effect). A preliminary uncorrected threshold of p < .001 was applied (Woo et al., 2014). Voxels surviving such threshold were further corrected for family-wise error (FWE) rate at cluster level with a threshold of p < .05 (Woo et al., 2014). The analysis was restricted to grey matter.

2.15. H3. Changes in mean GMBF will correlate with working memory performance changes more strongly in the exercise group than in the resting group

According to our hypotheses, changes in the n-back performance would correlate with GMBF changes. N-back accuracy was averaged across blocks, and the difference from pre- to post-test was calculated. The subjects who were extreme outliers on one or more of the measurements were excluded. Additionally, two extreme outliers on the average accuracy, both belonging to the resting group, were also excluded. The change in GMBF was also computed. The analyses were run in two steps. As a first step, and according to the pre-registration (osf.io/ve2j), we tested for associations between n-back performance change and GMBF change, and for an group×GMBF change interaction effect. We used a generalized linear model, with the change in n-back performance set as dependent variable and the changes in GMBF and group set as factors. In
this model, the interaction effect tests for between-groups differences in the association between two variables (n-back performance and GMBF) and represented the critical effect for testing our hypothesis, as we expected the association to be stronger in the exercise than in the resting group.

As a second step, we performed GLMM analyses to further explore whether the association between the variables changed differently over time in the two groups. To this purpose, and similarly to the previous analysis, we used a generalized linear model where the number of correct answers was used as target variable, and the number of trials was entered as denominator. A full factorial model including main effects, two-ways and three-ways interaction terms for the factors group, session and GMBF was carried out. The threshold for significance was set at \( p < .05 \).

### 2.16. RQ2. Is the correlation between GMBF and working-memory performance regionally localized?

Correlations between mean n-back performance and GMBF were assessed separately in each group at each session with linear regression models in SPm 12. A preliminary uncorrected threshold of \( p < .001 \) was applied (Woo et al., 2014). Voxels surviving such threshold were further corrected for family-wise error (FWE) rate at cluster level with a threshold of \( p < .05 \) (Woo et al., 2014). Voxel-wise GMBF was set as dependent variable; mean n-back performance was set as sole independent variable.

### 2.17. H4. Changes in pulse pressure will correlate with GMBF changes, more strongly in the exercise group than in the resting group

We had also hypothesized that GMBF changes would be associated with pre- to post-test changes in pulse. The analyses were run following the same steps as detailed above. We first tested the association between GMBF change (normally distributed) and pulse change and group*pulse change (critical effect). Pulse pressure change was calculated as the difference between the pulse pressure measured at baseline, before the start of the experimental session (first measurement), and after the exercising or resting session, before the second MRI acquisition (third measurement). As a second step, we performed GLMM analyses to further test for associations between GMBF and pulse by using a full factorial model including main effects, two-ways and three-ways interaction terms for the factors: group, session and pulse. The threshold for significance was set at \( p < .05 \).

### 2.18. RQ3. Is VO2 max associated with mean GMBF?

To better elucidate the mechanisms underlying our findings related to exercise-induced effects on GMBF (see below), we decided to also explore the relationship between GMBF and cardiovascular fitness, as measured by the VO2 max (hypothesis not preregistered). To this purpose, and similarly as to what was detailed above, we first tested the association between GMBF change and VO2 max and group*VO2 max (critical effect). We then used GLMM analyses to further test for associations between GMBF and session*group*VO2 max. The threshold for significance was set at \( p < .05 \).

### 3. Results

#### 3.1. Sample descriptives

The exercise and resting groups were equivalent regarding age, sex, physical activity reports (IPAQ), MMSE score, blood pressure and subjective memory evaluation (Table 1). The subgroup whose n-back data were available was comparable to the total sample, both before and after the exclusion of the outliers (Table S1). Pulse pressure was measured at four time-points: (1) before the first MRI; (2) after the first MRI and before the exercising or resting session; (3) after the exercise or resting session and before the second MRI; (4) after the second MRI (Fig. 2; Fig. S4, Supplementary material). In both groups, pulse pressure showed a statistically significant increase after the first MRI (2), probably due to stress in relation to the experimental session (Trapp et al., 2014). After resting (3), pulse pressure in the resting group had reduced and reached the baseline levels (1), as expected following the physiological recovery of blood pressure indices after stress, aided by the resting session (Kaushik et al., 2006; Chafin et al., 2004; Santaela et al., 2006). On the other hand, pulse pressure was still higher than the baseline in the exercise group, as expected as result of the physical exercise (Sharman et al., 2005). Pulse pressure then increased again in the resting group during the second imaging session (4), where it reached the same levels as after the first MRI (2); on the opposite, pulse pressure decreased in the exercise group (4) as part of the post-exercise recovery phase (Sharman et al., 2005), going back to the baseline levels (1).

#### 3.2. Pre- to post-test correlations

Pre- to post-test correlations showed overall a good stability of the measurements over time. N-back accuracy at pre-test showed a statistically significant correlation with accuracy at post-test in both the exercise (Spearman’s Rho = 0.636) and in the resting group (Spearman’s Rho = 0.901). A statistically significant pre- to post-test correlation on GMBF was also present in both groups (Spearman’s Rho = 0.710 in the exercise group and Spearman’s Rho = 0.869 in the resting group). Mean DVARS also showed a statistically significant correlation from pre- to post-test (Spearman’s Rho = 0.734 in the exercise group and Spearman’s Rho = 0.475 in the resting group). Pre- to post-test correlations are represented in Figs. S1–S3, in Supplementary materials.

#### 3.3. H1. Working memory performance will improve more after exercise than after resting

In total, 234 data-points were included in the analysis. Statistically significant main effects of session and load were found on n-back performance, while the main effect of group was not statistically significant (Table 2; Figs. 2 and 3). A statistically significant improvement in performance was observed from pre-test to post-test, though the overall difference in accuracy was rather small (1.4% gain in accuracy from pre- to post-test; Cohen’s \( d = 0.042 \)). As expected, the performance decreased with increasing difficulty load regardless of session. Concerning the interaction terms, a statistically significant session*load effect was detected, indicating that the improvement in performance from pre- to post-test was limited to the 1-back task (Cohen’s \( d = 0.333 \); medium effect). No statistically significant effects of the group*session, intervention*load, or group*session*load interactions were found, although a trend for a better performance on the 3-back task for the exercise group compared with the resting group was found at both pre- and post-test. However, the confidence intervals (C.I.) relative to the post-

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Exercise (mean (SD))</th>
<th>Resting (Mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>24</td>
<td>25</td>
</tr>
<tr>
<td>Age (years)</td>
<td>69.6 (2.8)</td>
<td>70.7 (3.1)</td>
</tr>
<tr>
<td>VO2 max (ml/min/kg)</td>
<td>31.4 (5.5)</td>
<td>32.3 (5.6)</td>
</tr>
<tr>
<td>MMSE</td>
<td>28.9 (1.2)</td>
<td>28.4 (1.0)</td>
</tr>
<tr>
<td>IPAG (MET/min/week)</td>
<td>2731.0 (1442.0)</td>
<td>3268.2 (2027.0)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>136.1 (16.4)</td>
<td>140.8 (16.7)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>87.8 (11.1)</td>
<td>86.5 (9.7)</td>
</tr>
<tr>
<td>Memory evaluation</td>
<td>3.6 (0.7)</td>
<td>3.6 (0.7)</td>
</tr>
<tr>
<td>Memory vs 20 years old</td>
<td>3.1 (0.9)</td>
<td>3.4 (0.7)</td>
</tr>
<tr>
<td>Plasticity belief</td>
<td>2.1 (0.9)</td>
<td>2.1 (1.2)</td>
</tr>
<tr>
<td>Sex (females; males)</td>
<td>50:50</td>
<td>40:60</td>
</tr>
<tr>
<td>Marital status (married)</td>
<td>66.7</td>
<td>56</td>
</tr>
</tbody>
</table>
test comparisons were very close to encompassing zero (95% C.I. (min, max)=[0.0005, 0.074]), suggesting that other factors might have influenced the baseline measurements, followed by a regression to the mean at post-test. When excluding mild outliers from the analysis, the results were mostly unchanged (Table S2), though the trend for a difference on 3-back performance at post-test was no longer observable. Overall, our hypothesis that n-back performance would improve more after exercise than after resting was not supported by our findings.

3.4. H2. GMBF will increase after exercise, compared with resting

After the removal of motion outliers, each group consisted of 23 individuals, for a total of 92 data-points. No statistically significant effects of group, session or group session were found on movement (mean DVARS). A statistically significant effect of the group session interaction on GMBF was found (Table 3, Fig. 4). In particular, reduced GMBF was found in the exercise group at post-test compared to pre-test (Cohen’s $d = 0.302$; medium effect), while no statistically significant pre- to post-test differences were observed in the resting group. Moreover, no between-groups differences were detected either at pre-test or post-test. No effects of group or session were found. These findings were conflicting with our initial hypothesis that GMBF would increase after exercise, compared with resting.

3.5. RQ1. What is the regional localization of GMBF changes?

At the exploratory, voxel-wise analysis, no statistically significant effects of group, group or of the group session interaction were found.
Fig. 3. Subject-level pre- to post-test changes on n-back performance at different loads. The figure represents the changes on n-back performance at different cognitive loads (1-back, 2-back, 3-back) in the exercise (upper panel) and resting (lower panel) groups, before (pre-test) and after (post-test) the group. The group’ load’ session interaction was not statistically significant (p = .466).

Table 3
Effects of group and session on GMBF.

<table>
<thead>
<tr>
<th></th>
<th>C.E.</th>
<th>S.E.</th>
<th>t</th>
<th>Adj. sig.</th>
<th>C.I. 95% (min, max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GROUP, F(1,88)=0.009; p=.524</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Exercise vs Resting</td>
<td>0.157</td>
<td>0.096</td>
<td>0.924</td>
<td></td>
<td>−3.116, 3.341</td>
</tr>
<tr>
<td>SESSION, F(1,88)=1.687; p=.197</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-test vs Pre-test</td>
<td>−0.71</td>
<td>0.543</td>
<td>−1.308</td>
<td>0.194</td>
<td>−1.788, 0.368</td>
</tr>
<tr>
<td>† GROUP*SESSION, F(1,88)=6.574; p=.012</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise, post- vs pre-test †</td>
<td>−2.122</td>
<td>0.784</td>
<td>−2.706</td>
<td>0.008</td>
<td>−3.681, −0.564</td>
</tr>
<tr>
<td>Resting, post- vs pre-test</td>
<td>0.688</td>
<td>0.734</td>
<td>0.937</td>
<td>0.351</td>
<td>−0.771, 2.146</td>
</tr>
</tbody>
</table>

C.E., contrast estimate; S.E., standard error; df, degrees of freedom; C.I., confidence intervals; †, statistically significant.

Fig. 4. Effect of the group’ session interaction on grey matter blood flow. The box plots represent GMBF in the exercise (left) and resting (right) groups, before (pre-test) and after (post-test) the exercise or resting sessions. GMBF had a statistically significant decrease at post-test compared with pre-test in the exercise group (p = .008), but not in the resting group (p = .351). Circles represent the mild outliers, defined as values lying between 1.5 and 3 interquartiles range (IQR).
The cluster showing a stronger interaction effect was located in the left inferior parietal gyrus (IPL), extending to the medial prefrontal cortex (p FWE-corr = 0.393, cluster extent: 179 voxels, MNI coordinates: −60, −56, 12).

3.6. H3. Changes in mean GMBF will correlate with working memory performance changes more strongly in the exercise group than in the resting group

The pre- to post-test change on average n-back performance was not correlated with the pre- to post-test difference on GMBF, nor was an effect of the group*GMBF difference found statistically significant. In the GLMM, a statistically significant group*GMBF interaction effect was found, indicating an overall difference in the regression effect between groups (Table 4). In particular, GMBF was inversely associated with n-back performance in the exercise group, and positively associated in the resting group (Fig. 5a). A statistically significant effect of the session*GMBF interaction was not statistically significant; nonetheless, in the post-hoc tests, a statistically significant change in the association between GMBF and n-back performance from pre- to post-test was detected in the exercise group (Fig. 5b) but not in the resting group (Fig. 5c). Thus, the between-group difference observed in the previous group*GMBF interaction seems to be mostly driven by the exercise-induced inverse association between GMBF and performance at post-test. Our hypothesis that changes in mean GMBF would correlate with working memory performance more strongly in the exercise group than in the resting group were thus partly supported by the data, although we expected the association to be positive rather than negative.

3.7. RQ2. Is the correlation between GMBF and working-memory performance specifically localised?

A statistically significant negative correlation was found between the mean n-back performance and post-test GMBF in the right hippocampus (cluster extent=1488 voxels; p FWE-corr=0.001; F = 62.3; MNI coordinates (x,y,z)= 32, −38, −6) and left medial frontal cortex (cluster extent=733 voxels; p FWE-corr=0.017; F = 35.3; MNI coordinates (x,y,z)= −8, 26, −14), largely extending to the contralateral orbitofrontal cortex in the exercise group only (Table 5; Fig. 5d).

3.8. H4. Changes in pulse pressure will correlate with GMBF changes, more strongly in the exercise group than in the resting group

No effects of either pulse difference or group*pulse pressure interaction on GMBF pre- to post-test change were found. At the mixed model analysis, no statistically significant effects of pulse pressure*group, pulse pressure*session or pulse pressure*group*session interactions were found on GMBF. The data did not support our hypothesis that changes in pulse pressure would correlate with GMBF changes more strongly in the exercise group compared with the resting group.

3.9. RQ3. Is VO2 max associated with mean GMBF?

No effects of either VO2 max or group*VO2 max interaction were found on the pre- to post-test GMBF change. At the GLMM, a statistically significant effect of the group*VO2 max interaction on GMBF was found; in particular, a stronger inverse association between GMBF and VO2 max was observed in the exercise group compared with the resting group (Table 5). A statistically significant effect of the session*VO2 max was also detected; however, post-hoc tests were not statistically significant, likely due to the small difference in the association between pre- and post-test. On the other hand, despite the interaction between group*session*VO2max was not statistically significant, the post-hoc test did nonetheless show a statistically significant difference between groups in the pre- to post-test regression effect (Table 5). In particular, the regression coefficient changed from pre- to post-test in the exercise group, while it remained stable in the resting group (Fig. 6).

4. Discussion

We assessed the effects of acute moderate intensity physical exercise on WM performance and GMBF in 23 older individuals, compared with 23 subjects who relaxed between the measurements. Confirmatory analyses were carried out to confirm our hypotheses concerning exercise-induced changes on n-back performance and GMBF. Overall, cognitive performance decreased with increasing difficulty load regardless of session and group, and slightly increased from pre-test to post-test, independent of group. GMBF decreased from pre- to post-test in the exercise group, but not in the resting group. Correlation analyses between GMBF and average n-back performance, and between pulse pressure and GMBF, consisted of a confirmatory part testing for associations between

| Table 4 |
| Assumption between GMBF and n-back performance. |
| | Coeff. | S.E. | t | Sig. | C.I. 95% (min, max) |
| † Group GMBF, F(1,60)=16.674; p<.001 | 0.043 | 0.0099 | −4.284 | <0.001 | −0.062, −0.023 |
| † Exercise vs Resting † | −0.043 | 0.0099 | −4.284 | <0.001 | −0.062, −0.023 |
| † Session-GMBF, F(1,60)=4.229; p=.044 | −0.016 | 0.0069 | −2.331 | 0.023 | −0.030, −0.002 |
| Post-test vs pre-test † | −0.016 | 0.0069 | −2.331 | 0.023 | −0.030, −0.002 |
| Group*session-GMBF, F(1,60)=2.759; p=1.102 | −0.017 | 0.0068 | −2.439 | 0.020 | −0.030, −0.003 |
| Exercise group, post- vs pre-test † | −0.017 | 0.0068 | −2.439 | 0.020 | −0.030, −0.003 |
| Resting group, post-test vs pre-test | −0.001 | 0.0052 | −0.219 | 0.829 | −0.012, 0.010 |
| S.E., standard error; †, degrees of freedom; C.I., confidence intervals; †, statistically significant. |

| Table 5 |
| Association between VO2max and GMBF. |
| | Coeff. | S.E. | t | Sig. | C.I. 95% (min, max) |
| † Group VO2max, F(1,84)=4.024; p=.048 | −0.027 | 0.0136 | −1.993 | 0.049 | −0.054, 0.001 |
| † Exercise vs Resting † | −0.027 | 0.0136 | −1.993 | 0.049 | −0.054, 0.001 |
| † Session*VO2max, F(1,84)=0.079; p=.047 | −0.010 | 0.0074 | −1.331 | 0.187 | −0.024, 0.005 |
| Post-test vs pre-test | −0.010 | 0.0074 | −1.331 | 0.187 | −0.024, 0.005 |
| Group*session*VO2max, F(1,84)=2.595; p=.111 | −0.022 | 0.0098 | −2.248 | 0.030 | −0.042, −0.002 |
| Exercise group, post- vs pre-test † | −0.022 | 0.0098 | −2.248 | 0.030 | −0.042, −0.002 |
| Resting group, post-test vs pre-test | −0.003 | 0.0073 | −0.350 | 0.728 | −0.017, 0.012 |
| S.E., standard error; †, degrees of freedom; C.I., confidence intervals. |
pre- to post-test changes on the variables, and of an exploratory (not preregistered) part testing the impact of the group on the association between the variables. The confirmatory analyses showed no statistically significant associations between the pre- to post-test difference in n-back performance and the difference in GMBF. However, the exploratory analyses showed that in the exercise group, lower GMBF was associated with higher n-back performance, while a positive association was found in the resting group. This pattern was likely driven by the inverse association between GMBF and n-back scores emerging in the exercise group at post-test, that was not observed in the resting group, where no change in the association strength was observed. Regional associations were found in the exercise group between lower GMBF in the right hippocampus, left medial frontal cortex and right orbitofrontal cortex and higher average n-back performance. An additional exploratory analysis revealed a negative association between VO2 max and GMBF that was stronger in the exercise group at post-test compared with the resting group.

4.1. Working memory performance

Contrary to our hypothesis, exercise did not influence the level of performance in a statistically significant way, in contrast with some previous reports of immediate positive effects of physical exercise on executive functions and WM (Volkers and Scherder, 2014; Langlois et al., 2013; Alves et al., 2012). Our statistical power is however low and the uncertainties around the estimates are high. We are thus hesitant to
make too much out this null finding, although we note that despite the large body of evidence in some reports in favor of the positive cognitive effects of acute exercise, the evidence is still considered inconclusive (Young et al., 2015; Angervaren et al., 2008; Gasquione and Chen, 2020). Recent meta-analyses of randomized controlled trials have argued that acute bursts of physical activity have minor effects on WM performance at best (effect size: 0.15; 95% CI: −0.33, 0.63) (Rathore and Lom, 2017), and that the suggested beneficial effects of aerobic physical activity are not supported by available evidence in cognitively healthy older adults (Young et al., 2015). However, the studies included in the meta-analysis had considerable heterogeneity in terms of instruments used to assess WM performance, unequal sample sizes, and age of the participants (Rathore and Lom, 2017). Age, in particular, moderates the effects of at least chronic physical activity interventions, with small positive effects (effect size: 0.324; 95% CI: 0.185, 0.463) only observable in individuals over 65 years (Rathore and Lom, 2017). However, older adults are underrepresented in investigations of immediate effects of physical activity on WM performance. The lack of studies investigating the immediate effects of physical activity in older adults might have masked any potential effect of acute physical exercise. Another potential explanation for null findings is that the gains in cognitive performance are not uniform across individuals, being rather influenced by their baseline WM performance (Sibley and Beilock, 2007; Yamazaki et al., 2018).

In particular, cross-over design studies have indicated that individuals starting with lower WM seem to gain the most in terms of cognitive performance after an acute bout of moderate intensity physical activity (Sibley and Beilock, 2007; Yamazaki et al., 2018). Accordingly, several studies reporting improvements in cognitive functions after a physical training program have been carried out in older individuals affected by mild or moderate cognitive impairment (Volkers and Scherder, 2014; Park et al., 2019).

A pivotal role in the inconsistent results across studies might also be played by the length of the exercise protocol (Rathore and Lom, 2017). In fact, the majority of studies have focused on long-term programs (Rathore and Lom, 2017). While these seem to be more efficient than acute physical exercise sessions in exerting beneficial effects on executive functions and cognition, the data relative to acute physical exercise are not sufficient for this claim (Rathore and Lom, 2017). Concerning acute exercise session, aerobic moderate intensity physical exercise lasting at least 20 min has been suggested to exert the most prominent effect on executive function (Chang et al., 2012), particularly in older adults (Ludyga et al., 2016). However, the moderator role played by the intensity and duration of the exercise session (Rathore and Lom, 2017), the time of day when the test is carried out, as well as by the age and fitness level of the participants, has to be noted (Chang et al., 2012; Ludyga et al., 2016). In such regard, our study protocol was designed in order to minimize the confounding effects of these factors. Our participants were matched for age and cardiovascular fitness and there were no differences in the time of day (morning vs afternoon) when the participants in the two groups were tested. We can therefore at least rule out that these known confounders might have impacted on the lack of effect of physical exercise on cognitive performance in our study. However, we cannot exclude that the timing of the post-test assessment of cognitive function might have played a role. In fact, one study has reported that the beneficial effects on cognitive performance start to be evident few minutes after the exercise, reaching their peak 11–20 min after and then starting to subside (Chang et al., 2012). While the post-test WM task started around 12 min after the end of the physical exercise (Fig. 2) and was thus within the expected peak window, the paucity of evidence concerning the timing of the cognitive effects warrants for further investigation on the matter.

Worth noting, relaxation exercises have also been reported to exert positive effects on cognition (Ma et al., 2017; Ferreira et al., 2015; Tang et al., 2007; Gard et al., 2014; Galvin et al., 2006; Siponkoski et al., 2019). Although our control group did not perform any active relaxation exercise (i.e. sustaining attentional focus on their body, breath, imagery or else), they were still instructed to rest and exposed to soothing sounds. This may have contributed to improve their cognitive performance, limiting the power to detect potential beneficial effects of the exercise bout.

4.2. Grey matter blood flow

GMBF was decreased from pre- to post-test in the exercise group only. The literature is quite consistent in suggesting that increases in cerebral blood flow occur during exercise (Querido and Sheel, 2007; Steventon et al., 2020; Kleinloog et al., 2019; Chaddock-Heyman et al., 2016; Joris et al., 2018), despite some scattered conflicting evidence (van der Kleij et al., 2018; Querido and Sheel, 2007). This discrepancy might be partly due to the different protocols of physical exercise used in different studies. The increase in cerebral blood flow in fact mirrors the intensity of the physical exertion up to 60% of maximal oxygen uptake (Ogoh and Ainslie, 2009; Querido and Sheel, 2007); at higher physical exertion, on the other hand, a plateau or even a reduction is reached, due to hyperventilation-induced cerebral vasoconstriction (Ogoh and

Fig. 6. Association between VO2max and GMBF. The figure shows the pre- to post-test change in the association between VO2 max and GMBF (a) in the exercise group (B=−0.653, p=0.009 at pre-test; B=−0.920, p<0.001 at post-test) and (b) in the resting group (B=−0.256, p=0.246 at pre-test; B=−0.286, p=0.213 at post-test). Coefficients were calculated separately in each group and session using linear regression.
Ainslie, Querido and Sheel, 2007). In fact, the hypocapnia induced by hyperventilation, coupled to the sympathetically-mediated protective mechanisms opposing excessive increases in cerebral blood flow, triggers cerebral vasoconstriction at high physical exertion (Querido and Sheel, 2007). Even at moderate intensity, already a few seconds after the cessation of the exercise bout, the cerebral blood flow starts to decrease due to post-exercise hypotension (Querido and Sheel, 2007). In addition to the post-exercise drop in cardiac output, this leads to a transient decrease in GMBF immediately after exercise (Querido and Sheel, 2007). According to other previous studies using ASL to assess blood flow in a supine position, this transient decrease in GMBF is still observable up to ten minutes after the completion of a single bout of aerobic physical exercise in young adults, resuming baseline levels forty minutes post-exercise (MacIntosh et al., 2014). In fact, the decrease in cerebral perfusion and the resulting hypoxia, as well as the normalization of the cardiac output after exercise cessation, induce a later re-boost in GMBF (Querido and Sheel, 2007; MacIntosh et al., 2014). Our findings indicate that the transient decrease on GMBF shortly after an acute bout of moderate intensity aerobic exercise is maintained in older age.

It is unlikely that any sustained effect of switching from a standing position to a lying position might have influenced the blood flow measurements in the scanner, as the time allowed between changing body posture and GMBF measurements should have been enough for the autoregulation of blood flow to occur (at least two minutes for preparing the subject in the scanner plus localizer acquisition). Studies measuring MCA-velocity have in fact indicated that around two minutes may be sufficient for autoregulatory mechanisms (Haubrich et al., 2004).

4.3. Associations between task performance, GMBF, and cardiovascular variables

While the pre- to post-test difference in GMBF did not correlate with the improvement on the n-back performance, the groups exhibited different correlational patterns between GMBF and average n-back performance over time. Consistently with previous literature, in the resting group higher GMBF was associated with better n-back performance. Previous studies performed in older adults using ASL, in fact, have reported cross-sectional positive associations between global cerebral blood flow and executive functions (Leeuwis et al., 2018), and GMBF has been reported to also predict longitudinal changes on fluid intelligence (De Vis et al., 2018). On the other hand, lower GMBF was associated with higher n-back performance in the exercise group. This finding may be ascribed to be the inverse association between GMBF and n-back emerging in the exercise group at post-test. Lower GMBF was thus associated with higher task accuracy after exercise, contrary to our expectations.

Most of the previous research carried out with ASL reporting positive associations between cerebral blood flow and cognitive performance was based on longer physical training interventions, lasting several weeks (Chapman et al., 2013). When a single bout of physical activity is performed, the relationship between cognitive performance and cerebral perfusion seems to progressively uncouple during exercise (Lucas et al., 2012). Indeed, the exercise-induced temporal dynamics of cerebral perfusion changes might help us elucidate the observed inverse relationship between n-back performance and GMBF after exercise. As mentioned above, we measured GMBF within the timeframe for the transient post-exercise reduction in cerebral blood flow. If we assume that lower GMBF in this context is indicative of higher cerebrovascular reactivity, as supported by the presence of the intercurrent negative correlation between GMBF and VO2 max found at the exploratory analysis, its association with task performance is then less surprising. In fact, higher cardiovascular fitness was associated with lower GMBF, and the association was stronger after exercise compared with resting. Negative associations between VO2 max and GMBF have been reported before in young adults (Foster et al., 2020; Furby et al., 2019), and possibly in healthy older adults (Intzandt et al., 2019). The strengthening of the association after exercise is consistent with the notion that a well-functioning cerebrovascular reactivity, as assessed with the combined use of Doppler and PET(CO2), is protective against cerebrovascular diseases associated with ageing (Murrell et al., 2013). Cerebral vasodilator responses to hypercapnia, as measured by transcranial Doppler, are also associated with higher cardiovascular fitness and VO2 max in healthy older adults (Barnes et al., 2013). In this framework, we might expect individuals with lower GMBF after exercise to have experienced a higher boost in flow following initial vasoconstruction. If we had measured GMBF a few minutes later, we might have then been able to observe a reversed association of GMBF with task performance, as reported in previous literature. Supporting this hypothesis, we have also found better post-test n-back performance to be unexpectedly associated with lower, rather than higher, post-test GMBF in the exercise group in the hippocampal and orbitofrontal regions, both well-known to be positively related with better WM (Barbey et al., 2011; Loprinzi et al., 2019; Nissim et al., 2016) and exercise-related effects on cognitive performance (Basso and Suzuki, 2017; Chapman et al., 2016; Den Ouden et al., 2018; Firth et al., 2018; Erickson et al., 2011; Brockett et al., 2015). Nonetheless, it must be noted that we did not have a direct measure of cerebrovascular reactivity, and only early measurements on GMBF were available. Though based on well-established literature on exercise-related cerebrovascular reactivity (Lucas et al., 2012; Foster et al., 2020; Furby et al., 2019; Intzandt et al., 2019; Murrell et al., 2013; Barnes et al., 2013), the proposed explanation is thus to be considered speculative.

We had hypothesized that pulse pressure would also be positively related to the GMBF. In fact, while persistently elevated pulse pressure has been related with arterial stiffness (Safar et al., 2011) and cerebrovascular events (Thorin-Trescases et al., 2018), when maintained within the physiological range pulse pressure contributes to an adequate cerebrovascular perfusion. The relationship between blood pressure and brain health is indeed quite complex in old age (Shang et al., 2016; Forte et al., 2019; McDeade et al., 2016), when low systolic blood pressure can be as detrimental for the brain (Foster-Dingley et al., 2015; Muller et al., 2010) and cognition (Shang et al., 2016; Forte et al., 2019; McDeade et al., 2016; Mossello et al., 2015) as high blood pressure. Indeed, individuals with Alzheimer’s Disease have lower pulse pressure and cerebral hypoperfusion compared with controls (Roher et al., 2012). Moreover, low pulse pressure, particularly when combined with low cerebral blood flow, has been associated with cortical (Muller et al., 2010) and subcortical (Foster-Dingley et al., 2015) atrophy. The relationship between pulse pressure and GMBF in our sample was however somewhat stronger in the resting group, rather than in the exercise group as we expected. Considering the GMBF reduction observed in the exercise group post-test, it is not surprising that such relationship would be weaker in this group. Given the complexity of the relationship between pulse pressure and GMBF, further studies will need to further elucidate this association.

4.4. Limitations

Some limitations in our study have to be acknowledged. First of all, the limited sample size did not allow for a stratification of the participants according to their baseline WM capacity, which has been indicated to moderate the gains in cognitive performance induced by physical exercise (Sibley and Bellocq, 2007; Yamazaki et al., 2018). Our protocol did not include a delayed measurement of GMBF, thus preventing us to fully describe the trajectory of blood flow changes after exercise. Moreover, the signal-to-noise ratio is inherently low for ASL acquisitions, as the signal from the labeled inflowing blood constitutes only 0.5–1.5% of the full tissue signal. Furthermore, arterial stiffness was not evaluated (Petcharunpaisan et al., 2010). Concerning the exercise protocol, measurements of blood gases during exercise were not acquired, potentially allowing for too high intensities of exercise being reached. Also, dehydration is known to affect cerebral blood flow measurements during exercise (Trangmar et al., 2015). Our participants were allowed to drink
as much water as they wanted. Though most of them only consumed the amount filling a regular gym water bottle (500 ml), their water intake was however not noted, and could not be controlled for in the GMFB analyses. Finally, the data were acquired prior to the prerегистration. Nonetheless, we had not looked into the data before preregistering the study hypotheses.

4.5. Conclusions

A decrease in GMFB was observed in healthy older adults shortly after a single session of 30 min of physical exercise at moderate intensity, compared with age and VO2 max-matched individuals relaxing for 30 min. This confirms and extends previous findings of transient decreases in GMFB after exercise observed in young adults. Higher cardiovascular fitness was also associated to lower GMFB after exercise, potentially supporting a link between cardiovascular fitness and cerebral vascular reactivity in older age. While WM performance was not affected by exercise, task accuracy was inversely associated with GMFB in the exercise group at post-test, more specifically with blood flow in the hippocampus and frontal regions, majorly affected by age-related decreases in perfusion and more sensitive to the effects of moderators. This would support the notion that the cognitive improvements attributed to exercise may depend on the beneficial effects on cerebralvascular reactivity, although longer interventions and plasticity mechanisms may be required to be put in place before they become appreciable. It also suggests that immediate effects of acute physical exercise might not be uniformly generalized to healthy old adults, but might rather be modified by other factors, such as baseline cognitive performance or genetic risk for accelerated cognitive decline and dementia.

Declaration of Competing Interest

The authors declare no conflicts of interest to disclose.

CRediT authorship contribution statement

Gaia Olivo: Formal analysis, Writing - original draft, Writing - review & editing. Jonna Nilsson: Conceptualization, Methodology, Writing - review & editing. Benjamin Garzón: Conceptualization, Methodology, Writing - review & editing. Alexander Lebedev: Conceptualization, Data curation, Methodology, Writing - review & editing. Anders Wåhlin: Methodology, Writing - review & editing. Olga Tarassova: Data curation, Methodology, Writing - review & editing. Maria Ekblom: Conceptualization, Data curation, Methodology, Funding acquisition, Writing - review & editing. Martin Lövdén: Conceptualization, Data curation, Methodology, Funding acquisition, Writing - review & editing.

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Supplementary materials

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